1 Recovery of aquatic and terrestrial populations in the context of
2 European pesticide risk assessment

3

4 Mira Kattwinkel ^{1,2,*}, Matthias Liess ², Maria Arena ³, Stephanie Bopp ³, Franz Streissl ³, Jörg
5 Römbke ⁴

6

7 ¹ Department of System Analysis, Integrated Assessment and Modelling, Eawag: Swiss
8 Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland

9 ² Department of System-Ecotoxicology, UFZ - Helmholtz Centre for Environmental
10 Research, Leipzig, Germany

11 ³ European Food Safety Authority (EFSA), Pesticides Unit, Parma, Italy

12 ⁴ ECT Oekotoxikologie GmbH, Flörsheim a. M., Germany

13

14 * mira.kattwinkel@gmx.net, +41 58 765 5262 (corresponding author)

15

16 Word count: 8354

This document is the accepted manuscript version of the following article:
Kattwinkel, M., Liess, M., Arena, M., Bopp, S., Streissl, F., & Römbke, J.
(2015). Recovery of aquatic and terrestrial populations in the context of
European pesticide risk assessment. Environmental Reviews, 23(4), 382-394.
<https://doi.org/10.1139/er-2015-0013>

17

18 **Abstract**

19 In the present review, we compiled and evaluated the available information supporting
20 the assessment of population and community recovery after pesticide application. This
21 information is crucial for the environmental risk assessment of pesticides. We reviewed more
22 than 3900 manuscripts on those organism groups relevant or likely to become relevant for the
23 risk assessment procedures in Europe, i.e. aquatic invertebrates, algae, aquatic plants, fish,
24 aquatic microbes, amphibians, as well as birds and mammals, non-target terrestrial arthropods
25 including honeybees, non-arthropod invertebrates, terrestrial microbes, non-target terrestrial
26 plants, nematodes, and reptiles. Finally, 106 aquatic and 76 terrestrial studies met our
27 selection criteria and were evaluated in detail.

28 We extracted the following general conclusions: (1) Internal recovery depends
29 strongly on reproduction capacity. For aquatic invertebrates recovery was generally observed
30 within a maximum of five generation times. (2) In cases where recovery occurred within one
31 generation, migration from uncontaminated areas was identified as the main pathway for
32 aquatic and terrestrial invertebrates, in particular for insect species with the ability for aerial
33 re-colonization. (3) Community composition in general did not recover within the study
34 duration in the majority of cases. (4) The ecological context, including factors such as food
35 resources, habitat quality and re-colonization potential, is a crucial factor for recovery from
36 pesticide effects. (5) Indirect effects acting through food chain processes, including predation
37 and competition, are highly relevant for increasing the magnitude of effect and for prolonging
38 recovery time.

39 Based on our findings, we recommend defining realistic scenarios for risk assessment
40 regarding exposure, taxa considered, environmental conditions and ecological context. In
41 addition to experimental studies, field monitoring was shown to yield valuable information to

42 identify relevant taxa, long-term effects and the conditions for recovery, and should therefore
43 be considered to validate approaches of risk assessment. Likewise, ecological modelling was
44 found to be a valuable tool for assessing recovery. Finally, both study design and
45 interpretation of results still often suffer from missing ecological information or from neglect
46 of the available knowledge. Hence, a more rigorous utilization of existing knowledge (e.g.
47 from general disturbance ecology) and the generation of systematic ecological knowledge on
48 the various factors influencing recovery are needed.

49

50 KEY WORDS

51 plant protection products; long-term effect; transient effect; European Food Safety Authority
52 (EFSA); environmental risk assessment; herbicides; insecticides; fungicides

53

1. Introduction

In Europe an estimated 5.1 kg of pesticides per ha of arable land is on average applied annually (active substance used divided by the area of arable land and permanent crops, based on the average between 2000 to 2009 (Eurostat 2015)). Their intentional release into the environment and their quality as active substances designed to efficiently control pests and weeds lay the foundation for the risk of widespread effects of pesticides on non-target organisms. For this reason the risks of pesticides have to be particularly carefully assessed before authorization to place them on the market is granted. In this context, the reliable assessment of population and community effects, including recovery, are crucial for the protection of biodiversity and ecosystems.

In the European Union, the authorization of pesticides is regulated by Regulation (EC) No. 1107/2009 (European Parliament and European Council 2009), which aims at preventing “unacceptable effects on the environment, having particular regard to [...] impact on non-target species, including on the ongoing behavior of those species” and “impact on biodiversity and the ecosystem”. For the evaluation and authorization requirements, Regulation (EC) No. 1107/2009 refers to the Uniform Principles laid down in Commission Regulation (EU) No. 546/2011. There it is stated that “[...] Member States shall ensure that use of plant protection products does not have any long-term repercussions for the abundance and diversity of non-target species.”

For each active substance an initial risk assessment is conducted by an EU Member State. After reception of the dossier from an applicant, the risk assessment is carried out according to evaluation procedures (guidance documents) which are implemented in the framework of the Standing Committee on Plants, Animals, Food and Feed of the European Commission. Guidance documents are usually developed by the European Food Safety Authority (EFSA) and/or its Panel on Plant Protection Products and their residues (PPR

Panel) and once implemented these documents become legally binding. EFSA, through its panels and groups of experts, can also produce scientific opinions addressing the state of the science on the risk assessment of plant protection products, providing the scientific background on specific topics. The risk assessment is peer-reviewed in a process organized by EFSA and which also involves other EU Member States. The final risk assessment for an active substance is summarized in the "EFSA conclusion on pesticides". This conclusion provides a basis for the legislative decision of the European Commission, the risk manager, on whether or not to include a substance in the Union's list of approved active substances (Regulation (EU) No. 540/2011). The risk assessment and authorization of specific plant protection products and uses of those products lies within the jurisdiction of the EU Member States.

For specific groups of organisms (vertebrates), where the regulation does not permit lethal effects or impacts on reproduction, the recovery option is void, while for other organisms some degree of impact is allowed if recovery is shown within a specific period of time. Hence, the assessment of recovery is very important for an informed and protective risk assessment for those organism groups. In detail, the current environmental risk assessment (ERA) of pesticides is based on a tiered approach. If laboratory tests (first tier) indicate a risk, higher-tier studies are required to show that no unacceptable long-term impacts are to be expected under field conditions. Such higher-tier studies consist of additional laboratory tests or micro- and mesocosm experiments as well as (semi-) field studies. Due to the complexity of such higher-tier tests, detailed guidance on what effects and recovery times are acceptable in higher-tier studies is highly desirable. Fortunately, some progress has been made in this area (e.g. EFSA PPR Panel 2013). In general, all relevant ecological factors influencing population and community recovery should be taken into account, including "dispersal ability, life-history, breeding season, number of breeding attempts per season, abundance in the

environment, spatial records, as well as the natural variability in population sizes and distributions" (European Commission 2001).

There is, however, scant information and guidance on how to quantitatively incorporate these ecological factors into the assessment process. Additionally, little is known about the recovery potential of different taxa and information on this matter is scattered throughout the scientific literature. Furthermore, the question arises how to extrapolate from laboratory experiments and semi-field studies (i.e. the level generally investigated in the current risk assessment) to recovery under field conditions (i.e. the relevant protection level). Moreover, despite the efforts of current ERA, a recent scientific opinion on the aquatic environment (EFSA PPR Panel 2013) summarizes that "available information on unacceptable effects of pesticides in the field indicates that care has to be taken using the RA [risk assessment] approach used until now". Likewise, effects of pesticides have been found in the terrestrial environment (e.g. Dietrich et al. 1995, Liess et al. 2005, Clark et al. 2009, Henry et al. 2012).

In the present study we summarize the results of a literature review of population recovery following pesticide application. It is based on a report prepared by the authors for EFSA (Kattwinkel et al. 2012), which is part of EFSA's activity to provide overviews of the current scientific knowledge on specific topics regarding the revision of the Guidance Documents situations (European Commission 2001, 2002). Here we address the following questions:

- (1) What scientific information is available on population and community recovery after contamination?
- (2) What are the main drivers of recovery that can be used for field level estimations based on laboratory or semi-field experiments?

(3) How can the current procedures to assess population and community recovery be improved for an effective risk assessment?

2. Approach and overview of investigated studies

2.1 Definition of Ecological Recovery

In the course of this study, we define recovery as the extent of return of a population or community to relevant aspect(s) of its previous condition or to the status of a control treatment or a reference site, either from outside or from within the affected system (EPPO 2003, US EPA 2015). Full recovery is reached when there is no or only a negligible difference in the properties of the previously affected population or community and that of the control treatment, reference site or the status before the pesticide application for a longer period of time. Recovery can be internal, i.e. from within the affected area by reproduction, or external (usually termed re-colonization), i.e. from outside the affected area by individuals immigrating and thereby increasing the affected or starting a new population.

Our aim was to assess recovery as the time point when there was no statistically significant difference observed to the control or to the status before the pesticide application after an initial effect, or as derived from no observed effect concentrations (NOEC) or lowest observed effect concentrations (LOEC) as given in the original study. However, a consistent assessment of recovery would not have been possible based on this approach for several reasons: (1) some studies did not include any statistical test, (2) the variety of statistical tests applied made the comparability among studies difficult, (3) often there was a clear deviation from the control although the statistical test indicated no difference, for example, due to low replicate numbers, and (4) in some cases the respective endpoint reached control levels after initial decline or increase but continued to rise or decrease, respectively. Therefore, we assessed population recovery based on the time point when the difference to the control (or to

the state before treatment) in the figures, tables, or as described in the text was negligible for consecutive sampling dates or when the population clearly seemed to develop towards that direction (Knauer and Hommen 2012). Furthermore, we investigated the time course of population development after application. To simplify the communication and comparison, we defined different classes of recovery and no recovery (Fig. 1).

Fig. 1 about here

2.2 *Organism groups*

The current ERA of pesticides relies on a few test species (Supplementary Material 1) out of some organism groups (European Commission 2013). For the aquatic compartment these organism groups are fish, aquatic and sediment dwelling invertebrates, algae, and aquatic macrophytes. For the terrestrial compartment certain mammals, birds, bees, non-target arthropods (NTAs), soil invertebrates (earthworms and other non-target macro- and mesofauna), soil microorganisms and plants are tested. In addition to these species groups, in this study we also collected information on aquatic micro-organisms, which are not yet included in the current risk assessment, as well as amphibians and reptiles, for which so far no risk assessment guidelines exist.

2.3 *Literature Review*

We conducted a detailed assessment of all manuscripts found in the literature search (for details see Supplementary Material 2) based on (1) the ecological relevance regarding the species monitored and the spatial aspects of the study design; (2) the study design and analysis regarding extent of replication (at least two), possible bias or confounding of variables, and, if applicable, the appropriate application of statistical tests; (3) the temporal sampling design (at least one sampling before and several samplings after the application to

enable an assessment of the time course of recovery and to go beyond a pure effect measurement). Although we collected information useable for the risk assessment in Europe we did not restrict our review to European studies or substances currently authorized in Europe as population and community dynamics follow general pattern.

The heterogeneity in experimental setups resulted in low numbers of directly comparable studies. However, we were able to perform some descriptive statistics. To this end, we define a “study” as one experiment comprising control and potentially different concentrations. If other treatment settings were changed (e.g. nutrient addition, shading) this was denoted as a different study. “Data series” refers to each single observed time series within one study per taxa and per concentration (but not per replicate as most often only averaged values were presented in the original studies).

2.4 Studies investigated

More manuscripts related to the aquatic compartment compared to the terrestrial one were considered in the final data analysis, 74 compared to 52, comprising 106 and 76 studies, respectively. In general, higher-tier studies conducted by the applicants for pesticide authorization (i.e. companies), which may also include valuable information on recovery, were underrepresented as most often these studies are treated as confidential and remain unpublished, in particular in terrestrial ecotoxicology (J. Römcke, personal communication). For instance, field studies with earthworms (ISO (International Organization for Standardization) 1999, de Jong et al. 2006, Kula et al. 2006), bees (EPPO 2001), and non-target arthropod (Candolfi et al. 2000) are regularly performed for authorization and are likely to contain data on recovery.

By far the most studies dealt with aquatic invertebrates (79) followed by algae (54), with several studies dealing with more than one species group in parallel (**Table 1**). For the terrestrial taxa groups, we found most studies on soil invertebrates and NTAs (**Table 1**). The

lack of experimental studies on vertebrates (amphibians, fish, reptiles, birds, and mammals) may result from the fact that such studies are much more demanding in terms of time, space and equipment than those on invertebrates, plants, microbes, or algae. This is due to the longer generation cycles, space demands and higher trophic level of these taxa. Moreover, for animal welfare reasons, vertebrate testing is reduced as much as possible.

Most often insecticides were investigated, followed by herbicides and fungicides (Table S3 in the Supplementary Material). Some of the substances investigated are not authorized in Europe anymore (e.g. pentachlorophenol, 2,4,5-T, atrazine or lindane). Nevertheless, the experimental findings on recovery from effects of such substances are valuable information and were therefore considered. Due to the broad variety of species tested and experimental setups, no general relationship between recovery and the mode of action of the substances investigated (Tables S4 and S5 in the Supplementary Material) or the physico-chemical parameters of the different substances could be found. Details on the experimental setups can be found in the Supplementary Material.

Table 1 about here

There was a major difference between aquatic and terrestrial studies with regard to the exposure scenarios investigated. In the aquatic compartment they were generally less complex than many studies of the terrestrial compartment. Most frequently a single or a few pulses or continuous exposure of one substance was tested in contrast to the terrestrial studies, where 15 out of 76 studies were conducted with mixtures of pesticides. Therefore, the aquatic studies were in general more comparable to each other than the terrestrial ones. On the other hand, terrestrial studies more often investigated exposure scenarios which are commonly found in

agricultural landscapes, including mixtures of different substances applied at different times of the year.

Most studies investigated systems with more than one species, hence included species interactions, resulting in competition, predation or grazing pressure. However, additional stressors including such interactions, low food conditions, pH or climate influence, as well as re-colonization due to migration, were rarely investigated systematically.

3. Population and community recovery

3.1 *Aquatic environment*

3.2.1 Fish

No studies on population or community recovery of fish were found. Fish are exposed to pesticides and can be affected in the wild (see, for example, the review of Schulz (2004)). However, to better understand the long-term effects of pesticide on fish, there is a need for studies going beyond the effects on single individuals. Using Pacific salmon as an example, Macneale et al. (2010) demonstrated the knowledge gaps regarding the complex interactions of pesticide effects, food webs and habitat availability. Additionally, rehabilitation studies might offer valuable insights into population recovery potential.

3.2.2 Aquatic invertebrates

This group was the one with the most studies (79) identified in our investigation. In general, if effects on aquatic invertebrates were observed, they were mostly negative irrespective of the substance class (Fig. 2). Negative effects of insecticides and fungicides on invertebrates were presumably most often direct (with additional potential indirect effects on predators), whereas herbicides effects were partly caused by a decline of algae as a food resource. The time for recovery varied greatly, ranging from 1 month (e.g. for emerging

insects in a stream mesocosm after pulse contamination with 3.2 µg/l thiacloprid, HC_5 0.65 µg/l (Beketov et al. 2008)) to 1 year (e.g. for arthropods after pulse contamination with 6 µg/l chlorpyrifos, $TU_{Daphnia magna}$ 0.78 (van den Brink et al. 1996)).

Based on changes of community composition analysis (as assessed by principle response curve (PRC) analysis that summarizes the dynamics of all taxa in one graph), more than 60% of the data series that showed an effect did not recover within the duration of the study (Fig. 2). In the majority of these investigations, pesticide application resulted in a decline of the species dominating the community, hence the time course after application was classified as “no recovery 1” or “recovery 1” (Fig. 1). In contrast, species diversity (measured as taxa richness) recovered in 70% of the data series within the study duration (Fig. 2), mostly following “recovery 1”. If herbicides were applied, in only 4 out of 10 cases was recovery observed, while for effects of insecticides, 21 out of 29 cases with negative effects recovered. This might indicate that indirect effects due to herbicide applications might last longer on species diversity compared to direct insecticidal effects.

Linking the physico-chemical properties of pesticides to recovery is difficult, in particular in complex communities. For instance, the abundance of Cladocera showed comparably negative effects following the application of high concentrations of malathion, diazinon and chlorpyrifos, respectively (Hua and Relyea 2014). Only for malathion, exhibiting high break-down rates, was recovery observed (after 9 weeks). However, chlorpyrifos was broken down similarly fast, but recovery did not occur within the study duration.

Predation was reported to modify effects as well as recovery. For instance, under low predatory pressure an indirect increase of Rotifera after carbaryl application was observed, but no such increase was similarly found under high predatory pressure (Chang et al. 2005). In populations of *Daphnia magna* with simulated predation, the distribution in body size was

reported to recover faster after fenvalerate application compared to non-predated ones (Liess and Foit 2010). Several studies reported indirect effects. For example, pesticide exposure released competition by Cladocera and resulted in an increase of Rotifera abundance (Hanazato and Kasai 1995, López-Mancisidor et al. 2008b). Likewise, contamination resulted in a decrease of grazing pressure by Cladocera and in an increase of algae (Hanazato and Kasai 1995). Interspecific competition increased recovery time after thiacloprid application; this effect was further prolonged by increased water temperature (Knillmann et al. 2013).

Fig. 2 about here

Most importantly, species with longer generation times (age at maturity) often showed increased absolute recovery times (Fig. 3). In 45% of all data series, population abundance recovered within one generation time after a decrease, after five generation times, recovery was present in most cases. The exceptions were a few data series of mainly Rotifera, which exhibit short generation times but recovered very slowly in some particular studies (e.g. López-Mancisidor et al. 2008a, López-Mancisidor et al. 2008b). This important influence of generation time was, for example, shown in a mesocosm experiment: the composition of univoltine and semivoltine species did not recover within 6 months after thiacloprid application, as opposed to multivoltine species (Beketov et al. 2008). However, another study found the opposite effect for a substance that affects molting, metamorphosis and hatching by chitin synthesis inhibition (lufenuron) (Brock et al. 2009): multivoltine arthropods were in general affected more strongly and recovered more slowly compared to univoltine ones due to the higher probability of exposure of sensitive life stages. Most of the taxa that recovered within one generation were insects (Fig. 3, light grey bars); hence taxa that are able to fly and can therefore re-colonize affected replicates from the control, from less affected replicates, or from external sources. For non-insect taxa, recovery took longer than one generation time in

the majority of cases and was completed within five generation times for most taxa (Fig. 3, dark grey bars). The important role of external recovery was, for example, reported by Caquet et al. (2007): for emerging insects long-term effects were still present one year after deltamethrin application when external recovery was prevented. Trekels et al. (2011) demonstrated the limitation of external recovery due to isolation, in particular for univoltine species after application of the insecticide endosulfan in a pond mesocosm. Likewise, immobile, sensitive species did not recover within the study duration (ten weeks after the last treatment) in a pond mesocosm experiment in contrast to mobile ones (Auber et al. 2011). This was also the only study on aquatic invertebrates that investigated realistic agricultural exposure regimes consisting of multiple applications of various pesticides.

We conclude that population recovery should also be evaluated in terms of the generation time of the respective species rather than on an absolute temporal scale. Additionally, the dispersal ability of the species needs to be taken into account. Both could be applied on a species basis but also on a community basis when taking into account that component with the largest ratio in recovery time vs. generation time and with the lowest dispersal ability. Furthermore, the distribution of recovery sources in the landscape should be considered. Recovery time also needs to be related to the time intervals between exposure events. This is especially relevant as low-dose pulses of exposure with minor effects may culminate in strong effects when successive pulses of exposure are present (Liess et al. 2013). Trait-based approaches can help to classify species according to their life cycle characteristics (Baird and van den Brink 2007, Rubach et al. 2011, van den Brink et al. 2011) and have already been successfully applied in the field (Liess and von der Ohe 2005) and in mesocosms (Liess and Beketov 2011).

Fig. 3 about here

3.2.3 Algae

In the studies investigated here, most often abundance of single species was assessed, followed by chlorophyll *a* content of the water, community composition (PRC) and diversity (either taxa richness or Shannon index). With regard to the different substance classes, fungicides nearly always had a positive effect on abundance, the amount of chlorophyll *a*, and community composition (i.e. resulted in an increase of individuals (of dominant taxa in case of PRC)), while the effect was always negative in the few cases where diversity was investigated (Fig. 4). In the majority of cases, herbicides had negative, presumably direct effects; for insecticides the outcomes are more diverse suggesting both direct and indirect effects. For insecticides and fungicides, negative effects were caused by significantly lower toxic units than positive effects (Kruskal Wallis rank sum test, $p < 0.001$) due to a higher effect threshold for indirect, positive effects.

Regarding the recovery pattern, in general recovery within the duration of the studies was observed in approximately 50% of all data series; only diversity recovered in fewer cases (Fig. 4). However, there was no significant positive correlation between recovery time and effect strength, as might be expected, for both positive and negative effects. Additional stress such as grazing pressure was reported to interact with atrazin effects on algae community structure and showed a tendency to prevent recovery (Muñoz et al. 2001). On a larger scale, effects on algae can have long-term effects on whole ecosystem status: in a pond mesocosm experiment (depth 1.2 m, area 25 m²) glyphosate application resulted in a shift from macrophyte dominated clear state to phytoplankton dominated turbid state (Vera et al. 2010). Such alternative stable states in lake status and shifts from one to the other are usually determined by nutrient status and exhibit strong hysteresis.

Fig. 4 about here

3.2.4 Aquatic plants

Only two manuscripts comprising five studies on the recovery of aquatic plants after pesticide exposure were found that met our criteria. Both reported effects on several endpoints and their recovery in pond mesocosm studies without animals. Cunningham et al. (1984) reported effects and recovery of density, biomass, and O₂ production of *Potamogeton perfoliatus* after atrazine application. Only for the highest tested concentration (TU based on *Lemna gibba* 1.8) did recovery not occur within the four weeks of study duration. Knauer and Hommen (2012) described relatively strong effects of the herbicides atrazine, diuron, isoproturon and a mixture of the three on photosynthetic rate of *Elodea Canadensis*, *Myriophyllum spicatum*, *Potamogeton lucens*. TU based on *Lemna gibba* ranged from 0.5 to -0.5. Recovery of the endpoint occurred within 2 weeks. In both studies the lack of animals feeding on the plants might be the reason for the fast recovery. The question remains whether such rapid recovery would be possible in natural, more diverse systems. Hence, clearly more research is needed to derive meaningful protection goals for aquatic plants (Thursby and Lewis 2013) and to elucidate their sensitivity and recovery potential.

3.2.5 Aquatic microbes

Twelve studies on the recovery of aquatic microbes were found. Often no effect was observed on microbial endpoints (e.g. bacteria abundance, herbicide simetryn: Chang et al. 2011; decomposition of organic matter, fungicide azoxystrobin: Zafar et al. 2012). If effects were detected, they were most often positive, i.e. showed an increase in abundance or biomass compared to the control (e.g. bacteria abundance, insecticide aldrin: López et al. 2002; pyrethroid insecticide cypermethrin: Friberg-Jensen et al. 2003). In nearly all cases, the endpoint did not decrease to control levels within the study duration, which ranged between 5 and 28 days. The apparent lack of effects indicates that this organism group might be highly resilient or may even profit by indirect effects (e.g. lack of predators) as suggested by the

mainly positive effects. Likewise, the missing recovery back to control levels may be based on long-term indirect effects as identified by Foit et al. (2012). The question arises, on the one hand, whether the insensitivity of the chosen microbial endpoints reflects reality. On the other hand, as recovery was not often observed in the study period, it seems appropriate to increase the observation time even for this generally rapidly reproducing species group.

With respect to ecological functions, López et al. (2002) reported an increase of denitrifying bacteria and other heterotrophic bacteria in response to the insecticide aldrin. However, simultaneously a decrease of denitrification activity was measured. Hence, even when the pesticide effect on the density of certain functional groups is measured, the effect on the actual function can be reverse. Therefore, it seems advisable to investigate effects on both functional groups of microbes as well as directly on microbial ecosystem function.

3.2.6 Amphibians

No study on amphibian recovery was found, but they are very likely to be negatively affected by pesticides, particularly in combination with other stressors (Blaustein and Kiesecker 2002, Davidson et al. 2002). For instance, Rohr et al. (2006) found complex, long-term effects on the streamside salamander (*Ambystoma barbouri*) of atrazine. As for fish, several factors including habitat destruction, diseases, climate change as well as contamination can have negative effects on amphibian populations (Collins and Storfer 2003). Disentangling and weighting these factors is still a question of on-going research. The procedures for assessing the role of pesticides would profit from better monitoring of both amphibian populations and field contamination levels.

As amphibians generally have long generation times, they might be particularly vulnerable. Additionally, due to their longer life span and high trophic level, they are likely to suffer from longer or repeated exposures, secondary poisoning as well as biomagnification (Rowe 2008). Therefore, the effects on single individuals are particularly important for

population development, and recovery is likely to take a long time. Additionally, amphibians are not only exposed in the aquatic environment but also when crossing through fields or field margins (Fryday and Thompson 2012), which might have substantial negative effects (Brühl et al. 2013).

3.2 Terrestrial environment

3.3.1 Birds and Mammals

Only one study was found in the literature describing the recovery of a bird community (23 species) after exposure to glyphosate and sulfometuron methyl herbicides at a forest site (Stoleson et al. 2011). However, the high species turnover, even within control plots, suggests that avian communities may be more appropriately assessed on larger spatial scales than those used in this study (6.5-8 ha).

Despite the lack of experimental studies, recovery of bird populations is one of the best examples of recovery after the long-term usage of pesticides. In the 1950s to 1970s strong effects of pesticides such as DDT occurred on populations of birds-of-prey, in particular hawks, ospreys and eagles (Henny et al. 2010). After banning these insecticides, monitoring data indicated population recovery, e.g. in the USA 1972 (Henny et al. 2010), in Germany 1972 (Wegner et al. 2005), in the UK 1984 (Walker and Newton 1998), and in Spain 1977 (Mañosa et al. 2001). Hence, population monitoring enabled the establishment of a clear link between the decreased exposure to the pesticide and the recovery of populations, but also indicates possibilities of how recovery of impacted species could actively be supported.

In general, the current testing practice for birds and mammals is problematic because laboratory test results are difficult to extrapolate to the field (Mineau 2005). In addition, changes (and recovery) in bird populations are often caused by indirect effects. An example is

the reduction of invertebrate food after the use of insecticides in English farmland regions (e.g. Morris et al. 2005). Recovery of affected bird populations (in this case the yellowhammer *Emberiza citronella*) was only possible when measures were taken to minimize applications of persistent broad-spectrum insecticides during the breeding season. In the current EU ERA schemes, no direct effects on the individuals are accepted (EFSA 2009), while there is a clear lack in the assessment of indirect or long-term effects. These could only be assessed if long-term monitoring programs for evaluating emerging contaminants using birds with a well-known ecology, such as the osprey, would be set up or continued (Henny et al. 2010).

No study on mammal population recovery after the impact of pesticides could be found.

3.3.2 Bees

Only one study specifically addressing recovery of bee populations was found (Faucon et al. 2005). Two groups of eight honey bee colonies were fed with two different concentrations of imidacloprid in saccharose syrup during summer. Among a variety of measurement endpoints, effects such as higher activity of adult bees or a significantly higher frequency of pollen carrying during the feeding period were observed compared to a control. When the pesticide was no longer applied, activity and pollen carrying were re-established at a similar level within three months.

3.3.3 Non-target-arthropods (NTAs)

In contrast to other terrestrial groups studied, a relatively high number of studies (15) on the recovery of NTAs after exposure to a wide range of pesticides were available. In almost all studies recovery was observed, most often following recovery class 1. This result

might be caused by the fact that the evaluated studies mainly focused on the effects of insecticides on a small number of arthropods.

One group of studies consists of those related to pesticide registration in Europe, mainly investigating the effects of insecticides on aphid predators or parasitoids (e.g. Shires 1985, Duffield and Aebischer 1994). It has been shown that the standard test species *T. pyri* reacts less sensitively to a pesticide (mancozeb) when it lives at a site where this fungicide had been applied previously (Auger et al. 2004); hence, recovery might be faster due to smaller effects. Several field studies focus on the distinction between internal (i.e. from remnants of the impacted population) and external (e.g. by reinvasion) recovery. For example, it was found that at least for highly mobile flying insects such as the aphid parasitoid *Aphidius ervi*, re-colonization after application of the insecticide deltamethrin was more important than vertical recruitment (Desneux et al. 2006). Another group of studies identified factors which may facilitate (or impact) recovery processes. For example, the distance between the center of treated areas and field margins acting as refugia and / or as sources for NTAs play an important role for recovery of populations living in-field (Jepson and Thacker 1990, Duffield et al. 1996, Longley et al. 1997). Even the properties of field margins influence recovery of different species of hover flies (syrphid Diptera) (Wratten et al. 2003). There were clear differences of fly movements between four types of field boundaries (fences, lines of poplars with or without gaps, and controls (i.e. no potential barriers)). These factors, being probably relevant for other organism groups too, have to be considered when assessing measures to facilitate recovery of species affected by pesticides.

Recently, an international working group discussed the improvement of the existing requirements for ERA of pesticides for NTAs (Alix et al. 2012) with a subgroup focusing on recovery (Brühl et al. 2012). The group concluded that when investigating recovery, endpoints should vary between different scales and areas: (1) in off-crop areas, recovery of

the communities (e.g., assemblage of arthropod species and their abundance living in a grassy margin) is the relevant endpoint; (2) in in-crop areas, the recovery of ecosystem functions (e.g., pollination, pest control) assessed for appropriate functional groups (e.g., pollinators and beneficial arthropods) is the relevant endpoint. Considering field studies for off-crop risk assessment, no effect or only transient effects (de Jong et al. 2010) are acceptable.

3.3.4 Soil invertebrates

In most studies recovery was observed (Fig. 5A), which might be explained by the fact that many of these studies were long-term field studies of duration of about one year; i.e. they were long enough to detect recovery.

Fig. 5 about here

3.3.4.1 Arthropoda (*springtails and mites*)

Only six studies addressing recovery were found – and three of them refer to the same British landscape project (SCARAB) (Frampton 1999, 2000b, 2002). This data set, containing the same sites, pesticides and species, was used to perform a community analysis (Frampton 2000a, Frampton and van den Brink 2007). Since in this study exposure regimes followed the usual application regimes of local farmers, it is hardly possible to determine the reaction to and recovery from individual pesticides. Nevertheless, the indirect effects of pesticides were proven; for instance the insecticide cypermethrin affected predators (e.g. carabid beetles, linyphiid spiders) stronger than springtails, allowing the latter to recover their abundance earlier than the predators. Furthermore, different Collembolan species reacted significantly differently (including recovery), indicating that an aggregation to higher than species level cannot adequately describe pesticide effects. It was also shown that clearly unexposed field margins play an important role as a source for re-invasion, meaning that buffer zones are an important measure to foster recovery.

Finally, Duffield and Aebischer (1994) showed that recovery assessment in small in-field areas may underestimate the pesticide effects on large predators but overestimate them on micro-arthropods. In the center of large plots the latter were able to already recover before predators were able to do so since they need time to colonize these areas from the field edges.

3.3.4.2 *Nematoda*

Only two manuscripts on recovery were found. Strong effects on various trophic groups, generally lasting for several months, have been found in the field, and recovery was observed after one year at the latest (Stirling et al. 2001). Due to the low number of sampling dates (just three) these findings have to be evaluated cautiously. Yardim and Edwards (1998) also found relatively strong effects of a mixture of different pesticides on various trophic groups mostly without recovery within the one month monitored after the last application.

3.3.4.3 *Oligochaeta (earthworms and enchytraeids)*

Based on the results of standard field tests, Jones and Hart (1998) concluded that the recovery of earthworm populations mainly depends on the persistence of the test compound. Even a reduction of 90% in population size was considered to be acceptable by the authors when the population reached the former total abundance in less than one year. However, recovery by re-colonization might be strongly overestimated since only small plots without barriers were considered.

Three studies performed under tropical conditions in India and Sri Lanka, using megascolecid earthworms, also showed close relationships between recovery and persistence of substance (Reddy and Reddy 1992, Panda and Sahu 1999, De Silva et al. 2010). Additionally, a strong correlation between recovery and application rate was demonstrated in the only study in which several (three) levels of concentrations were tested (De Silva et al. 2010). However, the difference in the start of recovery (five versus 46 weeks) between two

organophosphates compared to carbaryl and benomyl was much larger than expected from the difference in half-life time (DT_{50}) (Potter et al. 1990). Furthermore, different ecological groups of earthworms (mineral dwellers, vertical burrowers and litter dwellers) showed very different recovery patterns (Edwards and Brown 1982).

Re-colonization by earthworms can be active (i.e. by moving on the surface) or passive (e.g. cocoons via human transport of living plants, i.e. including their roots and soil (Lee 1985)). In general, dispersal rates are low and range between 6.3 and 9.0 m/y (Eijsackers 2011). However, in the literature the same species (*Aporrectodea longa*) was found to be a slow “wanderer” in an English grassland (Butt et al. 1997), but was classified as a “colonizer” in a Dutch polder soil (Ligthart and Peek 1997). In particular, cocoons are important for dispersal and recovery, since they are very well protected against a wide range of natural and anthropogenic factors. This can be used for practical recovery efforts, i.e. by collecting soil at sites well-inhabited by earthworms and introducing this soil at sites which have lost their earthworms (Butt et al. 1999). By organizing the agricultural landscape in such a way as to avoid large fields it should be possible to allow re-immigration of earthworms after a community has been negatively affected (Ehrmann 1996).

The main results concerning the recovery of soil invertebrates (exemplified for earthworms) in agricultural systems can be summarized as follows:

- Earthworm populations are usually able to recover from natural stress factors, but reaching pre-stress numbers is not sufficient since species number and composition (at least ecological groups) should also be back to a pre-application range (Lee 1985).
- Agro-ecosystems often lack structures known to be important for earthworm recovery (e.g. shelters) and are often so large that recovery by migration is not possible (or takes decades) (Edwards et al. 1995).

- Since anthropogenic factors can be beneficial (e.g. input of food) or harmful (ploughing, pesticides), their overall impact is difficult to identify. When assessing effects of anthropogenic stress, interaction with natural stress factors has to be considered (Holmstrup et al. 2010).
- Recovery from population declines is only possible if certain minimum requirements are fulfilled (in particular provision of enough food) (e.g. Marinissen and van den Bosch 1992).
- The amount of reliable biological data (e.g. life-cycle) for important species needs to be increased (e.g. Axelsen and Holmstrup 1998).
- Long-term studies of at least one year duration (depending on the generation time of the taxa under consideration) that enable assessment of recovery are required.

3.3.5 Soil microbes

Due to their short generation time, it is not surprising that the recovery of microbial populations or communities after exposure towards pesticides has been studied relatively often (Malone 1971). In contrast to the other terrestrial taxa groups discussed so far, these studies were almost completely performed in the laboratory (11 out of 15), while only three (Chen and Edwards 2001, Chen et al. 2001a, 2001b) were conducted in a semi-field test and just one in the field (an almost arctic site in Northern Sweden (Haugwitz et al. 2011)). Especially the semi-field method (Chen and Edwards 2001, Chen et al. 2001a, 2001b) seems to be a promising approach for an ecologically relevant but also practical assessment of recovery processes since several taxa groups can be studied under realistic conditions in parallel.

We ascertained that recovery occurred in only 50% of all data series (Fig. 5B) despite relative long study durations (often longer than two months, Table S2 in the Supplementary Material) and generally short reproduction cycles.

3.3.6 Terrestrial plants

Seven studies were identified which described the recovery of plants, mostly performed in the laboratory. The study of Carpenter and Boutin (2010) is the most important one in the context of this review since it addresses in a simple but well-designed way the question of recovery. This study confirmed that, even within the relatively short duration of OECD standard tests, plants are able to increase their growth after an initial impact of the herbicide glufosinate ammonium in such a way that they can make up the difference to the control. It indicates that the normal plant testing procedure seems to be protective, but it could not address the question of whether real plant communities in the field would react similarly because such studies on the individual level are difficult to relate to population recovery.

Structure and adaptation history of a plant community determine recovery after pesticide stress: For example, three years after the last application of different herbicides there was a higher resistance in an established community than in a recently disturbed one (Tomkins and Grant 1977). Interestingly, the extremely persistent herbicide paraquat hampered recovery in the recently disturbed site but had only transient effects at the field site with the established community. It was also shown that indirect effects of pesticides can be responsible for the lack of recovery: Low concentrations of pesticides inhibited the growth of legumes, probably because they affected the arbuscular mycorrhizal fungi living in close connection with these plants (Abd-Alla et al. 2000).

In summary, our knowledge on the recovery of plant populations or communities after exposure to pesticides is still very limited. In accordance with Boutin et al. (2012) it is therefore necessary that data on long-term effects and recovery be requested in upcoming

ERA documents, meaning that current test strategies and guidelines have to be modified accordingly.

3.3.8 Reptiles

Reptiles are a widely distributed and often highly endangered group of vertebrates (Todd et al. 2010). Most of what has been said for amphibians is true for reptiles as well, but due to their better physical protection, exposure might be less pronounced than in the case of amphibians. However, in Europe almost all reptilian species are protected under specific conservation acts, including that of the EU (European Commission 2013). In the literature, no study on the recovery of reptiles after being affected by pesticides has been found.

4. Recommendations and Conclusions

4.1 *Conclusions on Population and Community Recovery*

Based on the evaluated literature and the established ecological knowledge, general conclusions on the recovery of populations and communities can be given:

- Internal recovery of populations is directly linked to the generation time of species.
- External recovery is observed for mobile species, especially those with aerial stages.
- Community composition in general did not recover within the study duration in the majority of cases. Hence, more complex characteristics, which might describe ecosystem structure better than single species abundance, recover more slowly.
- The ecological context plays a crucial role for recovery as environmental stress generally acts in addition to or in synergy with pesticide stress; for instance, recovery is prolonged if certain minimum requirements are not fulfilled, including provision of enough food, sufficient density of mating partners, or suitable distance to unaffected sites.

- 614 - Indirect effects based on food web interactions can play an important role for the
615 magnitude and duration of effects and for recovery processes; this is especially true for
616 taxa on higher levels of the food web.

617 With regard to re-colonization, it is unclear if sufficient sources are present in agricultural
618 landscapes with a variety of spatially and temporally distributed pesticide applications and
619 additional stressors. For terrestrial exposures this might rarely be the case when the area of
620 buffer strips is very small related to the size of the treated field. Likewise, aquatic ecosystems
621 might be exposed from numerous directly and indirectly connected diffuse sources.

622 Additionally, it is important to mention that in agricultural landscapes pesticide
623 exposure re-occurs every year and consists of a mixture of different substances applied at
624 different times of the year (Liess et al. 1999, EFSA PPR Panel 2012, 2013, 2014, 2015).
625 Hence, even if a species can recover in experimental studies within one year, this has to be
626 related to realistic exposure scenarios in terms of short-term exposure profiles within a year
627 and also long-term exposure profiles over multiple years.

628 Some of these aspects are already included in the current guidance documents. In
629 general, all relevant ecological factors influencing population and community recovery should
630 be taken into account, including “dispersal ability, life-history, breeding season, number of
631 breeding attempts per season, abundance in the environment, spatial records, as well as the
632 natural variability in population sizes and distributions”(European Commission 2001).
633 Moreover, some rules of thumb for maximum recovery times are already given (European
634 Commission 2001, 2002, EFSA PPR Panel 2013). However, there is a lack of knowledge on
635 how to modify these rules by incorporating additional ecological factors into the assessment
636 and how to support them with sound scientific data.

4.2 Recommendations for Assessing Recovery in Risk

Assessment

The present study identifies several aspects that contribute to an improvement of the environmental risk assessment of pesticides in Europe with respect to recovery from pesticide effects.

1. The existing knowledge on the relationships between the individual populations and species, their main functions and services in aquatic and terrestrial ecosystems, and the recovery mechanisms is still limited. Therefore, it is necessary to generate systematic ecological knowledge on the various factors influencing recovery and, in particular, to utilize existing knowledge (e.g. from general disturbance ecology) more rigorously. In doing so, a better understanding of the processes and structures driving ecological recovery needs to be generated. The concept of biological traits could be useful to address this issue (Artigas et al. 2012). Additionally, it is essential to investigate in more detail the role of single species in ecological functions and also to derive more knowledge on the ecosystem services that are potentially affected by pesticides (Nienstedt et al. 2012). As one step in this direction, the data situation could be considerably improved if the reports (not just the summaries) of higher-tier studies prepared as part of the pesticide registration procedure in the EU would be made publicly available for further investigations.

2. Higher-tier tests often lack statistical power because of (i) low number of replicates, (ii) high variation between replicates and (iii) low abundance of vulnerable species with a long generation time and low recovery potential. These issues need to be addressed by increasing the number of replicates, providing conditions that foster vulnerable species (long pre-application periods) and applying statistical approaches capable of dealing with the limitations of higher tier-tests (e.g. Beketov et al. 2013). Therefore, we recommend defining minimum standards for higher tier studies that enable a comparable evaluation of

test results and maximize their value for ERA (de Jong et al. 2006, de Jong et al. 2010). In particular regarding higher tier studies, the standardization should be guided by agreed scenarios (see below). Such standards should also include technical guidance on the minimum number of replicates, relevant endpoints, suitable sampling methods, the interval between and the duration of sampling, concentration ranges to be tested, minimum requirements for community composition, and suggestions for statistical evaluation. For the aquatic environment, this is often already realized in aquatic mesocosms in the aquatic guidance document (EFSA PPR Panel 2013). In the terrestrial compartment, such improved studies could be performed with semi-field methods (e.g. Terrestrial Model Ecosystems (Schäffer et al. 2010)) or directly in the field. Currently there is no study that fulfills all these criteria, but some come close (e.g. Frampton 2000a). In addition, the standard earthworm field test (ISO (International Organization for Standardization) 1999), used for pesticides registration for more than a decade, could be improved accordingly: besides the inclusion of residue analysis and the usage of a dose-response-design (Kula et al. 2006), the addition of more organism groups in the same test has been proposed (Römbke et al. 2009).

3. For the reporting of results from higher-tier tests we also recommend identifying the length of generation time of relevant taxa, the degree of synchronization of life cycles and the potential for external recovery of relevant taxa, or better, the actual rate of external recovery. This information will enable an informed interpretation of test results.
4. Well-designed monitoring studies can be a valuable tool to identify the long-term effects of a pesticide and recovery of the affected organisms, as demonstrated by the experience with DDT. Such studies also might help separate pesticide effects from other (anthropogenic) disturbances if applied before and after application / registration. This

might be particularly helpful for birds and mammals but can also deliver valuable information for other species groups.

5. Ecological / ecotoxicological modeling studies are a promising tool for supplementary investigation of population and community level effects and recovery (e.g. fish: Baldwin et al. 2009, mammals: Dalkvist et al. 2009, aquatic invertebrates: Kattwinkel and Liess 2014). They may also help to disentangle the role of different environmental factors that influence population and community dynamics. However, such models can aid the assessment of effects and recovery only if the relevant ecotoxicological and ecological processes are known. Models lacking relevant components of the community only provide information of limited value for risk assessment as they can only describe the development of a population without its relevant context.

6. Finally, ERA would profit from the development of realistic and representative worst case scenarios for the environmental situation. Such scenarios are helpful as the multitude of potential environmental scenarios in terms of relevant taxa and also biotic and abiotic conditions cannot be covered by (standardized) test-systems. This also includes realistic size, distribution and re-colonization rates for refuges and reservoirs and a realistic magnitude of biological interactions (competition, predation) as these parameters can influence recovery considerably. Using such a scenarios-based approach can reduce the effort to generate meaningful data and to interpret and compare the results.

706 **Acknowledgement**

707 This study was funded by the European Food Safety Authority (EFSA).

708 **Disclaimer** The publication was drafted under the sole responsibility of the authors and is not
709 regarded as an EFSA output. The positions and opinions presented are those of the authors
710 alone and are not intended to represent the views of EFSA.

711

Draft

References

- Abd-Alla, M.H., Omar, S.A. and Karanxha, S. 2000. The impact of pesticides on arbuscular mycorrhizal and nitrogen-fixing symbioses in legumes. *Appl. Soil Ecol.* **14**(3): 191-200.
- Artigas, J., Arts, G., Babut, M., Caracciolo, A.B., Charles, S., Chaumot, A., Combourieu, B., Dahllof, I., Despreaux, D., Ferrari, B., Friberg, N., Garric, J., Geffard, O., Gourlay-France, C., Hein, M., Hjorth, M., Krauss, M., De Lange, H.J., Lahr, J., Lehtonen, K.K., Lettieri, T., Liess, M., Lofts, S., Mayer, P., Morin, S., Paschke, A., Svendsen, C., Usseglio-Polatera, P., van den Brink, N., Vindimian, E. and Williams, R. 2012. Towards a renewed research agenda in ecotoxicology. *Environ. Pollut.* **160**: 201-206.
- Auber, A., Roucaute, M., Togola, A. and Caquet, T. 2011. Structural and functional effects of conventional and low pesticide input crop-protection programs on benthic macroinvertebrate communities in outdoor pond mesocosms. *Ecotoxicology* **20**(8): 2042-2055.
- Auger, P., Kreiter, S., Mattioda, H. and Duriatti, A. 2004. Side effects of mancozeb on *Typhlodromus pyri* (Acari : Phytoseiidae) in vineyards: results of multi-year field trials and a laboratory study. *Exp. Appl. Acarol.* **33**(3): 203-213.
- Axelsen, J.A. and Holmstrup, M. 1998. Simulation of pesticide effects on populations of 3 species of earthworms. *In* *Advances in earthworm ecotoxicology. Edited by* S.C. Sheppard, J.D. Bembridge, M. Holmstrup and L. Posthuma. Pensacola, SETAC Press. pp. 281-294.
- Baird, D.J. and van den Brink, P.J. 2007. Using biological traits to predict species sensitivity to toxic substances. *Ecotoxicol. Environ. Saf.* **67**(2): 296-301.
- Baldwin, D.H., Spromberg, J.A., Collier, T.K. and Scholz, N.L. 2009. A fish of many scales: extrapolating sublethal pesticide exposures to the productivity of wild salmon populations. *Ecol. Appl.* **19**(8): 2004-2015.

- 735 Beketov, M.A., Kattwinkel, M. and Liess, M. 2013. Statistics matter: data aggregation
736 improves identification of community-level effects compared to a commonly used
737 multivariate method. *Ecotoxicology* **22**(10): 1516-1525.
- 738 Beketov, M.A., Schäfer, R.B., Marwitz, A., Paschke, A. and Liess, M. 2008. Long-term
739 stream invertebrate community alterations induced by the insecticide thiacloprid: Effect
740 concentrations and recovery dynamics. *Sci. Total Environ.* **405**(1-3): 96-108.
- 741 Blaustein, A.R. and Kiesecker, J.M. 2002. Complexity in conservation: lessons from the
742 global decline of amphibian populations. *Ecol. Lett.* **5**(4): 597-608.
- 743 Boutin, C., Aya, K.L., Carpenter, D., Thomas, P.J. and Rowland, O. 2012. Phytotoxicity
744 testing for herbicide regulation: Shortcomings in relation to biodiversity and ecosystem
745 services in agrarian systems. *Sci. Total Environ.* **415**: 79-92.
- 746 Brock, T.C.M., Roessink, I., Belgers, J.D.M., Bransen, F. and Maund, S.J. 2009. Impact of a
747 benzoyl urea insecticide on aquatic macroinvertebrates in ditch mesocosms with and without
748 non-sprayed sections. *Environ. Toxicol. Chem.* **28**(10): 2191-2205.
- 749 Brühl, C.A., Schmidt, T., Pieper, S. and Alscher, A. 2013 Terrestrial pesticide exposure of
750 amphibians: An underestimated cause of global decline? *Scientific Reports* **3**, DOI:
751 10.1038/srep01135
- 752 Brühl, C.A., Neumann, P., Aldershof, S., Bohan, D., Brown, K., Candolfi, M., Geiger, F.,
753 Kovalkovičová, N., Kula, C., Nienstedt, K., Pestanudo, S., Römbke, J., Schmidt, T., Topping,
754 C. and van Vliet, P. 2012. Recovery. *In Ecological Risk Assessment of Pesticides: Linking*
755 *Non-Target Arthropod Testing with Protection Goals (ESCORT 3). Edited by A. Alix, F.*
756 *Bakker, K. Barrett, C.A. Brühl, M. Coulson, S. Hoy, J.P. Jansen, P. Jepson, G. Lewis, P.*
757 *Neumann, D. Süßenbach and P. Van Vliet, SETAC Press. pp. 54-57.*
- 758 Butt, K.R., Frederickson, J. and Morris, R.M. 1997. The earthworm inoculation unit
759 technique: An integrated system for cultivation and soil-inoculation of earthworms. *Soil Biol.*
760 *Biochem.* **29**(3-4): 251-257.

- 761 Butt, K.R., Frederickson, J. and Lowe, C.N. 1999. Colonisation, survival and spread of
762 earthworms on a partially restored landfill site. *Pedobiologia* **43**(6): 684-690.
- 763 Candolfi, M., Blümel, S., Forster, R., Bakker, F.M., Grimm, C., Hassan, S.A., Heimbach, U.,
764 Mead-Briggs, M., Reber, B., Schmuck, R. and Vogt, H. 2000. Guidelines to evaluate side-
765 effects of plant protection products to non-target arthropods. Gent, IOBC-WPRS.
- 766 Caquet, T., Hanson, M.L., Roucaute, M., Graham, D.W. and Lagadic, L. 2007. Influence of
767 isolation on the recovery of pond mesocosms from the application of an insecticide. II.
768 Benthic macroinvertebrate responses. *Environ. Toxicol. Chem.* **26**(6): 1280-1290.
- 769 Carpenter, D. and Boutin, C. 2010. Sublethal effects of the herbicide glufosinate ammonium
770 on crops and wild plants: Short-term effects compared to vegetative recovery and plant
771 reproduction. *Ecotoxicology* **19**(7): 1322-1336.
- 772 Chang, K.H., Sakamoto, M. and Hanazato, T. 2005. Impact of pesticide application on
773 zooplankton communities with different densities of invertebrate predators: An experimental
774 analysis using small-scale mesocosms. *Aquat. Toxicol.* **72**(4): 373-382.
- 775 Chang, K.H., Sakamoto, M., Ha, J.Y., Miyabara, Y., Nakano, S., Doi, H. and Hanazato, T.
776 2011. Response of the plankton community to herbicide application (triazine herbicide,
777 simetryn) in a eutrophicated system: short-term exposure experiment using microcosms.
778 *Limnology* **12**(1): 11-16.
- 779 Chen, S.-K. and Edwards, C.A. 2001. A microcosm approach to assess the effects of
780 fungicides on soil ecological processes and plant growth: comparisons of two soil types. *Soil*
781 *Biol. Biochem.* **33**(14): 1981-1991.
- 782 Chen, S.-K., Edwards, C.A. and Subler, S. 2001a. Effects of the fungicides benomyl, captan
783 and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory incubations.
784 *Soil Biol. Biochem.* **33**(14): 1971-1980.

- 785 Chen, S.-K., Edwards, C.A. and Subler, S. 2001b. A microcosm approach for evaluating the
786 effects of the fungicides benomyl and captan on soil ecological processes and plant growth.
787 *Appl. Soil Ecol.* **18**(1): 69-82.
- 788 Clark, K.E., Zhao, Y. and Kane, C.M. 2009. Organochlorine Pesticides, PCBs, Dioxins, and
789 Metals in Postterm Peregrine Falcon (*Falco peregrinus*) Eggs from the Mid-Atlantic States,
790 1993-1999. *Arch. Environ. Contam. Toxicol.* **57**(1): 174-184.
- 791 Collins, J.P. and Storfer, A. 2003. Global amphibian declines: sorting the hypotheses. *Divers.*
792 *Distrib.* **9**(2): 89-98.
- 793 Cunningham, J.J., Kemp, W.M., Lewis, M.R. and Stevenson, J.C. 1984. Temporal responses
794 of the macrophyte, *Potamogeton perfoliatus* L., and its associated autotrophic community to
795 atrazine exposure in estuarine microcosms. *Estuaries* **7**(4 B): 519-530.
- 796 Dalkvist, T., Topping, C.J. and Forbes, V.E. 2009. Population-level impacts of pesticide-
797 induced chronic effects on individuals depend more on ecology than toxicology. *Ecotoxicol.*
798 *Environ. Saf.* **72**(6): 1663-1672.
- 799 Davidson, C., Shaffer, H.B. and Jennings, M.R. 2002. Spatial tests of the pesticide drift,
800 habitat destruction, UV-B, and climate-change hypotheses for California amphibian declines.
801 *Conserv. Biol.* **16**(6): 1588-1601.
- 802 de Jong, F.M.W., van Beelen, P., Smit, C.E. and Montforts, M.H.M.M. 2006. Guidance for
803 summarising earthworm field studies. RIVM Report No. 601506006: 46.
- 804 de Jong, F.M.W., Bakker, F.M., Brown, K., Jilesen, C.J.T.J., Posthuma-Doodeman, C.J.A.M.,
805 Smit, C.E., van der Steen, J.J.M. and van Eekelen, G.M.A. 2010. Guidance for summarising
806 and evaluating field studies with non-target arthropods. RIVM report 601712006: 73.
- 807 De Silva, P.M.C.S., Pathiratne, A., Van Straalen, N.M. and van Gestel, C.A.M. 2010.
808 Chlorpyrifos causes decreased organic matter decomposition by suppressing earthworm and
809 termite communities in tropical soil. *Environ. Pollut.* **158**(10): 3041-3047.

- Desneux, N., Denoyelle, R. and Kaiser, L. 2006. A multi-step bioassay to assess the effect of the deltamethrin on the parasitic wasp *Aphidius ervi*. *Chemosphere* **65**(10): 1697-1706.
- Dietrich, D.R., Schmid, P., Zweifel, U., Schlatter, C., Jennieiermann, S., Bachmann, H., Buhler, U. and Zbinden, N. 1995. Mortality of birds of prey following field application of granural carbofuran - A case-study. *Arch. Environ. Contam. Toxicol.* **29**(1): 140-145.
- Duffield, S.J. and Aebischer, N.J. 1994. The effect of spatial scale of treatment with dimethoate on invertebrate population recovery in winter-wheat. *J. Appl. Ecol.* **31**(2): 263-281.
- Duffield, S.J., Jepson, P.C., Wratten, S.D. and Sotherton, N.W. 1996. Spatial changes in invertebrate predation rate in winter wheat following treatment with dimethoate. *Entomol. Exp. Appl.* **78**(1): 9-17.
- Edwards, C.A., Bohlen, P.J., Linden, D.R. and Subler, S. 1995. Earthworms in agroecosystems. *In* Earthworm ecology and biogeography in North America. *Edited by* P.F. Hendirx, CRC Press. pp. 185-215.
- Edwards, P.J. and Brown, S.M. 1982. Use of grassland plots to study the effect of pesticides on earthworms. *Pedobiologia* **24**(3): 145-150.
- EFSA 2009. Risk assessment for birds and mammals. *EFSA Journal* **7**(12): 1438.
- EFSA PPR Panel 2012. Scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). *EFSA Journal* **10**(5): 2668.
- EFSA PPR Panel 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. *EFSA Journal* **11**(7): 3290.
- EFSA PPR Panel 2014. Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target terrestrial plants. *EFSA Journal* **12**(7): 3800.

- 835 EFSA PPR Panel 2015. Scientific Opinion addressing the state of the science on risk
836 assessment of plant protection products for non-target arthropods. EFSA Journal **13**(2): 3996.
- 837 Ehrmann, O. 1996. Regenwürmer in einigen südwestdeutschen Agrarlandschaften:
838 Vorkommen, Entwicklung bei Nutzungsänderungen und Auswirkungen auf das Bodengefüge.
839 Hohenheimer Bodenkundliche Hefte **35**: 1-135.
- 840 Eijsackers, H. 2011. Earthworms as colonizers of natural and cultivated soil environments.
841 Appl. Soil Ecol. **50**: 1-13.
- 842 EPPO 2001. Guideline for the efficacy evaluation of plant protection products – Side effects
843 on honeybees. OEPP/EPPO Bulletin **170**: 19-23.
- 844 EPPO 2003. EPPO Standarts: Environmental risk assessment scheme for plant protection
845 products: Chapter 8: Soil organisms and functions. OEPP/EPPO Bulletin **33**: 195-209.
- 846 European Commission 2001. Working document, Guidance document on aquatic
847 ecotoxicology in the context of the directive 91/414/EEC. SANCO/3268/2001 rev.4 (final) 17
848 October 2002.
- 849 European Commission 2002. Draft Working Document. Guidance Document on Terrestrial
850 Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 rev 2 final.
- 851 European Commission 2013. Commission Regulation (EU) No 283/2013 of 1 March 2013
852 setting out the data requirements for active substances, in accordance with Regulation (EC)
853 No 1107/2009 of the European Parliament and of the Council concerning the placing of plant
854 protection products on the market.
- 855 European Parliament and European Council 2009. Regulation (EC) No 1107/2009 of the
856 European Parliament and of the Council of 21 October 2009 concerning the placing of plant
857 protection products on the market and repealing Council Directives 79/117/EEC and
858 91/414/EEC.

- 859 Eurostat 2015. Statistics: Browse/Search Database. Available from
860 http://epp.eurostat.ec.europa.eu/portal/page/portal/statistics/search_database [accessed
861 10.02.2015].
- 862 Faucon, J.P., Aurières, C., Drajnudel, P., Mathieu, L., Ribière, M., Martel, A.C., Zeggane, S.,
863 Chauzat, M.P. and Aubert, M.F.A. 2005. Experimental study on the toxicity of imidacloprid
864 given in syrup to honey bee (*Apis mellifera*) colonies. *Pest Manage. Sci.* **61**(2): 111-125.
- 865 Foit, K., Kaske, O. and Liess, M. 2012. Competition increases toxicant sensitivity and delays
866 the recovery of two interacting populations. *Aquat. Toxicol.* **106**: 25-31.
- 867 Frampton, G.K. 1999. Large-scale monitoring of nontarget pesticide effects on farmland
868 arthropods in England. *Abstr. Pap. Am. Chem. S.* **218**(1-2): 56-AGRO 56.
- 869 Frampton, G.K. 2000a. Large-scale monitoring of non-target pesticide effects on farmland
870 arthropods in England: The compromise between replication and realism of scale. *In*
871 *Pesticides and Wildlife. Edited by J.J. Johnston.* Washington DC, American Chemical
872 Society. pp. 54-67.
- 873 Frampton, G.K. 2000b. Recovery responses of soil surface Collembola after spatial and
874 temporal changes in long-term regimes of pesticide use. *Pedobiologia* **44**(3-4): 489-501.
- 875 Frampton, G.K. 2002. Long-term impacts of an organophosphate-based regime of pesticides
876 on field and field-edge Collembola communities. *Pest Manage. Sci.* **58**(10): 991-1001.
- 877 Frampton, G.K. and van den Brink, P.J. 2007. Collembola and macroarthropod community
878 responses to carbamate, organophosphate and synthetic pyrethroid insecticides: Direct and
879 indirect effects. *Environ. Pollut.* **147**(1): 14-25.
- 880 Friberg-Jensen, U., Wendt-Rasch, L., Woin, P. and Christoffersen, K. 2003. Effects of the
881 pyrethroid insecticide, cypermethrin, on a freshwater community studied under field
882 conditions. I. Direct and indirect effects on abundance measures of organisms at different
883 trophic levels. *Aquat. Toxicol.* **63**(4): 357-371.

- 884 Fryday, S. and Thompson, H. 2012. Toxicity of pesticides to aquatic and terrestrial life stages
885 of amphibians and occurrence, habitat use and exposure of amphibian species in agricultural
886 environments. EFSA Supporting Publications. **EN-343**: 348.
- 887 Hanazato, T. and Kasai, F. 1995. Effects of the organophosphorus insecticide fenthion on
888 phytoplankton and zooplankton communities in experimental ponds. *Environ. Pollut.* **88**(3):
889 293-298.
- 890 Haugwitz, M.S., Michelsen, A. and Schmidt, I.K. 2011. Long-term microbial control of
891 nutrient availability and plant biomass in a subarctic-alpine heath after addition of carbon,
892 fertilizer and fungicide. *Soil Biol. Biochem.* **43**(1): 179-187.
- 893 Henny, C.J., Grove, R.A., Kaiser, J.L. and Johnson, B.L. 2010. North American Osprey
894 Populations and Contaminants: Historic and Contemporary Perspectives. *J. Toxicol. Env.*
895 *Heal. B* **13**(7-8): 579-603.
- 896 Henry, M., Beguin, M., Requier, F., Rollin, O., Odoux, J.-F., Aupinel, P., Aptel, J.,
897 Tchamitchian, S. and Decourtye, A. 2012. A Common Pesticide Decreases Foraging Success
898 and Survival in Honey Bees. *Science* **336**(6079): 348-350.
- 899 Holmstrup, M., Bindesbol, A.-M., Oostingh, G.J., Duschl, A., Scheil, V., Koehler, H.-R.,
900 Loureiro, S., Soares, A.M.V.M., Ferreira, A.L.G., Kienle, C., Gerhardt, A., Laskowski, R.,
901 Kramarz, P.E., Bayley, M., Svendsen, C. and Spurgeon, D.J. 2010. Interactions between
902 effects of environmental chemicals and natural stressors: A review. *Sci. Total Environ.*
903 **408**(18): 3746-3762.
- 904 Hua, J. and Relyea, R. 2014. Chemical cocktails in aquatic systems: Pesticide effects on the
905 response and recovery of > 20 animal taxa. *Environ. Pollut.* **189**: 18-26.
- 906 ISO (International Organization for Standardization) 1999. Soil quality – Effects of pollutants
907 on earthworms - Part 3: Guidance on the determination of effects in field situations., Geneva,
908 Switzerland. **ISO No. 11268-3**.

- 909 Jepson, P.C. and Thacker, J.R.M. 1990. Analysis of the spatial component of pesticide side-
910 effects on non- target invertebrate populations and its relevance to hazard analysis. *Funct.*
911 *Ecol.* **4**(3): 349-355.
- 912 Jones, A. and Hart, A.D.M. 1998. Comparison of laboratory toxicity tests for pesticides with
913 field effects on earthworm populations: a review. *In* *Advances in earthworm ecotoxicology.*
914 *Edited by* S.C. Sheppard, J.D. Bembridge, M. Holmstrup and L. Posthuma. Pensacola,
915 SETAC Press. pp. 247-267.
- 916 Kattwinkel, M. and Liess, M. 2014. Competition matters: Species interactions prolong the
917 log-term effects of pulsed toxicant stress on populations. *Environ. Toxicol. Chem.* **33**(7): 1458-
918 1465.
- 919 Kattwinkel, M., Römbke, J. and Liess, M. 2012. External Scientific Report: Ecological
920 recovery of populations of vulnerable species driving the risk assessment of pesticides, EFSA:
921 98.
- 922 Knauer, K. and Hommen, U. 2012. Sensitivity, variability, and recovery of functional and
923 structural endpoints of an aquatic community exposed to herbicides. *Ecotoxicol. Environ. Saf.*
924 **78**: 178-183.
- 925 Knillmann, S., Stampfli, N.C., Noskov, Y.A., Beketov, M.A. and Liess, M. 2013. Elevated
926 temperature prolongs long-term effects of a pesticide on *Daphnia* spp. due to altered
927 competition in zooplankton communities. *Global Change Biol.* **19**(5): 1598-1609.
- 928 Kula, C., Heimbach, F., Riepert, F. and Römbke, J. 2006. Technical recommendations for the
929 update of the ISO Earthworm Field Test Guideline (ISO 11268-3). *Journal Soil and Sediment*
930 **6**: 182-186.
- 931 Lee, K.E. 1985. *Earthworms - Their ecology and relationships with soils and land use,*
932 Academic Press.
- 933 Liess, M. and von der Ohe, P.C. 2005. Analyzing effects of pesticides on invertebrate
934 communities instreams. *Environ. Toxicol. Chem.* **24**(4): 954-965.

- 935 Liess, M. and Foit, K. 2010. Intraspecific competition delays recovery of population structure.
936 *Aquat. Toxicol.* **97**(1): 15-22.
- 937 Liess, M. and Beketov, M.A. 2011. Traits and stress: keys to identify community effects of
938 low levels of toxicants in test systems. *Ecotoxicology* **20**(6): 1328-1340.
- 939 Liess, M., Schulz, R., Liess, M.H.D., Rother, B. and Kreuzig, R. 1999. Determination of
940 insecticide contamination in agricultural headwater streams. *Water Res.* **33**: 239-247.
- 941 Liess, M., Foit, K., Becker, A., Hassold, E., Dolciotti, I., Kattwinkel, M. and Duquesne, S.
942 2013. Culmination of Low-Dose Pesticide Effects. *Environ. Sci. Technol.* **47**(15): 8862-8868.
- 943 Liess, M., Brown, C., Dohmen, P., Duquesne, S., Heimbach, F., Kreuger, J., Lagadic, L.,
944 Reinert, W., Maund, S., Streloke, M. and Tarazona, J. 2005. Effects of Pesticides in the Field
945 – EPIF. Brussels, Belgium SETAC Press.
- 946 Ligthart, T.N. and Peek, G. 1997. Evolution of earthworm burrow systems after inoculation of
947 lumbricid earthworms in a pasture in the Netherlands. *Soil Biol. Biochem.* **29**(3-4): 453-462.
- 948 Longley, M., Jepson, P.C., Izquierdo, J. and Sotherton, N. 1997. Temporal and spatial
949 changes in aphid and parasitoid populations following applications of deltamethrin in winter
950 wheat. *Entomol. Exp. Appl.* **83**(1): 41-52.
- 951 López-Mancisidor, P., Carbonell, G., Marina, A., Fernández, C. and Tarazona, J.V. 2008a.
952 Zooplankton community responses to chlorpyrifos in mesocosms under Mediterranean
953 conditions. *Ecotoxicol. Environ. Saf.* **71**(1): 16-25.
- 954 López-Mancisidor, P., van den Brink, P.J., Crum, S.J.H., Maund, S.J., Carbonell, G. and
955 Brock, T.C.M. 2008b. Responses of zooplankton in lufenuron-stressed experimental ditches
956 in the presence or absence of uncontaminated refuges. *Environ. Toxicol. Chem.* **27**(6): 1317-
957 1331.
- 958 López, L., Pozo, C., Gómez, M.A., Calvo, C. and López, J.G. 2002. Studies on the effects of
959 the insecticide aldrin on aquatic microbial populations. *Int. Biodeterior. Biodegrad.* **50**(2): 83-
960 87.

- 961 Macneale, K.H., Kiffney, P.M. and Scholz, N.L. 2010. Pesticides, aquatic food webs, and the
962 conservation of Pacific salmon. *Front. Ecol. Environ.* **8**(9): 475-482.
- 963 Malone, C.R. 1971. Responses of soil microorganisms to non-selective vegetation control in a
964 fescue meadow. *Soil Biol. Biochem.* **3**(2): 127-131.
- 965 Mañosa, S., Mateo, R. and Guitart, R. 2001. A review of the effects of agricultural and
966 industrial contamination on the Ebro delta biota and wildlife. *Environ. Monit. Assess.* **71**(2):
967 187-205.
- 968 Marinissen, J.C.Y. and van den Bosch, F. 1992. Colonization of new habitats by earthworms.
969 *Oecologia* **91**(3): 371-376.
- 970 Mineau, P. 2005. A review and analysis of study endpoints relevant to the assessment of "long
971 term" pesticide toxicity in avian and mammalian wildlife. *Ecotoxicology* **14**(8): 775-799.
- 972 Morris, A.J., Wilson, J.D., Whittingham, M.J. and Bradbury, R.B. 2005. Indirect effects of
973 pesticides on breeding yellowhammer (*Emberiza citrinella*). *Agr. Ecosys. Environ.* **106**(1): 1-
974 16.
- 975 Muñoz, I., Real, M., Guasch, H., Navarro, E. and Sabater, S. 2001. Effects of atrazine on
976 periphyton under grazing pressure. *Aquat. Toxicol.* **55**(3-4): 239-249.
- 977 Nienstedt, K.M., Brock, T.C.M., van Wensem, J., Montforts, M., Hart, A., Aagaard, A., Alix,
978 A., Boesten, J., Bopp, S.K., Brown, C., Capri, E., Forbes, V., Köpp, H., Liess, M., Luttik, R.,
979 Maltby, L., Sousa, J.P., Streissl, F. and Hardy, A.R. 2012. Development of a framework based
980 on an ecosystem services approach for deriving specific protection goals for environmental
981 risk assessment of pesticides. *Sci. Total Environ.* **415**: 31-38.
- 982 Panda, S. and Sahu, S.K. 1999. Effects of malathion on the growth and reproduction of
983 *Drawida willsi* (*Oligochaeta*) under laboratory conditions. *Soil Biol. Biochem.* **31**(3): 363-
984 366.

- 985 Potter, D.A., Buxton, M.C., Redmond, C.T., Patterson, C.G. and Powell, A.J. 1990. Toxicity
986 of pesticides to earthworms (Oligochaeta: Lumbricidae) and effect on thatch degradation in
987 Kentucky bluegrass turf. *J. Econ. Entomol.* **83**(6): 2362-2369.
- 988 Reddy, M.V. and Reddy, V.R. 1992. Effects of organochlorine, organophosphorus and
989 carbamate insecticides on the population structure and biomass of earthworms in a semi-arid
990 tropical grassland. *Soil Biol. Biochem.* **24**(12): 1733-1738.
- 991 Rohr, J.R., Sager, T., Sesterhenn, T.M. and Palmer, B.D. 2006. Exposure, postexposure, and
992 density-mediated effects of atrazine on amphibians: Breaking down net effects into their parts.
993 *Environ. Health Perspect.* **114**(1): 46-50.
- 994 Römcke, J., Schmelz, R.M. and Knaebe, S. 2009. Field studies for the assessment of
995 pesticides with soil mesofauna, in particular enchytraeids, mites and nematodes: Design and
996 first results. *Soil Organisms* **81**(2): 237-264.
- 997 Rowe, C.L. 2008. "The Calamity of So Long Life": Life histories, contaminants, and potential
998 emerging threats to long-lived vertebrates. *Bioscience* **58**(7): 623-631.
- 999 Rubach, M.N., Ashauer, R., Buchwalter, D.B., De Lange, H.J., Hamer, M., Preuss, T.G.,
1000 Töpke, K. and Maund, S.J. 2011. Framework for Traits-Based Assessment in Ecotoxicology.
1001 *Integr. Environ. Assess. Manage.* **7**(2, Sp. Iss. SI): 172-186.
- 1002 Schulz, R. 2004. Field studies on exposure, effects, and risk mitigation of aquatic nonpoint-
1003 source insecticide pollution: A review. *J. Environ. Qual.* **33**(2): 419-448.
- 1004 Shires, S.W. 1985. Effects of aerial applications of cypermethrin and demeton-S-methyl on
1005 nontarget arthropods of wheat. *Ecotoxicol. Environ. Saf.* **10**(1): 1-11.
- 1006 Stirling, G.R., Blair, B.L., Pattemore, J.A., Garside, A.L. and Bell, M.J. 2001. Changes in
1007 nematode populations on sugarcane following fallow, fumigation and crop rotation, and
1008 implications for the role of nematodes in yield decline. *Australas. Plant Path.* **30**(4): 323-335.

- 1009 Stoleson, S.H., Ristau, T.E., deCalesta, D.S. and Horsley, S.B. 2011. Ten-year response of
1010 bird communities to an operational herbicide-shelterwood treatment in a northern hardwood
1011 forest. *For. Ecol. Manage.* **262**(7): 1205-1214.
- 1012 Thursby, G.B. and Lewis, M.A. 2013. Protection goals for aquatic plants. *Integr. Environ.*
1013 *Assess. Manage.* **9**(1): 168-169.
- 1014 Todd, B.D., Willson, J.D. and Gibbons, J.W. 2010. The Global Status of Reptiles and Causes
1015 of Their Decline. *In* *Ecotoxicology of Amphibians and Reptiles*, Second Edition. Edited by
1016 D.W. Sparling, G. Linder, C.A. Bishop and S.K. Krest. CRC Press-Taylor & Francis Group;
1017 USA. pp. 47-367
- 1018 Tomkins, D.J. and Grant, W.F. 1977. Effects of herbicides on species diversity of two plant
1019 communities. *Ecology* **58**(2): 398-406.
- 1020 Trekels, H., Van de Meutter, F. and Stoks, R. 2011. Habitat isolation shapes the recovery of
1021 aquatic insect communities from a pesticide pulse. *J. Appl. Ecol.* **48**: 1480-1489.
- 1022 US EPA 2015. Terms of Environment: Glossary, Abbreviations and Acronyms. Available
1023 from <http://www.epa.gov/OCEPAt/terms/> [accessed 20.02.2014].
- 1024 van den Brink, P.J., van Wijngaarden, R.P.A., Lucassen, W.G.H., Brock, T.C.M. and
1025 Leeuwangh, P. 1996. Effects of the insecticide Dursban® 4E (active ingredient chlorpyrifos)
1026 in outdoor experimental ditches: II. Invertebrate community responses and recovery. *Environ.*
1027 *Toxicol. Chem.* **15**(7): 1143-1153.
- 1028 van den Brink, P.J., Alexander, A.C., Desrosiers, M., Goedkoop, W., Goethals, P.L.M., Liess,
1029 M. and Dyer, S.D. 2011. Traits-Based Approaches in Bioassessment and Ecological Risk
1030 Assessment: Strengths, Weaknesses, Opportunities and Threats. *Integr. Environ. Assess.*
1031 *Manage.* **7**(2, Sp. Iss. SI): 198-208.
- 1032 Vera, M.S., Lagomarsino, L., Sylvester, M., Pérez, G.L., Rodríguez, P., Mugni, H., Sinistro,
1033 R., Ferraro, M., Bonetto, C., Zagarese, H. and Pizarro, H. 2010. New evidences of Roundup®

- 1034 (glyphosate formulation) impact on the periphyton community and the water quality of
1035 freshwater ecosystems. *Ecotoxicology* **19**(4): 710-721.
- 1036 Walker, C.H. and Newton, I. 1998. Effects of cyclodiene insecticides on the sparrowhawk
1037 (*Accipiter nisus*) in Britain - a reappraisal of the evidence. *Ecotoxicology* **7**(4): 185-189.
- 1038 Wegner, P., Kleinstäuber, G., Baum, F. and Schilling, F. 2005. Long-term investigation of the
1039 degree of exposure of German peregrine falcons (*Falco peregrinus*) to damaging chemicals
1040 from the environment. *J. Ornithol.* **146**(1): 34-54.
- 1041 Wratten, S.D., Bowie, M.H., Hickman, J.M., Evans, A.M., Sedcole, J.R. and Tylianakis, J.M.
1042 2003. Field boundaries as barriers to movement of hover flies (Diptera: Syrphidae) in
1043 cultivated land. *Oecologia* **134**(4): 605-611.
- 1044 Yardim, E.N. and Edwards, C.A. 1998. The influence of chemical management of pests,
1045 diseases and weeds on pest and predatory arthropods associated with tomatoes. *Agric.*
1046 *Ecosyst. Environ.* **70**(1): 31-48.
- 1047 Zafar, M.I., Belgers, J.D.M., Van Wijngaarden, R.P.A., Matser, A. and Van den Brink, P.J.
1048 2012. Ecological impacts of time-variable exposure regimes to the fungicide azoxystrobin on
1049 freshwater communities in outdoor microcosms. *Ecotoxicology*: 1-15.
- 1050

1051 **Table 1:** Numbers of studies investigated for the different species groups.

Taxa group	Number of studies
Aquatic Environment	
Fish	-
Invertebrates	79
Algae	54
Plants	5
Microbes	12
Amphibians	-
Terrestrial Environment	
Birds	1
Mammals	-
Bees	1
Non-target-arthropods (NTAs)	24
Soil invertebrates	27
Microbes	17
Plants	8
Reptiles	-

1052

1053

1054 **Fig. 1.** Idealized visualization of the recovery classes. Black arrows indicate the time of
1055 contamination, gray arrows the time of recovery.
1056

Draft

Fig. 2. Percentage of data series with different direction of maximum effect after pesticide application for aquatic invertebrates for the endpoints abundance, community composition (assessed by principle response curves, PRC), and diversity (assessed by taxa richness) in relation to different substance classes. Shading lines indicate the fraction of recovered data series. The numbers of data series are shown above the bars. A *data series* refers to each single observed time series within one study per taxa and per concentration (but not per replicate). Adapted from Kattwinkel et al. (2012).

Draft

Fig. 3. Histogram of the ratio of population recovery time and generation time for data series with a decrease in abundance present after contamination (265 data series, 45 studies, 37 families). A value ≤ 1 indicates recovery within one generation time (marked by the dashed black line), while a value > 1 means that recovery takes multiple generation times. A *data series* refers to each single observed time series within one study per taxa and per concentration (but not per replicate). Adapted from Kattwinkel et al. (2012).

Draft

Fig. 4. Percentage of data series with different direction of maximum effect after pesticide application for algae for the endpoints chlorophyll a, abundance, community composition (PRC), and diversity (taxa richness or Shannon Index) of algae in relation to different substance classes. The numbers of data series are shown above the bars. A *data series* refers to each single observed time series within one study per taxa and per concentration (but not per replicate). Adapted from Kattwinkel et al. (2012).

Draft

1079 **Fig. 5.** Percentage of data series following the different no recovery / recovery classes. A: soil
1080 invertebrates (all effect endpoints), n = 99; B: soil microbes (all effect endpoints), n = 205. A
1081 *data series* refers to each single observed time series within one study per taxa and per
1082 concentration (but not per replicate).

Draft

no effect

recovery 1

recovery 2

recovery 3

Environmental Reviews

Page 52 of 52

recovery 4

no recovery 1

no recovery 2

no recovery 3

no recovery 4

no recovery 5

no recovery 6

■ abundance
■ community composition

□ diversity
▨ fraction recovery

data series [%]

insecta
non-insecta

<https://mc.manuscriptcentral.com/er-pubs>

recovery time / generation time

abundance
chlorophyll a
community composition

diversity
fraction recovery