Environmental Sciences Europe

Open Access

Check for updates

Food-related exposure to systemic pesticides and pesticides from transgenic plants: evaluation of aquatic test strategies

Rebecca Bundschuh[1,4], Mirco Bundschuh[1,2], Mathias Otto[3] and Ralf Schulz[1*]

Abstract

The aquatic Environmental Risk Assessment (ERA) for pesticides relies on standardized experimental protocols focusing on exposure via the water phase or the sediment. Systemic pesticides (e.g., neonicotinoids) or pesticides produced in transgenic plants (e.g., *Bt* proteins) can be introduced into aquatic ecosystems as part of plant residues. Consequently, they may be taken up by organisms as part of their diet. Here, we analyzed (i) whether standardized aquatic ecotoxicological test guidelines consider an exposure route via food and (ii) whether these tests can be easily modified to take this exposure route into account. From the 156 existing test guidelines, only those for fish and amphibians partly consider a potential route of uptake via food. From the remaining invertebrate guidelines, those focussing on chronic endpoints may be most suitable to cover this exposure path. We suggest assessing the food-related effects of systemic pesticides in a dose-dependent manner using standardized guidelines or methods developed from peer-reviewed literature. For transgenic plants, spiking uncontaminated leaf material with increasing concentrations of the test substances would allow to test for dose responses. After adaption to oral uptake, standard test guidelines currently available for the ERA appear, in principle, suitable for testing effects of systemic pesticides and transgenic plants.

Keywords: Risk assessment, Aquatic ecosystems, Genetically modified organisms, GMO, RNAi, *Bt* protein, Ecotoxicity

Background

The current aquatic Environmental Risk Assessment (ERA) for pesticides involves a tiered approach of acute and chronic exposure scenarios under well-controlled laboratory, semi-field or field conditions [1]. On this basis, a predicted no-effect concentration (PNEC) is derived by dividing the concentration of the product (i.e., its active ingredient) causing a defined effect in the respective environmental medium by a so-called assessment factor. The latter usually decreases with increasing biological complexity of the experimental design assuming that more complex systems do more likely represent the actual situation in the field reducing uncertainty [sensu 2].

In the course of this process, environmental risk assessors rely amongst others on standardized experimental protocols—especially during the first tier. These protocols have been developed to assess potential adverse effects caused by an exposure via the water phase [e.g., 3] or the sediment [e.g., 4]. The use of systemic pesticides or transgenic plants in agriculture, however, expands the relevant exposure pathways for some species that need to be considered during ERA. These exposure pathways relate to the presence of toxicants in the plant's tissues. Systemic insecticides are absorbed via cuticula or root system and distributed in the plant [5]. In transgenic plants, insecticidal proteins such as *Cry* or *Vip* proteins that originating from *Bacillus thuringiensis* (thus, often summarized as *Bt* proteins in the following) [6] or RNAi, which silence vital genes in the target organism, can be translated from

*Correspondence: schulz@uni-landau.de
[1] Institute for Environmental Sciences, University of Koblenz-Landau, Fortstrasse 7, 76829 Landau, Germany
Full list of author information is available at the end of the article

Bundschuh *et al. Environ Sci Eur*   (2019) 31:87

Page 2 of 13

the modified genes [7–10]. These options are the most relevant genetically modified organism applications for pest control. Both systemic pesticides and insecticidal proteins or RNAi from transgenic plants are designed to cause damage in target organisms after ingestion as some pests such as the larval stages of stem borers cannot be exposed to pesticides by direct spraying. For the risk assessment, the presence of these toxicants in the plant tissue implies that they can be introduced into aquatic ecosystems [11–15] and may subsequently leach into the water phase [12, 13, 16] or be taken up by detritivores as part of their diet [11, 16, 17]. Despite the fact that negative effects in aquatic invertebrates such as stone-, crane-, or caddisflies, and amphipods from the consumption of plant material have been described for both systemic insecticides [16–19] and *Bt* plants [11], the uptake of these toxicants with the diet seems not properly covered in standardized experimental protocols currently available and used in regulatory risk assessment.

The present study provides in a first step a detailed evaluation of pathways relevant to assess effects on aquatic life caused by systemic pesticides and *Bt* protein or RNAi from transgenic plants. In a second step, the suitability of current standardized experimental protocols to address these pathways is analyzed, experimental adaptation that may be needed is discussed, and suggestions for the development of target orientated experimental designs are provided.

Identification of relevant exposure pathways

Pesticides considered as systemic can be taken up by the crop plant and are distributed within this plant (see Fig. 1; [5]). Besides spraying or drenching on agricultural fields, which leads to an exposure pattern in aquatic ecosystems dominated by spray drift and surface run-off [20], seed treatment is another prevailing type of application [21]. This means that the seeds are coated with pesticides prior to planting and from there, the pesticides are released into the soil where they are taken up by plant roots [22] and distributed within the plant during growth [cf. 23]. However, not the entire amount of pesticides associated with the seeds is taken up by the crop plant and parts of it may remain in the soil and, although subject to degradation [24], may accumulate over time [25]. From soils treated with pesticide via this pathway, non-target plants from adjacent (non-agricultural) ecosystems may also take up these pesticides [21]. As pollen from target plants may carry a pesticide load [26], deposition of pollen may be another exposure path for
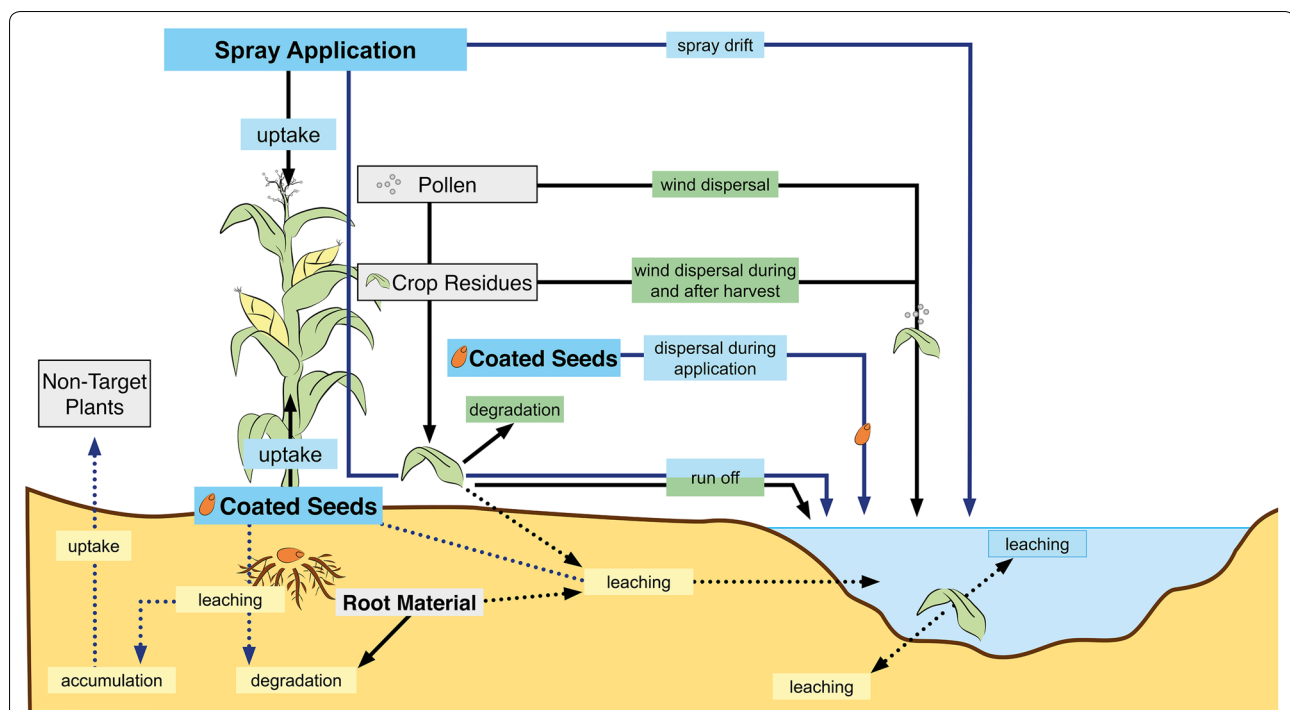

Fig. 1 Fate of systemic pesticides and *Bt* proteins. Schematic diagram on the fate of systemic pesticides (blue boxes) directly following their application (blue solid lines) and released from the coated seed (blue dotted lines). Fate of systemic pesticides and *Bt* proteins as part of plant material (green boxes in combination with black solid lines) and after degradation in water and soil (dotted black lines). Please note, that following uptake into non-target plants, the respective plant material (leafs) may be a source of exposure

Bundschuh *et al. Environ Sci Eur* (2019) 31:87

Page 3 of 13

systemic insecticides. Following the harvest or abscission [21], plant residues, and leaf litter [16], which may contain measurable concentrations of the systemic pesticides [27], can be transferred into freshwater ecosystems via wind or surface run-off. Leaching of the pesticides retained in the plant biomass may expose and affect organisms feeding on the suspended or deposited pollen and leaf material (detritus) [16, 17]. Since the sowing of seeds coated with systemic pesticides is not considered as a pesticide application procedure, regulating measures such as obligatory distances from surface waters do not apply and surface waters may be directly exposed to seed-coating toxicants during sowing. Together pollen, plant residues and coated seed represent—in addition to exposure to the pure toxin in the water phase—additional exposure paths, which should be considered in the ERA.

With the exception of seed coating and spray drift, exposure pathways of systemic insecticides and *Bt* proteins from GM crops are largely similar [see 11, 12, 14]. Figure 1 provides an overview of exposure pathways of aquatic systems to systemic insecticides and insecticidal *Bt* proteins from GM crops.

Those pathways are, however, not explicitly considered in the testing regimes of either the ERA of pesticides [cf. 28] or transgenic plants [14, 29]. Since test methods to evaluate effects from *Bt* plants on non-target organisms are derived from pesticide testing [30], it is sensible to evaluate the currently employed test guidelines for their general suitability to cover the exposure pathway of toxicants via plant material.

Toxic mode of action

The mode of toxic action of chemical pesticides is described in detail, for instance in Stenersen [31], which is, however, not necessarily determined by the pesticides systemic or non-systemic nature. The major difference between systemic and non-systemic substances is the potential of systemic pesticides to be taken up by and distributed within the treated plants [5]. This might lead to the overlooked exposure pathway for non-target species via the food [13].

Similar to systemic insecticides, *Cry* or *Vip* proteins are present within the plant tissues and designed to express toxicity after being ingested by the target organism. Compared to systemic pesticides, *Bt* proteins or RNAi approaches for pest control are supposed to be more specific and have a tighter spectrum of insecticidal activity. This specificity can be influenced by a number of processes such as the proteolytically transformation of *Cry* proteins to toxins in the insect gut and binding to receptors (such as aminopeptidases, involved in cell adhesion

and digestion) in the midgut [32]. Although the specificity of *Bt* proteins is challenged [33, 34] and the molecular mechanisms of the modes of action of *Bt* proteins are not fully understood [35, 36], differences in the activity spectrum of systemic insecticides, *Bt* proteins and even more so for RNAi constructs are obvious. Despite the joint potential to expose non-target species via their food, systemic pesticides and *Bt* proteins may require a different testing strategy to adequately reflect their respective characteristics. For systemic pesticides, for instance, it may be sufficient to understand the consequence of the food-related exposure path relative to the classical water phase exposure of sensitive aquatic species.

For the identification of potentially sensitive species, research and regulation can rely on published data though the overwhelming majority of publications only deal with waterborne exposure [see as an example 17]. For *Bt* proteins in contrast, studies identifying sensitive aquatic species are very limited [14].

Standardized test guidelines

To date, standardized test guidelines have not been developed specifically for systemic pesticides or transgenic plants [37]. To evaluate, therefore, the existing standardized test guidelines for their current and potential ability to cover the exposure paths via food, the respective documents used during the ERA of pesticides were compiled. The present study exclusively focuses on test protocols for freshwater organisms including amphibians.

In detail, approved guidelines—or those being in the progress for standardization—published by the Organization for Economic Co-operation and Development (OECD), the Official Journal of the European Communities (EU), the International Organization for Standardization (ISO), the American Society of Testing and Materials (ASTM), the Ministry of Agriculture, Forestry and Fisheries (MAFF) and the Environmental Protection Series published by Environment Canada (EC) provided the basis for the present evaluation. In addition, guidelines published by Office of Chemical Safety and Pollution Prevention (OCSPP) within the United States Environmental Protection Agency (EPA) were taken into account. For each of the 156 test guidelines considered (see Additional file 1: Table S1), the taxon of the test species as well as the test design, including the assessed endpoints, the study duration and the experimental conditions were abstracted (Table 1). On this basis, the suitability of the guidelines to cover the exposure path via the food was evaluated. Where possible, we suggest amendments to or alterations in the guideline that allow integrations of food intake as an exposure path.

Bundschuh *et al. Environ Sci Eur*   (2019) 31:87

Page 4 of 13

Table 1 Test guidelines for pesticides separated by systematic groups of test organisms with information on the number of test guidelines available for laboratory, microcosm and field experiments, the range of study durations, and the test item application

Systematic group	Number of guidelines			Study duration	Application
	Laboratory	Microcosms	Field		
Algae, cyanobacteria and macrophytes	24	2	1	24 h–14 days	Dissolved in water or spiked in sediment
Bacteria and microorganisms	15	0	0	0.5 h–3 h	Dissolved in water
Rotifers	4	2	0	24 h–63 days	Dissolved in water
Crustaceans	44	3	0		
Daphniidae	31	3	0	24 h–42 days	Dissolved in water
Gammaridae	5	0	0	48 h–8 days	Dissolved in water
Dogielinotidae	9	2	0	96 h–56 days	Dissolved in water or spiked in sediment
Cyprididae	1	2	0	6 days–63 days	Dissolved in water or spiked in sediment
Ostracoda	1	2	0	6 days–56 days	Dissolved in water or spiked in sediment
Decapoda (Cambridae sp., Astacidae sp. or Parastacidae sp.)	6	1	0		Dissolved in water
Atyidae	1	0	0	1 h–96 h	Dissolved in water
Thamnocephalidae	1	0	0	1 h–24 h	Dissolved in water
Insects	17	1	0	48 h–65 days	Dissolved in water or spiked in sediment
Diptera	15	0	0	48 h–65 days	Dissolved in water or spiked in sediment
Ephemeroptera	9	1	0	48 h–28 days	Dissolved in water or spiked in sediment
Plecoptera	3	0	0	24 h–8 days	Dissolved in water
Trichoptera	1	0	0	At least 21 days	Dissolved in water and/or in the diet
Sediment-dwelling	6	0	0	96 h–28 days	Spiked in sediment
Molluscs	5	1	1	24 h–30 days	Dissolved in water
Fish	54	1	0	24 h–120 days or full life cycle	Dissolved in water or spiked in food
Amphibians	7	0	0	48 h–30 days	Dissolved in water or spiked sediment
Others	4	3	1		

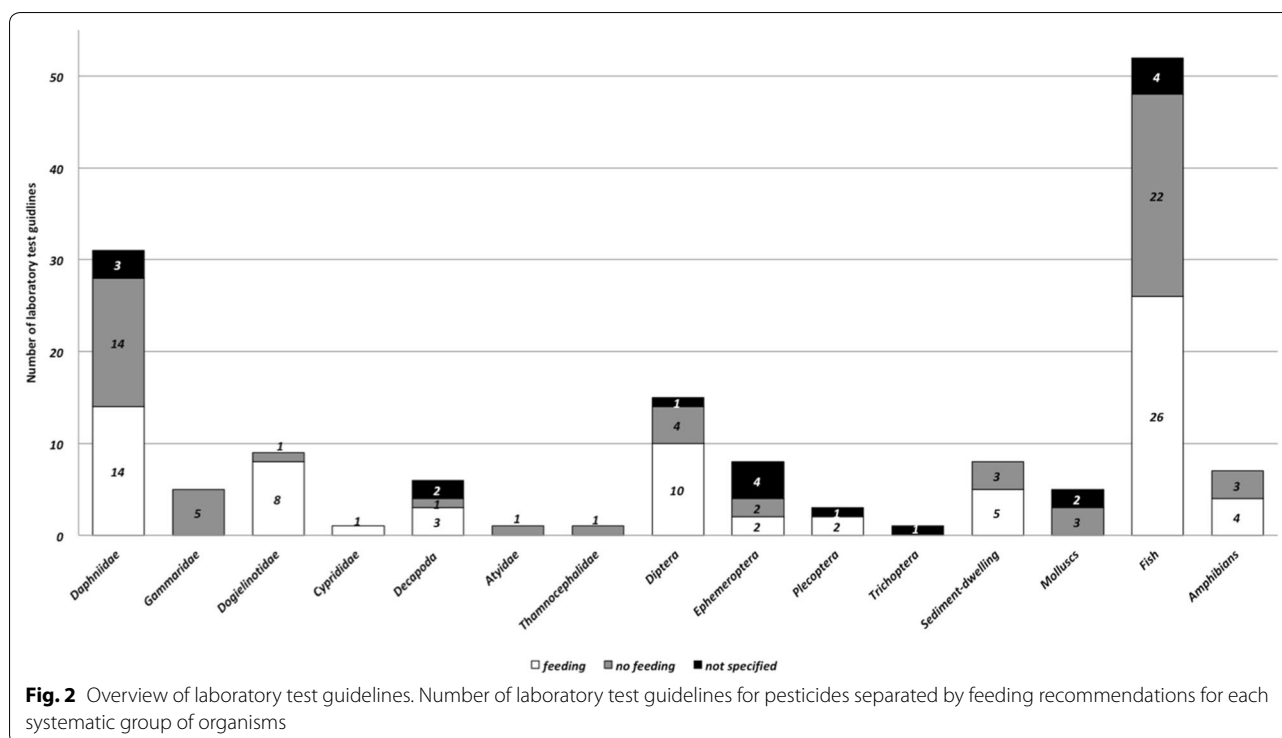## Analyses of standardized test guidelines

General characteristics of the guidelines

Most of the 156 evaluated test guidelines (Additional file 1: Table S1) describe single-species laboratory tests (148), whereas four are laboratory microcosms studies. Two recommend outdoor mesocosm or in situ studies, respectively, and two documents cover actual field studies (Table 1). Nine test methods evaluate the bioaccumulation of chemicals. As some guidelines describe test procedures for more than one species or trophic level, the overall sum of the described test procedures is exceeding the number of the evaluated test methods. We evaluated for each systematic group whether the test organisms' route of exposure occurs via the water phase or the ingestion of potentially contaminated food. For reasons of completeness, we also included autotrophic organisms in our evaluation, since they may act as carrier of contaminant entry into the food web. Moreover, we provide an overview on the guidelines major route of exposure (Table 1) and the number of test guidelines per systematic group that feeds the

test species during testing (Fig. 2) providing a potential avenue for adaptation to cover an exposure pathway via the food.

Algae, cyanobacteria and macrophytes

In total, 27 test guidelines have been identified that describe test procedures for algae, cyanobacteria and macrophytes, of which 24 are focusing on laboratory experiments with an acute or chronic experimental duration (Additional file 1: Table S1) ranging from 2 to 14 days. As neither algae nor cyanobacteria or macrophytes actively ingest plant residues containing either systemic pesticides or *Bt* proteins, the main exposure path for primary producers is via the water phase and hence as a result of spray drift and run-off from agricultural fields or leaching of the pesticides or *Bt* proteins from the plant residues. It should, however, not be ignored that the systemic pesticides or *Bt* proteins can adsorb to the surface of algae, cyanobacteria and macrophytes that are finally consumed by higher trophic levels not covered in these test guidelines. This

Bundschuh *et al. Environ Sci Eur*   (2019) 31:87

Page 5 of 13

Fig. 2 Overview of laboratory test guidelines. Number of laboratory test guidelines for pesticides separated by feeding recommendations for each systematic group of organisms

can ultimately provide an additional exposure path for these stressors along the aquatic food web.

Bacteria and microorganisms
Standardized experimental guidance for the testing of bacteria and microorganisms is available in 15 different documents (Additional file 1: Table S1), while six of these guidelines refer to the utilization of representatives from the class of Gammaproteobacteria (*Pseudomonas putida*, *P. phosphoreum* and *Salmonella typhimurium*) and the remaining nine refer to an undefined composition of microorganisms summarized as "activated sludge". The recommended test duration is between less than 1 h and 3 days, while mainly growth and respiration are assessed. Similar to algae, cyanobacteria and macrophytes, these microorganisms may mainly experience an exposure towards the systemic pesticides and *Bt* proteins via the water phase.

However, some microorganisms such as aquatic fungi, which are not covered by any of the test guidelines used during the ERA [38], can colonize allochthonous organic material [39] including crop plant residues. As a result of their activity (e.g., growth, release of enzymes) structural carbohydrates are degraded [40], which likely increases the leaching rate of systemic pesticides and *Bt* proteins into the water. Thus, the exposure concentrations may be higher for leaf-associated relative to pelagic microorganisms. In this context, one group of systemic pesticides, namely systemic fungicides, may be a particular concern

as they can directly affect the leaf-colonizing fungal community with knock-on effects for the whole heterotrophic food web [sensu 41, 42] [but see 43].

Rotifers
The next higher trophic level is represented by the rotifer *Brachionus calyciflorus*, which is the recommended test species in three single-species test guidelines (Additional file 1: Table S1) covering acute and chronic exposure durations of up to 48 h as well as one of the various rotifer test species in two guidelines employing laboratory microcosms with a duration of up to 63 days. As the *B. calyciflorus* is mainly feeding on algae [44], an exposure to systemic pesticides and *Bt* proteins via the ingestion of food seems relatively irrelevant, although pesticides can be taken up in algae and delivered to rotifers after ingestion. However, since *B. calyciflorus* is a suspension feeder [44], it is feasible that the species ingests pollen or fine particles from crop plant residues when present in the water column. Hence, amending the test guidelines to also consider potential implications of systemic pesticides and *Bt* proteins contained in these plant parts may be feasible and could assist ERA (Table 2). In this context, Bøhn et al. [45, 46] have developed a procedure to generate fine particles from leaf material, which ensures a lower sedimentation rate of the particles, and hence represents a worst-case scenario for the test species.

Table 2 Overview of relevant systematic groups assessed via standardized test guidelines in the laboratory indicating their current and potential ability to cover the exposure paths via food, the most relevant pathway for effects of systemic pesticides or transgenic plants

Systematic group	Suggested max. study duration (days)	Ability to assess for effects as a consequence of exposure through food
Rotifers	63	Adaptation possible through the application of fine particles (pollen and ground leaf material) as food
Crustaceans		
Daphniidae	21	Adaptation possible through the application of fine particles (pollen and ground leaf material) as food Need to revisit validity criteria
Gammaridae	8	Adaptation possible through the application of leaf litter as food Need to revisit exposure duration and response variables
Dogielinotidae	56	Adaptation possible through the application of leaf litter as food
Insects		
Diptera	65	Adaptation possible through the application of fine particles (pollen and ground leaf material) as food
Ephemeroptera	28	Adaptation possible through the application of fine particles (pollen and ground leaf material) as food if relevant for the feeding group of the selected species Need to revisit exposure duration and response variables depending on species life cycle
Plecoptera	8	Adaptation possible through the application of fine particles (pollen and ground leaf material) as food if relevant for the feeding group of the selected species Need to revisit exposure duration and response variables depending on species life cycle
Trichoptera	21	Adaptation possible through the application of fine particles (pollen and ground leaf material) as food if relevant for the feeding group of the selected species Need to revisit exposure duration and response variables depending on species life cycle
Molluscs	30	Adaptation possible through the application of fine particles (pollen and ground leaf material) as food Need to revisit exposure duration and response variables depending on species life cycle

The list focuses exclusively on chronic test guidelines considering the likely high relevance of sublethal effects

Crustaceans

A total of 47 guidelines were identified that describe standardized ecotoxicological methods to assess the effects of stressors on crustaceans (Additional file 1: Table S1) involving study durations of up to 65 days. The largest number of guidelines is available for the filter-feeding representatives of the family Daphniidae (Crustacea: Branchiopoda). Also, for daphnids, acute and chronic experimental designs are available. The descriptions for acute toxicity tests are nearly identical irrespective of which institution has published the document. Although this indicates a high consensus regarding the optimal experimental design, the current acute toxicity test guidelines exclusively focus on the exposure via the water phase and even avoid feeding the test species during the course of the experiment. This procedure is similar to the guidelines describing acute testing procedures for the two families Atyidae (i.e., *Neocaridina denticulata* and *Paratya compressa improvisa*) and Thamnocephalidae (i.e., *Thamnocephalus platyurus*) [e.g., 47, 48], which feed amongst others on algae. However, during chronic testing, daphnids are fed with algae (Fig. 2), which are non-selectively ingested by the filter-feeding test species if retained by their filter apparatus [49]. Therefore, and similar to the situation for the rotifers, it is feasible to modify the testing regime that either pollen or fine

particles originating from the crop plant residues can be ingested and tested (Table 2). In fact, Bøhn et al. [45, 46] have fed daphnids fine particles originating from genetically modified maize plants expressing the insecticidal protein *Cry*1Ab and compared their response with the isogenic counterpart. Although the reproduction of *Daphnia magna* was substantially lower relative to a control situation where algae served as food, the study indicated a negative impact of the *Cry*1Ab proteins on the reproductive performance of daphnids [45]. These insights were supported by follow-up studies showing adverse effects in the survival, fecundity and population growth rate of *D. magna* when fed with fine particles generated from genetically modified maize [46, 50]. These studies, hence, indicate the general feasibility to modify the experimental design of chronic tests with daphnids to cope with the challenges specific for the assessment of systemic pesticides and *Bt* proteins [see also 51] contained in plant material. At the same time, they also highlight that commonly employed validity criteria regarding the reproductive output may not be met if fine particles are provided as food source, as they usually exhibit a relatively low nutritious quality [45, 50].

In contrast to the filter feeders, representatives of the families Gammaridae (e.g., *Gammarus pulex* or *G. fossarum*), Dogielinotidae (i.e. *Hyalella azteca*) and

Bundschuh *et al. Environ Sci Eur*   (2019) 31:87

Page 7 of 13

Cyprididae (i.e., *Heterocypris incongruens*) are leaf-shredding organisms (more generally detritivores) or omnivores contributing substantially to the decomposition of allochthonous organic material—including crop plant residues—in freshwater ecosystems [e.g., 52–54]. Therefore, an adaptation of the experimental designs detailed in the respective acute and chronic guidelines may be an ecologically relevant and regulatory sensible procedure (Table 2). The available test guidelines either exclude feeding (i.e., Gammaridae) (Fig. 1) or use artificial food such as fish flakes (i.e., Dogielinotidae). In this context, Li et al. [55] demonstrated no substantial effects of *Cry* proteins extracted from GK-12 transgenic cottonseeds and spiked to the water or sediment using the (sub)acute 10-day sediment and 4-day water-only exposure with *H. azteca*. In the light of the exposure path of systemic pesticides and *Bt* proteins, which is expected to occur via the food, leaf material containing these stressors should be offered. At the same time, extending the exposure duration (from acute to chronic exposures) as a result of the expected sublethal effects [18, 56] should be considered. This extension is particularly relevant for the guidelines addressing the toxicity for Gammaridae after a short-term exposure of 4 days (Tables 1 and 2). Besides reproduction, which is according to current guidelines exclusively assessed for *H. azteca* over a study duration of 42 days, sublethal responses such as the feeding rate on leaf material can be quantified for both *Hyalella* [cf. 57] and *Gammarus* [cf. 17, 19, 58] already after a subacute exposure of 7 days. However, chronic exposure durations of several weeks with regular water exchanges and food renewal should be preferred, which would allow for a meaningful assessment of additional endpoints related to the energy availability and processing of the test species over longer study durations and may be particularly valuable for fungicides [59]. By following, for instance, the design of Bundschuh et al. [60], the feeding rate, feces production, assimilation, growth as well as the energy reserves of the test species may be determined that would foster the scientific understanding regarding the physiological reaction of organisms towards different types of chemical stress. This would likely increase the level of protection provided by the ERA. Finally, a procedure, which allows for the assessment of multiple sublethal energy processing related endpoints—as suggested here and below—seems particularly sensible for *Bt* proteins with unknown consequences for non-target species [61].

Insects

The class of insects is represented by 18 guidelines (Additional file 1: Table S1), of which one guideline describes a procedure for a 21-day-lasting sediment spiked survival and growth assay with *Hexagenia* sp. (Emphemeridae) and 14 focus on the family of Chironomidae with acute (48 h) and chronic (up to 65 days) test designs, including full life-cycle tests. The diversity of endpoints that can be assessed by employing these guidelines includes besides mortality also emergence rate, developmental rate, time to emergence, fecundity, fertility in the parental generation as well as the sex ratio in the parental and filial generation. Against this background, the aquatic insect community composition (e.g., Ephemeroptera, Plecoptera and Trichoptera) is an endpoint that responded to leaf litter containing insecticidal *Bt* proteins [62] and individual insect species showed a reduced feeding when offered leaf material containing the systemic pesticides [16, 19] or were exposed to *Bt* proteins [55, 63]. Consequently, insects are likely responsive and sensible test organisms to assess negative impacts of these stressors on aquatic ecosystems in general. Moreover, since chironomids and to some extent *Hexagenia* sp. are, similar to gammarids, detritivorous [64, 65], they represent a potentially suitable group of ecotoxicological test species for the ERA of systemic pesticides (particularly insecticides) and *Bt* proteins contained in crop plant residues; nevertheless, their test guidelines would need some modifications: Although the test organisms are regularly fed (Fig. 1)—at least during the chronic and full life-cycle tests—they usually receive ground artificial fish food (i.e. fish flakes), which needs to be replaced by the respective crop plant residue provided as fine particles (Table 2). As suggested above, the procedure detailed by Bøhn et al. [45] may provide an adequate guidance. It may also be desirable to assess the emerged insects for their energy reserves that may be affected by the food quality (i.e., the systemic pesticides and *Bt* proteins contained in crop plant residues) [sensu 65] and could result in implications in the filial generation. The guidance documents on full life-cycle tests with chironomids seem—due to the coverage of two subsequent populations—in general suitable for the characterization of sublethal environmental risks associated with the food-mediated exposure path of systemic pesticides and *Bt* proteins.

Besides the guidelines mentioned above, Ephemeroptera, Plecoptera, and Trichoptera are recommended as groups of test species in a total of ten, three and one test guideline, respectively. However, none of these guidelines have been developed specifically for any species belonging to these three orders and partly lack a clear description of the experimental design. In general, also these guidelines assess the sediment or waterborne exposure over up to 28 days while focusing on mortality, immobilization, growth and bioaccumulation as endpoints.

Bundschuh *et al. Environ Sci Eur*   (2019) 31:87

Page 8 of 13

Molluscs

Seven test guidelines refer to molluscs as test species (Additional file 1: Table S1), while these are divided into guidelines using the class of Bivalvia or Gastropoda (i.e., the family of Physidae and Amnicolidae). The two guidelines using Gastropoda are acute laboratory test guidelines with an experimental duration range between 2 and 8 days without feeding. The water-only exposure experiments assess mortality and immobilization as endpoints. Furthermore, these test guidelines are not specific for Gastropoda as a broad range of species including fish, macroinvertebrates and amphibians are listed as appropriate test organisms. Five test guidelines—including one for *in situ* bioassays—suggest bivalves as test organisms. These guidelines recommend exposure durations from 24 h up to 30 days and focus on bioaccumulation, growth, mortality and immobilization as endpoints. An exposure of freshwater mussels towards *Cry* proteins was documented in agriculturally influenced ecosystems in Canada [66]. Thus, their inclusion in a testing strategy seems sensible. Although molluscs cover a broad range of feeding strategies, including filter feeding of suspended fine particulate organic material [67], which may also be released by the decomposition of plant material potentially containing systemic pesticides or *Bt* proteins [68], the short study durations question thesuitability of this protocol to adequately cover this particular exposure pathway. Nonetheless, by elongating the study duration and focusing mainly on sublethal, including developmental endpoints, as suggested by one guideline targeting *in situ* and, thus, field-orientated approaches may be a sensible advancement (Table 2).

Other invertebrate species

Only eight guidelines (Additional file 1: Table S1) with an experimental duration between 4 and 28 days are dedicated to the assessment of chemical effects on sediment-dwelling organisms from various families including the species *Lumbriculus variegatus*, *Tubifex tubifex*, *Caenorhabditis elegans*, *Dugesia tigrina* and *Branchiura sowerbyi*. The endpoints of these tests include one or more of the following: bioaccumulation, mortality, growth, reproduction, fertility and sex ratio. Moreover, most of the test guidelines require the provision of standardized food (mainly fish food). Since these test species are consuming microorganisms likely associated with detritus, it may be feasible to experimentally assess the implications of systemic pesticides and *Bt* proteins contained in crop plant residues using a similar approach as suggested above for the experiments with chironomids and daphnids.

Fish

With 55 test guidelines, the class of fish is the most frequently considered taxonomic group (Additional file 1: Table S1), while the study duration ranges from 24 h to a full life cycle of a species, which can last several months. These guidelines focus on endpoints such as mortality, development, growth, reproduction, hatching success, swimming behavior as well as bioaccumulation. These endpoints in combination with the partly relatively long study duration suggest these guidelines as a suitable starting point for the development of some guidance for the determination of environmental risks associated with systemic pesticides and *Bt* proteins introduced into aquatic systems together with plant material on fish. However, most of the fish species covered by these guidelines are carnivores and do not primarily rely on the ingestion of detritus. Together with the general call to reduce animal (mainly vertebrate) testing during the registration process of chemicals [69], fish experiments may not be considered as an approach substantially improving the ERA of these substances in future.

Amphibians

The class of amphibians is represented by seven guidelines mainly focusing on *Xenopus laevis* and *Rana catesbeiana* (Additional file 1: Table S1), which are during their larval stages (=tadpoles) feeding on pelagic microorganisms such as bacteria and algae [70]. Although the recommended study duration (between 4 and 30 days) and the endpoints (i.e., development, growth, mortality) may be suitable to detect adverse effects caused by systemic pesticides and *Bt* proteins associated with plant material, the feeding ecology of the relevant species together with the ethical concerns regarding animal testing [69] suggest this group of organisms at the current stage as suboptimal to advance the ERA of these substances.

Improvement of test guidelines for testing systemic pesticides

As detailed above, the mode of toxic action of systemic pesticides is not expected to be substantially different from their non-systemic counterparts. However, the importance of the exposure pathway via the ingestion of contaminated food needs to be related to the exposure via the surrounding medium, namely water. Ideally, this should be realized using dose–response relationships allowing also for a direct inclusion in the ERA of these substances.

In a first step, plant material that contains increasing concentrations of these systemic pesticides should be generated. One feasible approach is to spike potted trees (or other plants such as maize or rapeseed) with different concentrations of a systemic pesticide either via soil or stem injection, while the application rates may be related to the recommendations of the respective producer [13]. Later during the year, more

Bundschuh *et al. Environ Sci Eur*   (2019) 31:87

Page 9 of 13

precisely at the time of abscission, the leaf material can be collected directly from the trees and stored either frozen or air-dried until being used for ecotoxicological experiments. The concentrations of the systemic pesticides within the leaf material—or other plant tissue such as pollen—need to be verified via chemical analysis, likely involving accelerated solvent extraction and liquid chromatography tandem mass spectrometry techniques [13]. In a second step, the acute and chronic toxicity of these plant residues can be estimated using the standard methods considering the amendments for the respective test organisms described above and in Table 2. From the evaluation of existing guidelines, however, it is apparent that only few test species can be classified as leaf-shredding invertebrates. Since shredders are the functional group which will be directly exposed to systemic pesticides contained in the plant material, we suggest expanding the list of test organisms with additional shredder species from insect taxa including trichopterans and plecopterans. These taxa are in the case of neonicotinoids also considerably more sensitive compared to standard crustacean test organism [71 but see 19]. The inclusion of insects as a further group appears also sensible since the enzyme activity, gut pH and other parameters may be deviating among representatives from different subphyla or classes [72], which may influence the digestion of the leaves, the release of the pesticide from the leaf material and ultimately their effects in the exposed organisms. Although, this physiological diversity in the group of insects complicates standard toxicity testing, their consideration during test development could inform about the magnitude of differences in sensitivity among subphyla or classes ultimately informing risk assessment.

The actual experimental design to test the effects of systemic pesticides depends also on the purpose of the study. If a dose–response relationship is to be established, the assessment of multiple concentrations is required and calls for rather short exposure durations to allow sufficient replication. Kreutzweiser et al. [16] indicates that at the recommended application rates of systemic pesticides, relatively low concentrations of the pesticide in the plant tissues can be anticipated. As a result, sublethal rather than lethal effects may be expected [19]. To account for sublethal effects, other experimental designs may be applied: Quantification of feeding rates of leaf-shredding invertebrates on standardized and uncontaminated leaf material has been done in various studies [58, 60]. Replacing the uncontaminated leaf material with leaves from trees treated with different concentrations of systemic pesticides would allow for the estimation of the potential effects associated with the introduction of such leaves into aquatic ecosystems. However, systemic

pesticides might leach from the leaf material into the surrounding medium generating a situation in which the test species is exposed to the test substance both via the water phase and its food [17, 19].

To disentangle the relative importance of both exposure pathways, a 2×2 factorial approach as proposed by Englert et al. [17] may be employed: The experimental design is composed of five treatments during one of which the test species receive leaves from an uncontaminated tree grown under the same conditions as those subjected to a treatment with systemic pesticides. In the second treatment, the test species will also receive leaves from the control trees but in the same replicate, an equivalent amount of leave material from a treated tree will be provided but inaccessible for the test species. This ensures the quantification of the effects caused by the leached pesticide. In a third treatment, the test species will be offered exclusively leaf material from a treated tree, while a continuous water exchange (flow through) will ensure that water phase concentrations, remain at negligible levels and thus enabling the quantification of effects caused via the ingested food exclusively. As this treatment likely deviates from the others with regard to the development of water quality parameters over time, a respective control treatment (fourth treatment) needs to be established relative to which the effects can be expressed. The fifth treatment, in which the test organism receives leaves from a treated tree and the medium will not be exchanged, ensures the exposure of test organisms to the systemic pesticide via both the water phase and food.

Improvement of test guidelines for testing *Bt* plant material

The estimation of potential environmental effects of *Bt* proteins has various experimental challenges that need to be solved during their ERA. One very important challenge is—in contrast to the testing of systemic insecticides—the establishment of a dose–response relationship. This appears difficult with any transgenic plant material such as tissues from *Bt* plants for several reasons: To date, the expression of *Bt* proteins in transgenic crops is in most cases governed by a constitutive promotor and *Bt* proteins are expressed in most, if not all, plant tissues. However, expression levels are usually tissue specific and vary with the growth stage [73, 74]. Although tissue specific concentrations are assumed to be rather constant at a given growth stage, the use of different genetic backgrounds and other factors [75, 76] can cause considerable variations in expression levels. Driven by the variation in *Bt* protein expression, GM plant parts that have an equal nutritious value and constant *Bt* protein concentrations can hardly be generated. At the same

Bundschuh *et al. Environ Sci Eur*   (2019) 31:87

Page 10 of 13

time, the maximum test concentration of *Bt* proteins within tissue is limited to the naturally expressed levels. Practically, worst-case assumptions including common safety factors similar to tests of chemical pesticides are difficult, if not impossible. Moreover, in *Bt* plants, the genetic modification may induce changes in the plant that go beyond the expression of genes initiating the production of *Bt* proteins. These changes may, for instance, influence the general nutritious quality for the organisms such as shredders in the aquatic ecosystem [77]. Current EFSA guidelines for genetically modified organism [78] advise to use a near-isogenic line that should be similar to the genetically modified plant. Although this addresses the issue of a similar nutritious quality, the difficulty to establish dose–response relationships remains.

During sensitivity testing of terrestrial non-target organisms microbially produced *Bt* proteins, instead of *Bt* plant material are frequently used [79]. A similar procedure may be applied during aquatic ecotoxicology testing by spiking GM plant material with increasing *Bt* toxin levels of microbial origin. This procedure would allow for a dose response testing of the *Bt* toxin considering also the changes in the crop metabolism induced by the genetic modification. At the same time, this procedure requires the availability of a non-GM isoline. Food quality and the fact that not all *Bt* plants (e.g. maize) may allow aquatic organism to perform a full life cycle are critical in this respect. To address this challenge, we suggest spiking conditioned leaf material of a tree species such as black alder—a highly nutritious food source—with increasing and known concentrations of the *Bt* proteins of microbial origin during the testing of aquatic shredders [see e.g., 60, 80]. The Enzyme-Linked Immunosorbent Assay (ELISA) technique can be used to measure the concentrations of *Bt* proteins in plant tissues. Subsequently, the material can, in principal, be tested as detailed for the systemic pesticides, namely by offering the spiked material to leaf-shredding invertebrates and monitor their response. The approach, however, does not allow testing the GM plant as a whole [79] and the toxins of microbial origin may not be (toxicologically) identical to those produced by a GM plant. Further uncertainties remain as to whether the toxicant availability is comparable between spiking and regular GM plant treatment in which the toxins are contained within cells and not on the plant material surface. Nonetheless, the illustrated procedure would allow for an establishment of a dose–response relationship for an individual *Bt* protein or a defined mixture. Moreover, risk assessment procedures of genetically modified organisms in Europe [78] are based on a case-by-case approach calling for test organisms representative for the receiving environment. As Hilbeck et al. [81] provided a decision support system

containing criteria for the selection of potential test species for aquatic GM testing, this will not be addressed in further detail in this document.

Although the experimental design suggested in the following still needs some verification and adjustments, it is recommended to offer the spiked leaf material of interest, e.g., to a caddisfly species for at least 6 weeks (ideally longer) under continuous aeration in a climate-controlled room ideally using a temperature gradient simulating the field situation. The caddisfly larvae may be kept in groups of around five individuals in glass vessels containing the preferred substrate of the selected test species, while each treatment should be replicated at least ten times ensuring an appropriate statistical power. At weekly intervals, the test item, namely the food of the test species, can be renewed, the feeding rate of the species determined [see 82] and the test medium, which may either be a standardized medium as, for instance, described in Borgmann [83] or stream water (the site where the organisms have been collected from), may be renewed. At the same time, the survival and the growth of the caddisfly larvae can be monitored. The latter will be determined by measuring the width of the head from a digital image, an endpoint correlating well with the biomass of the species [11] and providing a measure of the larval instar. At the termination of the experiments, the individuals may be analyzed for the energy reserves, which provides insights into the physiological implications caused by the ingestion of food containing *Bt* proteins. This endpoint was successfully established as a measure for stress caused by chronic exposures and allows inferences on the population development in the long run [e.g. 60]. Another option, which one could pursue is to assess the time until insect emergence, which is a sensitive endpoint [84] and indicative for implication in the subsidy of terrestrial ecosystems by aquatic resources [land–water coupling, 85]. Also, in the emerged adults, energy reserves can be determined which may allow for insights into potential effects in the reproductive output, and thus population development [sensu 86].

Conclusion

Differences in the exposure route, namely the uptake of the active ingredient as part of the food matrix, scientifically justify that the risk assessment of both systemic insecticides and transgenic insect resistant plants should include this path of exposure in their ERA prior to authorisation of the respective products. Significant challenges remain, which include the need to update or adjust the currently available test guidelines to provide a meaningful basis for ERA. Moreover, only few test species represent the functional group of shredders which

Bundschuh *et al. Environ Sci Eur*   (2019) 31:87

Page 11 of 13

are supposedly regularly exposed to potentially contaminated plant residues under field conditions. Thus, particularly for the ERA of Bt proteins from transgenic crops, further research is required to optimize test strategies and methods which would allow to assess the dose–responses relationships.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12302-019-0266-1.

> **Additional file 1: Table S1.** Overview of the Test guidelines covered in this review. The Table carries information on the guideline number, the year of publication, its title, the level of testing and the systematic group assessed.

Abbreviations
ERA: Environmental Risk Assessment; *Bt*: *Bacillus thuringiensis*; PNEC: predicted no-effect concentration; RNAi: ribonucleic acid interference; *Cry*: proteins crystal proteins; *VIP*: vasoactive intestinal peptide; OECD: Organization for Economic Co-operation and Development; EU: European Communities; ISO: International Organization for Standardization; ASTM: American Society of Testing and Materials; MAFF: Ministry of Agriculture, Forestry and Fisheries; EC: Environment Canada; OCSPP: Office of Chemical Safety and Pollution Prevention; EPA: Environmental Protection Agency; EFSA: European Food Safety Authority.

Authors' contributions
MB, RS and MO conceived the study, RB performed the guideline analyses, RB prepared the first version of the manuscript with contribution of MB, MO provided insights on the risk assessment background, all authors critically reviewed the document and agreed on the submitted version. All authors read and approved the final manuscript.

Availability of data and materials
Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
One author is managing director of a consultancy, while the authors do not see any competing interest arising from this or any other relationship.

Author details
[1] Institute for Environmental Sciences, University of Koblenz-Landau, Fortstrasse 7, 76829 Landau, Germany. [2] Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Lennart Hjelms väg 9, 75007 Uppsala, Sweden. [3] Department of Biosafety, Federal Agency for Nature Conservation (BfN), Konstantinstr. 110, 53179 Bonn, Germany. [4] Present Address: SETAC Europe, Brussels, Belgium.

References

1. EFSA (2013) Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA J 11(7):3290
2. Chapman PM, Fairbrother A, Brown D (1998) A critical evaluation of safety (uncertainty) factors for ecological risk assessment. Environ Toxicol Chem 17(1):99–108
3. OECD 219 (2004) Sediment-water chironomid toxicity test using spiked water. https://www.oecd-ilibrary.org/environment/test-no-219-sediment-water-chironomid-toxicity-using-spiked-water_9789264070288-en. Accessed Oct 2019
4. OECD 218 (2004) Sediment-water chironomid toxicity test using spiked sediment. https://www.oecd-ilibrary.org/environment/test-no-218-sediment-water-chironomid-toxicity-using-spiked-sediment_9789264070264-en. Accessed Oct 2019
5. Bennett SH (1957) The behaviour of systemic insecticides applied to plants. Annu Rev Entomol 2:279–296
6. Crickmore N, Zeigler DR, Feitelson J, Schnepf E, Van Rie J, Lereclus D, Baum J, Dean DH (1998) Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. Microbiol Mol Biol Rev 62(3):807–813
7. Ni M, Ma W, Wang X, Gao M, Dai Y, Wei X, Zhang L, Peng Y, Chen S, Ding L, Tian Y, Li J, Wang H, Wang X, Xu G, Guo W, Yang Y, Wu Y, Heuberger S, Tabashnik BE, Zhang T, Zhu Z (2017) Next-generation transgenic cotton: pyramiding RNAi and Bt counters insect resistance. Plant Biotechnol J 15(9):1204–1213
8. Christiaens O, Dzhambazova T, Kostov K, Arpaia S, Joga MR, Urru I, Sweet J, Smagghe G (2018) Literature review of baseline information on RNAi to support the environmental risk assessment of RNAi-based GM plants. EFS3 15:1424E
9. Paces J, Nic M, Novotny T, Svoboda P (2017) Literature review of baseline information to support the risk assessment of RNAi-based GM plants. EFS3 14:e391
10. Rissler J, Mellon M (2000) The ecological risks of engineered crops. The MIT Press, Cambridge
11. Rosi-Marshall EJ, Tank JL, Royer TV, Whiles MR, Evans-White M, Chambers C, Griffiths NA, Pokelsek J, Stephen ML (2007) Toxins in transgenic crop byproducts may affect headwater stream ecosystems. Proc Natl Acad Sci U S A 104(41):16204–16208
12. Tank JL, Rosi-Marshall EJ, Royer TV, Whiles MR, Griffiths NA, Frauendorf TC, Treering DJ (2010) Occurrence of maize detritus and a transgenic insecticidal protein (Cry1Ab) within the stream network of an agricultural landscape. Proc Natl Acad Sci U S A 107(41):17645–17650
13. Englert D, Bakanov N, Zubrod JP, Schulz R, Bundschuh M (2017) Modeling re-mobilization of neonicotinoid residues from tree foliage in streams—a relevant exposure pathway in risk assessment? Environ Sci Technol 51(3):1785–1794
14. Pott A, Otto M, Schulz R (2018) Impact of genetically modified organisms on aquatic environments: review of available data for the risk assessment. Sci Total Environ 635:687–698
15. Bundschuh R, Kuhn U, Bundschuh M, Naegele C, Elsaesser D, Schlechtriemen U, Oehen B, Hilbeck A, Otto M, Schulz R, Hofmann F (2016) Prioritizing stream types according to their potential risk to receive crop plant material - A GIS-based procedure to assist in the risk assessment of genetically modified crops and systemic insecticide residues. Sci Total Environ 547:226–233
16. Kreutzweiser D, Good K, Chartrand D, Scarr T, Thompson D (2007) Non-target effects on aquatic decomposer organisms of imidacloprid as a systemic insecticide to control emerald ash borer in riparian trees. Ecotoxicol Environ Saf 68(3):315–325
17. Englert D, Zubrod JP, Pietz S, Stefani S, Krauss M, Schulz R, Bundschuh M (2017) Relative importance of dietary uptake and waterborne exposure for a leaf-shredding amphipod exposed to thiacloprid-contaminated leaves. Sci Rep 7:16182
18. Kreutzweiser DP, Good KP, Chartrand DT, Scarr TA, Thompson DG (2008) Are leaves that fall from imidacloprid-treated maple trees to control

Bundschuh *et al. Environ Sci Eur*   (2019) 31:87

Page 12 of 13

Asian longhorned beetles toxic to non-target decomposer organisms? J Environ Qual 37(2):639–646

19. Englert D, Zubrod JP, Link M, Mertins S, Schulz R, Bundschuh M (2017) Does waterborne exposure explain effects caused by neonicotinoid-contaminated plant material in aquatic systems? Environ Sci Technol 51(10):5793–5802

20. Schulz R (2004) Field studies on exposure, effects, and risk mitigation of aquatic nonpoint-source insecticide pollution: a review. J Environ Qual 33:419–448

21. Goulson D (2013) An overview of the environmental risks posed by neonicotinoid insecticides. J Appl Ecol 50:977–987

22. Bonmatin JM, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, Marzaro M, Mitchell EAD, Noome DA, Simon-Delso N, Tapparo A (2015) Environmental fate and exposure; neonicotinoids and fipronil. Environ Sci Pollut Rese 22(1):35–67

23. Maienfisch P, Angst M, Brandl F, Fischer W, Hofer D, Kayser H, Kobel W, Rindlisbacher A, Senn R, Steinemann A, Widmer H (2001) Chemistry and biology of thiamethoxam: a second generation neonicotinoid. Pest Manag Sci 57(10):906–913

24. Horwood MA (2007) Rapid degradation of termiticides under field conditions. Aust J Entomol 46:75–78

25. Bonmatin JM, Moineau I, Charvet R, Colin ME, Fleche C, Bengsch ER (2005) Behaviour of imidacloprid in fields. toxicity for honey bees. In: Lichtfouse E, Schwarzbauer J, Robert D (eds) Environmental Chemistry. Springer, Berlin, pp 483–494

26. Whitehorn PR, O'Connor S, Wackers FL, Goulson D (2012) Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science 336(6079):351–352

27. Botias C, David A, Horwood J, Abdul-Sada A, Nicholls E, Hill E, Goulson D (2015) Neonicotinoid residues in wildflowers, a potential route of chronic exposure for bees. Environ Sci Technol 49(21):12731–12740

28. Zubrod JP, Englert D, Feckler A, Koksharova N, Konschak M, Bundschuh R, Schnetzer N, Englert K, Schulz R, Bundschuh M (2015) Does the current fungicide risk assessment provide sufficient protection for key drivers in aquatic ecosystem functioning? Environ Sci Technol 49(2):1173–1181

29. Venter HJ, Bøhn T (2016) Interactions between Bt crops and aquatic ecosystems: a review. Environ Toxicol Chem 35(12):2891–2902

30. Hilbeck A, Meier M, Römbke J, Jänsch S, Teichmann H, Tappeser B (2011) Environmental risk assessment of genetically modified plants - concepts and controversies. Environ Sci Eur 23:13

31. Stenersen J (2004) Chemical pesticides—mode of action and toxicology. CRC Press LLC, Boca Raton

32. Whalon ME, Wingerd BA (2003) Bt: mode of action and use. Arch Insect Biochem Physiol 54(4):200–211

33. van Frankenhuyzen K (2009) Insecticidal activity of *Bacillus thuringiensis* crystal proteins. J Invertebr Pathol 101(1):1–16

34. Van Frankenhuyzen K (2013) Cross-order and cross-phylum activity of *Bacillus thuringiensis* pesticidal proteins. J Invertebr Pathol 114:76–85

35. Vachon V, Laprade R, Schwartz J-L (2012) Current models of the mode of action of Bacillus thuringiensis insecticidal crystal proteins: a critical review. J Invertebr Pathol 111(1):1–12

36. Hilbeck A, Otto M (2015) Specificity and combinatorial effects of *Bacillus thuringiensis* Cry toxins in the context of GMO environmental risk assessment. Front Environ Sci 3:00071

37. Jänsch S, Bauer J, Leube D, Otto M, Römbke J, Teichmann H, Waszak K (2018) A new ecotoxicological test method for genetically modified plants and other stressors in soil with the black fungus gnat *Bradysia impatiens* (Diptera): current status of test development and dietary effects of azadirachtin on larval development and emergence rate. Environ Sci Eur 30:654

38. Maltby L, Brock TC, Van den Brink PJ (2009) Fungicide risk assessment for aquatic ecosystems: importance of interspecific variation, toxic mode of action, and exposure regime. Environ Sci Technol 43(19):7556–7563

39. Suberkropp K, Klug MJ (1976) Fungi and bacteria associated with leaves during processing in a woodland stream. Ecology 57:707–719

40. Bärlocher F (1985) The role of fungi in the nutrition of stream invertebrates. Bot J Linn Soc 91:83–94

41. Bundschuh M, Zubrod JP, Kosol S, Maltby L, Stang C, Duester L, Schulz R (2011) Fungal composition on leaves explains pollutant-mediated indirect effects on amphipod feeding. Aquat Toxicol 104(1):32–37

42. Zubrod JP, Englert D, Rosenfeldt RR, Wolfram J, Lüderwald S, Wallace D, Schnetzer N, Schulz R, Bundschuh M (2015) The relative importance of diet-related and waterborne effects of copper for a leaf-shredding invertebrate. Environ Pollut 205:16–22

43. Newton K, Zubrod JP, Englert D, Lüderwald S, Schell T, Baudy P, Konschak M, Feckler A, Schulz R, Bundschuh M (2018) The evil within? Systemic fungicide application in trees enhances litter quality for an aquatic decomposer-detritivore system. Environ Pollut 241:549–556

44. Jensen TC, Verschoor AM (2004) Effects of food quality on life history of the rotifer *Brachionus calyciflorus* Pallas. Freshw Biol 49(9):1138–1151

45. Bohn T, Primicerio R, Hessen DO, Traavik T (2008) Reduced fitness of *Daphnia magna* fed a Bt-transgenic maize variety. Arch Environ Contam Toxicol 55(4):584–592

46. Bohn T, Traavik T, Primicerio R (2010) Demographic responses of *Daphnia magna* fed transgenic Bt-maize. Ecotoxicology 19(2):419–430

47. Hatakeyama S, Sugaya Y (1989) A freshwater shrimp (*Paratya compressa improvisa*) as a sensitive test organism to pesticides. Environ Pollut 59:325–336

48. Maeda-Martinez AM, Obregon-Barboza H, Dumont HJ (1995) Laboratory culture of fairy shrimps using baker's yeast as basic food in a flow-through system. Hydrobiologia 298:141–157

49. Geller W, Müller H (1981) The filtration apparatus of cladocera: filter mesh-sizes and their implications on food selectivity. Oecologia 49(3):316–321

50. Holderbaum DF, Cuhra M, Wickson F, Orth AI, Nodari RO, Bøhn T (2015) Chronic responses of *Daphnia magna* under dietary exposure to leaves of a transgenic (event MON810) Bt-maize hybrid and its conventional near-isoline. J Toxicol Environ Health Part A 78:993–1007

51. Mendelsohn M, Kough J, Vaituzis Z, Matthews K (2003) Are Bt crops safe? Nat Biotechnol 21:1003–1009

52. Dangles O, Gessner MO, Guerold F, Chauvet E (2004) Impacts of stream acidification on litter breakdown: implications for assessing ecosystem functioning. J Appl Ecol 41(2):365–378

53. Hargrave BT (1970) The utilization of benthic microflora by *Hyalella azteca* (Amphipoda). J Anim Ecol 39(2):427–437

54. Rossi V, Benassi G, Belletti F, Menozzi P (2011) Colonization, population dynamics, predatory behaviour and cannibalism in *Heterocypris incongruens* (Crustacea: Ostracoda). J Limnol 70(1):102–108

55. Li YL, Du J, Fang ZX, You J (2013) Dissipation of insecticidal Cry1Ac protein and its toxicity to nontarget aquatic organisms. J Agric Food Chem 61(46):10864–10871

56. Andow DA, Hilbeck A (2004) Science-based risk assessment for nontarget effects of transgenic crops. Bioscience 54(7):637–649

57. Bundschuh M, Zubrod JP, Seitz F, Newman MC, Schulz R (2011) Mercury-contaminated sediments affect amphipod feeding. Arch Environ Contam Toxicol 60(3):437–443

58. Zubrod JP, Bundschuh M, Schulz R (2010) Effects of subchronic fungicide exposure on the energy processing of *Gammarus fossarum* (Crustacea; Amphipoda). Ecotoxicol Environ Saf 73(7):1674–1680

59. Baudy P, Zubrod JP, Konschak M, Weil M, Schulz R, Bundschuh M (2017) Does long-term fungicide exposure affect the reproductive performance of leaf-shredders? A partial life-cycle study using *Hyalella azteca*. Environ Pollut 222:458–464

60. Bundschuh M, Zubrod JP, Schulz R (2011) The functional and physiological status of *Gammarus fossarum* (Crustacea; Amphipoda) exposed to secondary treated wastewater. Environ Pollut 159(1):244–249

61. Hilbeck A, Schmidt JEU (2006) Another view on Bt proteins—how specific are they and what else might they do? Biopeptites International 2(1):1–50

62. Axelsson EP, Hjalten J, LeRoy CJ, Whitham TG, Julkunen-Tiitto R, Wennstrom A (2011) Leaf litter from insect-resistant transgenic trees causes changes in aquatic insect community composition. J Appl Ecol 48(6):1472–1479

63. Prihoda KR, Coats JR (2008) Aquatic fate and effects of *Bacillus thuringiensis* Cry3Bb1 protein: toward risk assessment. Environ Toxicol Chem 27(4):793–798

64. Cummins KW, Klug MJ (1979) Feeding ecology of stream invertebrates. Annu Rev Ecol Syst 10:147–172

65. Ward GM, Cummins KW (1979) Effects of food quality on growth of a stream detritivore, *Paratendipes albimanus* (Meigen) (Diptera: Chironomidae). Ecology 60(1):57–64

Bundschuh *et al. Environ Sci Eur*   (2019) 31:87

Page 13 of 13

66. Douville M, Gagne F, Andre C, Blaise C (2009) Occurrence of the transgenic corn cry1Ab gene in freshwater mussels (*Elliptio complanata*) near corn fields: evidence of exposure by bacterial ingestion. Ecotoxicol Environ Saf 72(1):17–25

67. Vaughn CC, Hakenkamp CC (2008) The functional role of burrowing bivalves in freshwater ecosystems. Freshw Biol 46(11):1431–1446

68. Bundschuh M, McKie BG (2016) An ecological and ecotoxicological perspective on fine particulate organic matter in streams. Freshw Biol 61:2063–2074

69. Höfer T, Gerner I, Gundert-Remy U, Liebsch M, Schulte A, Spielmann H, Vogel R, Wettig K (2004) Animal testing and alternative approaches for the human health risk assessment under the proposed new European chemicals regulation. Arch Toxicol 78(10):549–564

70. Kupferberg SJ (1997) Bullfrog (*Rana catesbeiana*) invasion of a California river: the role of larval competition. Ecology 78:1736–1751

71. Morrissey CA, Mineau P, Devries JH, Sanchez-Bayo F, Liess M, Cavallaro MC, Liber K (2015) Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: a review. Environ Int 74:291–303

72. Sinsabaugh LR, Linkins AE, Benfield EF (1985) Cellulose digestion and assimilation by three leaf-shredding aquatic insects. Ecology 66(5):1464–1471

73. Nguyen HT, Jehle JA (2007) Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize Mon810. J Plant Dis Prot 114(2):82–87

74. Szekacs A, Lauber E, Juracsek J, Darvas B (2010) Cry1ab toxin production of Mon 810 transgenic maize. Environ Toxicol Chem 29(1):182–190

75. Carstens K, Anderson J, Bachman P, De Schrijver A, Dively G, Federici B, Hamer M, Gielkens M, Jensen P, Lamp W, Rauschen S, Ridley G, Romeis J, Waggoner A (2012) Genetically modified crops and aquatic ecosystems: considerations for environmental risk assessment and non-target organism testing. Transgenic Res 21(4):813–842

76. Adamczyk JJ, Meredith WR (2004) Genetic basis for variability of Cry1Ac expression among commercial transgenic *Bacillus thuringiensis* (Bt) cotton cultivars in the United States. J Cott Sci 8:17–23

77. Parrott W et al (2008) Study of Bt impact on caddisflies overstates its conclusions: response to Rosi-Marshall et al. Proc Natl Acad Sci U S A 105(7):E10

78. EFSA (2010) Guidance on the environmental risk assessment of genetically modified plants. EFSA J 8:1879

79. Römbke J, Jänsch S, Meier M, Hilbeck A, Teichmann H, Tappeser B (2009) General recommendations for soil ecotoxicological tests suitable for the environmental risk assessment of genetically modified plants. Integ Environ Assess Manag 6(2):287–300

80. Maltby L, Naylor C (1990) Preliminary observations on the ecological relevance of *Gammarus* "scope for growth" assay: effect of zinc on reproduction. Funct Ecol 4:393–397

81. Hilbeck A, Bundschuh R, Bundschuh M, Hofmann F, Oehen B, Otto M, Schulz R, Trtikova M (2017) Procedure to select test organisms for environmental risk assessment of genetically modified crops in aquatic systems. Integ Environ Assess Manag 13(6):974–979

82. Maltby L, Clayton SA, Wood RM, McLoughlin N (2002) Evaluation of the *Gammarus pulex* in situ feeding assay as a biomonitor of water quality: robustness, responsiveness and relevance. Environ Toxicol Chem 21(2):361–368

83. Borgmann U (1996) Systematic analysis of aqueous ion requirements of *Hyalella azteca*: a standard artificial medium including the essential bromide ion. Arch Environ Contam Toxicol 30(3):356–363

84. Schulz R, Liess M (2001) Toxicity of aqueous-phase and suspended particle-associated fenvalerate: chronic effects after pulse-dosed exposure of *Limnephilus lunatus* (Trichoptera). Environ Toxicol Chem 20(1):185–190

85. Schulz R, Bundschuh M, Gergs R, Brühl CA, Diehl D, Entling M, Fahse L, Frör O, Jungkunst HF, Lorke A, Schäfer RB, Schaumann GE, Schwenk K (2015) Review on environmental alterations propagating from aquatic to terrestrial ecosystems. Sci Total Environ 538:246–261

86. Cargill AS, Cummins KW, Hanson BJ, Lowry RR (1985) The role of lipids, fungi, and temperature in the nutrition of a shredder caddisfly, *Clistoronia magnifica*. Freshw Invertebr Biol 4(2):64–78

Publisher's Note