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Protection of Biodiversity in the Risk Assessment and Risk Management of Pesticides (Plant Protection Products & Biocides) with a Focus on Arthropods, Soil Organisms and Amphibians

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Protection of Biodiversity in the Risk Assessment and Risk Management of Pesticides (Plant Protection Products & Biocides) with a Focus on Arthropods, Soil Organisms and Amphibians

by

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Abbreviations

a.i.	Active ingredient
BB	Brandenburg
BBCH	*Scale used to identify the phenological development stages of plants*
BfN	Bundesamt für Naturschutz (German Federal Agency for Nature Conservation)
BNatSchG	Bundesnaturschutzgesetz (German Federal Nature Conservation Act)
BVL	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (German Federal Office of Consumer Protection and Food Safety)
CAP	Common Agricultural Policy
CBD	Convention on Biological Diversity
DEFRA	Department for Environment, Food and Rural Affairs (UK)
EC	European Commission
EPM	Equilibrium Partitioning Method
ERA	Environmental Risk Assessment
ESCORT	European Standard Characteristics of Non-Target Arthropod Regulatory Testing
ESD	Emission Scenario Documents
EU	European Union
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
GIS	Geographic Information System
HQ	Hazard Quotient
IPM	Integrated Pest Management
IUCN	International Union for Conservation of Nature
LC_x	Lethal Concentration x (= *concentration causing x% mortality of the test organisms, e.g., LC_{50}*)
LR_x	Lethal Rate x (= *application rate causing x% mortality of the test organisms, e.g., LR_{50}*)
MTI	Margin Treatment Index
NEPTUN	Netzwerk zur Ermittlung des Pflanzenschutzmitteleinsatzes in unterschiedlichen, landwirtschaftlich relevanten Naturräumen Deutschlands
NOEC	No Observed Effect Concentration
NOER	No Observed Effect Rate
NTA	Non-target Arthropods
OECD	Organization for Economic Co-operation and Development
PEC	Predicted Environmental Concentration
PflSchG	Pflanzenschutzgesetz (German Plant Protection Act)
PNEC	Predicted No Effect Concentration
RLP	Rhineland-Palatinate
RMM	Risk Mitigation Measures
SCR	Soil-Climate-Region
TER	Toxicity Exposure Ratio
TGD	Technical Guidance Document on Risk Assessment
TI	Treatment Index
TNsG	Technical Notes for Guidance on Product Evaluation
ZALF	Leibniz-Zentrum für Agrarlandschaftsforschung (Leibniz Centre for Agricultural Landscape Research)

1. Summary

Croplands and pastures represent one of the greatest terrestrial biomes on earth. In Europe, agriculture is a leading land-use which constitutes nearly half of the EU-27 land area. Modern, intensified agriculture is among other factors characterized by an increasing use of pesticides, where pesticides are plant protection products and biocides (e.g. herbicides, disinfectants, insecticides, rodenticides, repellents). The conservation of biodiversity is a major legislative objective and recent evaluations indicated that biodiversity loss does not appear to be slowing down. One of the general protection goals in the authorization of pesticides asserts that no unacceptable effects on biodiversity can be accepted following the use of plant protection products or biocides. This report deals with the effects of pesticides on biodiversity in agricultural landscapes and focusses on two organism groups: arthropods and amphibians. Terrestrial arthropods and soil invertebrates represent the majority of biodiversity and animal biomass in the agricultural landscape and are recognized as major food items of vertebrates. They provide ecosystem services such as pollination, maintenance of nutrient cycling, regulation of micro climate and local hydrological processes as well as detoxification of environmental pollutants. Amphibians are a group of organisms suffering current population declines, observed with great concern by many experts on a global scale. Significant declines of amphibian and reptile populations and species are occurring also in Western Europe. Amphibians are more sensitive than birds or mammals to environmental changes and contamination because they may face alteration and contamination in both terrestrial and aquatic environments and their skin is highly permeable. This report additionally gives some information about reptiles, with a special focus on the likelihood and relevance of reptile species exposure to plant protection products in Germany.

This report deals with the effects of pesticides on biodiversity in agricultural and especially arable landscapes. The term “pesticide” describes plant protection products and biocides. The former are used in the agricultural production process to protect crop plants (or their plant products) against harmful organisms like insects, fungi, or other plants. The term ‘biocides’ is used for substances to control organisms that are harmful to humans, their activities or the products they use or produce, or for animals or for the environment in the non-agricultural sector. In a first step, the available habitat of so called non-target arthropod (NTA) species in the agricultural landscape was described using a quantitative approach elaborated in a Geographic Information System (GIS). This habitat characterized by field margin and hedges mainly comprises narrow margins, the majority being below 3 m wide. The exposure of plant protection products in these narrow margins is determined by spray drift but additionally they might be oversprayed near the field with 50% of the plant protection products field application rate. By combining drift values and Treatment Indices for specific crops, it is possible to calculate a Margin Treatment Index, in order to characterize the input intensity of plant protection products in different field margin types. In contrast to plant protection products, biocides are normally not directly applied to agricultural sites. Biocides are divided into 23 different product types and at least some of them can reach terrestrial habitats in agricultural landscapes. For instance, disinfectants and insecticides (product types 3 and 18) used in the animal housing are applied on agricultural sites via manure and sewage sludge.

Furthermore, wood preservatives (product type 8) can be applied on wooden piles in the cultivation of e.g. fruits or hops. Contrary to the environmental risk assessment for plant protection products, there is no distinction between in-field and off-field or in-crop and off-crop in the assessment of biocides. Thus, no specific scenario is available which quantifies biocide input in (narrow) field margins. Field margins may be however exposed to biocides if they are subjected to leaching and run-off.

In a next step, a meta-analysis of 132 studies concerning eight arthropod groups revealed that agricultural intensification and pesticide usage affects arthropods predominantly negatively while plant species richness, availability of floral resources, and the occurrence of semi-natural habitats had positive effects on the abundance or species richness of most groups. Arthropod groups showing a high susceptibility towards pesticides combined with the availability of extensive data from published literature are butterflies and moths (herbivores and pollinators), carabid beetles and spiders (both predators). Field margins and hedgerows can constitute a habitat for a wide range of species (nearly 2000 reported) including e.g. Coleoptera, Diptera, Lepidoptera, and Araneae. For the butterflies, beetles and spiders, where data were available, most analysed species either preferred the field margins/ conservation headlands or seemed to use field margins/ headlands and crop to a similar extent. Since life history trait data to characterise ecological sensitivity is not available for most arthropod species, we propose instead to focus on threatened species recorded on red lists. Of the recorded Macrolepidoptera and spiders in field margins and woody structures around 10% belonged to (nearly) threatened species.

The current approach in the assessment of the risk for non-target arthropods exposed to plant protection products is historically derived from biological pest control strategies. The test species used belong to so called 'beneficial insects' important in integrated pest control practices. The risk assessment of biocides addresses the effects of ecologically functional groups in relevant environmental compartments (water, sediment, soil, and air). The terrestrial part –the soil compartment – considers especially soil organisms (e.g. earthworms) while tests with other non-target arthropods are only needed in the risk assessment if a potential risk for non-target arthropods can be assumed. This can be the case when a specific mode of action of the active substances is to be assessed and/or in cases of high releases of the active substance into the environment. The proposed arthropod test species are also here ‘beneficial insects’. However, ‘beneficial insects’ only represent a restricted subset of arthropod biodiversity, since they are mostly predators or parasitoids.The sensitivity of the current test species might be similar to other arthropods but their life history does not reflect the range of life history strategies for the highly diverse arthropods of e.g. the “off-crop” habitat. Phytophagous insects might additionally be exposed to plant protection products by consumption, depletion of food source, and reduction of host plant quality. The assessment of recovery from effects of plant protection products used in the risk assessment approach needs to be separated from recolonisation processes. If, after a breakdown, a population increase occurs within a few weeks, recolonisation is a more likely process for species with only one reproductive phase per year. Recolonisation is largely dependent on the mobility (dispersal ability) of the species and the surrounding habitat (landscape context). In-field recolonisation might also draw from the arthropod population of the field margins and crop fields are therefore acting as sinks for these populations. Arthropods represent major food items for vertebrates and their abundance is especially important during the rearing phase of young and chicks. Even short time reductions in their biomass might affect the next trophic level. This aspect needs further consideration to link the different groups assessed separately in current risk assessment procedures of pesticides. Arthropod presence and biomass also in-field should be included as an endpoint in risk assessment. Current testing of plant protection products includes in-crop field studies as highest tier. Since arthropod community composition and life histories as well as exposure to plant protection products differ between fields and field margins, the application of an uncertainty factor or the conduct of specific off-crop studies is suggested in conclusion with the outcome of the ESCORT 3 workshop. However, the testing of off-crop arthropod communities needs further evaluation to account for the variability of arthropod communities throughout Europe and to select sensitive groups and quantitative sampling methods.

In the standardized tests to assess the effect of plant protection products and biocides on soil organisms only a few test species are used. An ecological relevance of the test organisms often plays a secondary role because of practicability considerations. Soil organisms below-ground and non-target arthropods above-ground are not independent from each other. In the risk assessment of plant protection products non-target arthropods (above ground) are separated from soil organisms (below ground). However, many above ground insects have below ground larvae (e.g. carabid beetles, Diptera) and this separation seems artificial. In the risk assessments of biocides such a separation does not really exist, since it considers the different environmental compartments. It is proposed to evaluate the sensitivity of life stages of so-called Non-Target-Arthopods that live in soil in comparison to the sensitivity of test species like earthworms or collembolan.

Any management of agricultural fields (e.g. tillage, plant protection products) impacts directly and indirectly above- and below-ground processes. In the risk assessment of soil organisms a mixed approach may be advisable which assesses the presence of key species (e.g. lumbricids) and species belonging to other relevant trophic levels..

The German risk management for plant protection products regarding terrestrial off-field areas is based on use restrictions (e.g. usage of low drift nozzles and/or requirements regarding buffer strips in-field). In many cases, these use restrictions have not to be implemented by farmers due to existing exceptions (e.g. next to narrow off-field structures < 3 m). To enhance the management of agricultural landscapes to support terrestrial biodiversity, it is proposed to pursue three additional goals: (1) the preservation and enhancement of the existing field margins and hedgerows including a reduction of inputs of plant protection products (e.g. in-field buffer strips), (2) the increase of plant species richness and the provision of adequate floral resources from the field to the landscape level, (3) the appropriate management of off-field habitats to create e.g. areas with varying structural complexity of the vegetation. If the assessment of biomass of arthropods is taken into account because of their function as food for many organisms in the landscape, risk assessment and management procedures also need to be established for the in-field area. Due to the allocation of a sufficient amount of high quality in- and off-field habitats, the abundance, species richness, diversity, and biomass of arthropods will be enhanced so that in-crop population losses could be compensated and an adequate food supply for arthropod-feeding species is provided. Not only the total amount of such in- and off-field habitat is crucial, these structures have also to be properly arranged in the landscape, to allow the emergence of habitat networks and to cover a range of several habitat types. No-tillage management practices enhance on the one side the diversity of soil fauna in cropped fields. On the other side, however, the influence of herbicides that are always used in combination with no-tillage systems on the biodiversity of agricultural landscapes is currently matter of debate. Indirect effects via food web disruption should be evaluated.

Amphibian species living in agricultural landscapes are at risk of exposure to pesticides both in fields and in neighbouring non-crop areas. They perform species specific migrations on crop fields which temporally coincide with the application of pesticides. Depending on the vegetation cover of field crops and their related interception values, amphibians are at different exposure risks. Direct overspray of plant protection products of amphibians depends on the activity of individuals during daytime and availability of shelter. Because of amphibians being mostly nocturnal species, the risk of receiving a full direct overspray is likely to be low. Amphibians resting in fields are slightly buried in the soil surface (digging species), use sites beneath the plants or enter animal burrows. Resting in fields without any type of cover is very unlikely. However, a higher exposure risk is caused by their movements on treated soil or vegetation, due to their potentially intense skin-soil or skin-vegetation contacts. Preferred habitats in crop fields are areas next to breeding ponds and wet spots. Under normal cultivation, there is a rather high risk for amphibians to be exposed to plant protection products because of their long sojourn in fields. Amphibians can be exposed to plant protection products outside crop fields by spray drift and run-off. This risk increases strongly with lower shares of non-arable land. Exposure of biocides in manure and sewage sludge might be other potential exposure scenarios in-field and on grasslands.

The results of a literature review indicated that the transport of plant protection products across the skin is likely to be a significant route of exposure for amphibians and that plant protection products can diffuse one or two orders of magnitude faster into amphibians than into mammals. Since only a few studies were published on terrestrial amphibian life-stages a study to assess the toxicity of plant protection products on juvenile frogs was conducted. We studied the effects of seven plant protection products on juvenile European common frogs (*Rana temporaria*) in a laboratory overspray scenario. Mortality ranged from 100% after one hour to 40% after seven days at the recommended label rate of currently registered products. Effects were not restricted to a specific class of plant protection products and seem to be influenced not only by the active substance but also the formulation additives. The demonstrated toxicity is alarming and needs further research to understand the underlying mechanisms. The results also indicate that existing risk assessment procedures for plant protection product regulation are not protecting amphibians. Even if plant protection products were tested, similar effects and consequences cannot be excluded for biocidal products as active substances and formulation additives might be identical.

Measures for risk management implemented to protect amphibian should include a) an overall reduction of plant protection product use, b) specific measures on hot spots of amphibian presence in crop fields, and c) modifying the mode and/or timing of plant protection product application. While sound amphibian management on wet spots or pond edges (buffer areas) is easily to apply and can be easily implemented, other measures are more difficult to implement but may offer some potential future prospects: e.g. short-term time shifting of plant protection product application dates, replacing a plant protection product, alternative application techniques like plant protection product injection into soil instead of spraying. In all cases, effects on other organisms groups have to be considered in an overall approach.

There are no systematic quantitative studies on reptile occurrences in cropped fields available, but from existing information and observations it can be assumed that they are common visitors in agricultural land and thus, their presence potentially coincide with plant protection product application. Based on a very scarce body of literature and own observations, we conclude on reptiles having a lower risk of plant protection product exposure than amphibians. They usually do not migrate that extensively between different habitats and cross crop fields to a less extent. If present on fields, they are likely to be close to field edges. However, own accidental findings show that some reptiles, for instance sand lizard, also may be sporadically active within crop fields at places with more than 100 m distance from the field edge. Reptiles, contrary to most amphibian species, are also active during daytime. On sunny days lizards often do sunbathing in grass-herb edges adjacent to crop fields providing open sandy soils or rocks. Under this scenario, the exposure risk by spray drift of plant protection products applied on neighbouring fields is presumably high. This is even more the case if we consider permanent crops like orchard or wineyards.

2. Introduction

Today, croplands and pastures constitute one of the greatest terrestrial biomes on earth (Foley et al. 2005) and in Europe agriculture is a leading land-use which constitutes nearly half of the EU-27 land area (Stoate et al. 2009). Therefore, its management has profound consequences for the environment and biodiversity (Benton et al. 2003). Modern, intensified agriculture is characterized by an increasing use of pesticides and mineral fertilizers as well as an enlargement of field size and fragmentation of semi-natural habitat (Stoate et al. 2001; Tilman et al. 2001).

Pesticides can be divided into plant protection products and biocides. The former ones are used in the agricultural production process to prevent crop plants (or their plant products) against harmful organisms like insects, fungi, or other plants, while the term 'biocides' or 'biocidal products' describes substances which are used to control organisms that are harmful to humans, their activities or the products they use or produce, or for animals or for the environment in the non-agricultural sector. Depending on their range of use, biocides were divided into 23 different product types (Regulation No 528/2012[1], Annex V), which includes for example human hygiene biocidal products (product type 1), wood preservatives (product type 8), rodenticides (product type 14), insecticides, acaricides, and products to control other arthropods (product type 18), or repellents and attractants (product type 19). However, the same active ingredients can be used in both pesticide groups, e.g. lambda-cyhalodrin is classified as plant protection product if it is used against aphids on cereal fields while it is classified as biocidal product if it is applied against the caterpillars of *Thaumetopoea processionea* (Oak Processionary) in forests to protect human health.

For the signatory countries of the Convention on Biological Diversity, the conservation of biodiversity is a major political objective (Secretariat of the Convention on Biological Diversity 2005). However, a recent evaluation evaluating various indicators of the state of biodiversity (covering species' population trends, extinction risk, habitat extent and condition, and community composition) revealed that the rate of biodiversity loss does not appear to be slowing (Butchart et al. 2010).

Arthropods

During the last decades, a decline of biodiversity of organisms associated with the agricultural landscape and its intensified management was recognized (Robinson & Sutherland 2002). One of the best documented examples is the decline of farmland birds in Europe (Krebs et al. 1999; Donald et al. 2001). Of the 36 classified farmland birds 20 species show declines and numbers of common species have fallen by 48%. Although the decline appears to have levelled off in recent years, Europe has still lost half of its farmland birds in the last quarter of a century (PECBMS 2009). The decline in diversity is discussed as being associated with

[1] Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and the use of biocidal products.
Verordnung (EU) Nr. 528/2012 des Europäischen Parlaments und des Rates vom 22. Mai 2012 über die Bereitstellung auf dem Markt und die Verwendung von Biozidprodukt

structural changes in the landscape and the use of plant protection products and their negative influences (Rands 1985; McLaughin & Mineau 1995; Wilson et al. 1999; Robinson & Sutherland 2002; Benton et al. 2003; Morris et al. 2005). An important factor influencing bird declines may be that management changes decrease the availability of insect food (Vickery et al. 2001) since parental birds may need to forage more intensively to get the same or a reduced amount of food for their chicks (Brickle et al. 2000; Morris et al. 2005). The increased costs of foraging can have immediate consequences (through chick starvation or smaller clutches) or the effect may be delayed (slower growth, reduced over-winter survival of both juveniles and adults, reduced fecundity the following year) (Siriwardena et al. 2000). A study in Scotland could show in a correlative approach a linked temporal decline of farmland birds, invertebrate numbers and agricultural practice (Benton et al. 2002).

In agro-ecosystems, biodiversity is essential for the maintenance of ecosystem services such as pollination and the breakdown of organic matter to improve soil fertility (Power 2010). Terrestrial arthropods and soil invertebrates represent the majority of biodiversity and animal biomass in the agricultural landscape and are recognised as major food items of vertebrates (Duelli et al. 1999; Morris et al. 2005). They provide ecosystem services such as pollination, maintenance of nutrient cycling, regulation of micro climate and local hydrological processes as well as detoxification of environmental pollutants (Dunger 1983; Gobat et al. 2004). Pollination is provided by a large suite of bees, flies, beetles, and butterflies and, furthermore, predatory and parasitoid arthropod species are relevant as pest control agents and many of them are recognised as ‘beneficial insects’.

Many arthropod groups are susceptible towards effects of agricultural intensification (Wilson et al. 1999). The decline of arthropod richness and abundance in intensively managed agricultural landscapes was recognised even on a global level for decomposers and predators (Attwood et al. 2008). However, the difference was not only obvious in comparison to natural vegetation but also compared to landscapes under extensive agricultural management. The reduction of soil biodiversity caused by inputs of pesticides and modern soil management methods is also discussed as a cause for the decline in soil quality in various regions (Dunger 1983).

Pollinators are especially well studied and declines in species richness on a national and global scale were reported (Biesmeijer et al. 2006; Kluser & Peduzzi 2007). There are few long-term data available, but a correlative link was shown between higher agricultural intensity and lower arthropod abundance over a 30-year period (Benton et al. 2002). A study of common, larger moths in Britain revealed that two thirds of the considered species declined in their population size in the past 30 years (Conrad et al. 2006) and one of the main causes is seen in the agricultural intensification (Fox et al. 2006). Pesticides have been shown to cause declines in non-target beetles (Lee et al. 2001; Geiger et al. 2010) and bees (Alston et al. 2007) and other insects (Poulin et al. 2010). On a landscape scale, a negative impact of insecticide application on wild bee species was revealed in Italy (Brittain et al. 2010b).

Soil organisms

Soils are complex micro-landscapes and provide a multitude of unique niches supporting soil-dwelling life-forms (Parker 2010). The high biodiversity of soil organisms is caused by the multitude of bacteria, fungi, algae and protozoans and the large numbers of nematodes, mites, collembolans and earthworms. The overall soil biodiversity is estimated at approximately 1.6 million species (Swift et al. 2008) and may be even higher especially due the unpredictably high diversity of microorganisms. Soil organisms contribute the majority of genetic diversity to terrestrial ecosystems, with levels of taxonomic diversity several orders of magnitude greater than those found in their aboveground counterparts on a per-area basis (Bardgett 2005). Species are more densely packed in soil communities than in any other environment on earth; a single gram of soil can contain millions of individuals and can harbour in excess of 10,000 unique taxa representing a dozen different phyla (Fierer & Jackson 2006).

The impact of agricultural intensification on biological diversity of soils is of particular concern, with intensively managed agriculture recognized as a major cause of loss of global biodiversity (Altieri 1999; Attwood et al. 2008). Practices such as the clearing of native vegetation, application of agrochemicals, monoculture, and overgrazing by livestock have all been implicated in the loss of biological diversity. Agriculture has an impact on biodiversity via two broad processes: the conversion of natural systems into production land and the intensification of management on land that is already highly modified and dominated by humans (Attwood et al. 2008). Despite the heavy human impacts on agricultural systems, these systems are still complex, and there is a lack of understanding about the multiple environmental and biotic factors interact to affect soil biodiversity and function (Neher & Barbercheck 1998).

In agro-ecosystems, biodiversity performs a variety of ecological services beyond the production of food, including recycling of nutrients, regulation of microclimate and local hydrological processes, suppression of undesirable organisms, and detoxification of noxious chemicals (Altieri 1999). In many systems, the causal relationship between composition, diversity, and abundance of soil organisms and the sustainability of soil fertility is still unclear (Giller et al. 1997). Soil-fauna diversity manipulations indicate that the number of trophic levels, species identity, and the presence of keystone species have a strong impact on decomposition, whereas the importance of diversity within functional groups is not clear at present (Hättenschwiler et al. 2005; Brussaard et al. 2007). In grassland, most profound impacts of soil fauna on soil properties appeared to be due to the presence of macrofauna in soil communities, and there was evidence for functional redundancy at the species level amongst some soil biota (Cole et al. 2006).

The study of the role of soil biodiversity for soil functions is complicated by the large variability in space and time of soil organisms (Ekschmitt & Griffiths 1998; Englund & Cooper 2003). Despite of the need for further research, biodiversity is considered to be the most important protection goal for the risk assessment of plant protection products to soil organisms.

This report is characterizing the available habitat of so called non-target arthropod (NTA) species in the agricultural landscape. In this first step, we chose a quantitative approach in a GIS. This is followed by a literature review and analysis of occurring arthropods in the margins and a characterisation for soil organisms. The current risk assessment procedures for plant protection products and biocides are described and potential improvements are suggested. Additionally, we tried to show ways for a risk management that could include existing management practices and subsidies and present an overview on the influence of soil-conserving practices on soil organisms.

Amphibians

Amphibians are another group of organisms where current population declines are observed with great concern by many experts (Blaustein et al. 1994b; Mendelson et al. 2006; Whitfield et al. 2007). The IUCN includes more than 30% of the amphibian species in one of the threat categories but ‘only’ 12% and 23% of birds and mammals, respectively (Stuart et al. 2004). Significant declines of amphibian and reptile populations and species are occurring also in Western Europe (Bosch et al. 2007). Twelve out of 20 amphibian species and eight out of 14 reptile species occurring in Germany are listed in Annex II and/or IV of the European Habitats Directive and all amphibians in Germany are specially protected under the German “Bundesartenschutzverordnung”. Different reasons for the decline of amphibians are discussed including competition with alien species, over-exploitation, land use changes, increased ultraviolet radiation and global warming, increased use of plant protection products and other toxic chemicals, and emerging infectious diseases such as the chytrid fungus *Batrachochytrium dendrobatidis* (Collins & Storfer 2003). In an evaluation of the factors of the ‘global amphibian decline’, pollution is seen as the most important threat to amphibian populations after habitat loss (Mann et al. 2003).

Amphibians are more sensitive than birds or mammals to environmental changes and contamination mainly for two reasons (Quaranta et al. 2009b): Firstly, most species spend the first part of their life in aquatic environments and the second part in terrestrial environments, and they may face alteration and contamination of both environments (Dohm et al. 2008; Mann et al. 2009). This double jeopardy of contaminant exposure stemming from terrestrial and aquatic environments was shown for mercury exposure of *Bufo marinus* in a recent study (Todd et al. 2011). Secondly, amphibian skin is highly permeable and physiologically involved in gas, water, and electrolyte exchange with the environment (Quaranta et al. 2009b) and therefore highly susceptible to physico-chemical stressors like UV-B radiation, pathogens or xenobiotics. Malformations, disturbed metamorphosis as well as reduced reproduction have been reported from areas with extensive agrochemical use (Denver 1997; Taylor et al. 2005; McCoy et al. 2008). In agricultural practice usually mixtures of plant protection products are applied resulting in synergistic effects on amphibians (Hayes et al. 2006; Relyea 2009).

Amphibians are more diverse than mammals or reptiles (6,894 species are presently known) and they have an important role in nutrient dynamics, in the cycling of energy flows between terrestrial and freshwater systems, and in controlling populations of pest insects (Wake & Vredenburg 2008; Alford 2011). Many European amphibian species are present in the agricultural landscape during their life cycle and come in contact with plant protection products (Berger et al. 2011a).

Amphibians are biphasic organisms depending on terrestrial and aquatic habitats. Adult frogs, toads and newts migrate to breeding ponds that are often situated within or in close proximity to agricultural fields. The larvae develop in the ponds that often receive inputs of plant protection products and the juveniles forage in the agricultural landscape after emergence together with the adults. They are therefore at risk of exposure to cultivation measures, e.g. plant protection products, fertilisers or mechanical crop management practice (Dürr et al. 1999; Hatch et al. 2001; Berger et al. 2011a). Since in temperate regions major migrations of adults take place prior to spawning of most amphibians in spring, they coincide strongly with plant protection product and fertilizer applications on fields (Berger et al. 2011a). Since amphibians are carnivores consuming mainly invertebrates, and especially arthropods, they may be also not only affected by plant protection products through food contamination but also by reduced food availability.

In a modelling approach of population vulnerability to contaminants (DDT and chlorpyrifos) of 144 species belonging to seven taxonomic vertebrate groups reptiles and amphibians were the groups with the most vulnerable species (De Lange et al. 2009). Assessment of vulnerability was based on ecological traits like life history, feeding biology, internal contaminant distribution, toxicokinetics, toxicological sensitivity, or behavioural characteristics. Davidson & Knapp (2007) assessed factors driving occurrence and decline of amphibian populations and revealed a correlation between windborne plant protection product deposition and amphibian population decline. Low doses of pesticides frequently observed in the environment were able to weaken the immune response of *Rana pipiens* (Albert et al. 2007) and UV radiation and contaminants may interact with one another synergistically (Blaustein et al. 2003). These findings of sublethal effects of plant protection products and synergistic interactions with other stressors like disease and UV radiation are not easy to disentangle and cause for concern. However, in an experimental study with terrestrial exposure of juvenile toads *(Bufo cognatus)* a mortality of almost 70% was observed after 24 h at recommended application rates for the registered fungicide Headline (a.i. pyraclostrobin) (Belden et al. 2010).

So far, amphibians are not specifically mentioned in the risk assessment procedures for pesticides in the EU (Regulation (EC) No 1107/2009, Regulation (EU) No. 528/2012). However, the opinion was expressed that "*an appropriate risk assessment approach for amphibians should be developed*" (EFSA 2007) and in a stakeholder workshop of the Panel on Pesticides and their Residues (PPR) on protection goals for environmental risk assessment of pesticides it was mentioned that "*furthermore, reptiles and amphibians should be considered*" (EFSA 2010). Additionally, a comment was recorded in the same document that "*amphibians are generally already covered (in the risk assessment) by the sensitivity of the fish ELS study*". This might be the case for aquatic life stages of some amphibians but surely does not cover terrestrial exposure of juvenile and adult individuals. Although the mentioned opinions and workshop results refer to plant protection products they might be also relevant for the risk assessment of biocides.

This report additionally mentions the situation for reptiles. The first part evaluates the likelihood and relevance of the exposure of amphibian and reptile species to plant protection products in agricultural landscapes of Germany by comparing migration activity and plant protection product spray interception by different crop canopy. It is followed by a review of the effects of other stressors on potentially exposed amphibian and reptile species such as climate, land use, and food quality/quantity. In order to understand the current situation of terrestrial life stages of amphibians and their treatment in risk assessment of plant protection products, we evaluated the literature on existing toxicity data and dermal uptake data. That lack of useful data led to the generation of toxicity endpoints for a few selected plant protection products. The risk assessment method (for bird and mammals) is described and recommendations and/or supplementary modules for amphibian specific assessments were developed. The report concludes with a chapter on potential risk management strategies for amphibians.

3. Pesticides and non-target terrestrial invertebrates

M. Hahn, T. Schmidt & C.A. & Brühl

3.1 Selection of adequate organism groups

Terrestrial arthropods and soil invertebrates form the major part of the biodiversity of agro-ecosystems and are essential components in the diets of many vertebrates like birds (Wilson et al. 1999) and bats (Vaughan 1997). Furthermore, they provide ecosystem services like biological pest control, pollination, and decomposition of organic matter (Power 2010).

In the current risk assessment of pesticides, effects are addressed for a few test species in standardized laboratory test systems (see chapter 3.3 for more details). The test species are often beneficial organisms for agricultural production (like aphid predators and earthworms) but they may not necessarily represent appropriate test organisms regarding the protection of terrestrial biodiversity or the maintenance of ecosystem-services. Hence, a literature research was conducted to get an overview about (i) organism groups of different taxonomical and functional (predators, herbivores,…) groups inhabiting agricultural landscapes, (ii) environmental and management factors influencing these groups with a special focus on pesticides, and (iii) gaps in current knowledge. Based on this information suggestions are made about organism groups which seem to be appropriate to assess the potential effects of pesticides on terrestrial biodiversity.

3.1.1 Non-target arthropods (NTA)

Agricultural landscapes constitute a habitat for a range of non-target arthropods like wild bees, spiders, butterflies/moths or beetles (Wilson et al. 1999; Roß-Nickoll et al. 2004; Wickramasinghe et al. 2004). These organisms belong to several functional groups (e.g. they are pollinators or predators) and vary in their habitat requirements (e.g. food resources, availability of semi-natural habitats) or susceptibilities to different agricultural practices (e.g. intensity of pesticide usage, soil cultivation). Arthropod groups and species considered in the literature research encompass pollinators (wild bees/bumblebees, adult butterflies/moths, and adult hoverflies), herbivores (bugs, cicadas, caterpillars of butterflies/moths) and predators (ground beetles, rove beetles, spiders, bugs, larvae of hoverflies).

Published literature was searched mainly within the database 'ISI Web of Knowledge'. A multiplicity of search terms were used, e.g. Coleoptera, Carabidae, Staphylinidae, Lepidoptera,… AND e.g. community, margin, hedge, boundary, pesticide, fertilizer, vegetation, agriculture. Furthermore, an internal database of the Institute of Environmental Science at University Landau was used[2]. In order to be considered, data from field studies had to be conducted in Europe while in laboratory studies the test organisms employed had to be native in Europe. If results of reviews (without meta-analysis) were used, the underlying original studies were not analysed separately.

For some of the organism groups, especially concerning beneficial organisms (ground beetles and spiders) or groups of nature conservation value like butterflies, extensive research has been done in the past and a large amount of literature is available (see Appendix 5.1). For

[2] Literature was examined carefully but - since the aim of this study was to get an overview of factors influencing different organism groups - it makes no claim to be complete.

other groups, like bugs or cicadas, only a few studies were found that had been conducted in the agricultural landscape (each group: 14 studies).

Overall, 132 studies were analysed and the potential factors affecting presence and/or abundance of the investigated arthropods were extracted and evaluated as positive, neutral, and negative relationship for each group:

Positive:	e.g. higher plant species richness leads to higher species diversity
Neutral:	e.g. the percentage of semi-natural habitat does not influence abundance
Negative:	e.g. species richness was reduced in plots with higher pesticide usage

In some cases, no distinct relationship was described in the analysed study, although a factor influencing the organism group was discernable in the presented data (for example: Mowing influences the community composition. Vegetation height influences abundance). These results were listed separately.

A study could contain information for several factors (e.g. plant species richness, mowing, pesticides) but was counted only once per organism group and relationship (positive, neutral, negative), even if this relationship comprise several parameters (e.g. abundance, diversity, mortality). For example, it was found that '*species richness and abundance of bees (but not wasps) were closely related to plant species richness of the habitat*' (Tscharntke et al. 1998). This example was classified as showing ***one*** positive relationship for the organism group wild bees according to the factor plant species richness even if there are ***two*** considered parameters to this relationship (species richness and abundance). An overview of the evaluated literature and detailed information on the relationships and parameters are given in Appendix 5.1.

Generally, it seems that factors influencing abundance, species richness, and distribution of arthropods could be roughly attributed to main aggregated factors regarding vegetation characteristics, landscape characteristics, and agricultural management.

Table 3.1-1: Factors influencing different arthropod groups and their relationships ((+): positive (green), (o): neutral (yellow), (-): negative (red); wr: influencing factor without clear relationship (blue)) on the basis of an analysis of 132 studies. Numbers in cells represent the number of studies in which the respective relationship was found; letters reference the studies in Appendix 5.1. Grey fields: no information regarding the respective organism group and factor was found in the studied literature. (n): number of analysed studies.

factors influencing arthropods	Hymenoptera Wild bees (n=29)				Coleoptera Carabidae (n=26)				Coleoptera Staphylinidae (n=13)				Diptera Syrphidae (n=11)			
	(+)	(o)	(-)	wr	(+)	(o)	(-)	wr	(+)	(o)	(-)	wr	(+)	(o)	(-)	wr
plant species richness/flower abundance or area	9[a]	0	0	0									3[a]	1[b]	0	0
vegetation structure/height	0	1[b]	1[c]	0									1[c]	0	0	1[d]
Presence/ Proportion of forests or woody habitats	0	1[d]	0	0									1[e]	0	0	0
Percentage of semi-natural habitat in agricultural landscapes	6[e]	1[f]	0	0	1[a]	0	0	0	1[a]	1[b]	0	0				
field margins/ hedges	5[g]	0	0	0	7[b]	1[c]	2[d]	0	6[c]	1[d]	0	0				
Isolation/ Fragmentation	0	1[h]	4[i]	0									0	1[f]	0	0
Crop					0	1[e]	0	2[f]	0	0	0	2[e]				
Mass flowering crops	2[j]	0	1[k]	0												
Agricultural intensification	0	0	4[l]	0	0	0	1[g]	0					0	0	1[g]	0
Organic Agriculture	3[m]	1[n]	0	0	6[h]	3[i]	2[j]	0	1[f]	1[g]	1[h]	0				
Pesticides	0	1[o]	4[p]	0	2[k]	0	5[l]	0	0	2[i]	1[j]	0	1[h]	2[i]	3[j]	0
Fertilizer	0	0	1[q]	0	1[m]	1[n]	0	0	2[k]	0	1[l]	0				
Soil cultivation					1[o]	0	2[p]	1[q]	1[m]	0	2[n]	0				
Mulching					1[r]	0	1[s]	0	1[o]	0	0	0				
Mowing/grazing	1[r]	1[s]	1[t]	0					0	0	1[p]	0				
"Ackerschon-streifen"/ Headlands					4[t]	2[u]	0	0	3[q]	1[r]	0	0	3[k]	1[l]	1[m]	0
"Blühstreifen"/ Beetle banks/ grass strips	1[u]	0	0	0	2[v]	0	0	1[w]	1[s]	1[t]	0	0				
Wild flower areas																

Table 3.1-1: Continued.

factors influencing arthropods	Arachnida				Hemiptera								Lepidoptera			
	Spiders (n=32)				Heteroptera (n=14)				Auchenorrhyncha (n=14)				Macrolepidoptera (n=41)			
	(+)	(o)	(-)	wr	(+)	(o)	(-)	wr	(+)	(o)	(-)	wr	(+)	(o)	(-)	wr
plant species richness/flower abundance	1[a]	0	0	1[b]	1[a]	1[b]	0	1[c]	1[a]	1[b]	0	1[c]	11[a]	2[b]	0	1[c]
vegetation structure/ height	6[c]	1[d]	0	2[e]	2[d]	0	0	2[e]	2[d]	0	0	1[e]	3[d]	1[e]	1[f]	2[g]
Presence/ Proportion of forests or woody habitats	1[f]	0	1[g]	0									1[h]	0	0	0
Percentage of semi-natural habitat in agricultural landscapes	2[h]	0	0	0									1[i]	1[j]	0	1[k]
field margins/ hedges	3[i]	2[j]	1[k]	0	2[f]	0	0	0					8[l]	1[m]	0	0
Isolation/ Fragmentation					0	0	1[g]	0	0	0	2[f]	0	0	0	1[n]	0
Crop																
Mass flowering crops																
Agricultural intensification					1[h]	1[i]	1[j]	0	0	1[g]	2[h]	0	1[o]	2[p]	4[q]	0
Organic Agriculture	4[l]	2[m]	0	0									7[r]	3[s]	0	0
Pesticides	0	1[n]	7[o]	0	0	1[k]	2[l]	0	0	0	1[i]	0	0	2[t]	9[u]	0
Fertilizer	0	1[p]	0	0					1[j]	0	1[k]	0				
Soil cultivation	1[q]	0	2[r]	0												
Mulching																
Mowing/ grazing	0	0	3[s]	0	0	1[m]	1[n]	1[o]	0	0	3[l]	0				
"Ackerschon-streifen"/ Headlands	4[t]	0	0	0	3[p]	0	0	0	2[m]	1[n]	0	0	5[v]	0	0	0
"Blühstreifen"/ Beetle banks/ grass strips	2[u]	0	0	2[v]	1[q]	0	0	0	1[o]	0	0	0	3[w]	1[x]	0	0
Wild flower areas	1[w]	1[x]	0	0												

The relevance of different factors as related to their impact on e.g. occurrence, abundance, or species richness of terrestrial arthropods may vary between the groups according to their life history traits. However, several investigated factors associated with unfavourable consequences (negative relationships, Table 3.1-1) for many arthropod groups are predominantly associated with agricultural practices.

On the basis of this literature research, the most relevant environmental factors influencing arthropods in the agricultural landscape are shown in Table 3.1-2.

Table 3.1-2: Overview for all arthropod groups to the investigated environmental and management factors. Only those factors are shown for which data were available at least for five organism groups. Green: factors with predominantly positive relationships, red: factors with predominantly negative relationships. Eight organism groups considered in total (see Table 3.1-1 above).

Factors influencing arthropods	positive	neutral	negative	Number of organism groups
field margins/hedges	31	5	3	6
plant species richness/flower abundance or area	26	5	0	6
"Ackerschonstreifen"/ Headlands	24	5	0	7
Organic Agriculture	21	10	3	5
vegetation structure/height	14	3	2	6
"Blühstreifen"/ Beetle banks/ grass strips	11	2	0	7
Percentage of semi-natural habitat in agricultural landscapes	11	3	0	5
Fertilizer	4	2	3	5
Pesticides	3	9	32	8
Agricultural intensification	2	4	13	6
Mowing/grazing	1	2	9	5
Isolation/ Fragmentation	0	2	8	5

Especially high pesticide input and agricultural intensification practices were often negatively related to the studied arthropod parameters (see Table 3.1-1). This tendency was consistent for pollinators (e.g. wild bees), herbivores (e.g. cicadas), and predators (e.g. spiders).

In contrast, headlands/ ”Ackerschonstreifen” and beetle banks/ flower strips as well as field margins and hedges – which are characterized by less intensive management and pesticide usage in comparison to “normal” agricultural sites – had mostly positive effects on abundance and/or species richness of beetles (Carabidae, Staphylinidae), spiders, bugs, cicadas, and butterflies/moths (Table 3.1-1, Appendix 5.1). Furthermore, organic agriculture seemed to be beneficial to wild bees, spiders and butterflies/moths but showed more differentiated results for ground and rove beetles.

The considered studies indicate a positive effect of plant species richness and flower abundance/area for most organism groups. Increasing flower abundance (often in accordance with greater plant species richness, see Ebeling et al. 2008) improves nectar and pollen availability for pollinators. In addition, predators can profit by higher prey occurrence due to the attraction of flower-visiting or herbivorous insects (Haddad et al. 2011; Diehl et al. 2012). The vegetation structure and height seemed to be especially important for spiders, bugs, cicadas, and butterflies/moths. These results coincide with the positive influences of field margins and flower strips which hold greater plant species richness/flower abundance (Marshall & Moonen 2002; Carvell et al. 2007; Haaland et al. 2011) and a more complex vegetation structure in relation to crops.

Landscape characteristics like the availability of semi-natural habitats are essential for terrestrial arthropods since many species depend on them (Duelli & Obrist 2003). Hence, most of the studied organism groups were affected by such structures. Higher percentages of grasslands, forests or other woody habitats, or proximity to those structures especially benefits wild bees, beetles (Carabidae, Staphylinidae), spiders, and butterflies/moths.

One of the aims of this literature study was to suggest organism groups which should be considered in the risk assessment of pesticides to improve the protection of biodiversity. Since organism groups fulfil different functional roles in agro-ecosystems (pollinators, herbivores, predators) associated with different life history traits and habitat requirements, more than one organism group should be evaluated to be able to analyse the effects of pesticides on terrestrial biodiversity.

Since pollination is a crucial ecosystem service for the preservation of biodiversity and agricultural production (Isaacs et al. 2009; Ssymank et al. 2009), we propose to include in further assessments at least one representative of ***pollinators***. In our literature research three pollinator groups were included: wild bees, hoverflies and butterflies/moths. For hoverflies relatively little information was available but a low sensitivity towards pesticides was indicated. In contrast, wild bees and butterflies/moths showed a clear susceptibility towards pesticides (Table 3.1-1, Appendix 5.1). For farmers, wild bees could be of economic importance due to their pollination of crops like for example species of the genus *Osmia* which are known to pollinate apple flowers (Matsumoto et al. 2009; Gruber et al. 2011). The species rich group of butterflies/moths is not only of interest since adults of many species are rather mobile flower visiting insects but also since their often specialized herbivorous juvenile stages (caterpillars) are relative immobile and could be affected via pesticides due to direct application/spray drift, consumption of contaminated plant material, and loss of host plants caused by herbicide usage. Additionally, adults of many Lepidoptera species (especially butterflies) can easily be identified in the field (Harding et al. 1995) and extensive literature has been published about this group.

Herbivorous organisms like caterpillars, cicadas, and bugs are of great importance in terrestrial ecosystems since they link different trophic levels (producers – consumers) and species of higher trophic levels depend on them for nutrition (birds: Wilson et al. 1999, bats: Vaughan 1997, spiders: Ludy 2007). As exemplified above, pesticides can affect ***herbivores*** in different ways (direct application, oral intake, and loss of food plants). Therefore, herbivores should be considered when effects of pesticides on biodiversity are assessed (see chapter 3.3.1.3). However, little information could be found about the responses of cicadas and bugs to pesticides. For caterpillars, a greater knowledge on their susceptibility toward pesticides is available (e.g. Tan 1981; Sinha et al. 1990; Cilgi & Jepson 1995; Longley & Sotherton 1997b) which may favour the choice of this group for further consideration.

Predators can reduce the abundance of pest species and they are objects of agricultural research since many years and hence an extensive body of literature is available dealing with their occurrence, distribution, species richness,... (e.g. Carabidae: Heneghan 1992, Holland & Luff 2000, Irmler 2003; Staphylinidae: Andersen 1997, Bohac 1999, Balog & Marko 2007, Balog et al. 2008; spiders: Holland & Reynolds 2003b, Baines et al. 1998, Bogya & Marko

1999, Clough et al. 2005). Ground beetles and spiders showed a clear susceptibility towards pesticides (see Table 3.1-1). Both groups seem to be appropriate for analysing the effects of pesticides on terrestrial biodiversity.

In short:

- Agricultural landscapes are potentially inhabited by a range of arthropod groups which differ in their feeding characteristics (pollinators, herbivores, predators) and habitat requirements.
- A review of literature concerning eight prominent arthropod groups was conducted. Results indicate that agricultural intensification and pesticide usage affects arthropods predominantly negatively while plant species richness, availability of floral resources, and the occurrence of semi-natural habitats revealed benefits for most groups concerning their abundance or species richness.
- In order to assess the potential effects of pesticides on terrestrial biodiversity, more than one arthropod group should be considered. Arthropod groups showing a high susceptibility towards pesticides combined with the availability of extensive data from published literature are Lepidoptera (herbivores and pollinators), Carabidae, and spiders (both predators).

3.1.2 Soil organisms

Soils are the earth's only meeting place for four crucial life-supporting realms: the atmosphere, lithosphere, hydrosphere, and biosphere (Parker 2010). These come together to form the soil (pedosphere) with a multitude of unique niches supporting soil-dwelling life-forms, which vary in body size and body length over several orders of magnitude (Table 3.1-3).

Table 3.1-3: Hierarchy and Size of Abundance of Soil Organisms (Neher & Barbercheck 1998)

Class	Examples	Biomass (g per m^2)	Lenght (mm)	Populations (m^2)
Microflora	bacterians fungi algae actinomycetes	1-100	not applicable	10^9-10^{12}
Microfauna	protozoans	1.5-6.0	0.005-0.2	10^9-10^{12}
Mesofauna	nematodes enchytraeidas mites spingtails	0.01-10	0.2-10	10^2-10^7
Macrofauna	insects	0.1-2.5	10-20	10^2-10^5
Megafauna	earthworms (ants, termites)	10-40	20	0-10^5

The functional group diversity of soil biota is also high. These functional groups cut across taxa and include ecosystem engineers, litter transformers, decomposers, and root herbivores, among others (Brussaard et al. 2007). Moreover, single soil organisms may fulfil several functions. So called ecosystem engineers, for instance, have significant impacts on structure and processes in terrestrial ecosystems at different spatial and temporal scales. Representative group hereof are earthworms in the temperate and ants and termites in the sub-tropical and tropical zones (Jouquet et al. 2006).

The mobility of soil organisms within their habitat is mainly dependent on body size and the available space. Unlike soil macro- and megafauna, the mesofauna does not have the ability to reshape extensively the soil and is dependent on the existing pore spaces, cavities, and channels for locomotion in soil. Therefore, soil compaction can have detrimental consequences on soil fauna diversity (Larsen et al. 2004). Soil organism communities become increasingly dominated by smaller specimen as average pore volume decreases. Within the habitable pore space, microbial and mesofaunal activity is influenced by the balance between water and air (Neher & Barbercheck 1998). Maximum aerobic microbial activity occurs when 60% of the pore volume is filled with water (Linn & Doran 1984). Populations and diversity of mesofauna are greatest in soil with high porosity, high organic matter content, and structured horizons (Andrén & Lagerlöf 1983). Most biological activity occurs within the top 20 cm of soil which may correspond roughly to the “plow layer” in agricultural soil. In uncultivated soils, mesofauna is more abundant in the top 5 cm than in greater soil depths (Neher & Barbercheck 1998).

Soil microflora is the most numerous and most diverse part of the soil community and the basis of any soil food web. Its outstanding role in the process of decomposition of organic matter and respiration in soil is well known (Petersen & Luxton 1982). Recent culture-dependent techniques (Biolog microtiter plates) and culture-independent techniques (denaturant gradient gel eletrophoris) allow the study of carbon substrate utilisation and community structure of microorganisms (Anderson et al. 2009; Gielen et al. 2011). Microorganisms show a distinct sensitivity to soil disturbance as well as may also exhibit some tolerance (Gielen et al. 2011). The shift of dominance from fungi in litter and soil of no-tillage fields to bacteria in tillage fields is a general phenomenon (Hendrix et al. 1986) with some variability dependent on soil type and season (Reeleder et al. 2006). There is a large debate on the existence of species trait redundancy in the process of degradation of organic matter (Hättenschwiler et al. 2005).

Soil mesofauna is mainly composed of four species groups (nematodes, enchytraeids, mites and springtails, Table 3.1-3). Soil nematodes are abundant ($6x10^4$ to $9x10^6$ per m^2) and small (300 µm to 4 mm) worms with a short generation time (days to a few weeks) that allow them to respond quickly to changes in soil conditions or in food supply (Bongers 1990). Relative to other soil mesofauna, trophic or functional groups can be identified easily by morphological structures associated with various modes of feeding (Ferris et al. 2004). Different trophic levels can be impacted by soil disturbances (Bongers 1990), e.g. herbivore nematodes are negatively impacted by mowing (Todd & Seastedt 1992). The nematode maturity index, calculated from the distribution of k- and r-selected species and species groups, is used for monitoring purposes (Bongers 1990). The soil nematode *Caenorhabditis elegans* is mainly known as model organism for cell biology and genetics, but is recently used for the study of physiological effects of plant protection products (Martin et al. 2009; Svendsen et al. 2010).

Enchytraeid earthworms are often found in lower number in arable soil than in comparable uncultivated soils, but their contribution to total biomass and respiration can be considerable even in arable soils (Andrén & Lagerlöf 1983). In grassland and field soils cultivated with different crops, the majority of enchytraeids is found up to a soil depth of 12 cm with a density of up to 28000 individuals per m^2 (Lagerlöf et al. 1989). *Enchytraeus albidus* is used as test species in ecotoxicological soil testing in the laboratory (OECD 2004a), recent studies were run to establish field studies in the environmental risk assessment of pesticides (Römbke et al. 2009b). The sensibility to specific soil constituents (Prendergast-Miller et al. 2009) make enchytraeids suitable for monitoring soil quality, e.g. in city parks (Schlaghamerský & Pizl 2009), vineyards and orchards (Vavoulidou et al. 2009), and accordingly also in other habitats.

Mites are one of the arthropod classes with the highest species numbers and with the most diverse feeding modes (Krantz & Walter 2009). In soil, the two major orders Parasitiformes and Acariformes are represented by the mainly predatory Mesostigmata (including the test species *Hypoaspsis aculeifer* (OECD 2008a)) and by the mostly saprophagous Oribatida (Walter & Proctor 1999), respectively. In the litter and soil of grassland and many other habitats, mites are the most diverse and abundant arthropods and catalyze primary decomposition and nutrient cycling by activating of fungi and bacteria (Behan-Pelletier & Kanashiro 2010). Oribatida are one abundant species group in leaf litter of tropical and temperate zones, but are found in especially high numbers in the litter of Northern zones (Heneghan et al. 1998), many species are k-strategists and sensitive to soil perturbations (Princz et al. 2010). Some soil parameters like pH are correlated with the abundance of mite species groups (Bedano et al. 2005). Despite of their important role for soil functions, correct sampling and taxonomical determination of mite species remain difficult (Lakly & Crossley Jr. 2000; Behan-Pelletier & Kanashiro 2010).

The springtails are besides the soil mites the most important group of mesofauna (Palissa 2000), classified as eu-edaphic (deep soil-living), hemi-edaphic (intermediate) or epi-edaphic (surface-living) with corresponding morphological and physiological adaptations in, e.g., body shape and body colour (Parisi et al. 2005). The collembolans are a frequently used species group for field studies examining the influence of landscape elements on soil organisms' distribution (e.g. hedgerows (Alvarez et al. 2000), field edges (Frampton 2002)), the long-term impact of plant protection products (Frampton & van den Brink 2007) or recolonisation of brown coal mine (Dunger & Voigtländer 2009). Laboratory tests with the mostly parthenogenetic *Folsomia candida* and the sexually reproducing *Folsomia fimetaria* are conducted for the evaluation of the ecotoxicity of chemicals in soils (OECD 2009). Furthermore, many additional laboratory experiments highlight the diversity of responses of these soil organisms (feeding behaviour (Krogh 1995; Domene et al. 2007), variability of reproduction (Jaensch et al. 2005), sensitivity to soil bulk density (Larsen et al. 2004), sensitivity to plant protection products (Krogh 1991; Martikainen 1996), sensitivity to different soil types (Coja et al. 2006; Domene et al. 2011), avoidance of plant protection products (Fabian & Petersen 1994), gene regulation after exposure to heavy metals (Liu et al. 2010)).

Earthworms are classified as macrofauna due to the large body size of some species (Table 3.1-3). They can be categorized by three different life-forms (epigeic, endogeic and anecic) according to their foraging and feeding behavior (Sims & Gerard 1999). Anecic species like *Lumbricus terrstris* and *Aporrectodea longa* create long vertical holes within the soils and improve soil aggregation and porosity, and hence associated hydraulic properties and organic matter availability for microorganisms (Jouquet et al. 2006) and plant growth (Haimi et al. 1992; Baker 1998). Therefore, anecic species act as ecosystem engineers and are considered as keystone species for terrestrial ecosystems in temperate zones as are termites and some ant species for the sub-tropical and tropical zones. The earthworms' importance for soil functioning was the reason for implementing the earthworm acute test as the first ecotoxicological test for soil risk assessment (OECD 1984), followed some years later by the earthworm field study design (ISO 1999b). A correspondence was found between laboratory LC_{50}-values and field effects for a number of plant protection products (Heimbach 1992) but the discussion on the relevance of laboratory data for the field and the potential of extrapolating laboratory data to the field is still ongoing. Diverging sensitivities to different plant protection products were found between field species and *Eisenia fetida* (Kula 1994). Many studies were conducted testing the influence of specific variables on the composition of the earthworm community in the field. These encompass the investigation of land use change in long-term monitoring sites (Beylich & Graefe 2009), the impact of sewage sludge (Booth et al. 2000), plant protection products (Capowiez et al. 2006; Das Gupta et al. 2010), soil type (Gormsen et al. 2004), soil tillage (Curry et al. 2002), organic farming (Irmler 2007), and heavy metals (Eijsackers et al. 2005)).

All of the above mentioned groups of soil organisms are species-rich and can be considered in the assessment of soil quality. However, due to the variability in abundances in different habitats and different soil types, no specific group for its own can be recommended but the composition of the specific soil community should be characterised first before selecting a species group (e.g. soil mites in soils with C-rich litter, earthworms in grassland soils, springtails in field margins). Especially, in agroecosystems a mixed approach may be advisable by assessing the presence of key species (e.g. lumbricids) and relevant trophic levels supported by multifactorial statistical analysis (Neher & Barbercheck 1998; Barbercheck et al. 2008).

In short:

- Soil organisms include microflora (e.g. bacterians), microfauna (protozoans), mesofauna (nematodes, enchytraeids, mites and springtails), macrofauna (insects), and megafauna (e.g. earthworms).
- The mobility of the soil organisms within their habitat is mainly dependent on body size and the available space.
- A mixed approach is recommended by assessing the presence of key species and relevant trophic levels supported by multifactorial statistical analysis

3.2 Characterization of exposed habitats and their biocoenosis

3.2.1 Exposed habitats

3.2.1.1 Plant protection products

Traditionally managed agricultural landscapes provide habitats for high species diversity (Kretschmer et al. 1997). Especially natural and semi-natural habitats in agricultural areas lead to a species-rich biocoenosis (Duelli & Obrist 2003). In the last decades, however, semi-natural habitats like hedges or field margins were removed or diminished due to agricultural intensification practices (Robinson & Sutherland 2002). Furthermore, habitats adjoining to agricultural sites are to some extent affected by fertilizer and plant protection product inputs, with smaller structures receiving higher proportions of the in-field application rates related to their size than wider ones. Habitats next to treated fields that can be exposed to plant protection products are described and classified in Table 3.2-1 (on the basis of Riecken et al. 1994; Kühne & Freier 2001; Riecken et al. 2006; Vickery et al. 2009) and are illustrated in Figure 3.2-1. The considered habitats can be divided into in-crop/off-crop and in-field/off-field habitats according to their position in or next to fields – as suggested by the terminology of the ESCORT 3-workshop (Alix et al. 2012).

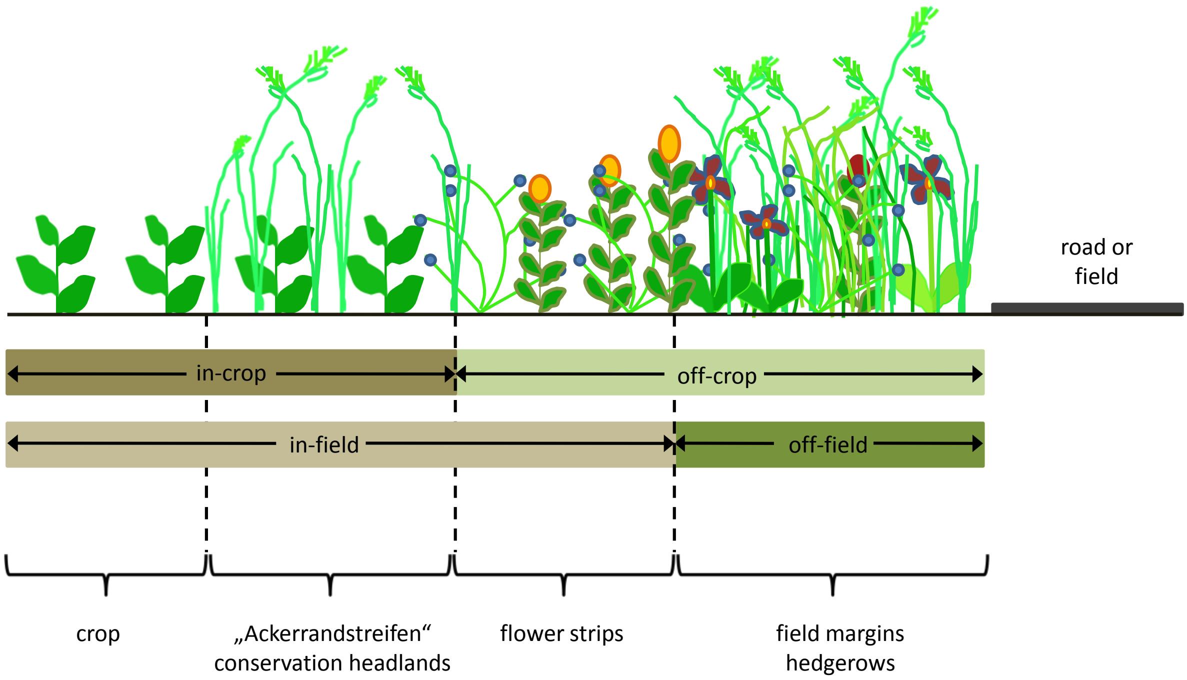

Figure 3.2-1: Habitats in agricultural landscapes that may be exposed to plant protection products classified in in-/off-crop and in-/off-field habitats. Examples for each habitat-type are given below the brackets. For further information see Table 3.2-1. Not all habitat-types shown have to occur in/next to an agricultural site

Table 3.2-1: Descriptions and classification of habitats in agricultural landscapes that may be exposed to plant protection products (based on Riecken et al. 1994; Kühne & Freier 2001; Riecken et al. 2006; Vickery et al. 2009).

	Shape	Description	in-crop	off-crop	in-field	off-field
woody structures (mostly associated with uncropped grassy and herbaceous structures)						
Hedgerow (Hecke)	linear	shrubs and/or trees growing in one- to multi-rowed structures adjoining to agricultural sites		x		x
Copse (Feldgehölz)	non-linear	small-scaled areas of bushes and trees (max. 2000 m²) within or next to agricultural sites showing a classification into tree- and shrub layer (height: more than 5 m)		x		x
Shrubbery (Gebüsch)	non-linear	small-scaled areas predominantly grown with shrubs (max. 2000 m²) within or next to agricultural sites (height: less than 5 m)		x		x
Edge of a wood (Waldrand)	linear	transitional zone between agricultural sites and woods/forests (without clear cutting)		x		x
uncropped grassy and herbaceous structures						
Field margin (Feldsaum)	linear	permanent vegetation strips (mostly grassy or herbaceous) adjoining to agricultural sites which were mown periodically		x		x
Margin of woody structures (Gehölzsaum)	linear	grassy and/or herbaceous vegetation strips adjoining to woody structures		x		x
Flower strip* (Blühstreifen)	linear	strips in the field which were sown with a seed mixture containing flowering plants to improve nectar-/ pollen availability		x	x	
cropped structures						
Conservation headland (Ackerrandstreifen)	linear	strips in the cropped area (mostly next to the crop edge) which receive a reduced input of (some) plant protection products	x		x	

* Flower strips or wildflower strips are only an example for a range of schemes farmers can use to support and enhance biodiversity in the in-field area. Further examples are bird cover strips, uncropped wildlife strips, or beetle banks (Marshall & Moonen 2002).

To quantify the occurrence of different habitats relevant for an exposure to plant protection products in agricultural landscapes (especially uncropped grassy/herbaceous structures and woody structures), a GIS-analysis (= Geographical Information System) was conducted. Two German regions were chosen, one in Rhineland-Palatinate and one in Brandenburg, with a special focus on information on the width of these habitats (see Research-BOX 1). Per region, 4,000 ha of predominantly agricultural landscape were analysed and habitats adjoining agricultural sites were digitized manually using digital orthophotos (ground resolution:

20 cm). The results reveal that narrow grassy/herbaceous field margins (width < 3 m) are the most common feature in Rhineland-Palatinate accounting for 85 % of the length and 65 % of the area of field margins. In Brandenburg, margin length was nearly evenly distributed across all width classes but highest in the lowest class < 1 m. However, the overall margin length was greatly reduced compared to Rhineland-Palatinate which is due to extremely large acreages in Brandenburg. But wider margins (5-10 m) occurred more often in Brandenburg and, hence, higher amount of the overall margin area could be attributed to these wider margins compared to Rhineland-Palatinate. Since woody structures like hedgerows were predominantly associated with wider margins, their availability was higher in Brandenburg than in Rhineland-Palatinate.

RESEARCH-BOX 1

Assessment of exposure-relevant habitats

BACKGROUND: In agricultural landscapes, semi-natural habitats adjoining to agricultural sites can be affected by inputs of plant protection products. Information on the shape of these exposure-relevant habitats is of special relevance since pesticide inputs decrease with increasing distance. Furthermore, in Germany (potential) restrictions for the conservation of non-target habitats during the application of plant protection products are only applied if the adjoining habitats are wider than 3 m. Few data to the width and amount (area and length) of semi-natural exposure-relevant habitats are published (mainly Kühne & Freier 2001). Therefore, these structures were quantified using a GIS-analysis (= Geographical Information System) in two agricultural landscapes in Germany (Hahn et al. in prep.).

METHODS: The study areas were located in Rhineland-Palatinate (RLP) and Brandenburg (BB) and differ in a range of characteristics (Table 3.2-2).

Table 3.2-2: Characterization of the study sites in Rhineland-Palatinate (RLP) and Brandenburg (BB).

	RLP	BB
study area [ha]	4,000	4,000
crops	vineyards arable fields orchards	arable fields
acreages	small scaled	large scaled

The data collection is based on digital orthophotos (ground resolution: 20 cm). Habitats adjoining to agricultural sites which were covered with permanent vegetation (including woody and grassy/herbaceous structures, but no cropped area) were digitized manually. Below, these predominately linear structures are entitled as **margins**.

During digitizing the margins were divided into sections with the same characteristics according to e.g. width class (0-1 m, 1-2 m…8-10 m), adjoining structures (e.g. fields, streets/country roads) and presence of woody structures (present, not present). Woody structures (normally hedgerows) should be built up of at least three shrubs/trees with overlapping branches. Length and area of each section were determined afterwards using ArcGIS (version 9.3.1, by ESRI). Structures of permanent vegetation with more than 10 m width could mostly be attributed to grasslands and could therefore be subject of several management practices (e.g. fertilizing). Hence, these structures were not included in the following analysis.

RESULTS: In the study region in **RLP** about 477 km of margins (119 m/ha) with a margin area of 91 ha (226 m²/ha) were recorded. The smaller margins comprised the main part of margin length and area (Figure 3.2-2). Especially margins with a width of 1-2 m account for a great amount of the length and area. According to the length 85% (102 m/ha) were smaller than 3m. These margins cover 65% of the margin area (145 m²/ha). Woody structures were only sparsely recorded (overall 8.3 km or 2.1 m/ha) and occurred almost exclusively on margins wider than 3 m.

The study region in **BB** was characterized by larger acreages in comparison to RLP. Hence, margin length was reduced (187 km or 47 m/ha). Margin length was nearly evenly distributed across all width classes but highest in the lowest class < 1m. The highest amount of margin area could be attributed to the wider margins (5-10 m). As in RLP, in BB woody structures were predominantly found in margins wider than 3 m (Figure 3.2-2). The amount of these structures was five-times higher than in RLP (nearly 40 km or 10 m/ha).

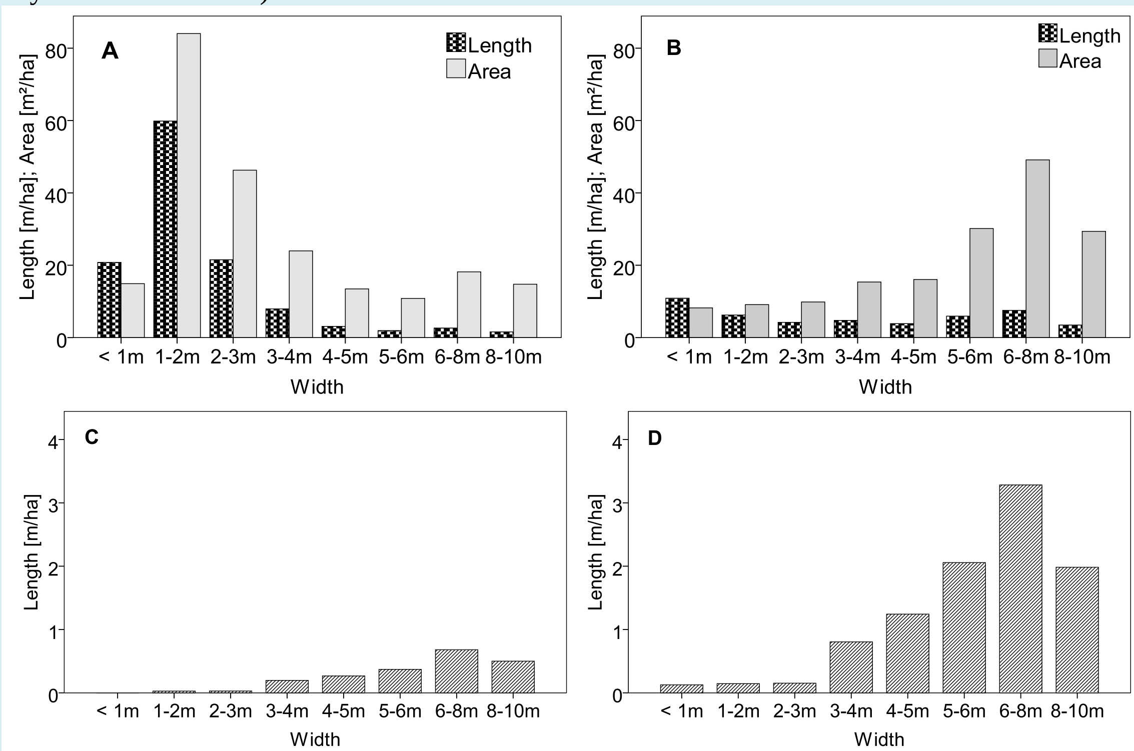

Figure 3.2-2: Length and area of the digitized margins in RLP (A) and BB (B) according to different margin width classes. Information on the occurrence of woody structures on margins of different width for RLP (C) and BB (D) is given below. Data based on an analysed area of 4,000 ha each in RLP and BB.

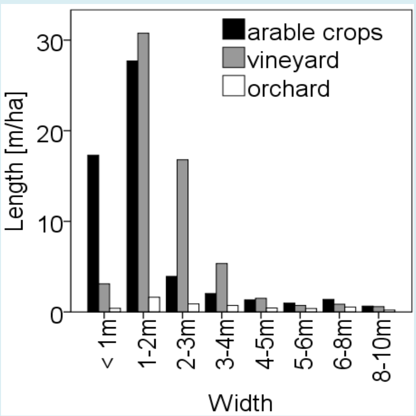

Figure 3.2-3: Margin length per hectare and width class for different crops in RLP. Data based on an analysed area of 4,000 ha.

Concerning the crop type, the studied field margins in RLP showed a different distribution to the width classes (Figure 3.2-3). Arable field margins were mostly smaller than 2 m, whereas vineyards were predominantly 1-3 m wide. Orchards represent a minor land usage with field margins found to be nearly evenly distributed to the different width classes.

Source: Hahn, M., Lenhardt, P., Vollmar, T. & Brühl, C. A. (in prep.). "Characterization of field margins in intensified agro-ecosystems – why narrow margins matter in terrestrial pesticide risk assessment and management."

3.2.1.2 Biocides

Biocides are used in various sectors of human life. In this report, we want to focus on biocidal product types which could be (indirectly) applied to terrestrial habitats in agricultural landscapes, especially agricultural sites. So, we concentrated on disinfectants (product type 3, see Introduction), insecticides (product type 18), and wood preservatives (product type 8).

Insecticides and disinfectants used as biocides are applied e.g. in animal housing for the control of manure-breeding house flies and other arthropods (like e.g. bloodsucking flies, lice, mites, louse flies and fleas) and of pathogens causing dysentery. The main destination of insecticides in animal housing is the manure (OECD 2006c). Spreading (liquid) manure (which could contain insecticides) to fields and grasslands for fertilizing them is a common practice in agriculture.

Wood used on agricultural sites might be treated with wood preservatives. This can involve wooden piles in the cultivation of fruits (viticulture, orcharding) or hops that are located at the agricultural sites or wooden fences at the field edges. Wood preservatives or their eluates found in the local environment might result of leaching out of treated wood-in-service which is in contact with soil and/or water (OECD 2000c).

3.2.2 Quantification of pesticide input

3.2.2.1 Plant protection products

The input of plant protection products in semi-natural structures next to agricultural sites depends e.g. on the adjoining crop (varying applications of plant protection products), the application technique, the weather situation during the application (especially wind speed), and the width of the margin. There are three pathways inputs of plant protection products can enter semi-natural terrestrial habitats: via run-off, via direct overspray, and via spray drift (see Figure 3.2-4):

- ***Run-off***:
  Plant protection products are applied in the cropped area, but caused by (heavy) rain they are flushed out into adjacent habitats. In contrast to aquatic systems, run-off is currently not considered as pathway for plant protection products inputs in terrestrial non-target habitats.
- ***Overspray***:
  Overspray is an application method in which aqueous solutions of plant protection products are applied on (crop) plants via nozzles in droplet form.
  To ensure a full application rate (100%), the spray cones of two nozzles have to overlap. When the last nozzle is placed at the field edge, parts of the adjoining non-crop habitat are sprayed with 50% of the in-crop application rate (see Figure 3.2 4).
- ***Spray drift***:
  Spray drift is the movement of smaller plant protection products droplets or particles through air to areas outside the intended target area. It occurs during or soon after the application of plant protection products and is present as fine mist like droplets.

Detailed information on drift rates is available for several crops (Ganzelmeier et al. 1995; Rautmann et al. 1999). In general, spray drift values as percentage of the field application rates are available from the first meter onwards. Drift deposition decreases with increasing distance to the field.

The input by overspray is especially relevant for smaller margins, since there the overspray area constitutes a large proportion of the whole field margin area.

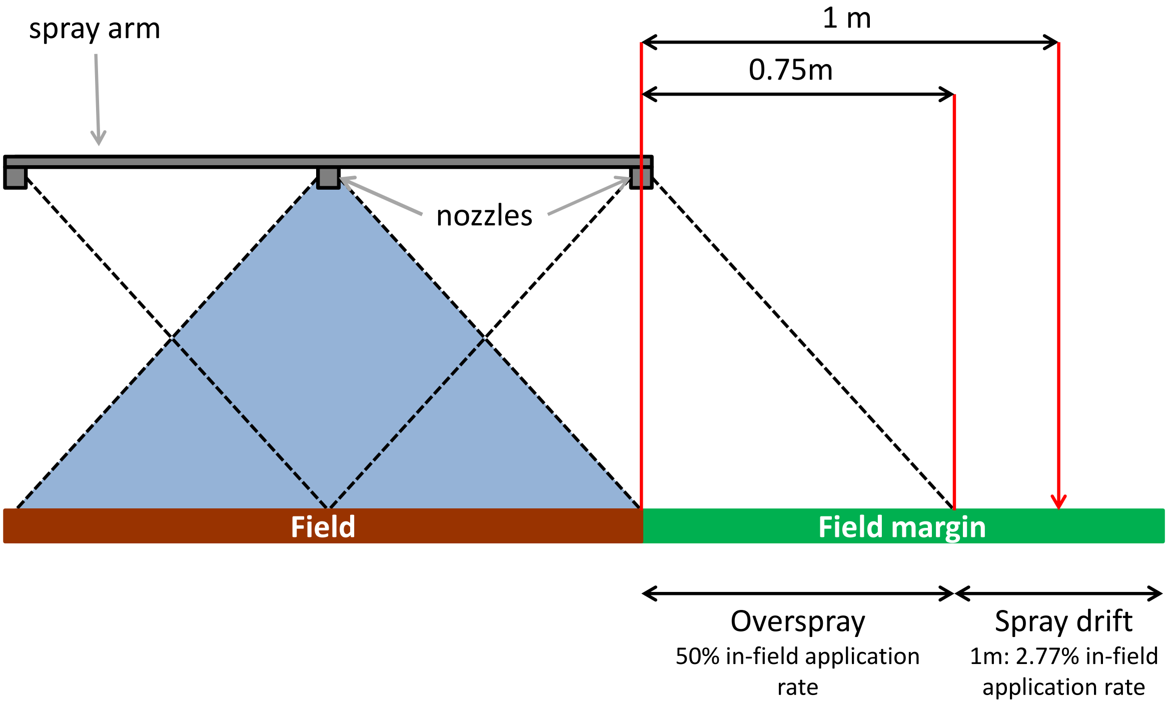

Figure 3.2-4: Scheme of the inputs of plant protection products via overspray and spray drift in cereal field margins. The blue coloured area illustrates the spray cone of one nozzle. (Based on a personal communication of Rautmann, D. with Brühl, C.A.)

Different cultivated crops also receive a different number of applications and a suite of various products depending on the management. The NEPTUN-project[3] holds data according to usage of plant protection products in Germany (e.g. treatment index, see Info-Box 1). When this information is combined with the inputs of plant protection products of drift and overspray, a ***Margin Treatment Index*** (MTI) can be calculated to characterize the intensity of plant protection products in different margin-types (see Info-BOX 1).

[3] NEPTUN: Netzwerk zur Ermittlung der Pflanzenschutzmittelanwendung in unterschiedlichen, landwirtschaftlich relevanten Naturräumen Deutschlands

INFO-BOX

Calculation of the Margin Treatment index (MTI)

Drift values *D* (in % of application rate) can be calculated using the following equation (1) with parameters (a, b) adjusted to the particular crop as shown in Table 3.2-3 (Rautmann et al. 1999).

$D = a \times x^b$ (Equation 1)

x: distance to the sprayed agricultural site [m]

Table 3.2-3: Parameters (a, b) for the calculation of the drift values in different crops according to Rautmann et al. (1999) and the particular Treatment Indices (TI) on the basis of the NEPTUN-Project (Roßberg et al. 2002; Roßberg 2007a; 2009b).

	a	*b*	TI
arable fields	2,7705	-0,9787	5,30 [1]
vineyard (early)	15,86	-1,6132	14,20
vineyard (late)	44,506	-1,5593	
orchard (early)	60,36	-1,2243	14,64 [2]
orchard (late)	66,686	-0,7517	

[1] Mean TI of cereals, vegetables, maize, potato, oil rape
[2] Mean TI of apple, pear, cherry, plum

For example in vineyards (late spraying) spray drift of plant protection products at a distance of 3 m to the agricultural field accounts for 8 % of the in-field application rate ($D = 44{,}506 \times 3^{-1{,}5593} = 8{,}02$).

An indicator for the intensity of an application of plant protection products is the **Treatment Index (TI)** (Roßberg 2007b). This index is crop specific (see Table 3.2-3) and based on the number of applied plant protection products in relation to the treated area and the percentage of applied to permitted rate (Roßberg et al. 2002). If one plant protection product is applied on the whole acreage with 100% of the permitted rate, this results in a treatment index of 1.

We propose to combine drift values and Treatment Indices to calculate the **Margin Treatment Index (MTI)**, in order to characterize the input intensity of plant protection products in different field margin types (see equation 2).

$MTI = D \times TI$ (Equation 2)

In vineyard margins, for example, at a distance of 3 m from the field edge the MTI is estimated to be:
0.08 x 14.2 = 1.14
Further results regarding the MTI for different crop types are given in Appendix 5.2.

Comments to the use of MTIs for small margins:

- For orchards and vineyards the parameters used for the calculation of the drift values (Table 3.2-3) are defined for distances of at least 3m. Due to the lack of adequate parameters we applied the same parameters also to smaller margins and therefore, these results should be interpreted carefully.
- The mentioned parameters for arable fields were defined for distances of at least 1m. However, since a part of the field margin is oversprayed with 50% of the in-field rate the plant protection product input (D) have to be set to 50%.

The MTI allows a comparison of the margins next to different crops and with varying width regarding their exposure intensity to plant protection products (Figure 3.2-5). In the following, a MTI ≥ 1 is used as threshold value for graphical representation since this is equivalent to one application with plant protection products at field rate.

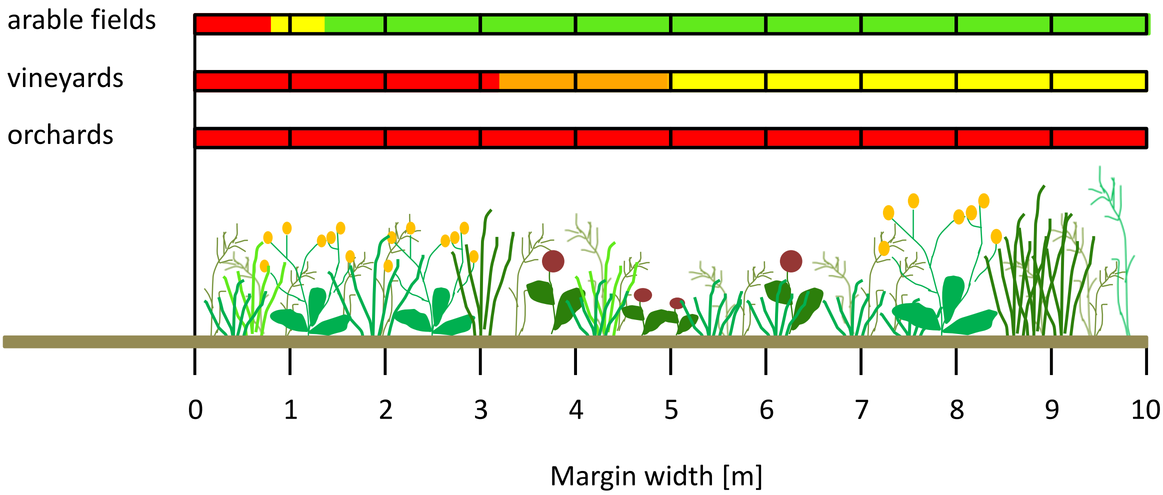

Figure 3.2-5: Estimated input of plant protection products in margins with a width up to 10 m adjoining to different crops. The calculations are based on the in-field treatment indices (Roßberg et al. 2002; Roßberg 2007a; 2009b) and the drift values (Rautmann et al. 1999) for arable fields (mean treatment index, including cereals, vegetables, potatoes, maize, oil rape), vineyards and orchards (mean treatment index, including apple, pear, cherry, plum). For vineyard and orchards, the calculations are based on application dates causing the highest drift rates. Red: margin treatment index (MTI) > 1, Orange: MTI ≥ 0.5, Yellow: MTI > 0.1, Green: MTI ≤ 0.1.

Lowest input of plant protection products is expected next to arable fields where the MTI falls below 0.1 after 1.4 m width. However, when this information is transferred to the studied agricultural landscape in the German region RLP (Research-BOX 1) – where most digitized margins were less than 2 m wide – it is evident that a high proportion of this margin habitat is potentially affected by inputs of plant protection products and that especially in the first meter the MTI is above 1. This means that a full field rate accumulates in narrow field margins over the year. In vineyards, over 80% of the margins were less than 3 m wide, but in these width classes calculated MTIs exceed the value of 1. In the region RLP, orchards represent a minor land use in comparison to arable fields or vineyards. However, in all respective margins up to a distance of 10 m from the field edge the calculated MTI was higher than 1 (e.g. in 3 m width: MTI = 4.27, in 10 m width: MTI = 1.73, for further examples see Appendix 5.2).

3.2.2.2 Biocides

In order to estimate the quantity of biocides released into the environment, emission scenarios have been developed for different biocide product types which are described in Emission Scenario Documents (ESDs) by the OECD.

According to the "Emission Scenario Document for Insecticides for Stables and Manure Storage Systems" (OECD 2006c) the fraction of the ***insecticide*** that is transferred with manure to the manure storage system depends on a variety of factors (e.g. animal species, type of housing, application methods, processes like degradation and volatinisation). Worst case scenarios mirror the biocidal application in the storage system itself, where the whole amount of insecticide reaches the manure, which is spread to the field after a certain amount of time (OECD 2006c). Within the scope of this project, the application of manure contaminated with insecticides to agricultural fields was considered to be a similar scenario to the in-field application of plant protection products. But the input of insecticides will differ depending on whether a plant protection product is freshly applied or manure is spread that may have been stored over a certain period of time and may additionally influence the fate and behaviour of active substances on field. The ESD estimates the biocide input in different environmental compartments, like soil or water. For biocides, there is no differentiation between in-field and off-field habitats in the environmental risk assessment. Hence, models to assess the input of manure contaminated with insecticides with a focus only on (terrestrial) non-target areas like field margins are not available.

The "Emission Scenario Document for Wood Preservatives" (OECD 2000c) is an approach to estimate the concentrations in the environment of specific active substances used in ***wood preservatives***. It includes two stages of the life cycle of wood (a) the application and storage of treated wood prior to shipment as well as (b) treated wood-in-service. Wood products used in agriculture like fences or wooden piles can be classified as wood-in-service and, therefore, we want to focus on this stage of the life cycle. For the purpose of this project, we considered the leaching of biocides and their eluates of treated wood-in-service as a route of entry into environment. The ESD for wood preservatives provides scenarios in which treated wood is exposed to the weather and in contact with the ground as occurring for example with transmission poles or fence posts. For these scenarios the soil compartment has been identified to be the primary receiving environmental compartment. A possible pathway might be the leaching of active substances analogue to run off (see chapter 3.2.2.1) of applied plant protection products from treated fields. As for the assessment of insecticides (see previous passage), there is no differentiation between in-field and off-field habitats to conclude on environmental risks, models to assess the input of wood preservatives with a focus only on (terrestrial) non-target areas like field margins are not available. As described for insecticides in manure, the amount of active substances (and eluates) reaching terrestrial habitats from woods treated with biocides will substancially differ from active substances transported there by run-off from whole fields treated with plant protection products.

3.2.3 Protection goals

Semi-natural habitats have been identified as important habitat types in agricultural landscapes especially in support of high biodiversity levels. Hence, several protection, preservation and development objective have been defined in German and European Union legislation. The objectives mentioned below focus on the preservation of semi-natural habitats due to their characteristics of linking habitats and/or the reduction of adverse effects of pesticides on biodiversity in agro-ecosystems.

One of the main objectives of the ***Convention on Biological Diversity (CBD)*** is the protection of biodiversity on genetic, species, and habitat level. Contracting parties shall develop national strategies, plans or programmes for the conservation and sustainable use of biological diversity (Article 6). Germany ratified the Convention in 1993. In order to fulfil the obligation mentioned in Article 6, Germany developed the ***National Strategy on Biological Diversity*** (Nationale Strategie zur Biologischen Vielfalt). In this strategy, the definition of a minimum density of linking landscape elements and a reduced input of plant protection products are aspired to improve the biodiversity in agricultural landscapes (chapter B 2.4):

Wir streben Folgendes an:

- *[…]*
- *Weiterführung des Reduktionsprogramms chemischer Pflanzenschutz mit dem Ziel, Risiken, die durch die Anwendung chemischer Pflanzenschutzmittel entstehen können, weiter zu reduzieren,*
- *[…]*
- *Definition einer naturraumbezogenen Mindestdichte von zur Vernetzung von Biotopen erforderlichen linearen und punktförmigen Elementen (Saumstrukturen, Hecken, Feldraine, Trockenmauern, Trittsteinbiotope) bis 2010 und Abbau ggf. bestehender Unterschreitungen,*
- *[…]*

(Extract from chapter B 2.4, National Strategy on Biological Diversity[4])

Furthermore, hedgerows and field margins are mentioned in the German ***Federal Nature Conservation Act*** (Bundesnaturschutzgesetz, BNatSchG), since these structures are valuable properties of the biotope network (BNatSchG §21). As linking landscape elements, they shall be preserved and – if necessary – established at a regional scale.

Auf regionaler Ebene sind insbesondere in von der Landwirtschaft geprägten Landschaften zur Vernetzung von Biotopen erforderliche lineare und punktförmige Elemente, insbesondere Hecken und Feldraine sowie Trittsteinbiotope, zu erhalten und dort, wo sie nicht in ausreichendem Maße vorhanden sind, zu schaffen (Biotopvernetzung).

(Extract from BNatSchG §21(6))

At EU level, the ***Regulation No 1107/2009 of the European Parliament and the Council***[5] was enforced in June 2011. This Regulation contains rules for the authorisation of plant protection products in commercial form and for their placing on the market, use and control within the European Community (Article 1). It aims to ensure a high level of protection of human and animal health as well as the environment (Article 1). In this regulation,

[4] Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit (2007): Nationale Strategie zur biologischen Vielfalt.
English version available on http://www.bfn.de/fileadmin/ABS/documents/Biodiversitaetsstragie_englisch.pdf

[5] Regulation (EC) No 1107/2009 of the European parliament and the council of 21 October 2009 concerning the placing of plant protection products on the market
Verordnung (EG) Nr. 1107/2009 des europäischen Parlaments und des Rates vom 21. Oktober 2007 über das Inverkehrbringen von Pflanzenschutzmitteln

biodiversity is explicitly considered as a protection goal (Article 4.3e) which shall not be unacceptably affected by plant protection products.

A plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use, shall meet the following requirements:
[…]

(e) it shall have no unacceptable effects on the environment, having particular regard to the following considerations where the scientific methods accepted by the Authority to assess such effects are available:
- *(i) its fate and distribution in the environment, particularly contamination of surface waters, including estuarine and coastal waters, groundwater, air and soil taking into account locations distant from its use following long-range environmental transportation;*
- (ii) *its impact on non-target species, including on the ongoing behaviour of those species;*
- (iii) *its impact on biodiversity and the ecosystem.*

(Extract from Regulation 1107/2009, Article 4.3)

The ***Directive 2009/128/EC of the European Parliaments and Council***[6] (enforced November 2009) establishes a framework to achieve a sustainable use of pesticides by reducing the risks and impacts of pesticide use on human health and the environment (Article 1). Furthermore, it shall promote the use of integrated pest management and of alternative approaches or techniques such as non-chemical alternatives to pesticides (Article 1). In Germany, the directive was implemented into national law by the amendment of the ***Plant Protection Act*** (Pflanzenschutzgesetz, PflSchG, enforced February 2012). One objective of the Plant Protection Act is the prevention of unacceptable risks originating from the usage of plant protection products. This covers the health of humans, animals, and ecosystems (PflSchG §1). Application of plant protection products is only allowed if the ***Good Plant Protection Practice*** (Gute fachliche Praxis im Pflanzenschutz) is fulfilled and the principles of the ***Integrated Pest Management*** (Integrierter Pflanzenschutz) are followed (PflSchG §3). Furthermore, the application of plant protection products is not permitted if it can have unacceptable impacts on ecosystems (PflSchG §13).

Within the Directive 2009/128/EC it is claimed that member states should pass ***National Action Plans*** (Nationale Aktionspläne) to set up "*their quantitative objectives, targets, measures and timetables to reduce risks and impacts of pesticide use on human health and the environment and to encourage the development and introduction of integrated pest management and of alternative approaches or techniques in order to reduce dependency on the use of pesticides*" (Article 4). These National Action Plans shall be communicated to the European Commission and to other member states until 14 December 2012 (Article 4). In the

[6] Directive 2009/128/EC of the European parliament and of the council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of plant protection products
Richtlinie 2009/128/EG des Europäischen Parlaments und des Rates vom 21. Oktober 2007 über einen Aktionsrahmen für die nachhaltige Verwendung von Pflanzenschutzmitteln

Plant Protection Act it is stated that the Federal Government of Germany enact an Action Plan on sustainable usage of plant protection products (PflSchG §4):

Die Bundesregierung beschließt einen Aktionsplan zur nachhaltigen Anwendung von Pflanzenschutzmitteln im Sinne des Artikels 4 Absatz 1 der Richtlinie 2009/128/EG (Aktionsplan). Der Aktionsplan wird unter Mitwirkung der Länder und Beteiligung von Verbänden, die sich mit Pflanzen oder Pflanzenerzeugnissen, dem Pflanzenschutz, dem Verbraucherschutz, der Wasserwirtschaft oder dem Umwelt- und Naturschutz befassen, erstellt. Der Aktionsplan umfasst auch unter Berücksichtigung bereits getroffener Risikominderungsmaßnahmen quantitative Vorgaben, Ziele, Maßnahmen und Zeitpläne zur Verringerung der Risiken und Auswirkungen der Anwendung von Pflanzenschutzmitteln auf die Gesundheit von Mensch und Tier sowie auf den Naturhaushalt. Die Zielvorgaben betreffen die Bereiche Pflanzenschutz, Anwenderschutz, Verbraucherschutz und Schutz des Naturhaushaltes.

(Extract from PflSchG §4(1))

In Germany, the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) takes the lead concerning the ***National Action Plan on Sustainable Use of Plant Protection Products*** (Aktionsplan zur nachhaltigen Anwendung von Pflanzenschutzmitteln), supported by the Federal Office for Agriculture and Food (BLE), the Federal Office for Consumer Protection and Food Safety (BVL), and the Julius-Kühn-Institute (JKI), Federal Research Centre for Cultivated Plants. At the time of the preparation of this report, a draft of the German National Action Plan on Sustainable Use of Plant Protection Products was published (version: 27 September 2012, available on http://www.nap-pflanzenschutz.de/fileadmin/SITE_MASTER/content/Dokumente/Grundlagen/NAP2012/NationalerAktionsplan120927Kopierschutz.pdf). With reference to the protection of biodiversity, aims of the German National Action Plan Draft include, inter alia, an increase in the proportion of area managed under organic farming, an increase in the number of farms using integrated pest management strategies, and an increase in the availability of habitats and refuges for beneficial organisms and non-target species in agricultural landscapes for example via the creation of hedgerows and flower strips.

Environmental concerns are also integrated into the ***Common Agricultural Policy*** (CAP; Gemeinsame Agrarpolitik) of the EU. Biodiversity is considered as one of the priority areas for action to protect and enhance the EU's rural heritage (European Commission, available on http://ec.europa.eu/agriculture/envir/index_en.htm, accessed 04.12.12). There are two principle tools in the CAP to integrate environmental aspects:

(1) ***Cross-compliance***:
Direct payments to farmers are linked to their compliance concerning basic standards e.g. in terms of the environment. Mandatory cross-compliance was introduced by the 2003 CAP reform. Rules of the direct support schemes for farmers are established in ***Council Regulation No 73/2009***[7]. For instance, member states shall ensure that

[7] Council Regulation (EC) No 73/2009 of 19 January 2009 establishing common rules for direct support schemes for farmers under the common agricultural policy and establishing certain support schemes for farmers,

agricultural land is maintained in good agricultural and environmental conditions (Article 6). These conditions are defined in Annex III and include, inter alia, the retention of landscape features like hedgerows and field margins.

(2) ***Agri-environment measures***:
Farmers are paid for the provision of environmental services like the adoption of environmentally-friendly farming techniques that go beyond legal obligations. Details are given in ***Council Regulation No 1698/2005***[8], Article 39. An overview of agri-environment measures available in Germany is provided by Thomas et al. (2009).

In reference to biocides, the ***Directive 98/8/EC***[9] of the European Parliament and the Council is established as a regulatory framework for the placing of biocidal products on the market. The Directive states that member states shall authorize a biocidal product only if it

[…] has no unacceptable effect itself, or as a result of its residues, on the environment having particular regard to the following considerations:

- *its fate and distribution in the environment; particularly contamination of surface waters (including estuarian and seawater), groundwater and drinking water,*
- *its impact on non-target organisms;*

(Extract from Article 5, Directive 98/8/EC)

The new ***Regulation No 528/2012***[10] will replace the current Directive in September 2013. This Regulation states in Article 19 ("conditions for granting authorisation"):

1. A biocidal product other than those eligible for the simplified authorisation procedure in accordance with Article 25 shall be authorised provided the following conditions are met:

[...]

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amending Regulations (EC) No 1290/2005, (EC) No 247/2006, (EC) No 378/2007 and repealing Regulation (EC) No 1782/2003
Verordnung (EG) Nr. 73/2009 des Rates vom 19. Januar 2009 mit gemeinsamen Regeln für Direktzahlungen im Rahmen der gemeinsamen Agrarpolitik und mit bestimmten Stützungsregelungen für Inhaber landwirtschaftlicher Betriebe und zur Änderung der Verordnungen (EG) Nr. 1290/2005, (EG) Nr. 247/2006, (EG) Nr. 378/2007 sowie zur Aufhebung der Verordnung (EG) Nr. 1782/2003

[8] Council Regulation (EC) No 1698/2005 of 20 September 2005 on support for rural development by the European Agricultural Fund for Rural Development (EAFRD)
Verordnung (EG) Nr. 1698/2005 des Rates vom 20. September 2005 über die Förderung der Entwicklung des ländlichen Raums durch den Europäischen Landwirtschaftsfonds für die Entwicklung des ländlichen Raums (ELER)

[9] Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market
Richtlinie 98/8/EG des Europäischen Parlaments und des Rates vom 16. Februar 1998 über das Inverkehrbringen von Biozid-Produkten

[10] Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and the use of biocidal products.
Verordnung (EU) Nr. 528/2012 des Europäischen Parlaments und des Rates vom 22. Mai 2012 über die Bereitstellung auf dem Markt und die Verwendung von Biozidprodukt

(b) *it is established, according to the common principles for the evaluation of dossiers for biocidal products laid down in Annex VI, that the biocidal product, when used as authorised and having regard to the factors referred to in paragraph 2 of this Article, fulfils the following criteria:*

[…]

(iv) the biocidal product has no unacceptable effects itself, or as a result of its residues, on the environment, having particular regard to the following considerations:

- *the fate and distribution of the biocidal product in the environment,*
- *contamination of surface waters (including estuarial and seawater), groundwater and drinking water, air and soil, taking into account locations distant from its use following long-range environmental transportation,*
- *the impact of the biocidal product on non-target organisms,*
- *the impact of the biocidal product on biodiversity and the ecosystem.*

(Extract from Article 19, Regulation No 528/2012)

In Annex VI ("common principles for the evaluation of dossiers for biocidal products") it is stated further under terms and definitions that 'Effects on the environment' corresponds to criterion (iv): '*has no unacceptable effects itself, or as a result of its residues, on the environment, having particular regard to the following considerations:*

- *its fate and distribution in the environment,EN L 167/108 Official Journal of the European Union 27.6.2012*
- *contamination of surface waters (including estuarial and seawater), groundwater and drinking water, air and soil, taking into account locations distant from its use following long-range environmental transportation,*
- *its impact on non-target organisms,*
- *its impact on biodiversity and the ecosystem*'.

3.2.4 Biocoenosis in exposed habitats

One major aim of the project was to acquire information on the species composition of arthropod communities in habitats exposed to pesticides. Hence, a literature search was conducted focusing on the identification of invertebrate species using semi-natural habitats in agricultural landscapes as (temporal) habitats. On the basis of previous results on the occurrence of semi-natural habitats in agro-ecosystems (see Research Box 1), we considered especially studies addressing the species richness and species distribution in field margins and woody structures (hedgerows). In a second step, studies were analysed which were dealing with habitat preferences of different insect groups to assess if there are species that prefer either semi-natural habitats or cropped field areas or if the occurring species use both habitats in a similar intensity.

The following analysis included ten studies recording invertebrate species in field margins and woody structures. A few of these studies included comprehensive surveys of invertebrates including different organism groups and functional groups (predators, pollinators, …)

(Tischler 1948; Lewis 1969; Zwölfer et al. 1984; Ross-Nickoll et al. 2004). The other studies assessed the occurrence of single organism group(s) like butterflies (Feber et al. 1996), moths (Merckx et al. 2010), ground beetles (Stachow 1988), spiders (Barthel 1997), hoverflies (Molthan 1990), and predatory arthropods valuable for biological pest control like ground beetles, rove beetles, and spiders (Welling et al. 1994). The following is a brief description of the analysed studies and an overview is given in Table 3.2-4.

Comprehensive surveys

Tischler (1948) assessed the species occurring in two hedgerow types ("Eichen-Hainbuchen-Knick", "Eichen-Birken-Knick") in the federal state of Schleswig-Holstein (Germany) over several years. He detected more than 1,000 invertebrate species belonging to Araneae, Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera, Orthoptera, and Pulmonata.

The study of Zwölfer et al. (1984) also deals with arthropod species inhabiting hedgerows. As study sites hedgerows in the federal state of Bavaria (Germany) were chosen. A special focus of this assessment was the survey of phytophagous insects, like for example caterpillars of Microlepidoptera, and their predators and parasitoids. Overall, 260 species belonging to Araneae, Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, and Neuroptera were identified in this study.

Roß-Nickoll et al. (2004) provided a comprehensive overview of invertebrate species occurring in field margins. The analyzed field margins belong to the vegetation type 'ruderalized tall oat grass meadow' ("ruderale Glatthafer-Wiese") which is described as the dominant vegetation type of grassy and hardly track-charged field margins in agricultural landscapes. Invertebrates were assessed in three study regions in Germany located in the federal states of North Rhine-Westphalia, Saxony, and Bavaria. At large, nearly 550 invertebrate species were detected belonging to Araneae, Coleoptera, Collembola, Diptera, Hymenoptera, and Orthoptera.

Single organism groups

In a study of Feber et al. (1996) the abundance and species richness of butterflies were measured on expanded uncropped field margins (2 m width) in an experimental design that focused on the effects of different management schemes. The study was conducted at different fields in Wytham (UK). Overall, 20 butterfly species were recorded using a monitoring program based on transect routes.

Stachow (1988) studied the Carabidae of three hedgerows and adjoining fields in the federal state of Schleswig-Holstein (Germany). All together 47 species could be identified. All species occurred in both habitat types - the hedgerows and the agricultural sites.

The study of Barthel (1997) investigated the influence of land-use intensity on foliage-dwelling spider fauna. The composition of the spider fauna in different habitats like field margins, fallows, and arable fields was surveyed in Bavaria, Germany, using standardized visual searching. In field margins, more than 70 species were registered.

Molthan (1990) assessed the composition, community structure, and seasonal abundance of Syrphidae (Diptera) in field margins of different width (<1 m, 1.5-2 m, 8 m width) in Hesse, Germany. He identified more than 60 species using sweep netting and standardized visual searching.

Some further studies are included in our analysis although they do not primarily deal with the survey of species compositions in field margins or hedgerows. However, common species are mentioned in these studies and so they are used as additional information sources to enlarge the data base.

The study of Welling et al. (1994) focused on the relationship of field margins and cropped fields regarding the occurrence and distribution of ground beetles and spiders. Arthropods were sampled in one field in a rather small-scaled agricultural landscape in the federal state of Hesse (Germany). Sample sites were located in the cropped area, an adjoining small field margin (0.5 m), and a wide field margin (4 m) next to a hedgerow.

Merckx et al. (2010) conducted a mark-recapture experiment in an agricultural landscape (Oxfordshire, UK) using 23 moth species to assess the impacts of prominent landscape features, grassy field margins (1-2 m width, 6 m width) and hedgerows with higher hedgerow trees on moth abundance. Since adult moths are a rather mobile organism group and it was not clearly mentioned if some species preferred the field margin or the hedgerow (tree) habitat, the listed species were attributed to both habitat types in our analysis.

Lewis (1969) sampled insects in a hedgerow and the neighbouring field using suction traps and vacuum sampler. Unfortunately for our data collection, taxa were mostly identified to family or order, and just a few species are listed. However, these species are also included in our analysis.

Based on the species listed in the above mentioned studies, a database was created containing about 2,100 entries (Table 3.2-4). A document containing all this information has been handed over to the German Federal Environment Agency (Umweltbundesamt) as part of this project.

Table 3.2-4: Number of invertebrate species of different organism groups listed in the mentioned studies. Only those entries containing the full taxonomic information were included (entries like *Dolomedes spec.* were not considered). H: hedgerow, FM: uncropped field margin, FM(H): field margin adjoining to hedgerow, A: agricultural (cropped) site.

	Araneae	Coleoptera	Collembola	Diptera	Hemiptera	Hymenoptera	Lepidoptera	Neuroptera	Orthoptera	Pulmonata	others	trapping method	habitat
Tischler (1948)	58	216		237	143	199	112	9	8	27	48	not mentioned in detail	H
Zwölfer (1984)	21	17		9	55	52	98	2			6	various (e.g. pitfall trapping, light trapping,…)	H
Roß-Nickoll et al. (2004)	163	139	46	70		106			15			various (e.g. pitfall trapping, sweep netting,…)	FM
Barthel (1997)	73											standardized visual searching	FM, A
Molthan (1990)				62								standardized visual searching, sweep netting	FM
Stachow (1988)		47										pitfall trapping	H
Feber et al. (1996)							20					butterfly transects	FM
Merckx et al. (2010)							23					light trapping	FM, H
Welling et al. (1994)	6	6										pitfall trapping	FM(H), A
Lewis (1969)		2				1	1					suction traps, vacuum sampler	H, A

About 650 species were observed in field margins and more than 1,250 species in woody habitats (results for the most common organism groups are shown in Table 3.2-5). 146 species were found in both habitat types. Due to the different aims and methods of the evaluated studies (Table 3.2-4), their results were not always directly comparable, especially regarding different habitat types (mostly only one habitat type was surveyed per study). Nevertheless, the results give an indication of the (possible) species richness of the two habitat types ‘field margin’ and ‘woody structure’ (Table 3.2-5).

Table 3.2-5: Number of observed species in field margins and woody structures based on ten studies (Tischler 1948; Lewis 1969; Zwölfer et al. 1984; Stachow 1988; Molthan 1990; Welling et al. 1994; Feber et al. 1996; Barthel 1997; Ross-Nickoll et al. 2004; Merckx et al. 2010). One species could be trapped in several studies. Results are shown for the most common organism groups.

	field margin	woody structure	both habitats
Araneae	211	81	34
Coleoptera	141	261	48
Collembola	46	-	-
Diptera	103	241	22
Hemiptera	-	175	-
Hymenoptera	106	249	13
Lepidoptera	43	216	25
Neuroptera	-	10	-
Orthoptera	15	8	4
Pulmonata	-	27	-
Total	665	1,268	146

In order to identify species that occur predominantly in field margins / conservation headlands or directly in the crop area, studies were selected that assessed the abundance of species in field margins / conservation headlands ***and*** in ‘normally’ treated agricultural sites. The following four studies (Rands & Sotherton 1986; Felkl 1988; de Snoo et al. 1998; Meek et al. 2002) and own unpublished data were analysed.

Meek et al. (2002) compared five types of field margins (cropped field margins, uncropped field margins sown with different seed mixtures, uncropped field margins vegetated by natural regeneration) in a replicated field experiment in terms of their invertebrate biodiversity. Study sites were located at four fields in New Yorkshire (UK). Overall, the occurrence and abundance of about 70 arthropod species (including Araneae, Coleoptera, and Lepidoptera) were assessed.

Felkl (1988) focused on the effects of conservation headlands (cropped cereal field margins, not sprayed with herbicides) on the abundance of predatory arthropods, especially Carabidae, Staphylinidae, and spiders. In this study, abundances of 32 arthropod species were compared between cropped field margins treated with or without herbicides. Arthropods were trapped at four study sites in the federal state of Hesse, Germany.

In the study of Rands & Sotherton (1986), occurrence of 17 butterfly species was compared between cropped field margins, which were either normally sprayed or left unsprayed with herbicides, fungicides, and insecticides (conservation headland). The butterflies were monitored using transect walks. The study took place in southern England.

A similar study was conducted by de Snoo et al. (1998) in the Netherlands. The study also focused on the occurrence and abundance of six butterfly species regarding sprayed and unsprayed cropped cereal and potatoe field margins.

Additionally, own data resulting from pitfall trapping and suction sampling of uncropped (permanent) field margins and adjoining maize fields located in Rhineland-Palatinate, Germany, were included. Overall, 25 species belonging to ground beetles, rove beetles and spiders were identified.

Since these studies were dealing predominantly with butterflies, beetles, and spiders, we focused our analysis on these three organism groups. Varying responses of species between the studies might be caused by different local conditions or temporal population changes which reflect the complexity of field data. But, overall, it could be shown that 20 of 21 butterfly species, 30 of 48 recorded beetle species, and all 15 spider species recorded clearly preferred the field margins/conservation headlands to the crop area or seem to use both the field margins/conservation headlands and the crop to a similar frequency. These species are summarized below (Table 3.2-6) and the complete species lists are given in Appendix 5.3.

Table 3.2-6: Summarized butterfly, beetle, and spider species that prefer field margins/conservation headlands to the crop area or showed no preference to one of both habitat types. Species that showed a preference for the crop area (at least in one study) were not shown here.

butterflies	beetles	spiders
Aglais urticae	*Agonum dorsale*	*Alopecosa pulverentata*
Anthocaris cardamines	*Agonum muelleri*	*Bathyphantes gracilis*
Aphantopus hyperantus	*Amara aenea*	*Diplostyla concolor*
Celastrina argiolus	*Amara familiaris*	*Enoplognatha ovata*
Coenonympha pamphilus	*Amara plebeja*	*Erigone atra*
Colias croceus	*Amara similata*	*Erigone dentipalpis*
Gonepteryx rhamni	*Bembidion aeneum*	*Lepthyphantes tenuis*
Inachis io	*Cantharis nigricans*	*Microlinyphia pusilla*
Lasiommata megera	*Clivinia fossor*	*Oedothorax apicatus*
Maniola jurtina	*Coccinella septempunctata*	*Oedothorax fuscus*
Melanargia galathea	*Drusilla canaliculata*	*Pachygnatha degeeri*
Ochlodes venata	*Harpalus affinis*	*Pardosa amentata*
Pararge aegeria	*Harpalus rufipes*	*Pardosa palustris*
Pieris brassicae	*Lathrobium fulvipenne*	*Pardosa pullata*
Pieris napi	*Notiophilus substriatus*	*Pisaura mirabilis*
Pieris rapae	*Oxytelus rugosus*	
Polyommatus icarus	*Philonthus fuscipennis*	
Pyronia tithonus	*Philonthus varius*	
Thymelicus lineola	*Platynus dorsalis*	
Thymelicus sylvestris	*Pterostichus niger*	
	Pterostichus strenuus	
	Rhagonycha fulva	
	Tachinus rufipes	
	Tachyporus hypnorum	
	Tachyporus obtusus	
	Tachyporus nitidulus	
	Tachyporus solutus	
	Trechus discus	
	Trechus quadristriatus	
	Xantholinus semirufus	

For example, *Maniola jurtina* (“Großes Ochsenauge”) is a butterfly species whose caterpillars feed on several grasses while diurnal adults need nectar for their nutrition. This species occurred more often in the conservation headlands of cereal field than in the normal sprayed crop (Rands & Sotherton 1986; de Snoo et al. 1998) and also statistically significantly favoured most uncropped margins over cropped margins (Meek et al. 2002). *Amara familiaris* (“Gelbbeiniger Kanalkäfer“) is a diurnal ground beetle species which feeds predominantly on plant material (Ribera et al. 1999). It could be trapped more often in conservation headlands (Felkl 1988) and uncropped margins (Meek et al. 2002), although no statistically significantly differences between cropped and uncropped margins were observed (Meek et al. 2002). In addition, it could be shown that field boundaries were the only habitat *Amara spp*. occurred (Thomas et al. 2001). The spider *Pachygnatha degeeri* is found throughout Europe and feeds predominantly on aphids (Harwood et al. 2005). It was trapped in high numbers exclusively in uncropped margins in comparison to cropped margins with statistically significantly differences both in spring and autumn (Meek et al. 2002).

In summary, field margins and hedgerows have been documented as (temporary) habitat for several hundred invertebrate species including phytophagous organisms like butterflies and moths and predators like spiders. Hence, the biocoenosis of these semi-natural habitats can (potentially) be rather diverse. However, preferences for habitat types can vary between species even within specific organism groups (for example beetles). Nonetheless, our results indicates that the majority of the analyzed species either prefers field margins/conservation headlands to the crop area or seems to use both the field margins/conservation headlands and the crop to a similar frequency.

As essential part of all terrestrial habitats, soil organisms are exposed to various natural soil conditions as well as to soils modified by human activity. The biocoenoses of these habitats are only partly known regarding some species groups (Table 3.2-7) but the impact on all relevant species groups remains to be investigated in most cases.

Table 3.2-7: Soil organisms in exposed habitats

Exposed habitat	Identified stressor	Species group	Impact	Source
Agricultural fields	Conventional methods	Lumbricidae	-	Irmler (2007)
	Organic farming	Lumbricidae Carabidae Staphylinidaeae	+ + +	Mäder et al. (2002)
Pastures	Ivermectin in dung	Collembola	-	Jensen et al. (2009)
Grassland	Amendment with sewage sludge	Oribatidae Enchytraiidae	+ +	Adesodun et al. (2005)
Smelter-impacted soils	Heavy Metals	Microbial community	-	Anderson et al. (2010)
Urban areas	Grassland soil in parks	Nematoda	+	Schlaghamerský & Pizl (2009)
Soils near roads	Heavy metals	Not yet studied		Malkoc et al. (2010)

The first indicator for detecting differences from an “undisturbed status” is the reduction of diversity but interpretation of diversity indices on the one hand and the definition of an ”undisturbed status” on the other hand are discussed strongly for a long time. Therefore, from a practical point of view, after the measurement of relevant soil parameters (e.g. soil particle distribution, pH, carbon content) and the identification of relevant soil organisms´ groups, structural (e.g. species lists) and functional (e.g. capability of degradation of organic material) can be used to determine the risk of soil organisms in exposed habitats. Unfortunately, the already known number of species in soil and the knowledge of their ecological sensitivity are still in a large discrepancy indicating to the important demand for further research.

3.2.5 Ecological sensitivity

Ecological characteristics or life history traits of species like their reproduction strategy (simplified as K-, r-strategist), the dispersal ability, and the degree of habitat specialization influence their extinction risks. We tried to characterize these traits exemplarily for the identified 15 spider species (see above). We could derive the relevant information for habitat type from the selected publications (Table 3.2-8), however, data on dispersal ability and reproduction potential was not available there.

Table 3.2-8: Spiders and their preferred habitats (based on abundances/ frequencies). Species were listed several times if the results vary in different studies.
Uncropped field margin/ conservation headland preferring species: statistically significant response or (if significance is not determined) species were at least twice more abundant in the margins/ headlands than in (normal) cropped areas. Field preferring species: statistically significant response or (if significance is not determined) species were at least twice more abundant in (normal) cropped areas than in the uncropped field margins/ conservation headlands. No preference: no statistically significant differences or abundances between the habitats differ less than the factor two. Used Literature: Felkl (1988), Meek et al. (2002), own unpublished data.

species	preferred habitat		
	field margin / conservation headland	field	no preference (moving species)
Alopecosa pulverentata			x
Bathyphantes gracilis			x
Diplostyla concolor	x		x
Enoplognatha ovata			x
Erigone atra	x		x
Erigone dentipalpis	x		x
Lepthyphantes tenuis			x
Microlinyphia pusilla	x		x
Oedothorax apicatus			x
Oedothorax fuscus			x
Pachygnatha degeeri	x		
Pardosa amentata	x		x
Pardosa palustris			x
Pardosa pullata	x		x
Pisaura mirabilis	x		

Trait data for spiders is not easily available and is published often in taxonomic publications in specialist journals. We discussed the assignment of life history traits with an expert for spiders in agricultural landscapes (Prof. Dr. Martin Entling, University Koblenz-Landau) and were informed that this information is not available in a summarized form. One group of researchers is currently reviewing the dispersal ability of these agrobiont spiders. Regarding the reproductive output it is not possible, even for an expert, to characterize a species as a K- or r- strategist. The same spider species may have between 2 to 5 life cycles in Germany depending on location and weather conditions during the year. Additionally the number of eggs laid is also not known for many of these spiders.

Since similar problems arose when getting into detail within the other arthropod groups, we concluded that it is an unfeasible task within this project to categorize species according to selected traits. To address life history traits for arthropods in the agricultural landscape and use them in a risk assessment approach, it would be essential to involve a range of experts of the relevant groups to establish a robust database.

As an alternative approach, we focus on threatened species. The population trends of a wide range of arthropod species are the basis for national red lists which evaluate the extinction risk of each species (Binot et al. 1998). Threatened species are characterized by a (strong) population decline. For example, an analysis of ecological characteristics of 23 threatened and 72 non-threatened butterfly species in Finland revealed that threatened species exhibit a narrow niche breadth, restricted resource distribution, poor dispersal ability, and short flight period (Kotiaho et al. 2005), life history traits that indicate ecological sensitivity.

For this reason, we propose to concentrate on red lists to identify ecologically sensitive species. We used our species database (see chapter 3.2.4) to compare the arthropod species recorded in field margins and woody structures (Table 3.2-5) to those listed in the national red list (Binot et al. 1998). We focused on Araneae and Lepidoptera since the former showed high species richness associated with field margins and the latter with woody structures.

The national red list of Araneae comprises entries for 385 spider species (including near threatened species, excluding species with geographical restriction). In the analysed literature (see above), information is available for 258 species existing in field margins and woody structures and 31 (12%) thereof are mentioned as (nearly) threatened (see Appendix 5.4).

The national red list for Lepidoptera refers only to Macrolepidoptera (information for Microlepidoptera are not available until now) and comprises entries for 635 butterfly and moth species when including near threatened species but excluding species with geographical restriction. In our database, 132 Macrolepidoptera species are recorded in field margins and woody structures and 10 species (8%) thereof are mentioned as (nearly) threatened (see Appendix 5.4).

In both cases, about 10% of the species surveyed in field margins and woody structures belong to (nearly) threatened species. Therefore, referring to both organism groups would include a range of ecologically sensitive species. To what extent other organism groups are suitable to represent ecologically sensitive species needs further evaluation.

In short:

- In agricultural landscapes various terrestrial habitat types can be found: woody structures (e.g. hedgerows), grassy and herbaceous uncropped structures (e.g. field margins, "Blühstreifen"/ flower strips), and cropped structures (e.g. "Ackerrandstreifen"/ Conservation headlands). The majority of field margins can be found in the smaller size classes (< 3 m width) regarding their length as revealed by a GIS analysis for RLP.
- In dependence on the cultivated crop, off-field habitats can receive high inputs of plant protection products due to overspray and spray drift.
- Scenarios in which biocides were (indirectly) applied to agricultural sites include the application of manure contaminated with disinfectants and insecticides as well as the usage of e.g. wooden piles or fences treated with wood preservatives. However, there are no models available which estimates the fraction of biocides reaching terrestrial off-field habitats separately.
- Field margins and hedgerows can constitute a habitat for a wide range of species (nearly 2000 reported) including e.g. Coleoptera, Diptera, Lepidoptera, and Araneae.
- According to Lepidoptera, Coleoptera, and Araneae most analysed species either preferred the field margins/ conservation headlands or seemed to use field margins/ headlands and crop to a similar extent.
- Since life history trait data is not available for most arthropod species, we propose instead to focus on threatened species recorded on red lists.

3.3 Pesticide risk assessment practice and proposals for further developments

3.3.1 Non-target arthropods (NTA)

3.3.1.1 Current situation for plant protection products

Risk assessment of plant protection products is described in the Regulation No 1107/2009 of the European parliament and the council[11] (enforced June 2011, formerly Council Directive 91/414/EEC). For arthropods (excluding bees) the "Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC" (SANCO 2002) refers to the results of the ESCORT 2 Workshop (Candolfi et al. 2000a). The recommended schemes for plant protection products comprise a tiered approach which, additionally, distinguishes between an in-field assessment and an off-field assessment:

At tier 1, LR_{50} [12] values are determined in laboratory studies under worst case scenarios for two indicator species: the parasitoid wasp *Aphidius rhopalosiphi* and the predatory mite

[11] Regulation (EC) No 1107/2009 of the European parliament and the council of 21 October 2009 concerning the placing of plant protection products on the market
Verordnung (EG) Nr. 1107/2009 des europäischen Parlaments und des Rates vom 21. Oktober 2007 über das Inverkehrbringen von Pflanzenschutzmitteln
[12] Lethal rate 50: application rate causing 50% mortality of the test organisms

Typhlodromus pyri. Subsequently, a hazard quotient (HQ) is calculated by dividing the crop-specific application rates (in-field assessment) or drift rates (off-field assessment) by the LR_{50} (Candolfi et al. 2000a). If the HQ-value is higher or equal than 2, a potential hazard to non-target arthropods can be assumed and higher-tier testing is necessary.

In the higher-tier testing, laboratory studies on one further species (HQ-value exceeded or equalled in the in-field assessment) or two further species (HQ-value exceeded or equalled in the off-field assessment) should be tested on a more natural substrate. Preferred species are the predatory bug *Orius laevigatus*, the lacewing *Chrysoperla carnea*, or the ladybird beetle *Coccinella septempunctata*. Furthermore, extended laboratory studies, aged residues studies, semi-field or field studies may be included in the higher tier testing. In Appendix 5.5 the proposed test systems for tier 1 and higher tier tests are shortly described (based on Candolfi et al. 2000b).

Both tier 1 test species and all higher tier test species can be classified as predators or parasitoids (mostly on aphids) and, therefore, this group of arthropods seems to be appropriately considered. As proposed before (chapter 3.1), beetles and spiders seem to be adequate predatory organism to assess the effects of plant protection products on biodiversity. Higher tier test systems are available for beetles (the staphylinid beetle *Aleochara bilineata*, the ladybird beetle *Coccinella septempunctata*, and the carabid beetle *Poecilus cupreus*) and spiders (wolf spiders *Pardosa spec.*). Candolfi et al. (2000a) compare the sensitivity of these organisms with the recommended tier 1 test species *A. rhopalosiphi* and *T. pyri* and state that both are good indicators for these other species. However, a re-evaluation of the data of Candolfi et al. (1999) and Vogt (2000) by DEFRA (2007) concluded "that based on these datasets it is potentially misleading to refer to *T. pyri* and Aphidius spp. as "sensitive indicator species".

3.3.1.2 Current situation for biocides

The risk assessment of biocides is based on Directive 98/8/EC[13] (until September 2013) and Regulation (EU) No 528/2012[14] (valid from September 2013), respectively. Detailed information on data requirements and waiving arguments for biocidal active substances and products is provided in the "Technical Guidance Document on data requirements for active substances and biocidal products" (EC 2008a) as well as the "Technical Notes for Guidance (TNsG) on product evaluation" (EC 2008b). The environmental risk assessment for biocides is described in "Technical Guidance Document on Risk Assessment" (TGD) (EC 2003).

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[13] Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market
Richtlinie 98/8/EG des Europäischen Parlaments und des Rates vom 16. Februar 1998 über das Inverkehrbringen von Biozid-Produkten

[14] Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and the use of biocidal products.
Verordnung (EU) Nr. 528/2012 des Europäischen Parlaments und des Rates vom 22. Mai 2012 über die Bereitstellung auf dem Markt und die Verwendung von Biozidprodukt

For the risk assessment of biocides core data are needed for active substances and biocidal products, irrespective of the product type. The core data for active substances include ecotoxicological studies in which the ability of the active substance or its degradation product(s) to negatively affect the function and structure of biotic systems is to be clarified (see EC 2008a). The tests should address the short-term effects in three ecologically functional groups, the producers, the consumers, and the decomposers, in relevant media (water, soil, and air).

Next to the core data, specific additional data requirements are defined for each of the 23 product types (e.g. long-term aquatic tests, tests with soil organisms). Product type specific additional data for the soil compartment are needed if high releases to the terrestrial compartment are possible. These tests should be based on three initial tests in the first step, an acute test with plants (producers), an acute test with earthworms or other soil non-target macro-organisms (consumers), and a test on the inhibition of microbial activity (decomposers).

As described above, we focus on three product types which can (potentially) enter the soil of agricultural sites: (a) disinfectants (product type 3, see Introduction) and insecticides (product type 18) used in animal housing which are applied with manure on fields and (b) wood preservatives (product type 8) used for the treatment of wooden piles or fences present on agricultural sites. For the product type 8 (wood preservatives) tests with terrestrial organisms are required, if high releases to the terrestrial compartment are possible. No tests with non-target arthropods are requested, unless a risk for NTAs is assumed because of the mode of the action of the active substance. The same applies for the product types 3 (disinfectants) and 18 (insecticides). For products used outside buildings as well as for products to be used by gassing, fogging, or fumigation a release to soil is possible and it is necessary to perform initial terrestrial tests. Tests with bees and other beneficial arthropods are necessary if it can be assumed by the mode of action of the active substance that these organisms are likely to be at risk and in cases of large-scale outdoor applications like fogging. In the TNsG it is stated that, "*at least one test on bees and one on another beneficial arthropod may be generally required for insecticides, acaricides and substances in products to control other arthropods which are used outdoors (product type 18)*" (EC 2008a). Such tests are usually not needed for other biocidal product types. Species proposed to be tested in addition to honeybees are the lacewing *Chrysoperla carnea*, the predatory wasp *Trichogramma cacoeciae*, the ladybird beetle *Coccinella septempunctata* or the staphylinid beetle *Aleochara bilineata*.

In general, the environmental risk assessment of biocides is based on the PEC/PNEC-approach. ***PEC*** describes the ***P***redicted ***E***nvironmental ***C***oncentration, which is calculated in an exposure assessment considering the fate and behaviour of the biocide in the environment. Emission scenarios have been developed for different product types to value the emission of biocides in different environmental compartments. The calculated PEC-value is compared to the ***P***redicted ***N***o ***E***ffect ***C***oncentration or ***PNEC*** which is calculated by dividing the lowest available valid effect value (EC_x [15] or NOEC [16]) by an assessment factor (AF) according to the

[15] Effective concentration X: concentration of a substance causing x% response of the test organisms
[16] No Observed Effect Concentration

TGD. The selection of the AF is based on the amount and type of available data. Together, the information on exposure assessment and the effect assessment can be used to conclude on the likelihood of adverse effects in the exposed compartment. The risk for the environment has been defined to be acceptable if the PEC/PNEC ratio is below 1.

3.3.1.3 Consideration of phytophagous insects

Currently no phytophagous insect is included in the established test systems. Phytophagous insects represent the first level in the trophic food web in agricultural landscapes since they transform plant biomass and represent food for many arthropod predators, including the species mentioned in the previous chapter, as well as vertebrates such as amphibians and reptiles, birds and mammals. Phytophagous insects can be affected by pesticides in three different ways: (1) via direct contact due to direct overspray or spray drift, (2) by consumption of contaminated plant material or ingestion of plant sap (sucking insects such as true bugs and cicadas), and (3) possibly also by reduction of host plant quality through previous contact with e.g. herbicides and induced plant defence (see Research Box 2).

RESEARCH-BOX 2:

Herbicide-effects on host plant quality

BACKGROUND: Herbicides are widely used plant protection products which can affect herbivorous organisms for example via direct toxicity or indirect via a decrease in host plant availability. Furthermore, herbicides might influence host plant quality for arthropods, but currently there is less information available on this topic (see e.g. Kjaer & Elmegaard 1996). Hence, this research project focused on herbicide-effects on host plant quality.

METHODS: Young cabbage moth (*Mamestra brassicae*) caterpillars (5 days old) were reared on different host plants (*Plantago lanceolata*, *P. major*, *Ranunculus acris*) treated, beforehand, with sublethal (and field margin relevant) rates of two herbicides (Atlantis® WG: a.i. Iodosulfuron + Mesosulfuron, Roundup® LB Plus: a.i. Glyphosat). The weight of the caterpillars and their development time to adults were assessed for each plant-herbicide combination. Additionally, herbicides were tested for direct toxicity effects towards *M. brassicae* caterpillars.

RESULTS: Caterpillars feeding on *R. acris* treated with the herbicide Atlantis® WG showed statistically significantly lower weights in comparison to caterpillars feeding on untreated control plants ($p<0.001$, Wilcoxon-Test, Figure 3.3-1). Since Atlantis® WG showed no direct toxicity towards the caterpillars the results indicate a reduced host plant quality of *R. acris* possibly caused by defence components produced in the plants following the herbicide application.

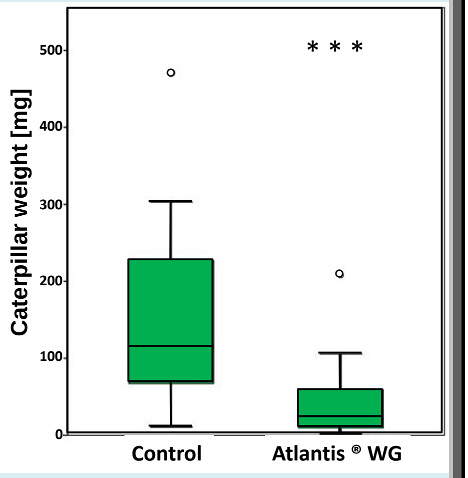

Figure 3.3-1: Comparison of the weights of *Mamestra brassicae* caterpillars (17 days old) reared on untreated control plants and plants treated with the herbicide Atlantis® WG (10% in-field application rate). ***: $p<0.001$ (Wilcoxon-Test).

SOURCE: Geisthardt, M., Hahn, M. & Brühl C. A. (2011): Effekte von Herbiziden auf die Futterpflanzen-Qualität phytophager Insekten. Poster presentation at the SETAC GLB 16th Annual Meeting 2011, Landau, Germany.

It seems questionable in the risk assessment of plant protection products if testing only the species *A. rhopalosiphi* and *T. pyri* is sufficient to cover the risk for species with completely different feeding strategies and exposure pathways. A laboratory study with *Chorthippus sp.* grasshopper nymphs using five insecticides (see Research Box 3 below) revealed a similar sensitivity than the standard test species (Bundschuh et al. 2012). However, in a monitoring study grasshopper densities were lower in narrow field margins next to cereals and vineyards compared to meadows but increased to similar levels in margins exceeding 9 m width (see Research Box 7, p. 66). Low grasshopper densities with no increase in individual number with increasing field margin width were recorded over all field margins (0.5 – 20 m) bordering apple orchards, that are characterized by a comparatively high input of plant protection products and three dimensional application techniques causing high drift rates (Bundschuh et al. 2012).

RESEARCH-BOX 3:

Toxicity endpoints for grasshoppers

BACKGROUND: Herbivorous species are not included as test species in the current risk assessment for non-target arthropods exposed to plant protection products although they can potentially be affected by direct contact (e.g. spray drift or moving through contaminated plants) or consumption of contaminated food plants. Therefore, the aim of this research project was to estimate the sensitivity of grasshoppers to plant protection products compared to the standard test species.

METHODS: Sensitivity of *Chorthippus* nymphs to five insecticides was tested using a simple test design: the surface of a test container was sprayed with different rates of various insecticides of different classes and mortality was evaluated 48 h after exposure to obtain comparable data to the tier 1 standard tests.

RESULTS: For the five insecticides tested, *Chorthippus* sp. showed a similar sensitivity as the standard toxicity test species *Aphidius rhopalosiphi* and *Typhlodromus pyri* (Table 3.3-1). The only difference seems to concern pirimicarb where no data were available for tier 1 tests.

Table 3.3-1: Comparison of LR_{50} concerning the tested substances. a.i.: active ingredient. NC: no calculation possible. LR_{50} values of *A. rhopalosiphi* and *T. pyri* referred to literature. (after Bundschuh et al. 2012, table modified)

a.i. (chemical class)	exposure scenario	*Chorthippus sp.* LR_{50} 48h [g a.i./ha]	*A. rhopalosiphi* LR_{50} 48h	*T. pyri* LR_{50} 48h
Dimethoate (Phosphodithioc acid)	contact	11.41	0.01	2.24
Pirimicarb (Carbamate)	contact	7.87	620 †	835 ‡
Imidacloprid (Neonicotinoid)	contact	2.09	0.02	4.23
Lamda-Cyhalothrin (Pyrethroid)	contact	0.35	0.50	0.2
Deltamethrin (Pyrethroid)	contact	0.10	0.55	0.01

† higher tier 48h barley seedlings test, ‡ higher tier 7 d bean leaf discs test

SOURCE: Bundschuh, R., Schmitz, J., Bundschuh, M. & Brühl C. A. (2012): Does drift of insecticide adversely affect grasshoppers (Orthoptera: Saltatoria) in field margins? A case study combining laboratory acute toxicity with field monitoring data. *Environmental Toxicology and Chemistry* **31**(8): 1874-1879.

RESEARCH-BOX 4:

***Drosophila* ssp. as test species**

BACKGROUND: Current ecotoxicological studies for plant protection products in tier 1 focused on only two test species (*Aphidius rhopalosiphi* and *Typhlodromus pyri*). In this research project, the suitability of two *Drosophila* species as test organisms were assessed and their sensitivity to different plant protection products[17] were compared to *A. rhopalosiphi*.

METHODS: Acute LR_{50} values for *Drosophila funebris* and *D. melanogaster* were established using 12 active substances contained in plant protection products (fungicides, herbicides and insecticides). Each test was carried out with five concentrations and four replicates with ten flies each (five males, five females) using the test design available for *A. rhopalosiphi* (Mead-Briggs et al. 2000).

RESULTS: The obtained LR_{50} values for *Drosophila spp.* were compared to literature data as well as *A. rhopalosiphi* tests that were carried out in parallel (Table 3.3-2).

Table 3.3-2: Comparison of LR_{50} concerning all tested substances. a.i.: active ingredient. (after Kimmel & Brühl 2012, table modified)

a.i.	**LR_{50}*** ***D. funebris*** **[g a.i./ha]**	**LR_{50}*** ***D. melanogaster*** **[g a.i./ha]**	**LR_{50}*** ***A. rhopalosiphi*** **[g a.i./ha]**	**LR_{50}*** ***T. pyri*** **[g a.i./ha]**
Dimethoate	0.04	0.01	0.05	2.24
Pirimicarb	1.95	1.05	3.68	---
Deltamethrin	0.83	0.54	0.55	0.0081
Lamda-Cyhalothrin	0.18	0.15	0.5	0.2
Imidacloprid	2.86	2.19	0.02	4.23
Trifloxystrobin	1176.4	336.42	<30% at 500	< 30% at 500
Mancozeb	32.5% at 10000	55% at 10000	>75% at 3600	0.4% at 2600
Kresoxim - Methyl	20% at 4000	3810	1071	45% at 900
2,4 - D	10% at 3000	32.5% at 3000	5.3% at 3000	7.5% at 3000
Pendimethalin	2292.62	1083.96	38% at 3200	18% at 2400 100% at 3200
Isoproturon	12.5% at 5000	27.5 % at 5000	16% at 2500 3% at 120	7% at 2500 21% at 1800
Glyphosat	27.5% at 5000	35% at 5000	100% at 3600	100% at 5760 25% at 3720

*= average values of multiple test results and/or available literature data; if no LR_{50} calculation was possible, the mortality at the highest testing rate is shown

Referring to the presented results, both tested *Drosophila spp.* are mostly equally sensitive compared to *A. rhopalosiphi* and *T. pyri* for most of the tested substances. As an example, the average Dimethoate LD_{50} of *D. melanogaster* was located at 0.01 g ai/ha, whereas the *A. rhopalosiphi* LD_{50} concerning Dimethoate was found to be at 0.038 g ai/ha, which points at a lower sensitivity.

SOURCE: Kimmel, S. & Brühl, C. A. (2012): Risk Assessment in Terrestrial Ecotoxicology: the Sensitivity of *Drosophila spp.* Towards Pesticides. Poster presentation. In: Alix, A., Bakker, F., Barrett, K., Brühl, C. A., Coulson, M., Hoy, S., Jansen, J. P., Jepson, P., Lewis, G., Neumann, P., Süßenbach, D., van Vliet, P. (Eds.): *ESCORT 3 - Linking Non-Target Arthropod Testing and Risk Assessment with Protection Goals.* 1- 151. CRC SETAC Press.

[17] Some active ingredients are also used in biocidal products: Deltamethrin (product type 18), Lamda-Cyhalodrin (product type 18), Imidacloprid (product type 18), Isoproturon (product type 7 + 10).

The evaluation of the toxicity of plant protection products within the environmental risk assessment (ERA) towards arthropods currently focuses on a selection of a few beneficial species. Strangely enough this historic approach is continued although there are other insect species that are amongst the best studied organisms in the world. The fruit flies in the genus *Drosophila* are probably the most studied insects in modern biology and for many species standard laboratory cultures and a wealth of information on its biology, developmental organisation even including the resolved genome structure, are available. This organism would therefore be highly suitable to evaluate effects of pesticides also on specific endpoints concerning endocrine disruption. Compared to the tested predatory species and parasitoids, *Drosophila* spp. shows a completely different lifestyle, feeding on rotten fruit and microorganisms without any tendency of parasitism. A first screen of the sensitivity of two *Drosophila* species to plant protection products revealed similar or higher sensitivity than the current test species (see Research Box 4).

3.3.1.4 Consideration of life history traits: Reproduction

The currently selected test organisms for the determination of the ecotoxicity of plant protection products (and the non-target arthropod species that are proposed to be tested in the risk assessment of biocides of the product types 3 and 18, see chapter 3.3.1.2) do show high reproductive rates as life history traits. These so called ‘benefical insects’ are used in integrated pest management schemes since they are capable of rapid population build-up that follow the pest population and control it. This life history strategy is not found in all arthropod species since many of them are producing a low number of eggs and are also comparatively long lived with only one generation present per year. The carabid beetle *Carabus granulatus* for example produces 40 eggs per year, and lives more than 2 years (Turin et al. 2003), whereas the green lacewing *Chrysoperla carnea*, a current test species, has a life cycle of 4 weeks in summer and lays between 400-700 eggs. The differences in life history traits such as reproductive capacity and number of generations per year between the current test species and the diverse arthropod species occurring in the agricultural landscape should be kept in mind when deducing from the reproduction rates determined in a standard laboratory test. If in a higher tier test for plant protection products a so called ‘potential for recovery’ is recorded, this does not necessarily implicate that the results hold true for the entire arthropod community as suggested, for example, for aged residue studies in the outcome of the ESCORT 3-Meeting (Alix et al. 2012). This is certainly not the case for the majority of arthropods taking the difference in life history traits into account. We support therefore the suggestion to carry out further sensitivity tests on guilds representative of field margin habitats and chick food groups, including a lepidopteran larva, a sap-feeding bug, a tipulid, and an orthopteran (Boatman et al. 2004).

3.3.1.5 Vegetation distribution factor in the risk assessment of plant protection products

In the current exposure scenario for the assessment of the impact of plant protection products, a vegetation distribution factor (‘vdf’) is applied to correct the estimated exposure in ‘2D’ glass plate tier 1 tests for dispersion of spraydrift droplets caused by the vegetation structure in the off-field. The mean vegetation distribution factor under field conditions has been recently re-evaluated by the German Federal Environment Agency (Umweltbundesamt, UBA

2006b). The evaluation indicated that a factor of 5 is more appropriate than a factor of 10 as used in the current European scheme. This led to the convention of using a vdf of 5 in the national authorization procedures in Germany and this decision was confirmed by a detailed analysis of 2d and 3d test data for the two standard tier 1 test species (Swarowsky et al. 2012, Research Box 5).

RESEARCH-BOX 5:

Comparison of different test systems in the testing of plant protection products

BACKGROUND: In the current risk assessment scheme of plant protection products, data for non-target arthropods were determined in a tiered approach using test systems with different exposure designs, e.g. '2D laboratory tests' on glass plates ('2D glass'), 'extended laboratory tests' on excised leaves ('2D ext') or whole plants ('3D plant') as substrate. To transfer these results to off-field-areas, a 'vegetation distribution factor' ('vdf') of 10 (EU concept) or 5 (German national concept) is applied to correct the estimated exposure for dispersion of spray drift droplets caused by the vegetation structure. Here we analyse whether extended laboratory tests for *Aphidius rhopalosiphi* and *Thyphlodromus pyri* could be substituted by a numerical safety factor applied on standard laboratory tests on inert substrate

METHODS: Acute toxicity endpoints (LR_X) of plant protection products in the mentioned standard tests (2D versus 3D) and to both standard test species (A. *rhopalosiphi* versus *T. pyri)* were compared. Valid test data were gathered from an internal database of the German Federal Environment Agency (Umweltbundesamt).

RESULTS: In the comparison of tests with inert and natural substrate, 90% of the ratios between LR values from '2d ext'-tests and '2d glass'-tests were in a range of 1.76 to 13.6 (*A. rhopalosiphi*) and 1.31 to 30.93 (*T. pyri*) (Figure 3.3-2). Hence, results of '2D'-tests on natural substrate ('2d ext') cannot be extrapolated with an additional assessment factor from the results of tier 1 tests on inert substrate ('2d glass'). The median ratio between LR-values from '2D'- and '3D'-tests was 12.9, i.e. slightly higher than the established EU vdf of 10. In this context, it should be noted that current data (UBA 2006b) indicate that the mean distribution factor under field conditions is 5 rather than 10. Hence, '3D'-tests probably overestimate the reduction in exposure caused by dispersion of spray drift droplets in the vegetation under field conditions. As this can lead to underprotective risk management decisions, it is strongly recommended to reassess the 'vdf' on the basis of all available data and accordingly revising the risk assessment concept based on '3D'- and '2D'-tests.

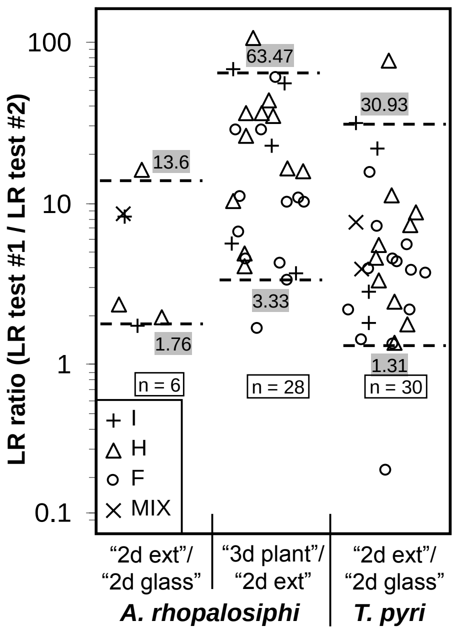

Figure 3.3-2: Scatter plats of the ration between the toxicity in different test systems. 'F', 'H', 'I', and 'MIX' represent fungicide-, herbicide-, insecticide-, and mixture-products. Dashed lines indicate the 5th and 95th percentiles of the distributions. The x-axis has no scale, data points fluctuate in the horizontal (Swarowsky et al. 2012, figure modified)

SOURCE: Swarowsky, K., Brühl, C. A., Süßenbach, D. & Wogram, J. (2012) Comparison of standard laboratory tests and extended laboratory tests for the non-target arthropod species *Aphidius rhopalosiphi* and *Typhlodromus pyri*. In: Alix, A., Bakker, F., Barrett, K., Brühl, C. A., Coulson, M., Hoy, S., Jansen, J. P., Jepson, P., Lewis, G., Neumann, P., Süßenbach, D., van Vliet, P. (Eds.): *ESCORT 3 - Linking Non-Target Arthropod Testing and Risk Assessment with Protection Goals.* 1- 151. CRC SETAC Press.

3.3.1.6 Recovery in the risk assessment of plant protection products

The concept of recovery is used to address short time effects of plant protection products (mostly insecticides) in higher tier studies. Here a ‘potential for recovery’ can be demonstrated if no adverse effects in reproduction of a selected test species or similar arthropod densities in control and treatment plots are recorded in large field studies. The term recovery in this respect is mostly used as ‘reaching similar abundances as before / or in a control’.

In a more ecological perspective, ***recovery*** of a population in a certain location should occur on site due to the reproductive capacity of the decreased population. This means that an affected population, e.g. in a field that was treated with an insecticide, recovers by reproduction of the remaining individuals. Depending on the life history pattern of the species, this is rarely the case in the short time course of a field trial if reproduction is low or if there is only one generation per season. In contrast, if a population decreased - or even became locally extinct - there might be ***recolonisation*** of the habitat by individuals from outside. Depending on the surrounding of the study field site and the mobility and density of the species in the surrounding landscape, this process can be very rapid or rather slow.

In most cases when ‘recovery’ is reported in non-target arthropod field studies, this effect is due to recolonisation of the majority of species and recovery of only a few. The species that are likely to recover are the pest species (e.g. aphids) and very often their predators and parasitoids (the ‘benefical’ insects), since they track their pests and have great reproductive capacity (e.g. the lacewing *Chrysoperly carnea*, see above). Therefore, it is not correct to conclude from non-existing reproductive effects in one of the current test species that recovery will occur in the majority of arthropods. The results from field studies should therefore be considered carefully. As previously mentioned, the observed population increase after a recorded effect of applied chemicals is mostly due to recolonisation. This can be from the surrounding and therefore is faster if there are many natural structures in the area (like e.g. large field margins, meadows, hedges). Immigration to the treatment plots can also occur from the control plots on the treated fields or orchards. If the treatment site had a high diversity of arthropods, we expect to see a large effect after the application of the tested product and then a relatively quick recolonisation because of immigration from neighbouring control sites.

Also the comparability of a higher tier field study with the situation after product registration changes dramatically: Whereas at the time of testing all surrounding area was untreated with the product, after registration of the product a large proportion of the landscape might also be treated – even simultaneously due to local pest control recommendations – and, therefore, the recolonisation potential on a landscape scale decreases.

Since the observed population increase after an effect of a plant protection product is mainly due to recolonisation and directly correlated to the mobility of a species and the surrounding of a treated area, the treated field or orchard becomes a major sink for arthropod populations that occur in the agricultural landscape and are moving from field margins and hedges into cropped areas that are treated with plant protection products. Results of a mark and release study for seven ground beetle species revealed that four species moved at least 150-200 m in the cropped area (*Carabus granulates, Platynus dorsalis, Pterostichus melanarius, Poecilus cupreus*), one species at least 100 m in the cropped area (*Carabus auratus*), and two species at least 50 m in the cropped area (*Harpalus aeneus, Loricera pilicornis*) (Welling et al. 1994). A range of further species using field margins as well as crops as habitats is listed in Appendix 5.3 (see also chapter 3.2.4).

This critical view of the 'recovery' concept so far used in non-target arthropod risk assessment of plant protection products was shared at the ESCORT 3 meeting. As stated there, "*for mobile taxa, observed return to the control levels or its absence is not considered to be a robust predictive indicator for the likelihood of recovery under larger scale use of pesticides: it does not consider e.g. applications of different products or different ecological conditions such as the size and distribution of refugia/reservoirs or life cycle parameters of species. […] Field studies can be used to answer specific questions e.g. magnitude of effects*" (Brühl et al. 2012).

3.3.1.7 Arthropods in the food web

The magnitude of the effect of a pesticide evaluates how many arthropod species were affected (e.g. a few or the majority, a specific guild: sap sucking insects,...), to what degree (proportion of the population) and for how long the decrease could be observed after the application since many products show their effect after days or even weeks. Assessing this magnitude of effect might be useful for the risk assessment of plant protection products, since arthropods represent important food resources for other organisms in the agricultural landscape and therefore their presence is a key for their survival and reproduction.

The decline in arthropods in agricultural landscapes is revealed for many different groups including decomposers, predators (Attwood et al. 2008), and pollinators (Biesmeijer et al. 2006; Kluser & Peduzzi 2007). Unfortunately there are few long-term quantitative data available, but a correlative link was shown between higher agricultural intensity and lower arthropod abundance over a 30-year period (Benton et al. 2002). A study of common, larger moths in Britain revealed that two thirds of the considered species declined in their population size in the past 30 years (Conrad et al. 2006) and one of the main causes is seen in the agricultural intensification (Fox et al. 2006).

An analysis of 16 years of butterfly transect count data from The Netherlands revealed that 55% (11 of 20 species) of these species suffered severe declines in distribution and abundance with farmland species showing the largest declines (Van Dyck et al. 2009). In an analysis of carabid populations in Europe, the highest declines were recorded for large beetles associated with open grassland habitats including many agricultural habitats (Kotze & O'Hara 2003).

Most bat species in Europe have suffered severe population declines during the 20th century (e.g. Stebbings 1988) with one of the main drivers believed to be the loss of roosting and foraging habitats through agricultural intensification (Walsh & Harris 1996). However, data about the occurrence of bats in European agricultural areas are relatively few compared to other mammals. Recent radio-tracking studies and acoustic surveys performed with bat-detectors revealed high foraging activity of bats in different agricultural crops (Arlettaz 1999;

Drescher 2004; Stahlschmidt et al. in prep.). Current evidence from radio-tracking by the Central Science Laboratory (UK) suggests that both the crop and field margins may be important for bats in agricultural areas (Boatman et al. 2004). In an acoustic survey in southern Germany, bat activity was measured on various sites of different crops (Stahlschmidt et al. in prep.). It was found that high foraging activity can occur on agricultural sites, dependent on crop type, food availability, and existence of nearby roost sites. Thirteen bat species have been recorded in agricultural crops representing 60% of the species recorded for Germany.

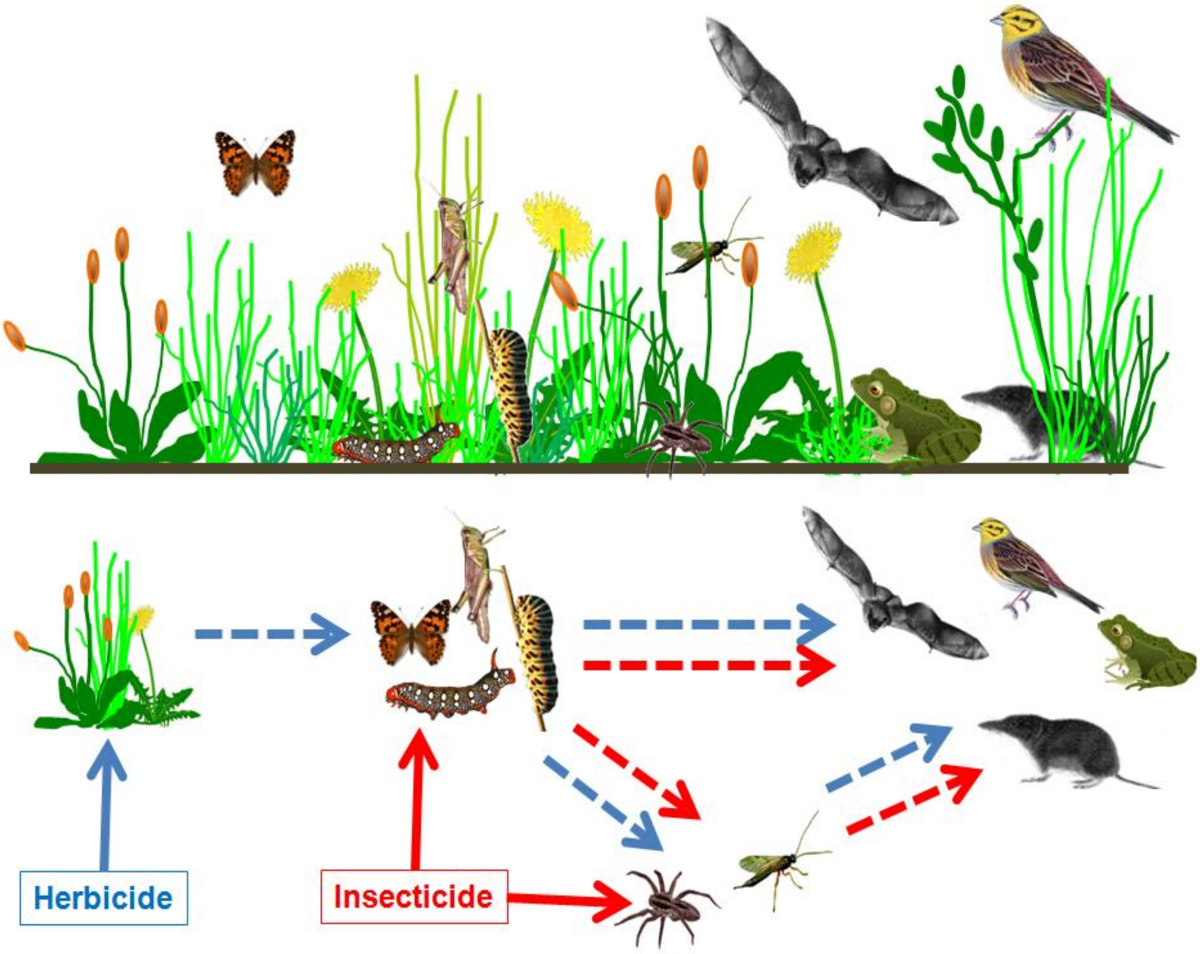

Figure 3.3-3: Food web in a field margins. Direct toxicity effects (continuous arrows) and indirect effects caused by reduced food availability (dashed arrows) are shown for herbicides (blue) and insecticides (red).

In intact food web structures, biomass of arthropods as a food source for invertebrates and vertebrates at a higher tropic level is critical, especially in the reproductive time of many vertebrates. Grasshoppers (Acrididae), sawflies (Symphyta), spiders (Araneae) and leaf-beetles (Chrysomelidae) are associated with the diet of declining bird species during the breeding season (Wilson et al. 1999). All mentioned arthropod groups are sensitive to insecticide applications but might also be affected by a reduction of food plant density caused by herbicides (Figure 3.3-3). It should be noted that especially the predatory and parasitoid arthropod species can be affected indirectly by a depletion of their arthropod prey. They, again, are important food sources for vertebrates. This effect was measured in the field when an application of *Bacillus thuringiensis israelensis* targeted at mosquitoes also reduced the abundance of dragon flies and other large arthropod predators (Poulin et al. 2010).

A few studies demonstrated the effects of plant protection product induced decreased invertebrate food availability on the population declines for birds. In a carefully designed field study it could be shown for the grey partridge *(Perdix perdix)*, a bird species that shows dramatic declines all over Europe, that brood size and the abundance of insects as food for chicks was significantly higher where cereal field headlands were left unsprayed with herbicides and fungicides than on completely sprayed fields (Rands 1985). Brigitte Poulin and coworkers (Poulin et al. 2010) assessed the effect of *Bacillus thuringiensis israelensis* (*Bti*) spraying for mosquito control on foraging rates and chick diet of house martins *(Delichon urbicum)* prior to and during three years in the Camargue, France. At treated sites, arthropod communities revealed lower densities and smaller sizes than in unsprayed sites. Moreover, lower foraging rates of house martins were recorded together with significantly lower clutch size and fledgling survival at treated sites compared to controls. The compelling evidence provided for *Bti* affecting vertebrate populations following the suppression of prey species should support more attention to indirect effects of pesticides through food web interactions. However studies on a larger scale in an intensified agricultural landscape are difficult to perform, since large, untreated control areas are not so often present and, therefore, arthropod densities are already low due to previous pesticide treatments.

Decreases of arthropod densities in spring time when reproductive activity of birds and also of many – if not most – other vertebrates in the agricultural landscape is high, are detrimental for the survival of the newly born / hatched offspring since these depend in many cases on arthropods as a first food source. This is also the case for granivorous birds such as grey partridges, where the adult feeds on seeds but the chicks depend on insect protein for the first few days. Although long-time studies are scarce, Benton and his group (Benton et al. 2002) could show in a correlative approach a linked temporal change between farmland birds, invertebrate numbers and agricultural practice in Scotland. The results are consistent with the view that agricultural change has influenced birds through changes in food quality or quantity.

So far, the assessment of the risk from plant protection product use for birds and mammals does not concern the depletion of food as a risk but only the ingestion of contaminated food. Since the different parts of risk assessment scheme that include plants, arthropods and birds & mammals are building up on each other to protect the entire ecosystem, the assessment of the risk for arthropods exposed to plant protection product should include the need to guarantee a remaining high biomass of arthropods that are food for other animals. Boatman et al. (2004) also recommended further evaluation of biomass as an endpoint in arthropod risk assessment in a report to DEFRA (Boatman et al. 2004).

The document on ‘Guidance for summarising and evaluating field studies with non-target arthropods’ by Frank De Jong and other experts (de Jong et al. 2010) is a useful source to evaluate non-target arthropod field studies on the effect of plant protection products. They propose various effect classes and describe effects as ‘pronounced’ if observable for 2 months and ‘slight’ if below this level and occurring only for a few species. This approach should be developed further to also take biomass fluctuations of major arthropod groups after a plant protection product induced decrease into account. However, additional studies would be needed to confirm the relationship between arthropod biomass and bird survival for some key farmland bird species.

3.3.1.8 Arthropods in field margins and current testing of plant protection products

The arthropod community of field margins (‘off-field habitat’) differ in their composition from the in-field communities (see chapter 3.2.4). Consequently this difference should also be recognised in the risk assessment procedure of pesticides, especially with regard to plant protection products for which the environmental risk assessment is divided in in-field and off-field assessment and which could reach off-field habitats in relatively high amounts (chapter 3.2.2.1). Currently, the highest tier studies that are provided for plant protection products for assessing the risk to arthropods are large scale field studies usually conducted in crops. However, the magnitude of the effects in the off-crop habitat might be underestimated, since only a subset of the field margin arthropods occur in the field. To account for this, the participants of the ESCORT3 workshop recommended: “*One option identified by the group to account for the uncertainty when extrapolating from in-crop to off-crop was the application of an uncertainty factor on in-crop based endpoints; the dimension of the actually applied assessment factor should account for the relevance and the quality of the in-crop study*” (Nikolakis et al. 2012). So far, no specific factor can be suggested and this gap can be identified as a research need.

In an earlier document (Candolfi et al. 2000b), the use of in-crop field tests with the off-crop drift rates is suggested for the risk assessment of plant protection products. However, this approach is problematic for several reasons. First, the differences between in-crop and off-crop regarding arthropod community composition, different life histories as well as differences in exposure due to vegetation structure are again not taken into account. Secondly, an off-crop drift rate corresponding to the drift expected at 1 m (2.77% in field crops) is usually used. However, inputs of plant protection products small field margins can be much higher, since there is partial overspray in the first meter (see Figure 3.2-4) and, therefore, the result of a study with a 2.77% drift rate would largely underestimate the effects occurring in the field margin. If we include also the variability of the off-crop habitats compared to a crop structure that is investigated in these studies, it is questionable whether such a field study might show the absence of unacceptable effects for the off-crop situation. De Jong et al. (2010) therefore recommend developing off-crop higher tier methods for studies from which a No Observed Effect Rate (NOER) can be derived.

Further options for an improvement of the test methods that assess the effect of plant protection products on field margin arthropod communities should be included in specific studies: “*For the off-field situation, field trials with a dose-response design are preferred in order to account for mitigation options (as different risk mitigation options can lead to different drift rates)*” (Nikolakis et al. 2012). However, only a few studies of this type were conducted so far (for an example see De Jong et al. 2010), and the design needs development and testing by different research groups and laboratories before being generally used in risk assessment procedures. This could be done in a ring-test approach at different locations in Europe to account for local differences in arthropod diversity and vegetation cover.

Additional points that require consideration are for example the timing of the application of the plant protection product and the state of the vegetation in the field margins since higher vegetation provides shelter from plant protection products and reduces exposure. The application of plant protection products should therefore ideally be as close to the earliest proposed field application rates for the plant protection product that needs to be registered. Also the target groups and sampling methods need to be selected. Sensitive arthropod groups that use field margins as a habitat are for example cicadas and caterpillars. Both groups were studied in an experimental design and revealed pronounced effects after a realistic insecticide application with a pyrethroid (see Research Box 6). Both groups include species that are using the higher strata of the vegetation and are therefore receiving a higher exposure. To account for the magnitude of effect (number of taxa affected and their abundances) one would only need to sample for a comparatively short time but preferably many different groups. Again, an important endpoint should be biomass to account for the fact that arthropods represent a major food source of vertebrates.

RESEARCH BOX 6:

Agrochemicals in field margins – an experimental field study

BACKGROUND: Field margins can be affected by plant protection products and fertilizer through direct overspray and spray drift from the adjacent field applications. This multi-year field study (started in 2010) mimicked the inputs of agrochemicals in the first meter of a winter wheat field margin to investigate the direct and indirect effects as well as the cumulative effects (due to the annual application sequence) of the misplacement of plant protection products and fertilizer on the flora and fauna of field margins.

METHODS: A randomized block design with seven treatments (I: insecticide, H: herbicide, F: fertilizer, H+I, F+H, F+I, F+H+I) and one control was established on a low productive meadow (Figure 3.3-4). Each treatment was replicated eight times in plots of 8 m x 8 m with 2 meter distance from each other. The used fertilizer concentrations (25% of the field rate) and pesticide concentrations (30% of the field rate of the insecticide Karate Zeon (a.i.: Lambda-Cyhalothrin) and the herbicide Atlantis WG (a.i. Iodosulfuron + Mesosulfuron)) were consistent with their inputs (drift + overspray) in the first meter of a field margin directly adjacent to the field under Good Agricultural Practices.

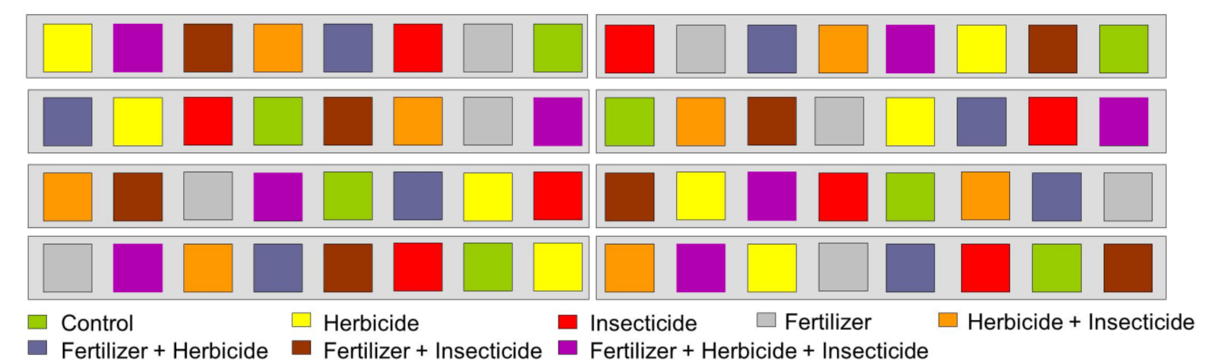

Figure 3.3-4: Randomized block design (after Schmitz et al. (accepted), figure modified)

Arthropod communities in each plot were assessed using various sampling techniques like suction sampling (cicadas) and sweep nets (caterpillars). The results of two groups (cicadas and caterpillars) are exemplarily presented.

RESULTS: Cicadas were analysed in 2010. Two species *Arthaldeus pascuellus* and *Philaenus spumarius* showed reduced abundances (partly statistically significant) in plots receiving an insecticide treatment in comparison to control plots (Figure 3.3-5). Both species are known to use predominately higher vegetation strata and, therefore, they were probably affected due to direct contact to the insecticide.

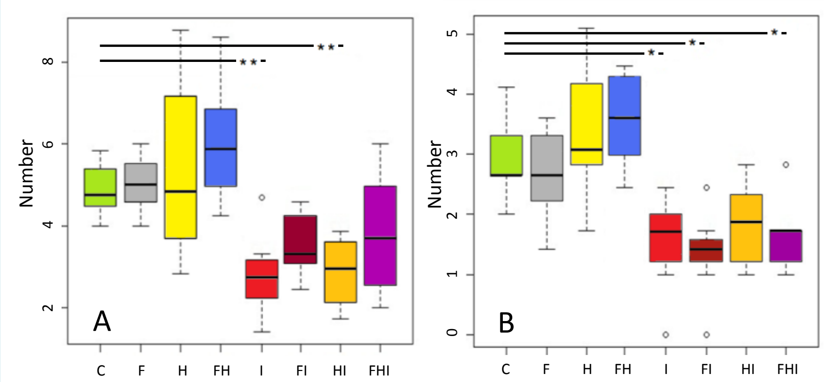

Figure 3.3-5: Abundance of *Arthaldeus pascuellus* (A) and *Philaenus spumarius* (B) in all treatments (C: control, F: fertilizer, H: herbicide, I: Insecticide, F+H, F+I, H+I, F+H+I, each treatment with n=8). Data square-root transformed. Sampling date: 23.06.2010. *: p<0.05, **: p<0.01 (Dunnett test). (after Felix (2011), figure modified)

Caterpillars were surveyed in 2011. Individual numbers per plot were low and therefore the analysis was based on the family rather than the species level. The most abundant families (Noctuidae and Geometridae) showed statistically reduced abundances in plots receiving an insecticide treatment (Figure 3.3-6).

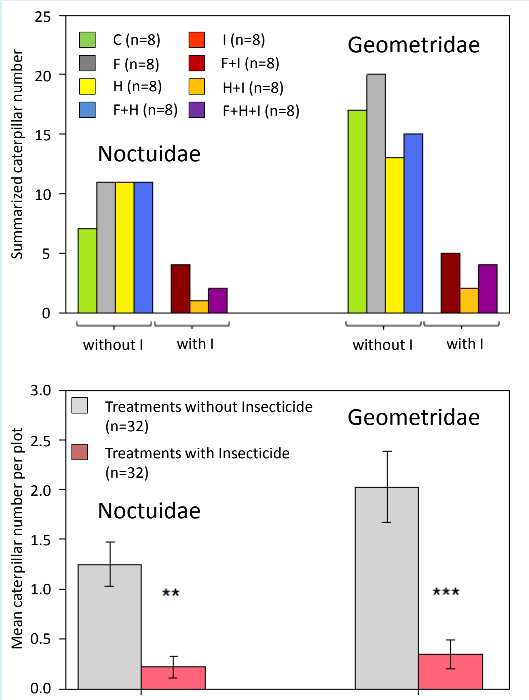

Figure 3.3-6: Summarised caterpillar number per treatment (above) and mean caterpillar number per plot (below, on the basis of all plots treated with/without insecticide) of Noctuidae and Geometridae. Results based on pooled data of two sampling dates: 30.05.2011 and 27.06.2011. **: p<0.01, ***: p<0.001 (permutational ANOVA, number of permutations: 999). (after Schotthöfer (2012), figure modified)

SOURCES:

Schmitz et al. (accepted): Agro-chemicals in field margins - Assessing the impacts of herbicides, insecticides, and fertilizer on the common buttercup (*Ranunculus acris*)." *Environmental Toxicology and Chemistry*.

Felix, T. (2011): Untersuchung zum Einfluss des Dünger-, Herbizid- und Insektizideintrags auf die Biodiversität von Feldsäumen am Beispiel von Zikaden (Auchenorrhyncha). Diploma thesis. University Landau.

Schotthöfer, A. (2012): Schmetterlingsraupen (Lepidoptera) in der Agrarlandschaft: Welche Rolle spielen Feldsäume verschiedener landwirtschaftlicher Kulturen als Entwicklungshabitat? Diploma thesis. University Landau.

In short:

- The decision, which test species should be employed to assess the effect of plant protection products to non-target arthropod is historically derived. The species used belong to so called 'beneficial insects' important in integrated pest control strategies. However, they only represent a restricted subset of arthropod biodiversity (predators / parasitoids). The sensitivity of the 'beneficial insects' might be similar to other arthropods but their life history does not reflect the range of life history strategies for the highly diverse arthropods.
- To assess the effects of biocides on the terrestrial compartment the tests focus on plants (producers), soil organisms (consumers), and/or the inhibition of microbial activity (decomposers). Specific tests with non-target arthropods are only requested in the risk assessment if it can be assumed by the mode of action of the active substance that these organisms are likely to be at risk and in cases where a high release to the terrestrial compartment is possible. The proposed test species belong to the 'beneficial insects', too.
- Phytophagous insects might additionally be exposed to pesticides by consumption and reduction of host plant quality.
- The assessment of recovery from effects of plant protection products needs to be separated from recolonisation processes. If, after a breakdown, a population increase occurs within a few weeks, recolonisation is a more likely process for species with only one reproductive phase per year. Recolonisation is largely dependent on the mobility (dispersal ability) of the species and the surrounding habitat. In-field recolonisation might also draw from the arthropod population of the field margins and fields are therefore acting as sinks for these populations.
- Arthropods are also major food items for vertebrates and especially important during the rearing phase of young and chicks. Even short time reductions in their biomass might affect the next trophic level. This aspect needs to be considered further to link the different groups assessed separately in current risk assessment procedures of plant protection products.
- Current testing of plant protection products includes in-crop field studies as highest tier. Since arthropod community composition and life histories as well as plant protection product exposure differ between fields and field margins, the application of an uncertainty factor or the conduct of specific off-crop studies is suggested. However the testing of off-crop arthropod communities needs further evaluation to account for the variability of arthropod communities throughout Europe and select sensitive groups and quantitative sampling methods.

3.3.2 Soil organisms

3.3.2.1 Current situation for plant protection products

In the early 1990ies, harmonized requirements for placing of plant protection products on the market were published within the European Union. Later, details of the testing requirements and the risk assessment procedure were elaborated further (Römbke et al. 2009a). In practice, the environmental risk assessment is carried out separately for each environmental compartment (water, sediment, soil, and air). Within the terrestrial compartment, the risk assessment for non-target arthropods, honey bees and soil organisms were and are still handled differently due to historical reasons.

For the risk assessment of plant protection products to soil organisms (EC 2002a; OEEP/EPPO 2002), the concentration of the test substance expected in soil (predicted environmental concentration = PEC) and the concentration with a specific effect in the environment is determined. Dependent on the test system, the specific effect is measured as no observed effect concentration (NOEC) or calculated as 50% lethal concentration (LC_{50}) or 50% effective concentration (EC_{50}). The impact of the plant protection product on individual species is measured as mortality or as a sub-lethal effect (e.g. growth or reproduction) in laboratory tests. To quantify the risk of a test substance, the quotient between the toxicity value (NOEC, LC_{50}, or EC_{50}) and the exposure value (PEC) is calculated and expressed as a toxicity exposure ratio (TER). With the assumption that the plant protection product is used according to the principles of good agricultural practice, including manufacturer's intended use, and depending on the comparison of the TER and certain safety or assessment factors, it is decided whether the use of plant protection products can be considered safe. If there are concerns about the safe use, the authorities can require safety measures (e.g. lower application rates, no application during specific time periods). If risks cannot be ruled out despite of safety measures, the plant protection product may not be granted authorization.

For the standardized tests, usually only a few test species are used. Most of them are easy to cultivate, genetically uniform, and of medium sensitivity to a wide range of plant protection products. An ecological relevance of the test organisms is desirable but often plays a secondary role because of practicability considerations. The main test organism for soil organisms is the compost worm *Eisenia fetida* (OECD 1984) that rarely occurs in soils of arable fields.

Standardized test guidelines are used over a wide range of concentrations following a hierarchical (tiered) order (see worm tests in Table 3.3-3). The first tier consists of simple, short-term, and low-cost single-species tests. Tests are performed under assumed worst-case conditions, e.g. the test organisms in the earthworm toxicity test are exposed to the test substance in artificial soil under continuous illumination in order to force the test organisms not to leave the treated substrate (OECD 1984; ISO 1993). At the first tier, a minimum set of test species is required (earthworms, microorganisms, plants; EC 2002a; OEEP/EPPO 2002). Depending on the study results of the acute toxicity tests at tier 1, in the next higher-tier tests sub-lethal effects can be tested (Table 3.3-3). Furthermore, testing of additional soil test species is an alternative (Table 3.3-3 and Table 3.3-4).

Table 3.3-3: Common Standardised Soil Test Systems

Test system	Substrate	Duration	Endpoint	Guideline(s)
Compost worm *Eisenia fetida*	Paper	48 hours	Mortality	OECD (1984)
	Artificial soil	14 days	Mortality (Body weight)	OECD (1984) ISO (1993)
		48 hours	Avoidance	ISO (2008)
		56 days	Reproduction (Body weight, Mortality)	ISO (1998) OECD (2004b)
		21 days	Bioaccumulation	OECD (2010)
Earthworms	Natural soil on grassland	1 year	Changes in species number and abundances	ISO (1999b) ISO (2006)
Enchytraeid worm *Enchytraeus albidus*	Artificial soil	56 days	Reproduction, Mortality	OECD (2004a)
Springtails *Folsomia candida*/*F. fimetaria*	Artificial soil	28 days	Reproduction (Mortality)	ISO (1999a) OECD (2009)
Predatory soil mite *Hypoaspis aculeifer*	Arificial soil	14 days	Reproduction (Mortality)	OECD (2008a)
Litter bag study	Field soil	3-12 months	Straw degradation	OECD (2006a)
Soil microflora	Natural soil	28 days	Nitrate formation	OECD (2000a)
Soil microflora	Natural soil	28 days	Respiration rate	OECD (2000b)
Plant seedling emergence test	Natural soil	21 days	Mortality (symptoms of toxicity, growth)	OECD (2006b)

Table 3.3-4: Less Common Standardised Soil Test Systems

Test system	Substrate	Duration	Endpoint	Guideline
Poecilus cupreus, adults	Natural soil	14 days	Mortality (Feeding rate)	Candolfi et al. (2000b)
Poecilus cupreus, larvae	Natural soil	28 days	Mortality (Body weight)	Heimbach et al. (2002)
Aleochara bilineata	Natural soil	8-10 weeks	Reproduction	Candolfi et al. (2000b)
Nematode *Steinernema carpocapsae*	Water	3 weeks	Mortality, infectivity, propagation	Peters (2003)

On the level of higher-tier studies, semi-field or field studies follow. The results of these field studies (i.e. earthworm field study, field litter degradation study) are of greater ecological relevance, but are often difficult to interpret because of high variability of the data as well as of the singularity of the study due to specific site and weather conditions. Depending on the tiers covered, the number of tests submitted or required for the registration of one active ingredient of a plant protection product and its formulations can vary considerably.

Tests with soil organisms are wide-spread and also used as test species for the risk assessment of pharmaceuticals (Reiss et al. 2009) or sewage sludge on fields (Staples et al. 2010). For the risk assessment of birds and mammals (EFSA 2009b), bioaccumulation studies with *Eisenia fetida* (OECD 2010) or with the earthworm community in the field are conducted.

The tiered test-procedure for plant protection products is currently under discussion (EC 2010). The main improvements to be planned are the use of only worm reproduction studies instead of acute worm tests (determination the LC50 at often very high and unrealistic test concentrations) and the confinement of the microflora study to only the nitrification as endpoint. The study type of straw degradation in litter bags (OECD 2006a) is not included furthermore since the endpoint is not considered to be sufficiently sensitive.

3.3.2.2 Current situation for biocides

The environmental risk assessment for active substances and biocidal products considers the different environmental compartments to which the substance may be exposed. As described in chapter 3.2.1.2, the initial requested data on active substances for the terrestrial compartment should comprise amongst others an acute test with earthworms (OECD 1984) or other soil non-target macro-organisms (like collembola). For insecticidal substances, testing of collembolan (ISO 11267) is favoured to tests with earthworms (BAUA 2009). Soil organisms might be exposed to the three product types focused on in this project, the insecticides and disinfectants appearing in manure and wood preservatives used for e.g. fences or wooden piles on agricultural sites.

In generel, the environmental risk assessment for biocides is based on PEC/PNEC-ratios for the respective environmental compartments water, sediment, and soil (see chapter 3.2.1.2). The risk has been defined to be acceptable if the PEC/PNEC ratio is below 1. However, the availability of toxicity data for the calculation of PNECs might be scarce for some substances. In such cases, the equilibrium partitioning method (EPM) can be applied to derive the PNEC for soil and sediment based on the PNEC for the aquatic compartment (EC 2003). Nontheless, if there are concerns about the safe use, the authorities can prescribe the application of risk mitigation measures (RMM, see chapter 3.4.1).

Additionally, as in the risk assessment of plant protection products, earthworms can be used to investigate biomagnification and the potential of secondary poisoning via terrestrial food chains (EC 2003). Especially birds and mammals might feed on worms.

3.3.2.3 Separate below-ground from above-ground organisms?

Based on the actual literature survey, the most obvious observation is the historical and artificial separation between soil organisms below-ground and non-target organisms above-ground in the risk assessment of plant protection products. Both groups do not act independently from each other.

Plants need the soil organisms for mineralizing the organic matter and the non-target arthropods for pollination and seed dispersal. Many insect groups use the soil surface as foraging habitat and soil organisms as prey (e.g. Carabidae, Lycosidae). The soil up to 30 cm depth is the habitat for the larvae of many species (e.g. Carabidae, Diptera, Scarabaeidae) and is used as overwintering habitat (e.g. Carabidae, Staphylinidae). Soil organisms benefit from an accelerated nutrient transfer by nutrient-rich faeces produced by herbivores (e.g. larval Lepidoptera, Barbosa & Wagner 1988). Any change within agricultural fields (e.g. tillage, fertilisation, crop rotation, plant protection products) impacts directly and indirectly the processes above- and below-ground. Therefore, the risk assessment of plant protection products, the design of the required tests and possible mitigation measures should be planned taking into account both soil organisms and non-target arthropods (Smith et al. 2007a). (In the environmental risk assessment of biocides this is already realized, dependent on different factors like product type, exposition and/or risk.)

3.3.2.4 Drawbacks in the current soil risk assessment

Concerning the current soil risk assessment of soil organisms, there are some drawbacks:

- The number and ecological significance of test species so far used is limited and does not include e.g. different earthworm life-strategies or saprophagous oribatid mites.
- Affordable semi-field higher tier study systems are missing which fill the gap between laboratory tests and the expensive highest tier field studies.
- If higher tier tests are needed, the number of available test systems is rather low. The field litter degradation test system (OECD 2006a) is the only test which examines the functional response of the soil to a plant protection product (i.e. the time-dependent decrease of organic matter) but is regarded as not sufficiently sensitive. The earthworm field study (ISO 1999b) is the remaining field alternative but is applicable only for structural community parameter of one single species group. Structural diversity and soil functions are aimed to be linked with terrestrial ecosystem models (Weyers et al. 2004; Jaensch et al. 2005) but the study design and the interpretation of data are still matter of debate.
- The realistic exposure of soil organisms to plant protection products is not clearly resolved. Using the equilibrium partitioning method (EPM), the pore water concentration can be linked with effect concentrations of the aquatic ecotoxicology (Bezchlebovà et al. 2007). However, in many cases, the analytically measured soil concentration does not correlate with the soil pore water concentration (Crommentuijn et al. 1997).

3.3.2.5 Indicator species

For field studies testing the influence of a plant protection product or for monitoring studies, the selection of appropriate endpoints is important. For soil organisms, some species were characterized as sensitive to a specific parameter. For example, in Table 3.3-5 different springtail species or species groups are listed which can be used as indicators for specific parameters.

Table 3.3-5: Examples for indicator species and species groups of Collembola for specific habitats/treatments

Parameter	Indicator species/ indicator species group	Response	Literature
mechanical cultivation on fields	*Lepidocyrtus paradoxus* *Orchesella celsa* *Schoettella banksi*	reduction in abundance in up to two of three years	Bitzer et al. (2002)
conventional cultivation on fields	*Bourletiella rustica*		
conventional field	*Entomobrya nicoleti* *Lepidocyrtus* ssp.	reduction in abundance over six years	Frampton (2002)
field edge	*Orchesella cincta* *Tomocerus* spp.	occurrence only in field edges	
recently disturbed area	*Ceratophysella* sp. *Hypogastrura* sp.	increase in abundance	Cole et al. (2006)
11 years old shelterbelt	*Schoetella ununguiculata*	high dominance in the respective habitat	Olejniczak (2007)
170 years old shelterbelt	*Onychiurus armatus*		
field	*Friesea mirabilis* *Proisotoma minuta* *Isotoma notabilis* *Onychiurus armatus*		
meadow	*Isotomus productus*		Troxler (1990)
field	*Proisotoma tuberculata*		
meadow	*Lepidocyrtus cyaneus*	sensitive to plant protection products	Ross-Nickoll et al. (2004)

Next to springtails other invertebrates like Nematoda, Carabidae, and Lumbricidae have also been characterized as sensitive to specific habitats or treatments of which some are described in Table 3.3-6.

Table 3.3-6: Examples for indicator species and species groups of soil community, Nematoda, Carabidae, Enchytraeidae, Lumbricidae for specific habitats/treatments

Parameter	**Indicator species/ indicator species group**	**belonging to**	**Literature**
soil disturbance	Secernentea Adenophorea	Nematoda	Bongers (1990)
no tillage	fungivorous nematodes mites springtails earthworms	Nematoda Acarina Collembola Lumbricidae	Hendrix et al. (1986)
conventional tillage	bacterivorous nematodes enchytraeids	Nematoda Enchytraeidae	
disturbed habitat	*Buchholzia appendiculata*	Enchytraeidae	Jänsch et al. (2006)
soil acidity	Lumbricidae	Lumbricidae	Beylich & Graefe (2009)
soil clay content	Lumbricidae		
sandy soils	*Fridericia* sp./*Enchytraeus* sp.	Enchytraeidae	
loamy soil	*Fridericia* sp./*Lumbricus* sp.	Enchytr./Lumbr.	
disturbed habitat	*Buchholzia* spp. *Enchytraeus* spp. *Henlea ventriculosa*	Enchytraeidae	Schlaghamerský & Pizl (2009)
conventional tillage	*Bembidion quadrimaculatum*	Carabidae	Cárcamo et al. (1995)
conventional agricultural fields	*Pterostichus melanarius,* *Poecilus cupreus* *Harpalus rufipes* *H. affinis* *Platynus dorsalis* *Agonum mülleri* *Bembidion lampro* *Trechus quadristriatus*	Carabidae	Krompp (1999)
organic fields	*Poecilus versicolor* *Dyschirius globosus* *Harpalus affinis*		
weeds	*Amara* sp.		
sensitive to plant protection products	*Poecilus versicolor* *Brachinus crepitans* *Pterostichus madidus*	Carabidae	Ross-Nickoll et al. (2004)

Furthermore, indices were established to describe and simplify the high species diversity of some groups of soil organisms (Table 3.3-7).

Table 3.3-7: Examples for Indices, calculated for soil organisms and other groups.

Index	Principle	developed for	Literature
arthropod acidity index	pH preferences of 8 springtail, 8 mite and 4 woodlice species	Arthropod community	Van Straalen (1997)
Maturity index	value of colonizer/persister	Nematoda	Bongers (1990)
Maturity index	proportion of K-values to the sum of the r and K-values	Gamasine mites (Mesostigmata: Gamasida)	Čoja & Bruckner (2006)
Vulnerability index	Calculated from 4 categories (external and internal exposure, effect on organism and population)	Vertebates	De Lange et al. (2010)
Quality index	Proportion of protected, intermediate and insensitive species	Spiders	Ruzicka & Bohac (1994)
AcariCollembola ratio	Proportion of mites and springtails	Arthropod community in grassland soil	Menta et al. (2011)
QBS-index	“Qualità Biologica del Suolo”: sum of species-specific eco-morphological scores (20=eu-edaphic, 1=epi-edaphic)	Collembola, soil arthropods	Parisi et al. (2005)

However, the relationship between species diversity in soil and soil functioning is not yet clear (Ekschmitt & Griffiths 1998; Hättenschwiler et al. 2005; Brussaard et al. 2007; Parker 2010). Especially, in agro-ecosystems functional dissimilarity within species community may be more important than soil biodiversity per se (Brussaard et al. 2007). Therefore, a mixed approach may be advisable by assessing the presence of key species (e.g. lumbricids) and relevant trophic levels supported by multifactorial statistical analysis (Neher & Barbercheck 1998; Barbercheck et al. 2008).

In short:

- In the standardized tests to assess the ecotoxicity of pesticides to soil organisms only a few test species are used. An ecological relevance of the test organisms often plays a secondary role because of practicability considerations.
- Soil organisms below-ground and non-target organisms above-ground are not independent from each other. Many above ground insects have below grund larvae (e.g. carabid beetles, diptera)
- Any change within agricultural fields (e.g. tillage, plant protection products) impacts directly and indirectly the processes above- and below-ground.
- In the risk assessment a mixed approach may be advisable by assessing the presence of key species (e.g. lumbricids) and relevant trophic levels.

3.4 Management of terrestrial habitats in support of biodiversity: current situation and suggestions for improvement

In order to ensure the maintenance of high biodiversity levels in agricultural landscapes the proper management of terrestrial habitats is of great concern. In the previous chapters it has been shown that especially uncropped areas like field margins, hedgerows, or flower strips are used by many organisms for e.g. foraging and reproduction. Agricultural management can affect these habitats and the organisms relying on them. In most cases, biocides are not specifically intended to be applied to agricultural sites. Instead, they reach the agricultural landscape in an indirect way, for example via the application of manure or sewage sludge. Hence, there are only few risk mitigation measures focusing on the management of the agricultural landscape. Plant protection products are directly applied to the agricultural sites and there are two types of management schemes which can improve biodiversity in agricultural landscapes: On the one hand, there are management schemes including a reduction of inputs of plant protection products in off-field areas. On the other hand, there are management schemes which aim to provide an appropriate amount of non-crop habitat in combination with a proper spatial arrangement of these habitats (biotope network) at a field and landscape level.

3.4.1 Risk mitigation measures (RMM) for biocides

Per definition (see chapter 2) biocides are not used for the protection of crops but they are e.g. applied in stables to reduce the occurrence of unwanted insects (product type 18, see Introduction) or pathogens (product type 3) which might harm livestock. Hence, biocides are normally not directly applied to agricultural sites and reach the field indirectly, for example via the fertilizing of fields with manure containing insecticides and/or disinfectants or via eluates leaching from wooden piles treated with wood preservatives (product type 8). As mentioned previously, the risk assessment of biocides considers different environmental compartments and for the terrestrial part (soil compartment) there is no differentiation done between in-field and off-field habitats. Similarly, there are no risk mitigation measurements with regard to in-field or off-field habitats. In order to reduce inputs of biocides in the environment, risk mitigation measures are available dealing with the placing on the market (e.g. user restrictions, area of application), the application of biocidal products (e.g. equipment) and/or the post-application time (e.g. storage of treated wood and manure, waiting periods, wastewater treatments) (Gartiser & Jäger 2011).

In the following, we focus on risk mitigation measures that could be relevant for biocides reaching agricultural sites (product types 3, 8, and 18, see Introduction).

For insecticides (product type 18) and disinfectants (product type 3) used in the animal housing mearsures that might influence the (indirect) application of these biocides to agricultural fields via manure include for example restrictions concerning the maximum amount of phosphate and/or nitrogen per area of agricultural soil. According to Directive 91/676/EEC[18] the amount of manure applied to soil is limited to 170 kg N per hectar. Furthermore, manure treatment (e.g. anaerobic digestion) prior to or instead of land spreading might reduce the biocide inputs applied with manure (Gartiser & Jäger 2011). In addition, possible risk mitigation measures concerning the agricultural practice may be included, e.g. '*[…] the size of the animal housings that are treated and the number of animals kept in the housing*', '*manure application rates*', or '*number of manure application events*' (OECD 2006c)

The implementation of appropriate waiting periods after the application of wood preservatives might be a relevant measure to reduce leaching of wood preservatives used on wooden piles on agricultural sites.

However, for biocides reliable data on the efficiency of RMM are not available (Gartiser & Jäger 2011). To characterize the efficiency of RMM, '*data on quantities of biocidal active substances and products produced or sold, as well as a quantitative description on the main emission sources*' are required (Gartiser & Jäger 2011).

3.4.2 Current risk management for terrestrial off-field habitats concerning inputs of plant protection products

If an unacceptable risk is predicted from the application of a plant protection product at intended field rates, the risk management for terrestrial off-field areas is based on risk mitigation schemes in form of use restrictions in Germany. These may comprise the usage of low drift nozzles and/or distance requirements from the field edge for each authorized plant protection product ("NT-Auflagen") to reduce inputs of plant protection products in adjacent off-field habitats. Many use restrictions have not to be followed in particular situations, for example when the width of adjacent off-field areas (e.g. field margins, hedges) is less than 3 m or when the applied field is located within a region which holds a sufficient percentage of

[18] Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources
Richtlinie 91/676/EWG des Rates vom 12. Dezember 1991 zum Schutz der Gewässer vor Verunreinigung durch Nitrat aus landwirtschaftlichen Quellen

small structures (“Kleinstrukturen”) like field margins, roadside vegetation, or parks (see Table 3.4-1 for an overview).

Table 3.4-1: Overview of several German use restrictions for the application of plant protection products next to terrestrial off-field areas ("NT-Auflagen") considering their exceptions according to the width of the off-field habitats and the regions listed in the register of regionalized small structures (requested small structure percentages vary between 5-20% per district, depending on the cultivated crops). DR: Distance Requirements, LDN: Low Drift Nozzles, class: spray drift reduction class of the nozzles. Use restrictions start at the field edge inwards.

off-field habitat	width < 3m		width ≥ 3m			width ≥ 3m	
register of small structures			coverage below requested percentage			coverage above requested percentage	
use restrictions	DR	LDN (class)	DR		LDN (class)	DR	LDN (class)
NT-Auflage 101					min. 20m (50%)		
NT-Auflage 102					min. 20m (75%)		
NT-Auflage 103					min. 20m (90%)		
NT-Auflage 104			5m	or	min. 20m (50%)		
NT-Auflage 105			5m	or	min. 20m (75%)		
NT-Auflage 106			5m	or	min. 20m (90%)		
NT-Auflage 107			5m	and	min. 20m (50%)		min. 20m (50%)
NT-Auflage 108			5m	and	min. 20m (75%)		min. 20m (75%)
NT-Auflage 109			5m	and	min. 20m (90%)		min. 20m (90%)
NT-Auflage 111			5m				
NT-Auflage 112			5m				

The small structures – recorded on a regional scale – should ensure the recovery of the arthropod populations in the fields after applications of plant protection products. The percentage of requested small structures per region to settle an exemption from the need of implementing the specific management restrictions is dependent on the intensity of chemical plant protection activities and varies between 5-20% per district area (Enzian & Gutsche 2004). All districts which fulfil the requirement are listed in the register of regionalized small structures (“Verzeichnis der regionalisierten Kleinstrukturanteile”) published by the Julius-Kühn-Institute[19]. In 2004, nearly 75% of the districts fulfilled the claimed minimum requirements to be listed in the register of regionalized small structure percentages (Enzian & Gutsche 2004) and the register has been further extended since. Therefore, in most areas of Germany, use restrictions must not be followed by farmers or have to be followed only in an extenuated form (Table 3.4-1). The increase of the percentage of small structures in a region can be seen as a positive development but it seems questionable if e.g. the vegetation strips

[19] For detailed information see: http://www.jki.bund.de/de/startseite/fachinformationen/pflanzenschutz/pflanzenschutzverfahren/kleinstrukturen.html

next to motor-ways (which are also considered in the calculation) can ensure an appropriate recovery of arthropod populations after applications of plant protection products on a larger scale. For example, several studies indicate an avoidance of the crossing of traffic routes by some invertebrates like bumblebees (Bhattacharya et al. 2003) or ground beetles (Mader 1981; Mader et al. 1990). Furthermore, none of the use restrictions mentioned in Table 3.4-1 needs to be followed next to field margins or hedgerows with a width of less than 3 m. Such smaller structures constitute a high percentage of the available off-field habitat next to agricultural fields in Germany (see Research Box 1). Additionally, it is assumed as underlying concept that all farmers apply the principles of Good Agricultural Practice. Results of a study of the Federal Environment Agency (Umweltbundesamt) indicate a high percentage of misuse during the application of plant protection products (UBA 2006a). Such misuses (e.g. applications at high wind speeds) further increase the inputs of plant protection products in off-field habitats next to agricultural sites.

3.4.3 Risk management of plant protection products for non-target arthropods

3.4.3.1 Proposals for the enhancement of the off-field risk management

Field margins and woody structures adjacent to agricultural sites potentially constitute habitats for a wide range of species (see Table 3.2-5) including threatened or near threatened species (see chapter 3.2.5, Research Box 7 and 8).

RESEARCH-BOX 7:

Caterpillars in field margins

BACKGROUND: Butterflies and moths (Lepidoptera) are a species rich order with many species inhabiting agricultural landscapes. In this research project it was assessed if narrow field margins represent suitable habitats for the development of caterpillars.

METHODS: Caterpillars were surveyed in field margins and meadows nearby Landau, Germany, using sweep nets (300 beats per site). All field margins had a width of 1-2 m and were located next to cereal fields or orchards.

RESULTS: Small field margins constitute caterpillar habitats for a range of Lepidoptera species including three species (*Polyommatus semiargus*, *Emmelia trabealis*, *Cucullia chamomillae*) characterized as near threatened on the German Red List (Binot et al. 1998). However, next to orchards less caterpillar individuals could be found in comparision to cereal field margins and meadows. This could probably be related to higher insecticide inputs in orchards (see Table 3.4-2).

Table 3.4-2: Number of caterpillars found in three habitats (meadows, cereal field margins and orchard margins) surveyed during 3 phases. N: Number of study sites per habitat, S: summerized number of individuals, M: mean number of individuals (grey), SD: standard derivation.

	Meadows				Cereal field margins				Orchard margins			
	N	S	M	SD	N	S	M	SD	N	S	M	SD
Phase 1 (May)	12	139	11.6	9.1	14	86	4.9	3.4	0	-	-	-
Phase 2 (June)	11	199	18.1	11.9	9	105	11.7	4.8	8	31	3.9	5.4
Phase 3 (July-Aug.)	10	102	10.2	5.7	9	56	6.2	5.9	8	25	3.1	2.3

SOURCE: Schotthöfer, A. (2012): Schmetterlingsraupen (Lepidoptera) in der Agrarlandschaft: Welche Rolle spielen Feldsäume verschiedener landwirtschaftlicher Kulturen als Entwicklungshabitat? Diploma thesis. University Landau.

RESEARCH-BOX 8:

Grasshoppers in field margins

BACKGROUND: Grasshoppers have been shown to be sensitive to insecticides (see Research Box 3). Since this organism group includes a number of Red List species and is furthermore an important food item in e.g. bird diets, the occurrence of grasshoppers in field margins was assessed.

METHODS: The study was conducted in an agricultural landscape around Landau, Germany. Grasshoppers were recorded in field margins of different crops (cereal fields, vineyards, orchards) and grasslands using a trapping cage quickly placed on the margin vegetation. Numbers of individuals were noted and species determined in the field when possible.

RESULTS: Overall, twelve grasshopper species were trapped in field margins. One of these species, *Chrysochraon dispar*, is classified as vulnerable (‘Kategorie 3: Gefährdet’) according to the German Red List (Binot et al. 1998). With respect to the abundance, the lowest number of grasshoppers was recorded in orchard field margins (Figure 3.4-1). Furthermore, next to cereal fields and vineyards the abundance of grasshoppers increased with increasing field margin width.

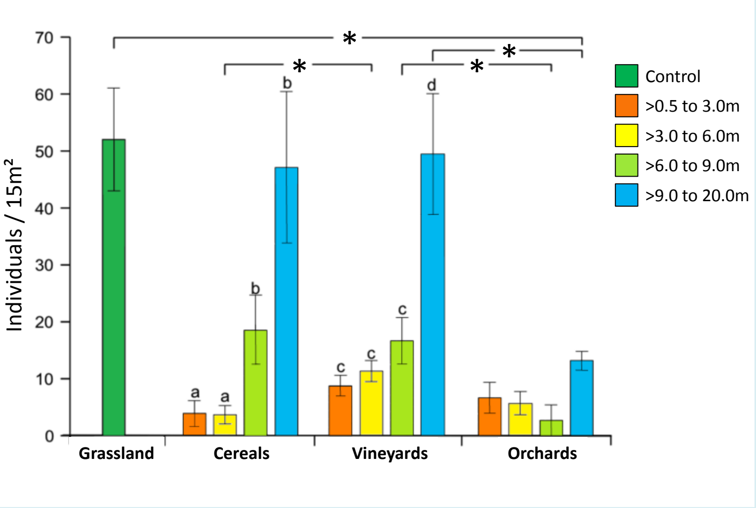

Figure 3.4-1: Mean grasshopper density (± standard error) in grasslands (control sites, n=10) and field margins (width classes: >0.5 to 3m, >3 to 6m, >6 to 20m) next to cereal fields (n=34), vineyards (n=46) and orchards (n=20). Different letters indicate statistically significantly differences between size classes within one crop. *:statistically significant differences between crops within one width class. (after Bundschuh et al. 2012, figure modified).

SOURCE: Bundschuh, R., Schmitz, J., Bundschuh, M. & Brühl C. A. (2012): Does drift of insecticide adversely affect grasshoppers (Orthoptera: Saltatoria) in field margins? A case study combining laboratory acute toxicity testing with field monitoring data." *Environmental Toxicology and Chemistry* **31**(8): 1874-1879.

As mentioned above, there are two types of management schemes – those targeting a reduction of inputs of plant protection products in off-field habitats at a field level and those focusing on an appropriate amount, position, and quality of non-crop habitats at a field and landscape level. However, there are overlaps between both types of schemes since for example in-field buffer strips for the protection of off-crop habitat from inputs of plant protection products can provide valuable habitat for several species. For this reason, both types of management schemes are discussed here jointly. However, an overview of the proposed management schemes including information on the management types is given in Table 3.4-3.

Table 3.4-3: Overview of the proposed management schemes that can be used to reduce inputs of plant protection products (ppp) in off-field habitats and/or are useful for the design of agricultural landscapes to enhance habitat availability and habitat quality for arthropods.

goal	proposed management schemes	reduce ppp inputs in off-field habitats	landscape design
preservation and enhancement of the existing field margins and hedgerows in quality or size	preservation of existing off-field habitats		x
	extension of small structures	x[1]	x
	unsprayed in-field buffer strips (cropped and uncropped)	x	x
	no overspraying of off-field habitats	x	
increase of plant species richness and the provision of adequate floral resources	extension of small field margins	x[1]	x
	unsprayed in-field buffer strips (cropped and uncropped)	x	x
	creation of e.g. conservation fallows	x[2]	x
	sowing of seed mixes (in-field buffer strips or conservation fallows)		x
	no overspraying of off-field habitats	x	
appropriate management of off-field habitats	annual and perennial mowing rhythms		x

[1] ppp input is not reduced due to width extensions, but the wider the field margin the lower is the ppp input via ppp spray drift so that some parts of the field margins are less affected.

[2] Depending on the position of the conservation fallow in the field (in the middle of the field, next to the field margin)

A first goal for the protection of the (analysed) arthropod groups in an agricultural landscape would be the **preservation and enhancement in quality or size of the existing field margins and hedgerows** adjacent to agricultural sites. As shown in Table 3.1-2 these off-field structures can be described as highly beneficial for many arthropod (and vertebrate) groups. They provide a (temporary) habitat (Research Box 7 and 8) and can link greater non-linear habitats to form a biotope network. Thereby, especially the enhancement of small linear field margins can be of importance since they represent a high percentage of the linear structures available in (some) agricultural landscapes (see Research Box 1). For example, the connection of (small) grass strips to non-linear forest habitats enhances the abundance of wasps in the grass strips and might be beneficial for the colonization of nesting sites possibly due to facilitated movements (Holzschuh et al. 2009). Furthermore, even a small (1.5 m wide) field margin can provide resources like flowers which support different Syrphidae species (and probably other flower visiting insects) (Molthan & Ruppert 1988). For this reason, the width of these structures should not be a criterion for use restrictions of plant protection products. Nonetheless, an enlargement of small structures towards wider margins should be beneficial for the plant species richness (Link & Harrach 1998) which is a positive influencing factor for most analysed arthropod groups (see next section). Furthermore, wider field margins provide habitats with a lower proportion contaminated with plant protection products.

Plant protection products lead predominantly to negative effects on all assessed arthropod groups. For this reason a reduction of the inputs of plant protection products in cropped as well as non-cropped habitats should be aspired. We propose that overspraying should be always restricted to the in-field area and not include parts of the field margins (see Figure 3.2-4) since otherwise great percentages of off-field habitats next to arable fields receive high plant protection product inputs (50% of the in-field rate). Another (or additional) possibility to insure lower inputs of plant protection products in off-field habitats is the creation of ***in-field buffer strips*** (cropped: "Ackerrandstreifen"/ conservation headlands, uncropped: e.g. "Blühstreifen"/ flower strips). Moreover, both the cropped and the uncropped buffer strips have been shown to be beneficial for most analysed organism groups (Table 3.4-4, Navntoft et al. 2009; Vickery et al. 2009).

As second goal, risk management should include schemes which **increase the plant species richness and the provision of adequate floral resources** from the field scale to the landscape scale. As shown in Table 3.1-2, the analysed arthropod groups benefit from higher plant species richness and/or flower occurrence. Most herbivore species and pollinators are more or less specialized to a range of host plants, sometimes they feed on only a single plant species. Studies indicate that current agricultural practices (e.g. herbicide usage, improved seed cleaning) have led to a decline of many plant species combined with a decline in the seed bank of the soils (Andreasen et al. 1996; Spahillari et al. 1999; Wilson et al. 1999; Sutcliffe & Kay 2000; Robinson & Sutherland 2002). Associated arthropod species disappear if they do not find suitable host plants. Even sublethal herbicide dosages can deplete the flowering of plant species (Feber & Smith 1995; Schmitz et al. accepted) and reduce food sources for flower-visiting arthropods. For this reason, the extension of narrow field margins as well as the creation of in-field buffer strips is proposed to enhance plant species richness and floral resources (Vickery et al. 2009).

A summary of effective management schemes using conservation fallows ("Naturschutzbrachen") to enhance the value of arable sites for plants and invertebrates is given in Berger & Pfeffer (2011). In addition, several organizations like "Netzwerk Blühende Landschaft"[20] or "Lebensraum Brache"[21] (current project: "Energie aus Wildpflanzen" (Energy from wild plants)) provide seed mixes and information material to farmers and other landowners to improve the food sources and habitat quality for e.g. pollinating insects in agricultural landscape.

The third goal includes the **appropriate management of off-field habitats**. A complex and/or high vegetation structure seems to be also positively correlated to high densities of most arthropod groups while mowing and grazing affect at least some of the tested organism groups negatively (Table 3.1-2). Therefore, we propose for field margins at the farm scale a

[20] For more information see: http://www.bluehende-landschaft.de/ (last access: 19.01.2012)
[21] For more information see: http://www.lebensraum-brache.de/Projekte/Biogas/index.php (last access: 17.01.2012)

mowing strategy based on a mixture of annual and perennial mowing rhythms. This also should ensure the seed production of non-weed plant species which would be beneficial for granivorous invertebrates (e.g. some ground beetles of the genus *Amara*) and vertebrate (e.g. birds) species.

To ensure an adequate amount of off-field (field margins, hedgerows) as well as in-field (buffer strips, fallows) arthropod habitats, it might be useful to establish such habitats on a defined percentage of the land area per farm. In Switzerland, ecological compensation areas ("ökologische Ausgleichsflächen") have to be established at 7% of the agriculturally used area per farm according to national legislation. Current proposals of the European Commission for the CAP reform also include the maintaining of “ecological focus area” of at least 7% of farmland (Ciolos 2011). But not only the total amount of habitat is crucial: These structures have to be proper arranged in the landscape to allow a habitat network and cover a range of several habitat types (Jenny et al. 2002).

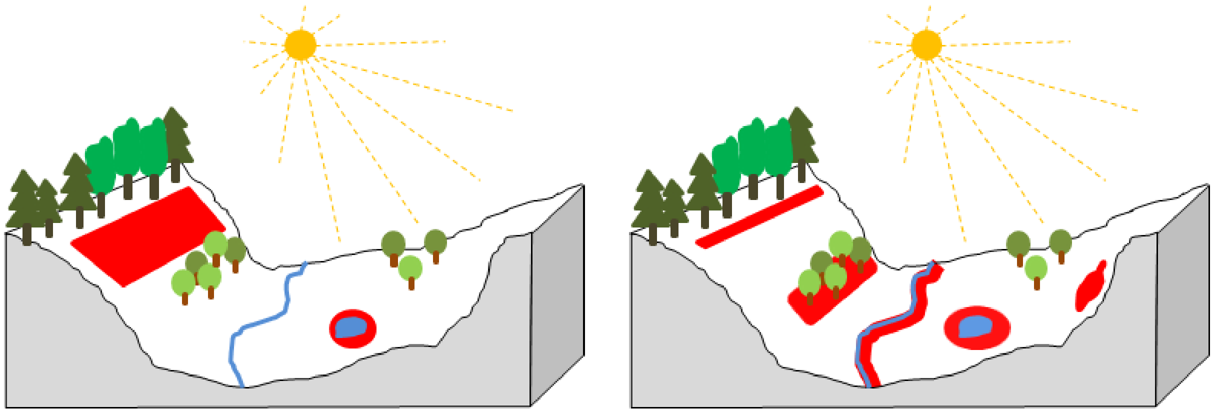

Figure 3.4-2: Negative (left) and positive (right) example for the arrangement of ecological compensation areas (in red) in a landscape. (after Jenny et al. 2002, figure modified)

3.4.3.2 Benefits of management schemes when including non-arthropod organisms

The enrichment of the landscape with habitats mentioned above can – next to arthropods – benefit further organism groups, especially those feeding on arthropods or seeds (e.g. birds), but also those who will preferentially colonize the new sites. For farmers, the enlargement of off-field habitats as well as the creation of new (permanent) field margins or hedgerows on previously farmed areas is associated with a long-lasting reduction of in-field area. However, in-field schemes like the creation of conservation headlands, grass strips, or flower strips are normally of short duration (one to several years) and, hence, can be applied more easily by farmers since they do not reduce their field size in the long run. In the following, organism groups which are known to benefit from the creation of two types (cropped and uncropped) of in-field buffer strips are summarized (Table 3.4-4).

Table 3.4-4: Benefits arising from two in-field management schemes (cropped and uncropped in-field buffer strips) for different organism groups.

Organisms	Benefits	Literature
Cropped in-field buffer strips		
Plants (especially: rare weeds)	Less herbicide input than in fully sprayed field edges	Schumacher (1980); Göttlicher-Göbel (1988); Chiverton (1994); de Snoo (1999); Vickery et al. (2009)
Flower-visiting insects	Less insecticide input than in fully sprayed field edges and good foraging habitat	Rands & Sotherton (1986); Cowgill et al. (1993); Dover (1997)
Arthropods	Less insecticide input and higher plant species richness than in fully sprayed field edges	Hassall et al. (1992); Chiverton (1994); Welling et al. (1994); de Snoo (1999); Vickery et al. (2009)
Mammals (wood mice)	Good foraging habitat	Tew et al. (1992); MacDonald et al. (2007)
Birds	Good foraging habitat	Chiverton (1994); de Snoo (1999); Vickery et al. (2009); Ewald et al. (2010)
Uncropped, sown in-field buffer strips and fallows with a (high) flower percentage		
Flower-visiting insects	Good foraging habitat	Meek et al. (2002); Carvell et al. (2007); Vickery et al. (2009); Haaland & Gyllin (2010); Haaland et al. (2011)
Herbivores	Good foraging habitat	Zurbrugg & Frank (2006); Haaland et al. (2011)
Arthropods	Good foraging habitat	Holland & Luff (2000); Zurbrugg & Frank (2006)
Predatory arthropods	Overwintering sites and (foraging) habitat	Holland & Luff (2000); Pfiffner & Luka (2000); Lemke & Poehling (2002); Zurbrugg & Frank (2006); Haaland et al. (2011)
Mammals (voles)	Habitat	Aschwanden et al. (2007)
Birds	Good foraging habitat	Vickery et al. (2009)

In addition, there are strong coherences with regard to other legislations and settlements (chapter 3.2.3), e.g. biotope network (BNatSchG, National Strategy on Biological Diversity), reduction of plant protection product inputs (National Strategy on Biological Diversity) or the preservation of threatened species (BNatSchG, Habitats Directive).

3.4.3.3 Practicability of management schemes supporting terrestrial biodiversity

Information on agricultural issues (e.g. costs of conservation headlands on heavy soils, Boatman et al. 1999), supply of food resources (plants, seed, and insects), and/ or accessibility is predominantly provided for in-field management schemes (de Snoo 1994; 1999; Boatman et al. 2004; Berger & Pfeffer 2011). In general, these schemes (cropped/ uncropped buffer strips, fallows) are easy to establish although local conditions (e.g. soils, weed problems) have to be considered since they can influence the costs or efforts for farmers to manage the habitats. Such schemes are also available as agri-environmental schemes which can offer an additional incentive for farmers to create in-field habitat structures. However, not all agri-environment schemes are available in all federal states of Germany (for an overview see:

Thomas et al. 2009). A review of the environmental benefits of current agri-environment schemes in the UK revealed that invertebrates and other organism groups profit by e.g. uncropped wildlife strips and conservation headlands, although benefits may differ between the groups (Boatman et al. 2008). An overview is given in Table 3.4-5.

Table 3.4-5: Overview of some proposed management schemes and their practicability based on literature statements. (+): low practicability, (+ +): mean practicability, (+ + +): good practicability, ?: no information found. AES: financial support via agri-environment schemes (for details see: Thomas et al. 2009). Grey background: management scheme proposals resulting from this R+D-project.

management scheme	crop	practicability	literature	AES
enlargement of off-field habitat (*permanently uncultivated*)	all	(+) to (+ +)	Boatman et al. (2004)	no
in-field buffer strips (cropped and uncropped) or conservation fallows *Creation at the field edge*	arable crops	(+ +) to (+ + +)	Berger & Pfeffer (2011) Boatman et al. (2004) Boatman et al. (1999) de Snoo (1999)	yes
in-field conservation fallows or beetle banks (uncropped) *Creation not at the field edge*	arable crops	(+ +) to (+ + +)	Berger & Pfeffer (2011) Boatman et al. (2004)	yes
avoidance of overspraying of off-field habitats	arable crops	?		no
annual and perennial mowing rhythms of field margins	all	?		no

3.4.3.4 In-field risk assessment and management of plant protection products

Unlike the assessment of potential risks arising from biocide use, where no differentiation is done beween in-field and off-field scenarios, for plant protection products the scenarios in-field and off-field are separately assessed. To date there is no evaluation of risk for the arthropod communities in the cropped fields in Germany regarding plant protection products. Only an evaluation of the pest control capacity is performed by the BVL (BVL 2011b). Here the toxicity of a plant protection product on a group of selected beneficial arthropods is used to recommend the product to growers as beneficial friendly. This is especially important if integrated pest management (IPM) is used by the farmer.

However, if the assessment of biomass of arthropods because of their function as food for many organisms in the landscape is taken into account (see chapter 3.3.1.7), risk assessment and management procedures also need to be established for the in-field area. This is simply because the majority of area in the agricultural landscape is cropped and only a low percentage is un-cropped. Although this un-cropped area is proposed to reach 7% on a farm scale (see above), it is possibly not enough to actually improve the food basis for many arthropods and vertebrates and to effectively restore biodiversity.

In-crop management is even more controversial than any changes in the off-crop area scheme. The main argument for its unfeasibility is that the costs for such 'nature conservation actions' are high and should be paid by the public (and not the farmer or industry) and decided democratically. However, this debate is heavily influenced by stakeholders that are interpreting the facts to support specific interests. We refer for example to a publication on 'Agriculture and biodiversity'[22] published in 2010 by e.g. the European Landowners Organization ELO and the European Crop Protection Association ECPA where the word 'pesticide' as a factor for the decrease of biodiversity in agricultural landscapes is not even mentioned (Riffel et al. 2010).

In-crop arthropod risk assessment should evaluate the magnitude of the plant protection product effect and measure biomass as criteria for the registration process as was proposed for the off-crop area. In our opinion, authorization of plant protection products should shift from single product assessments towards entire management packages (e.g. fungicides, herbicides and potential insecticide for a wheat farm) that can be evaluated in a total risk assessment approach for the crop in question. In this aspect, even landscape features for a regional registration could be taken into account. Management options could include changes in regional use patterns that do not allow simultaneous use of similar products on a landscape scale, adoptions of in-field no spray zones and a general reduction of plant protection products according to the reduction of plant protection product inputs (BMU 2007).

In cropped areas, methods of organic agriculture or integrated pest management (e.g. crop rotation, cultivation of crop varieties with resistances towards plant diseases, mechanical weed control, encouragement of beneficials) can be useful schemes to reduce the need for the application of e.g. insecticides (Dedryver et al. 2010) and herbicides (Chikowo et al. 2009). However, some of these methods (e.g. more frequent soil tillage to reduce weeds) can also affect several organism groups negatively (Deytieux et al. 2012). Nonetheless, a reduced plant protection product application in the cropped area decreases the plant protection product inputs in adjacent non-crop habitats.

3.4.4 Risk management of plant protection products for soil organisms – no-tillage soil cultivation

Conservation tillage means the crop planting system which leaves at least 30% or more of the crop residues on the soil surface instead of plowing them under (Hendrix et al. 1986). This procedure is soil conserving and results in a litter layer that influences the community of soil organisms (Hättenschwiler et al. 2005). From the farmer's point of view, the reduction of potential weeds by plowing is skipped, leading to the need for handling the growth of weeds before or during the emergence of the crop. Routinely, herbicides are applied, and the question arises whether this input of plant protection products on-top of the already employed affects the environment negatively.

In a literature survey, 20 studies were available dealing with tillage, soil organisms and their quantitative responses. Nine studies were conducted with soil surface active arthropods, namely carabids, spiders and ants (Table 3.4-6). In the remaining eleven studies, soil organisms were examined regarding their response to tillage and no-tillage (Table 3.4-7). Both for surface active arthropods and for soil organisms, no-tillage - with or without the use of herbicides – resulted to an increased abundance in the vast majority of cases. However, the taxonomic resolution is low in most studies and may hide large species-specific differences.

[22] Available on http://www.ecpa.eu/files/gavin/ECPA_agriculture_and_biodiversity.pdf (last access: 10.12.2012)

Concerning the use of herbicides, all studies – with the exception of one study (House & Parmelee 1985) – used the herbicide treatment only under no-tillage conditions. Therefore, the effects of herbicide and no-tillage are confounded, and a clear-cut distinction between herbicide and no-tillage effect cannot be made. To clarify this point, further research is needed.

Table 3.4-6: Influence of Tillage on Selected Arthropod Groups

Crop	Soil treatment	Taxonomic group	Endpoint	Change relative to tillage-treatment	Literature
sorghum	herbicides	Spiders	species number	>	Blumberg & Crossley (1983)
sorghum	herbicides	Carabidae	abundance	>>	House & Parmelee (1985)
		Araneae		>>	
barley	herbicides	Carabidae	species number	>	Cárcamo et al. (1995)
faba bean				>	
grain	---	Carabidae	abundance	<	Huusela-Veistola (1996)
rotation	---	Carabidae	abundance	>	Hummel et al. (2002)
		Staphylinidae		>	
		Lycosidae		>	
wheat	---	Carabidae	abundance	>	Holland & Reynolds (2003a)
		Araneae		>	
maize	herbicides	Formicidae	species number	>	Badji et al. (2006)
wheat	---	Carabidae	mean capture rate	>	Hatten et al. (2007a)
peas				<	
spring pea	---	Carabidae	abundance	>>	Hatten et al. (2007b)
spring wheat	herbicides			>	
spring barley				>>	
winter wheat				>>	

Table 3.4-7: Influence of Tillage on Selected Soil Organisms

Crop	Soil treatment	Taxonomic group	Endpoint	Change relative to tillage-treatment	Literature
sorghum	herbicides	Lumbricidae	abundance	>>	House & Parmelee (1985)
		Enchytraeidae		<	
maize	herbicides	Lumbricidae	abundance	>>	Mackay & Kladivko (1995)
soybean				>>	
rape	---	Lumbricidae	abundance	>	Daugbjerg et al. (1988)
wheat-soybean-corn	herbicides	soil arthropods	abundance	>>	House (1989)
	---			>>	
barley	---	Enchytraeidae	abundance	>	Lagerlöf et al. (1989)
rotation	herbicides	Lumbricidae	abundance	>	Parmalee et al. (1990)
		Enchytraeidae		>	
rotation	herbicides	Oribatida	abundance	>	Perdue & Crossley Jr. (1989)
		Mesostigmata		>	
maize	---	Lumbricidae	abundance	>>	Reeleder et al. (2006)
		Acarina		>	
		Collembola		no difference	
maize	herbicides	Collembola	abundance	>>	Rodríguez et al. (2006)
		Acarina		>	
maize	herbicides	Oribatida	abundance	>>	Badji et al. (2007)
		Gamasida		>	
		Collembola		>	
maize	---	Acarina	abundance	>	Tabaglio et al. (2009)
		Collembola		>	
		Coleoptera larvae		>>	

In short:

- For biocides, risk mitigation measures consider the placing on the market (e.g. user restrictions, area of application), the application of biocidal products (e.g. equipment), and/or the post-application time (e.g. storage of treated wood and manure, waiting periods, wastewater treatments). Specific risk mitigation measurements with regard to in-field or off-field habitats do not exist as there is no distinction between these habitats in the environmental risk assessment of biocides.
- For plant protection products, the German risk management for terrestrial off-field areas is based on use restrictions (usage of low drift nozzles and/or distance requirements). In many cases, these use restrictions have not to be implemented by farmers due to existing exceptions (e.g. next to off-field structures smaller than 3m).
- To enhance the management of agricultural landscapes to support terrestrial biodiversity, it is proposed to include three additional goals: (1) the preservation and enhancement of the existing field margins and hedgerows including a reduction of inputs of plant protection products (e.g. in-field buffer strips), (2) the increase of plant species richness and the provision of adequate floral resources from the field to the landscape level, (3) the appropriate management of off-field habitats to create e.g. areas with varying structural complexity of the vegetation.
- If the assessment of biomass of arthropods is taken into account because of their function as food for many organisms in the landscape, risk assessment and management procedures for plant protection products also need to be established for the in-field area (as already existent in Germany).
- Due to the allocation of a sufficient amount of high quality in- and off-field habitats, the abundance, species richness, diversity, and biomass of arthropods will be enhanced so that in-crop population losses could be compensated and an adequate food supply for arthropod-feeding species is provided.
- Not only the total amount of such in- and off-field habitat is crucial, these structures have also to be properly arranged in the landscape, so to allow the emergence of habitat networks and to cover a range of several habitat types.
- No-tillage management practices enhance on the one side the diversity of soil fauna in cropped fields. On the other side, however, the influence of herbicides that are always used in combination with no-tillage systems is difficult to evaluate from the current literature. Further research is required on this topic.

4. Assessment of pesticide exposure of amphibians and reptiles in agricultural landscapes in Germany and evaluation of the present pesticide risk assessment practice in EU

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4.1 Exposure of amphibian species to pesticides in Germany

4.1.1 Amphibians in agricultural landscapes

Of 89 amphibian species existing in Europe (Glandt 2010), 20 are endemic in Germany (Kühnel et al. 2009). Some species are widely distributed and are present both in lowland and mountain areas. Other species are characterised by a more regional presence (Figure 4.1-1). Seventeen species occur in rich structured agricultural landscapes consisting of a mosaic of arable fields, forests and grasslands (Table 4.1-1).

Table 4.1-1: Landscape types used as habitats by amphibians (Berger et al. 2011a, modified)

	landscape type				
species	open, pioneer character	open agricultural landscape	rich structured agric. landscape; mosaic of arable fields, forest, grassland	floodplain, rich in grassland	forests
Mountain newt (*Triturus alpestris*, Laurenti 1768)		xx	xxx	xx	xxx
Common toad (*Bufo bufo*, Linnaeus 1758)		x	xxx	xx	xxx
Palmate newt (*Triturus helveticus*, Razoumow. 1789)		x	xx		xxx
Fire salamander (*Salamandra salamandra*, L. 1758)					xxx
Yellow-bellied toad (*Bombina variegata*, Linnaeus 1758)	xxx		x		x
Midwife toad (*Alytes obstetricans*, Laurenti 1768)	xxx		x	x	x
Common frog (*Rana temporaria*, Linnaeus 1758)	x	xx	xxx	xx	xxx
Great crested newt (*Triturus cristatus*, Laurenti 1768)[23]		xx	xxx	xxx	x
Pool frog (*Rana lessonae*, Camerano 1882)		x	xx	xx	xxx
Common spadefoot (*Pelobates fuscus*, Laurenti 1768)	xx	xxx	xx	x	
Natterjack toad (*Bufo calamita*, Laurenti 1768)	xxx	xx	x	x	
Tree frog (*Hyla arborea*, Linnaeus 1758)		x	xxx	xx	x
Moor frog (*Rana arvalis*, Nilsson 1842)		x	xx	xx	xxx
Fire-bellied toad (*Bombina bombina*, Linnaeus 1761)	x	xx	xx	xxx	
Marsh frog (*Rana ridibunda*, Pallas 1771)		x	x	xxx	xxx
Agile frog (*Rana dalmatina*, Bonaparte 1840)		x	xx	x	xxx
Edible frog (*Rana kl. esculenta*, Linnaeus 1758)	x	xx	xx	xxx	x
Smooth newt (*Triturus vulgaris*, Linnaeus 1758)	x	xx	xxx	xx	x
Green toad (*Bufo viridis*, Laurenti 1768)	xxx	xx	xx	xx	

x = little relevance; xx = medium relevance; xxx = high relevance

[23] Following referred as crested newt

In open agricultural landscapes with higher propotions of arable land, at least seven amphibian species are relevant and one species, the spadefoot toad, has its main distribution in this type of landscape (see “xxx” in columns “open agricultural landscapes” and “rich structured agric. landscape; mosaic of arable fields, forest, grassland” of Table 4.1-1). These seven species at minimum are to be considered when dealing with potential threats to amphibians caused by pesticides. More amphibian species, however, can be assumed to be at risk too.

Most results presented in the following chapters refer to four typical species: The crested newt and spadefoot toad are more or less widely distributed throughout Germany as well as the moor frog and fire-bellied toad present in the pond rich areas of the northern and north-eastern part of Germany respectively (Figure 4.1-1).

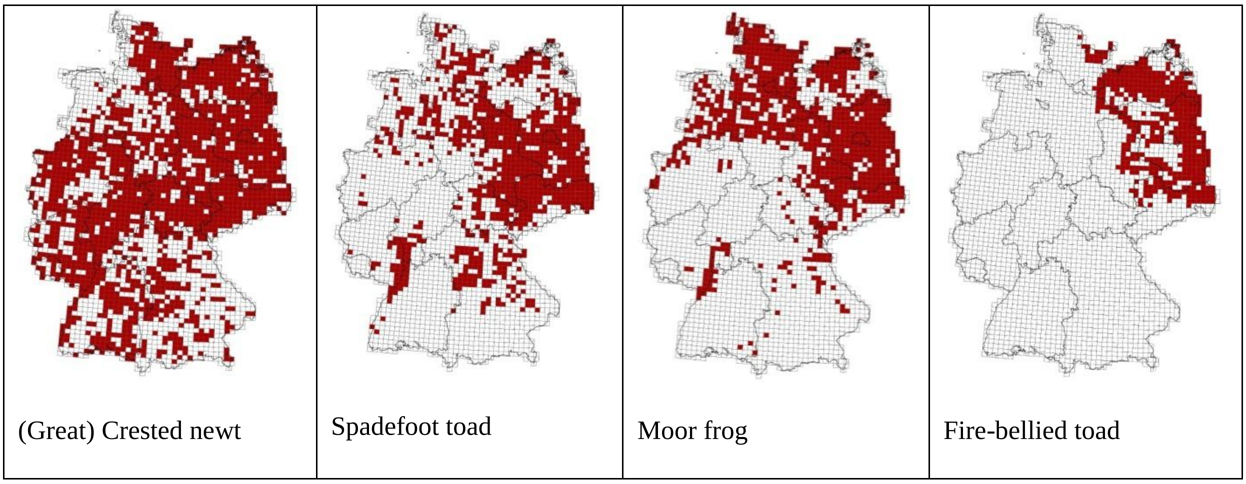


Figure 4.1-1: Spatial distribution of four species of amphibians with higher presence also in open landscapes with substantial share of arable land (BfN 2008)

Principle habit/behaviour of amphibians and species specific deviations

Amphibians are biphasic organisms with life stages requiring aquatic and terrestrial habitat (Semlitsch & Bodie 2003). Because of possible spatial distances between these elements, migrations over land are typical. All species leave terrestrial hibernation sites in spring (mainly from March to May) and migrate towards breeding water bodies (Figure 4.1-2). The time and duration of migration periods vary between species and years. The prevailing migration activity often depends on the weather situation. Males usually migrate slightly earlier than females. Subadults can have a behaviour comparable to adults, but it may be also very different (Günther 1996b; Laufer et al. 2007b).

There are differences between species with regard to the duration of the aquatic phase and the time of leaving ponds. Adults of species like the fire-bellied toad and newt species usually stay in ponds until the end of summer – or even later –, partly also migrating between water bodies. Most species, e.g. the moor frog and spadefoot toad, leave the breeding ponds immediately after spawning, stay for a short period in the environment of ponds and/or migrate towards terrestrial summer habitats. In these habitats, they often have a more or less stationary behaviour with feeding, resting and daily migration activities of only some 10 metres. Some of these summer habitats can be used for hibernation too. But often, autumn migrations towards separate hibernation sites take place. Species leaving ponds late in the year often migrate directly towards hibernation sites where feeding activity and resting takes place until the beginning of the winter period (Günther 1996b; Laufer et al. 2007b).

Juveniles usually leave water bodies from June until September/October. They partly stay next to their breeding pond for some weeks (moor frog, fire-bellied toad). Most species' juveniles perform a longer lasting emigration period successively leaving the ponds. Other species like moor frog or spadefoot toad, however, have large migration activities during only few days leading to explosive emigrations from ponds and migrations over land. Long time presence on fields is avoided by most species. Exceptions count for spadefoot toad which often has terrestrial (summer and winter) habitats with loose substrates and for other species which use specific micro-habitats in fields (see chapter 4.1.5). Amphibians on land are mostly nocturnally active. During specific periods, however, activity during day time may occur. During land sojourn, amphibians hide in burrows of small mammals, below plant shelter and under wooden or other items covered by soil. Some species, in particular the spadefoot toad, rest some centimetres deep in loose soil (Nöllert 1990).

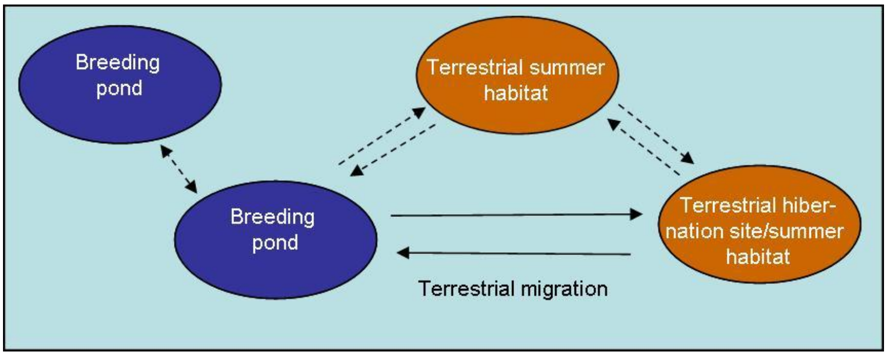


Figure 4.1-2: General biphasic behaviour of amphibians: Both terrestrial and aquatic habitats are used leading to more or less extensive migration on terrestrial sites (Berger et al. 2011a, p.40[24])

Regional differences in the behaviour of amphibians

Knowledge on the regional variability of amphibian activity and in particular during important terrestrial phases is essential to estimate coincidence with pesticide application in agriculture, which also has a regional variability (chapter 4.1.2). We surveyed existing regional data sources on amphibian terrestrial activity mainly during migration from hibernation sites into breeding ponds and partly also the return journey. We checked via www.herpetofauna.de (10.01.2012) for people/organisations having this kind of data and providing them. We ended up with a total of 11 respondents delivering data sets from 27 sites in Germany for a time span of all together 10 years. Going more into detail, however, we identified the following limitations in the collected data sets:

- no species data set was largely covering the German territory in the same year,
- investigation years were very different not allowing valid in-year analyses,

[24] We partly refer to specific pages in Berger et al. (2011a) with further explanations. A copy is sent to UBA separately.

- most data were recorded by volunteers of environmental organisations or private persons who did not check the traps daily but mostly under “migration conditions”. The purpose of this was not to investigate animal behaviour but to help individuals safely pass roads.
- gender and age of the animals –which is important because of specific migration behaviour– very often were not recorded,
- data collected by professional landscape planners usually could not be verified and completed because of staff fluctuations.
- Only one data set from the BfN research project “Drachenfelser Ländchen” from 2000-2003 would have been appropriate but did not cover any regional variability.

Due to the lack of suitable data, we were not able to analyse the regional variability of amphibian behaviour. This is a significant data gap and we therefore recommend conducting appropriate scientific field investigations in different regions of Germany and Europe in future.

4.1.2 Crop management measures involving plant protection product application, its variability across Germany and new management systems

The exposure of amphibians to plant protection products present on agricultural fields mainly depends on the crop specific management of plant protection products and the canopy development influencing the retention of chemicals (interception) in the crop layer. Both the presence of crops on fields and the development of their vegetation cover over time vary between crop species. The field management in total as well as the application of plant protection products is conducted depending on the crop and its canopy development. Hence, the number and timing of plant protection product applications vary strongly among crops – also as the proportion of applied herbicides, fungicides, insecticides and growth regulators differs. Furthermore, regional differences in the use of plant protection products are to be considered.

Timing of crop presence on fields and crop specific plant protection product applications

Suitable data on regional management of plant protection products of field crops at field and farm level are scarce. The most detailed information on the application of plant protection products surveyed on the national scale is available from investigations done in 2000 (Roßberg et al. 2002). Since then, no systematic survey of plant protection products use on farms on the national level has taken place. Furthermore, the usage of products and related active ingredients as well as the number of applications is assumed to have largely changed over the last 10 to 15 years (Roßberg 2011, Julius Kühn Institute JKI, personal communication). Because of this rapid development in agriculture (chapter 4.1.3) the data from Roßberg et al. (2002) are not appropriate to depict the today’s management of plant protection products. Since 2007, an annual survey of plant protection products has been done on a “Network of Reference Farms for Plant Protection” in Germany (Freier et al. 2010; 2011, Appendix 5.6). Because of data protection issues, no information on the crop specific timing and other characteristic of plant protection product applications on the field level is available. We therefore relied on expert knowledge (Dr. Reinhold Roth, retired from working at Leibniz

Centre for Agricultural Landscape Research, pers. comm.) to create typical and representative plant protection product management schemes of field crops under normal/average conditions –excluding extreme events leading to higher application rates (Table 4.1-2). These estimations are representative for the lowland area of Middle Germany except for Western and Northern regions with Atlantic climate. We validated them using own investigations of farm management measures on a daily base from 2006 to 2008 on 3,100 ha farmland and seven farms in East-Brandenburg (Berger et al. 2011a, p. 161ff).

Table 4.1-2: Crop presence and management of plant protection products on fields (decadal values) representative for the low land area of Middle Germany (Roth 2011, ZALF, personal communication). Legend: head line: month and number of decade per year; solid coloured bars: regular presence of crops on fields; strips left or right to solid coloured bars: non-regular presence of crops on fields; H … herbicide application, F … fungicide application, I … insecticide application; GR… growth regulator application; letters in (): non regular applications depending on the specific situation; letters with numbers: single application possible in more than one decade.

crop		July			August			September			October			November			December			January			February			March			April			May			June			July			August			September			October			November			December	
	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
winter rape					H		(H) + (I)		GR																		GR		I	I	I	I + F					I + F																	
winter barley										H + (I)																			(GR)		F + GR																							
winter rye											H + (I)																	GR		F + GR						(F)																		
winter triticale											H																(H)		GR		F																							
winter wheat											H + (I)																H1	(GR) + (H1)		F1	F1 + GR		(I)			F			(I)															
silage maize																															H		(H)						(I)															
corn																															H		(H)						(I)															
sun flowers																												H						I									(F)											
blue lupine																												H						(I)																				
field bean																									H				(H)				F + I																					
field pea																									H				(H)	(I)			(I)	I																				
sugar beet																											H					H							(I) + F															
potatoes																															H			F		F + I	F		F + I				(H)											
oat																										H					GR + (H)				(I)																			
summer barley																									(H)			H			GR + F			F																				

4.1.3 Number of plant protection product applications to field crops and its regional variability across Germany

Treatment index and its variability across Germany

Differences in application of plant protection products between regions and years may influence the likelihood of amphibians' exposure to plant protection products. Plant protection product types, application rates and timing vary depending on, inter alia, the agro-ecological environment, crop species, plant protection product availability and price, and the farmers' respective pest management strategies (Freier et al. 2010; Roth & Rosner 2011b). Hence, there is a multi-factorial variability in space and time. For a representative characterisation of plant protection product use, a spatial segmentation procedure is required that uses a stratified sampling design integrating different information layers. Roßberg et al. (2007) established a soil-climate-region (SCR) classification of Germany based on soil, climate and community border information (Appendix 5.6). We use the treatment index (TI) for discussing regional variability of plant protection product application (for details on the method, see Appendix 5.6).

Appendix 5.7 shows the treatment index (TI) for different crops and the associated plant protection products in Germany. The figures illustrate the average TI in winter wheat, winter barley and winter oilseed rape in Germany from 2007 to 2010. It shows a similar intensity in herbicide use for the three crops and four years despite large variations between replicate fields. High intensities are found for fungicides in winter wheat. Insecticides are applied in winter canola more than twice as often compared to winter wheat and winter barley, especially in 2009 and 2010. In winter barley, the insecticide applications in 2009 and 2010 were particularly low. Growth regulators were relatively uniformly applied in all three crops. For other crops (potato, corn, triticale, winter rye and sugar beet), there are less data available (data not shown). TI is highest for potatoes. Maize has the lowest TI with only herbicides used. In triticale, the intensity of treatment was similar to that in the winter barley, and varied hardly between years. Winter rye showed a very similar level as triticale, with major differences between years, especially with the fungicide applications. The plant protection intensity in sugar beet is determined by herbicide applications, with little annual differences.

Appendix 5.7 (Table 5.7-2) exhibits in more detail the differences between regions and years based on the calculations for winter wheat. It provides an overview of the TIs and informs about the significant differences between the major regions between 2007 and 2010. The mean TIs (all categories of plant protection products) ranged between 5.4 and 6.2. The overall TI in 2008 (6.2) was significantly higher compared to other years. In all four years, the treatment differed significantly between the major regions with reduced total indices in the South and East regions of Germany towards those in the North and West, except for 2010. There were significant differences in the individual plant protection product categories among the major regions and years. The South had lowest TIs in insecticides and growth regulators. In the North and partly in the West the TIs for fungicides were significantly higher than those in other major German regions. In the various plant protection product categories, there were mostly minor, non-significant differences between the years (Freier et al. 2011). Altogether, there was a large variance for all plant protection product categories in all four years both within Germany and within the large regions indicating a greater variability in the potential exposure of amphibians to plant protection products.

Plant protection product application to field crops in the investigation area of Eggersdorf

The following quantitative analyses on the temporal coincidence of amphibian presence and plant protection product application are based on field investigations in the area "Eggersdorf" (North-East Germany). From 2006 to 2008, we conducted an intensive monitoring programme on amphibian activity in terrestrial habitats on 800 ha of arable landscape, using 52 daily checked trap fences of 3,600 m length in total (Berger et al. 2011a p.81ff). We additionally recorded the field management measures of 10 summer and winter crops on a daily base on 3,100 ha of arable land and on 131 fields (Appendix 5.7, Table 5.7-2). For every crop, we calculated the average frequency of the application of herbicides, fungicides, insecticides, and growth regulators and other plant protection products (e.g. effect improver or micro fertiliser applied with plant protection products). Because of differences in the analysed number of fields of winter crops in the time period before July and starting from August onwards, we analysed these two periods separately. Then we calculated the frequency of plant protection product applications per year. This value is comparable to the treatment index (TI) used by Freier (Freier et al. 2011). However, we did not consider any reduction in quantity for both active substance and treatments if only subparts of field were treated. Thus, real TI values for plant protection product application in "Eggersdorf" may be slightly lower.

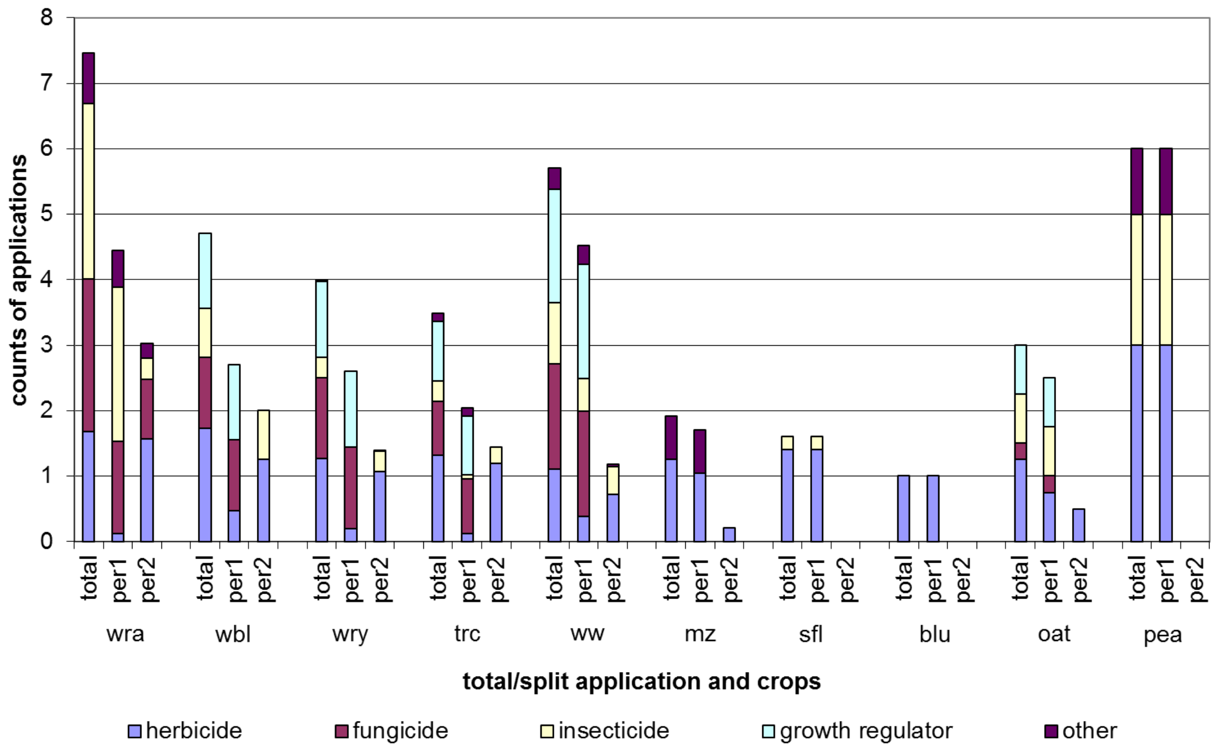


Figure 4.1-3: Total and periodically split application of plant protection products, growth regulators and other related chemicals to field crops (per1: 01.01.-31.07; per2: 01.08.-31.12., data from 2006 and 2007, investigation area Eggersdorf; wra winter rape, wbl winter barley, wry winter rye, trc triticale, ww winter wheat, mz maize, sfl sunflower, blu blue lupine).

The plant protection product application frequencies for Eggersdorf shown in Figure 4.1-3 are generally consistent with the TI values of Freier et al. (2011). The values ranging between 6.8 and 5.3 for winter rape and winter wheat, respectively, correspond well to average TI for the East German region as presented by Freier et al. (2011) and thus are considered appropriate and representative for analysis of the temporal coincidences of amphibians with plant protection product applications. The differences in frequencies between the two periods (until and after July) indicate much higher application frequencies for all crops in the first period. Summer crops like sun flowers, blue lupine and field pea are only treated with plant protection products in the first period.

Changes in crop share and application of plant protection products over time and new developments in cultivation of crops in Germany

The potential exposure to plant protection products of amphibians present in agricultural fields depends on the field crops and their specific vegetation development and plant protection product management, respectively. Changes in the share of the crops grown and their plant protection product management as well as new developments of plant protection product use may influence this potential.

Changes in crop share over time. During the last 15 years and in particular within the last five years, significant changes occurred in the cultivation of different field crops over the year (Appendix 5.8). While the share of arable land slightly increased to the disadvantage of grassland, the relative share of cereals grown on arable land achieved a climax in 2005 and decreased since then. From 2005 until now there was a strong increase of the relative share of green plants predominantly used as renewable energy plants, particularly silage maize. This development may be of importance for amphibians, because they might be affected by the different vegetation development of crops providing cover and shelter and their crop specific application schemes of plant protection products. Important is also the decreasing share of grassland, a preferred terrestrial habitat type of amphibians during summer and autumn and usually with no or very little application of plant protection products.

Changes in plant protection product use over time may lead to changes in the exposure risk of amphibians to these chemicals. Current data on employed quantities for specific plant protection product categories and rankings of their active ingredients is restricted to sales statistics, which can be assumed to largely picture the real usage of plant protection products in Germany (Seng 2011, Bundesamt für Verbraucherschutz (BVL), personal communication, Appendix 5.9: Table 5.9-1). Data indicate a slight increase in herbicide and fungicide application of less than 10 %, but a significant increase in insecticide usage by nearly 60 % over the last 12 years.

According to BVL (2011a), the total amount of authorised plant protection products has decreased by 35 % since 2001 and adds up to 644 products in 2010. To account for the increased sales, authorized plant protection products are used to a greater extent. Appendix 5.9 (Table 5.9-2) lists the most important active ingredient quantities sold in 2010 in Germany. It shows that Glyphosate and Isoproturon dominate among the herbicides; Mancozeb and Sulfur dominate among the fungicides; and Methiocarp, Thiacloprid and Dimethoat are most common among the insecticides. The comparison of the present data with the data of Roßberg et al. (2002) in 2000 shows that the changes in the application of single active ingredients tremendous within ten years.

While the amount of herbicides increases only slightly since 1999, there is a considerable and statistically significant shift towards increasing relative share of glyphosate (Appendix 5.9: Figure 5.9-1). This indicates both rapid changes in agricultural practices in total but in particular with regard to the usage of plant protection products. Application schemes of plant protection products are currently changing. Particularly Glyphosate is becoming more relevant for controlling not only competitive herbs and grasses (target organisms) but also for the entire production process –including reduced soil cultivation and seed bed preparation, erosion prevention and/or controlled ripening and harvesting of crops (see also next chapter and Griffin et al. 2010).

Important developments in crop cultivation and implications for usage of plant protection products

Agricultural science and machine industry provide new technologies leading to more economic efficiency. Often they are also linked or dedicated to environmental demands. In the following we present some technologies leading to modified plant protection product application schemes potentially changing the plant protection product exposure risk to amphibian populations –as well as of other non-target organisms.

Reduced soil tillage and no till cultivation: Ploughing is a typical feature of soil tillage. Increasing expenses linked to high fuel and time consumption and disadvantages with regard to soil fertility and erosion lead to new soil tillage techniques or even no till measures in agriculture (DLZ spezial 2010; Ackerplus 2012). Ploughing also controls herbs, but increasingly total herbicides mainly based on Glyphosate take over this function in low till systems. They are applied to the weeds some weeks before the following crop is sown. For summer crops this is done in spring and for winter crops between summer and early autumn. This means that at least one additional plant protection product application is occurring.

Reduced soil tillage of silage maize under conditions of water shortage in spring: In some agricultural regions of Germany, water supply during spring and early summer limits crop production. In particular regarding crops producing much green biomass (silage maize), water saving technologies are required. Therefore, total herbicides are applied very early in the year to green manure crops preceding maize so to stop them transpiring. Thus, “normal” application dates are moved from April to March, matching more amphibian species and higher population shares migrating from hibernation sites to breeding ponds.

Increasing share of only few crops, partly in monoculture: Because of economic interest and necessities, the crop rotation principles leading to appropriate and effective phyto-sanitary conditions are partially abandoned. This leads to critically high relative shares, for instance of winter rape of 30-40 % in some farms of North-East Germany, and promotes increasing application rates of insecticides due to insect resistance (Heidel 2010; Makowski 2011). Likewise, the monocultures of corn and silage maize in some regions of Germany are to be critically reflected. Treating with plant protection products *Diabrotica virgifera* presently invading Germany leads to intensive insecticide applications during summer and/or spring (LfL 2011; GAU 2012). These loads of plant protection products increase the exposure risk to amphibians and of other non-target organisms.

Simultaneous "ripening" of crops by Glyphosate application: Ripening of crops can be homogenised by herbicide application more or less independently from weather conditions. This way harvesting is easier and the expenses for technical drying of harvested grain can be reduced (Griffin et al. 2010). But one additional herbicide application mainly during wet summer increases the exposure risk to e.g. amphibians.

4.1.4 Linking migration activity and duration of stay of amphibians in terrestrial habitats to plant protection product applications and crop interception

Currently, most information on linkages between amphibians and plant protection product applications to field crops is based on literature reviews or published survey reports. For the present study, we performed the following analyses mainly based on our "Eggersdorf" investigation site (see previous chapter): a) the frequency of plant protection product application to winter rape, winter wheat and silage maize during main terrestrial phases of amphibians, b) the range of interception values of crops during these phases and c) the population share during field passage temporally coincident with plant protection product applications and the concurrent crop interception values.

4.1.4.1 Linking crop vegetation development, plant protection product interception and amphibian behaviour

The exposure risk to plant protection products of the amphibians present on the fields' soil surface depends not only on the applied field rate but also on the retention potential of the chemical by the crop canopy. In order to characterise the soil cover by field crops canopies, we used data obtained from field experiments conducted from 2006-2008 on five experimental stations in Germany (Vetter et al. 2011). During the vegetation period, both the canopy soil cover and the crop development stages (BBCH) were determined every 2 to 4 weeks. The dates were assigned to decades of the year. For decades without field investigations, lacking data were interpolated. To generalise this information for field crops throughout Germany, we pooled the canopy soil cover data from the experimental stations and years.

We combined canopy soil cover data and BBCH of three field crops (winter rape, winter wheat and maize) and assessed the interception of field crops by applying the crop interception values of FOCUS (2002). Appendix 5.12 shows that the soil coverage of winter rape and maize match well with the interception estimates. The interception values for winter wheat differed slightly. Hence, we estimated the interception values for a given combination of plant protection product application and crop development stage. All interception figures are based on data from intensive agriculture on fertile soils with a normal supply of fertilisers to crops. Less dense vegetation stands – because of low soil fertility, no fertilisation to crops or extreme site spots due to flooding etc. – were not considered (see chapter 4.1.5, wet spots as suitable habitat). We linked the vegetation development (soil cover and interception) with the expected plant protection product application and the major behaviour of adult and juvenile individuals of amphibian species, derived from literature and own investigations (Appendix 5.11).

4.1.4.2 Frequency of plant protection product application to field crops during terrestrial life phases of amphibians

Analysed species and their terrestrial phases

Based on data of Berger et al. (2011a), we determined terrestrial phases of spring migration of amphibian adults from hibernation sites into breeding ponds and of the terrestrial phase of adults and juveniles after leaving ponds until the end of the vegetation period. We assigned the dates of migration start and end to decades of the year (Table 4.1-3).

Table 4.1-3: Activity periods of adult and juvenile amphibians in terrestrial habitats. Data based on Berger et al. (2011a),* expected end of vegetation period.

species	age level	activity period	start		end		duration
			date	decade nr.	date	decade nr.	decades
crested newt	adult	spring migration	22.2.	6	18.4.	11	5
		after leaving ponds	21.8.	24	7.11.	31	7
	juvenil	after leaving ponds	21.8.	24	31.10.*	30	6
spadefoot toad	adult	spring migration	24.3.	9	10.5.	13	4
		after leaving ponds	26.4.	12	31.10.*	30	18
	juvenil	after leaving ponds	1.7.	19	31.10.*	30	11
moor frog	adult	spring migration	22.2.	6	29.3.	9	3
		after leaving ponds	10.4.	10	31.10.*	30	20
	juvenil	after leaving ponds	17.6.	17	31.10.*	30	13
fire bellied toad	adult	spring migration	10.3.	7	19.5.	14	7
		pond changing	15.5.	14	31.7.	21	7
		after leaving ponds	15.8.	23	5.10.	28	5
	juvenil	after leaving ponds	29.7.	21	31.10.*	30	9

The terrestrial phase of spring migration of adults into breeding ponds is usually shorter than the period after leaving ponds until the end of the vegetation period. Species like crested newt and fire-bellied toad perform a comparably short terrestrial phase after leaving ponds. In contrast, species leaving ponds early in the year, like spadefoot toad or moor frog, have long terrestrial phases of up to 20 decades during late spring, summer and autumn. With the exception of fire-bellied toad, the terrestrial phases of juveniles after leaving ponds are mostly shorter than those of adults.

Frequency of plant protection product applications to field crops

During spring migration of adults into breeding ponds, there are substantial differences in the frequency of plant protection product applications between crops and species (Appendix 5.13). Species active later in spring, like spadefoot toad and fire-bellied toad, more often face plant protection product applications than those migrating earlier. The application of fungicides (also acting as growth regulators in winter rape) and insecticides to winter rape showed the highest frequency compared to any other crops. For all species apart from the moor frog, similar risk from being exposed to plant protection products emerged showed similar trends: Fungicides were more frequently applied to winter cereals and winter rape, and herbicides more frequently to summer crops.

Adult spadefoot toad and moor frogs after leaving ponds faced maximum frequencies of plant protection product applications during their terrestrial phases, mostly exceeding the values found for spring migration into breeding ponds (Appendix 5.13). This was particularly true for winter cereals and winter rape, where the full set of plant protection products was applied in spring. But also summer crops like oat or field pea showed high application frequencies. Crested newt and fire-bellied toad staying longer in water bodies faced fewer plant protection product applications.

The numbers of plant protection product applications during the phase of juveniles “after leaving ponds” (Appendix 5.13) were much lower compared to those for adults. The application frequencies during this phase showed the same patterns for all species. Except for moor frog migrating through fields cultivated with pea, the plant protection product application to winter rape showed maximum values of plant protection product applications during juvenile migration, followed by winter barley and the other winter crops. The exception regarding the coincidence of pea cultivation and moor frog migration can be explained by the very early starting date of juveniles emigrating from ponds and high herbicide and insecticide application to pea.

4.1.4.3 Interception of crops during amphibians’ terrestrial activity periods

In Table 4.1-4, we show the range of interception values of the three field crops winter wheat, winter rape, and maize during terrestrial phases of amphibians. The red cells indicate low average interception values <=50% for herbicides affecting all amphibian species and age levels in nearly all crops. Particularly adults and juveniles after leaving ponds face high deposition of applied herbicides due to low interception values by the crops. Especially the fire-bellied toads migrating towards ponds in spring face high herbicide soil deposition in silage maize fields. Fungicides applied to field crops were retained by more than 50% by the growing crop. Low insecticide interception by the crops in autumn was only relevant to amphibians leaving ponds and partly for adults or juveniles. Other chemicals were usually applied in mixtures with plant protection products and thus not separately considered.

Table 4.1-4: Ranges of interception of plant protection products by crop canopy during terrestrial activity phases of amphibians (crops: wra … winter rape, wwt … winter wheat, mze … maize; data interception: FOCUS (2002); plant protection product application: Eggersdorf; BBCH: Vetter et al. (2011); orange: average values <= 50 % interception)

species	age level	activity period	crop	interception during period: minimum/average/maximum value (%)				
				herbicides	fungicides	insecticides	growth regulator	other
crested newt	adult	spring migration	wra	40/ 72/ 80	40/ 75/ 80	40/ 68.4/ 80		40/ 64/ 80
			wwt	50/ 50/ 50	50/ 50/ 50		50/ 50/ 50	50/ 50/ 50
			mze					
		after leaving ponds	wra	0/ 17.8/ 40	40/ 40/ 40	0/ 21.3/ 40		40/ 40/ 40
			wwt	0/ 7.8/ 25		0/ 5.7/ 25		0/ 0/ 0
			mze	75/ 75/ 75				
	juvenil	after leaving ponds	wra	0/ 17.8/ 40	40/ 40/ 40	0/ 21.3/ 40		40/ 40/ 40
			wwt	0/ 5.3/ 25		0/ 2.2/ 25		0/ 0/ 0
			mze	75/ 75/ 75				
spadefoot toad	adult	spring migration	wra	80/ 80/ 80	80/ 80/ 80	80/ 80/ 80		80/ 80/ 80
			wwt	50/ 57.5/ 70	50/ 60.5/ 70		50/ 55/ 70	50/ 50/ 50
			mze					
		after leaving ponds	wra	0/ 20.7/ 90	40/ 61.2/ 80	0/ 68.7/ 80		0/ 64.3/ 90
			wwt	0/ 20.8/ 90	50/ 77.8/ 90	0/ 52.5/ 90	50/ 62.8/ 90	0/ 53/ 70
			mze	25/ 32.1/ 75				25/ 25/ 25
	juvenil	after leaving ponds	wra	0/ 19.2/ 90	40/ 40/ 40	0/ 21.3/ 40		0/ 40.8/ 90
			wwt	0/ 11/ 90		0/ 2.2/ 25		0/ 0/ 0
			mze	75/ 75/ 75				
moor frog	adult	spring migration	wra	40/ 40/ 40	40/ 72.5/ 80	40/ 54.8/ 80		40/ 40/ 40
			wwt	50/ 50/ 50			50/ 50/ 50	
			mze					
		after leaving ponds	wra	0/ 23.5/ 90	40/ 62.7/ 80	0/ 72/ 80		0/ 66.9/ 90
			wwt	0/ 27.1/ 90	50/ 77.3/ 90	0/ 52.5/ 90	50/ 59.2/ 90	0/ 52.7/ 70
			mze	25/ 32.1/ 75				25/ 25/ 25
	juvenil	after leaving ponds	wra	0/ 19.2/ 90	40/ 40/ 40	0/ 21.3/ 40		0/ 40.8/ 90
			wwt	0/ 11/ 90	90/ 90/ 90	0/ 2.2/ 25		0/ 0/ 0
			mze	75/ 75/ 75				
fire bellied toad	adult	after leaving ponds	wra	40/ 74.2/ 80	40/ 78.3/ 80	40/ 74.4/ 80		40/ 74.2/ 80
			wwt	50/ 55/ 70	50/ 64.2/ 70	70/ 70/ 70	50/ 56.5/ 70	50/ 58/ 70
			mze	25/ 25/ 25				25/ 25/ 25
		pond changing	wra		80/ 80/ 80	80/ 80/ 80		
			wwt	90/ 90/ 90	70/ 84.5/ 90	70/ 83.3/ 90	70/ 75.4/ 90	70/ 70/ 70
			mze	25/ 25/ 25				25/ 25/ 25
		after leaving ponds	wra	0/ 17.8/ 90	40/ 40/ 40	0/ 20/ 40		0/ 40.8/ 90
			wwt	0/ 0/ 0		0/ 0/ 0		0/ 0/ 0
			mze	75/ 75/ 75				
	juvenil	after leaving ponds	wra	0/ 19.2/ 90	40/ 40/ 40	0/ 21.3/ 40		0/ 40.8/ 90
			wwt	0/ 5.3/ 25		0/ 2.2/ 25		0/ 0/ 0
			mze	75/ 75/ 75				

4.1.4.4 Population based coincidences of amphibians and plant protection product applications during main migration periods

Terrestrial phases of amphibians do not automatically imply that all individuals show a permanent presence on fields. They may rather pass directly through crop fields or stay there for some time. The relative daily migration activity of amphibian populations (relation of the activity per day to the total activity during the entire migration period) was used to calculate the share of the population temporally coinciding with the application of herbicides, fungicides insecticides and growth regulators during main migration periods. We applied the following settings

Migration parameters: The passage of fields lasts three days because of an average daily migration ability of 100 m and a distance to cross fields of 300 m (Berger et al. 2011a).

Exposure to plant protection products: To consider the persistence of plant protection products in fields and to calculate potential amphibian exposure duration we used substance related DT50 values. We assumed plant protection products with DT50 below and equal to 50 days to be present in the field for three days and those with DT50 above 50 days to be present for 14 days which can be considered conservative (for further information see Berger et al. 2011a, p. 163ff). In Appendix 5.16, we show figures of the population share of different amphibian species temporally coinciding with plant protection product applications from 2006 to 2008.

All figures were taken from Berger et al. (2011a, pages 172 ff). The resulting data were categorised within different coincidence levels (Table 4.1-5). The three classes illustrate values from low (green) over intermediate (yellow) to high (orange) coincidences (temporally coincident population shares in %).

Furthermore, we analysed interception values weighted by relative daily activity during migration periods of amphibians for the three crops winter rape, winter wheat and silage maize (Table 4.1-5). Days with large migration events thus contribute most to the weighted interception values. We selected this approach in order to achieve more realistic data, particularly during longer migration periods accompanied by strong vegetation development of crops. During the various migration periods of amphibians, there are large differences between interception values of crop canopy. Orange coloured boxes of both interception and plant protection product exposition indicate high species specific exposure risk.

This was true particularly in 2006, when high population shares of juvenile crested newt were facing after leaving ponds high deposition rates of herbicide and fungicide applied to winter rape. A similar situation -but to a somewhat lower extent- occurred in 2007.

Herbicide application to maize was particularly critical for spadefoot toad after leaving ponds in spring. In all three investigation periods from 2006 to 2008 high population shares were linked to very low interception values (Appendix 5.15).

Cases with high population shares during plant protection product application (fungicides and insecticides) coinciding with high interception values occurred in winter rape and winter wheat during spring migration of spadefoot and fire-bellied toad (Appendix 5.14).

Table 4.1-5: Interception of plant protection products in crop canopy during migration of amphibians from or to breeding ponds crossing fields and population share temporally coincident with plant protection product application (coloured boxes with numbers: interception weighted by relative daily activity: large migration events contribute most to population activity; data Eggersdorf 2006-2008; see also Berger et al. 2011a, p. 161ff)

species	age level	activity period	first date	final date	nr. individuals	winter rape					winter wheat					maize				
						intercept	herb.	fung.	insect.	gr	intercept	herb.	fung.	insect.	gr	intercept	herb.	fung.	insect.	gr
crested newt	adult	spring migration	08.03.2007	29.03.2007	44	59,4				included in fungizide application	44,2					out of gp	n.r.	no application	no application	no application
			22.02.2008	18.04.2008	142	56,8					38,7					out of gp	n.r.			
		after leaving ponds	26.08.2006	07.11.2006	109	31,2					10,8					65,7				
			21.08.2007	19.10.2007	312	31,3					0,0					75,0				
	juvenil	after leaving ponds	27.08.2006	10.10.2006	471	24,2					0,0					74,9				
			21.08.2007	02.10.2007	507	17,7					0,0					75,0				
spadefoot toad	adult	spring migration	24.03.2007	10.05.2007	464	79,9					58,0					0,0				
			29.03.2008	22.04.2008	1.347	80,0					50,0					0,0				
		after leaving ponds	04.04.2006	31.05.2006	2.184	79,9					67,8					14,7				
			11.05.2007	10.06.2007	318	80,0					78,9					25,0				
			26.04.2008	19.05.2008	470	80,0					65,8					12,5				
	juvenil	after leaving ponds	01.07.2006	08.08.2006	59.366	87,9					90,0					50,0				
moor frog	adult	spring migration	22.02.2007	29.03.2007	295	47,4					36,9					out of gp	n.r.			
			22.02.2008	18.03.2008	727	40,0					28,1					out of gp	n.r.			
	juvenil	after leaving ponds	17.06.2006	20.09.2006	14.831	42,1					40,5					63,5				
			05.07.2007	10.09.2007	240	69,3					70,4					55,4				
fire bellied toad	adult	spring migration	10.03.2007	07.05.2007	59	74,7					48,5					0,0				
			13.04.2008	19.05.2008	184	80,0					62,1					11,7				
		pond changing	15.05.2007	31.07.2007	224	81,1					89,5					30,7				
		after leaving ponds	15.08.2007	05.10.2007	178	36,9					0,0					75,0				
	juvenil	after leaving ponds	29.07.2006	08.09.2006	554	6,4					6,4					73,2				

Legend: gr … growth regulator; gp … growing period

interception (%):
- [green] > 66,6
- [yellow] > 33,3 and <= 66,6
- [orange] <= 33,3

population share min/max-values (%):
- [green] <12,5/25
- [yellow] >=12,5/25 and <25/50
- [orange] >=25/50

Methodological criticism:

The results in Table 4.1-5 show hot spots of potentially higher exposure risk of amphibians to plant protection products during field passage (red/red or red/yellow combinations of high population shares and interception).

It should be taken into account that:

a) we analysed the local crop management in East Brandenburg which may differ from other parts of Germany. Furthermore,

b) only three crops were included into the quantitative analyses. As shown by Berger et al. (2011a) and in Appendix 5.16, summer crops like sunflower, blue lupine or peas may also lead to high herbicide exposure risks for amphibians because of the animals' high population shares and very low crop interception values during plant protection product application.

c) The "migration model" used does neither take into account the species specific ability to migrate nor the roughness of field surface and canopy leading to modified migratory speed.

d) Individuals leaving ponds may stay in parts of the field such as wet spots and/or sandy micro-sites. In our approach, we assumed every individual to take strictly three days to pass the crop field, not resting there for a longer period. We also assumed the same migration speed for adult individuals during spring migration to ponds and spring/summer/autumn migration from water bodies, knowing that the demand for pairing and spawning leads to a more intensive migration activity towards ponds.

e) We did not discriminate between the migratory speed of adults and juveniles.

f) With regard to the "population", we did not distinguish between males and females, knowing the gender specific time shift. Thus, males and females may be at different risk to plant protection product exposure.

Case study on quantitative estimations of amphibians' exposure to herbicides applied in summer crops as pre-emerging application

This chapter aims at achieving some quantitative estimation on the potential exposure of adult amphibians to herbicides. We identified high relative amphibian population exposure shares to herbicide applications in summer crops during spring migration of adults into breeding ponds (Appendix 5.16). Sunflowers, field pea and blue lupine were treated regularly with herbicides based on Aclonifen (DT_{50}: 80.4 days). Because of the pre-emerging application on bare soils we assume a high exposure risk to migrating individuals.

We analysed as far as possible own real data on the amphibian morphology. Our calculations are based on the following scenario assumptions:

- no vegetation cover on field, no interception
- flat soil surface after seed bed preparation and sowing
- distance from field edge to breeding pond: 300 m
- spray application immediately after individuals entering fields
- direct overspray during field stay (no shelter)

- species specific skin contact to soil during crossing (Table 4.1-7)
- no absorption or infiltration of the herbicide into soil; 100% remains on the soil surface “spray film”
- no degradation of herbicide during field passage

Amphibian morphological characteristics and moving patterns

Morphological parameters of adult individuals of males and females of three amphibian species were measured and/or calculated according to Matthé et al. (2008) (Appendix 5.17).

Table 4.1-6: Morphological parameters of adult individuals of three amphibian species (investigation area Eggersdorf, 2006 to 2008, period from January to May).

Species	Gender	Number of individuals investigated	Weight in [g] (average, SD, maximum value)	Head-body length [cm] (average, SD, max. value)	Average body width (cm)[1]	dorsal skin area (cm^2)[2]	Belly area with soil contact (cm^2)[3]	Total track area on 300m field passage (m^2)
Crested newt	Male	122	5.8/1.8/11.3	6.5/0.6/7.9	1.1	7.2	?	3.3
	Female	130	7.0/2.2/12.8	6.8/0.7/8.7	1.2	8.2	?	3.6
Spadefoot toad	Male	3839	11.6/2.1/23.3	4.5[1]	2.5	8.5	6.8	7.5
	Female	2827	16.4/4.4/36.9	5.1[1]	2.6	9.9	8.0	7.8
Fire-bellied toad	Male	189	4.8/1.7/10.2	3.8/0.5/5.0	2.0	7.6	6.1	6.0
	Female	138	5.3/1.5/9.4	3.9/0.4/5.1	2.1	8.2	6.6	6.3

Legend: [1] 10 individuals with average weight were measured; [2] calculated by product of average head-body-length and body width corrected by estimated form factor, relevant for direct overspray; [3] calculated using dorsal skin surface corrected by estimated migration/behavioural factor, relevant for skin-soil contact during migration

In Table 4.1-6, the averages of the animals' weight, head body length and maximum body width as well as the calculated dorsal skin area relevant for direct overspray, and the belly area with potential soil contact are given. Because of the uncertain moving patterns of crested newt, we computed no contact area of skin and soil. The total track area for the 300 m field passage is calculated by incorporating the skin-soil contact (Appendix 5.18). Depending on the moving pattern of species, the share of soil touched by the skin is estimated separately (Table 4.1-7). Amphibians jump or walk on the soil surface (Table 4.1-7). The crested newt walks on extremities, usually not much touching the soil except for parts of the tail. If the soil surface is rough or covered with vegetation, there may be also belly skin contact (Schönbrodt 2011, and pers. comm.). Spadefoot toad and fire-bellied toad belong to mainly jumping species. Because of the kinetic energy connected to jumping, parts of their belly skin regularly touch the soil surface.

Depending on the jumping distance and belly area with soil contact per jump (Table 4.1-6), the totally touched soil area during migration can be estimated. The share of the soil area touched in relation to the track area is an estimation of the level of contact (Table 4.1-7). While spadefoot toad and fire-bellied toad touch between 25 and 50 % of their track area, we assume values below 10 % for crested newt, probably even 1 % or less.

Table 4.1-7: Moving pattern of adult amphibians and relative skin-soil contact during migration on uncovered and flat soil surfaces (expert estimation by Schönbrodt 2011).

species	predominant moving type	jumping distance (range in cm)	Share of belly skin of individual on soil surface when landing (%)	Relative skin-soil surface contact compared to the overriding track area (%)
crested newt	walking, tail partly touching soil	no jumping	no jumping	<< 10 (1 or 0.1?)
spadefoot toad	jumping	5-7	60	30-36 (for cal. ≈30%)
fire-bellied tad	jumping	6-12	80	26-51 (for calc. ≈30%)

Estimating the potential exposure of adult amphibians to the active ingredient Aclonifen

The herbicide Bandur is applied with a maximum of 4 l /ha, containing 600 g Aclonifen per litre. The deposition of Aclonifen amounts to 240 mg a.i./m^2 on the uncovered soil surface. For spadefoot toad and fire-bellied toad we assume the potential exposure to the soil surface (skin-soil contact) to be around 30 % of the track area (Table 4.1-7). The potential exposure for fire-bellied toad was calculated to be about 90 mg/g body weight (see exemplary calculation below), which is twice as high as for spadefoot toad (Table 4.1-8). For the crested newt (with assumed contact of 1% to 0.1% of the track area), we found potential exposure values between 0.1 and 1.4 mg/g body weight. It is assumed for all species that the males are more exposed than females because of their size and weight, especially for the spadefoot toad.

Exemplary calculation for male spadefoot toad:
Body width (2.,5 cm) x distance (300 m) x relative skin-soil surface contact (30 %) x a.s. deposit (240 mg/m²) / Body weight (11.6 g) = 46.,55 mg a.s./ g body weight

Compared to the potential exposure of amphibians touching the soil surface during migration, the exposure by direct overspray is assumed to be rather low. For example, even with low estimated soil surface contacts for crested newt, the potential exposure by soil contact is assumed to be at least four times higher than from direct overspray. The soil surface exposure of spadefoot toad and fire-bellied toad can be assumed to be at least 2000 times higher than through direct overspray.

Table 4.1-8: Exposure of amphibians to the herbicide Bandur by direct overspray and skin-soil-contact during migration of adults into breeding ponds (herbicide is applied pre-emerging to summer crops, bold numbers are estimations recommended for use)

species	gender	direct overspray	skin-soil-contact on 30% of the track area		skin-soil-contact on 10% of the track area		skin-soil-contact on 1% of the track area		skin-soil-contact on 0,1% of the track area	
		mg/g body weight	mg/g body weight	rel. to overspray	mg/g body weight	rel. to overspray	mg/g body weight	rel. to overspray	mg/g body weight	rel. to overspray
crested newt	male	0,03	40,68	1356	13,56	452	**1,36**	**45**	**0,14**	**5**
	female	0,03	36,77	1226	12,26	409	**1,23**	**41**	**0,12**	**4**
spadefoot toad	male	0,02	**46,55**	**2328**	15,52	776	1,55	78	0,16	8
	female	0,01	**34,24**	**3424**	11,41	1141	1,14	114	0,11	11
fire bellied toad	male	0,04	**89,44**	**2236**	29,81	745	2,98	75	0,30	7
	female	0,04	**86,07**	**2152**	28,69	717	2,87	72	0,29	7

Methodological criticism and risk assessment requirements

There is some uncertainty in our estimations. The scenarios selected can be regarded as worst case situations but they are realistic since they take parameters of real individuals from the field into account. However, we did not consider the following possible relevant processes: a) The plant protection product infiltration into the soil; b) the effect of the micro relief for skin-soil contact and c) the duration of amphibians' sojourn on the soil surface briefly after jumping. d) Particularly the expert estimation on moving parameters of amphibians needs validation. There is a wide range of the estimated skin-soil contact on the track area (30-0.1%). Risk assessors may in future adjust our assumptions easily in case more validated data are presented. But even if only a ratio skin-soil contact of 0.1% of the track area is considered, the potential exposure over soil contact is considerably higher than calculated for direct overspray. Hence, we consider that not only direct overspray but also the soil surface contact should be included in the risk assessment of the plant protection product used on agricultural fields.

4.1.5 Hot spots of amphibian presence in fields

Most amphibians except for spadefoot toad avoid longer stays on crop fields. They usually pass fields between terrestrial and aquatic habitats more or less directly. However, there are some areas within crop fields providing favourable conditions for amphibians and leading to higher numbers of individuals staying there for longer periods. The wet spots in crop fields are of particular importance for a wide range of amphibians. It may be a periodically flooded area of some hundred square meters or bigger or even a single tractor track of 3 m length and 10-30 cm depth filled with water. These spots provide suitable microclimate, food and shelter. Berger et al. (2011a, page 133), reported more than 1,300 individuals in a wet spot of about 1000 m² living there from April to the end of the vegetation period.

Since wet spots are part of the crop field, the farmers cultivate them regularly, including plant protection product application. Only if the passage is not feasible for tractors and machinery due to wet and instable soils, the farmers stop cultivating and applying plant protection products. Small wet spots and tractor tracks of only few square meters can be assumed to be regularly treated with plant protection products leading to a higher exposure risk. However, large wet spots with difficult carrying conditions are usually not treated in normal plant protection product application practice, leading to low exposure risk. Usually, every summer the farmers try to take the wet field spots into cultivation again. At this time of year, the wet spots are inhabited by high numbers of amphibians with high exposure risk.

Even entire crop fields can be used temporally as terrestrial habitat by amphibians as shown by Berger et al. (2011a, pages 148ff), for stubble fields of winter rape. During August and September, emerging winter rape seeds lead to soil coverage of more than 90 %. Microclimate, food supply and shelter characterized this stubble field to be “suitable” as terrestrial habitat for amphibians. For seedbed preparation of the follow crop, these stubble fields with a considerable share of amphibian populations are usually treated with total herbicides, such as Glyphosate. Hence, the coincidence of amphibians and plant protection product application is assumed to be high, but the exposure risk is reduced by the interception of the crop canopy.

4.1.6 Temporal coincidence of amphibian populations with the application of slurry to field crops

The application of slurry to fertilize field is common practice in agriculture. However, in the housing of animals biocides – especially insecticides (product type 18, see Introduction) and disinfectants (product type 3) – can be applied to stables or directly to the manure and, thus, reach agricultural fields on this pathway.

Slurry application to field crops

In the investigation area Eggersdorf slurry was applied to a wide range of field crops. The relative share of treated fields in relation to the total field number differed between crops. Maize predominated in 2007 and 2008 and was almost completely treated with slurry. In contrast, on the winter cereals and winter rape fields slurry was applied to 0 -30% of the fields (Table 4.1-9).

Table 4.1-9: Total number of investigated fields per crop and relative share of fields treated with slurry (investigation area Eggersdorf 2006 to 2008, yellow: population share >50%)

crop	harvesting year	total number of fields	relative share of treated fields (%)
winter rape	2006	28	21,4
	2007	19	21,1
	2008	28	25,0
winter barley	2006	5	0,0
	2007	12	25,0
	2008	15	46,7
winter rye	2006	23	17,4
	2007	34	14,7
	2008	24	4,2
triticale	2006	7	14,3
	2007	9	11,1
	2008	7	0,0
winter wheat	2006	21	23,8
	2007	13	30,8
	2008	16	18,8
maize	2006	5	20,0
	2007	19	94,7
	2008	18	94,4

Depending on the type of field crop the time of slurry applications differed widely throughout the year (Figure 4.1-4). Maize was treated from February to May usually prior to sowing. For winter cereals and winter rape the slurry was applied before crop sowing during summer and autumn. Slurry was also applied into growing crop stands. Winter wheat was treated in May. To all field crops investigated slurry was applied in October (e.g. into growing winter crops, to maize prior to sowing in next spring).

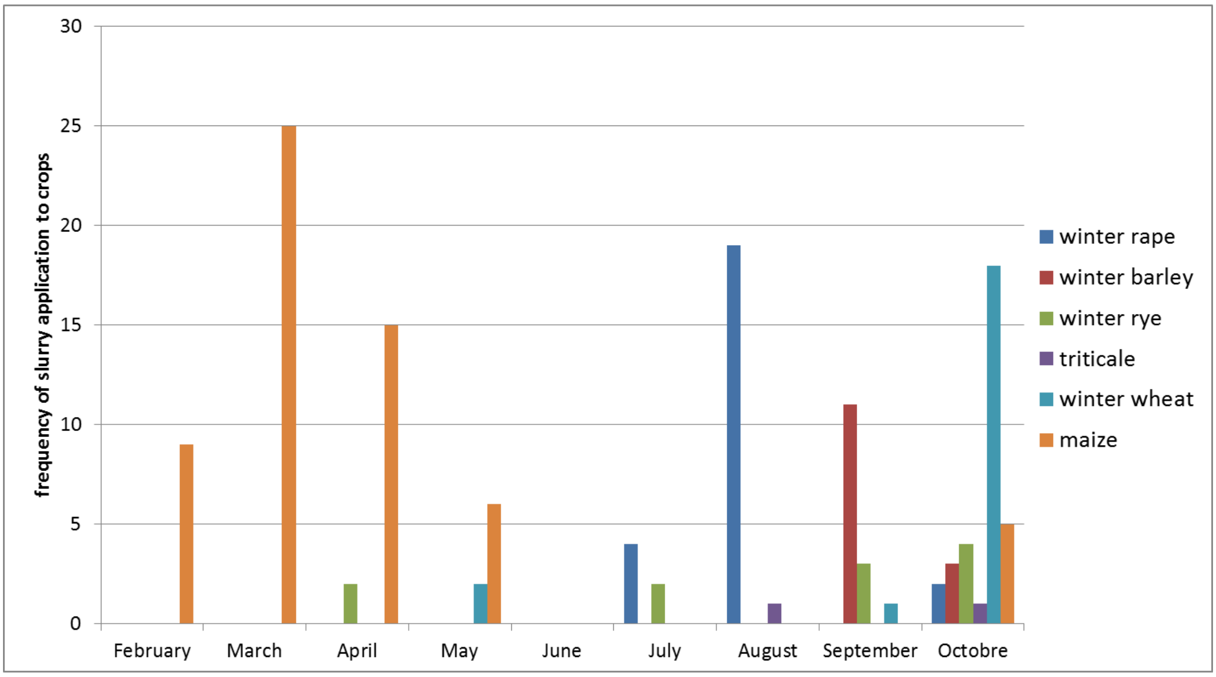


Figure 4.1-4: Frequency of slurry application to winter crops and maize (Eggersdorf 2006-2008).

Temporal coincidence of amphibian populations with slurry application

Our calculations of individuals active on fields two days before and three days after application revealed the following temporal coincidences of amphibian populations with slurry applications (Figure 4.1-5).

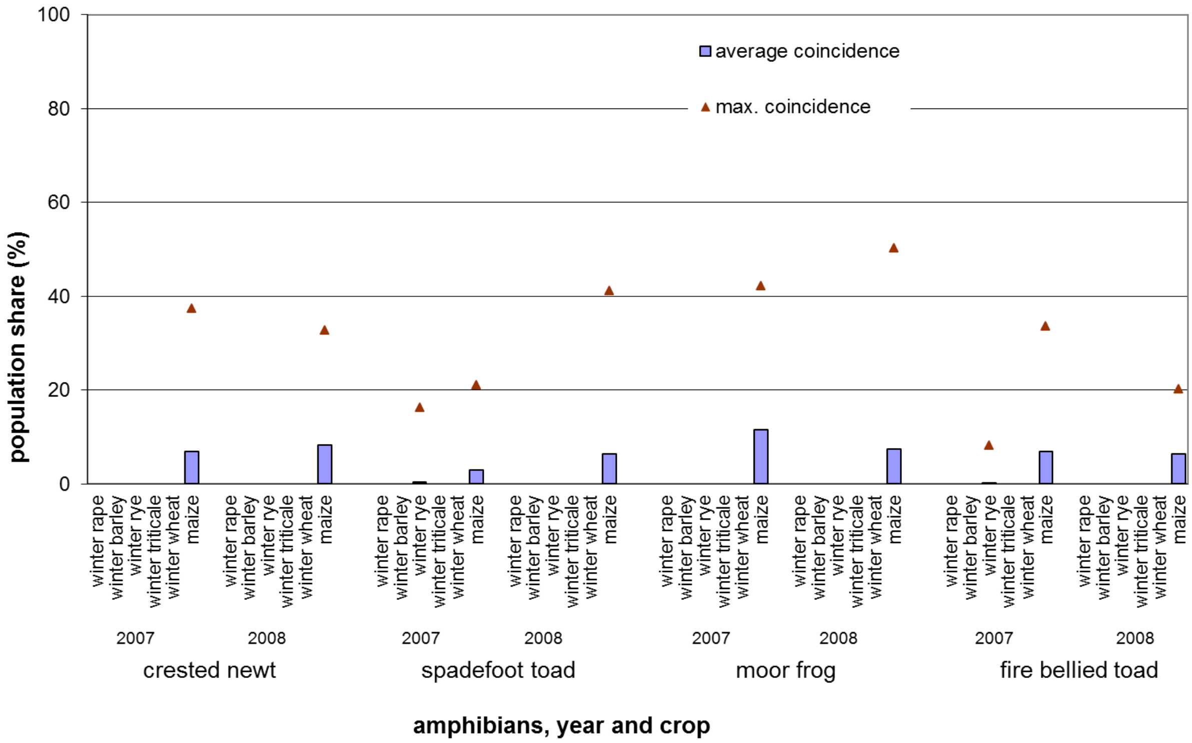


Figure 4.1-5: Population share of adult amphibians coinciding with slurry application during migration from hibernation sites into breeding ponds during spring.

During migration of adult amphibians into breeding ponds in spring the slurry applications to maize and winter rye were temporally coincident with parts of the populations (Figure 4.1-5). Slurry application to maize showed average values ranging from 5 to 12% with maximum values of 50 %. During the application from February to May all amphibian species investigated coincided with slurry application. Differences between early and late migrating species did not occur. Application to winter rye showed much lower average temporal coincidences with maximum values below 20%.

Juveniles leaving ponds during late summer and autumn also faced slurry application (Figure 4.1-6). The average as well as the maximum population share showed comparably low coincidence values. Differences between species did not occur.

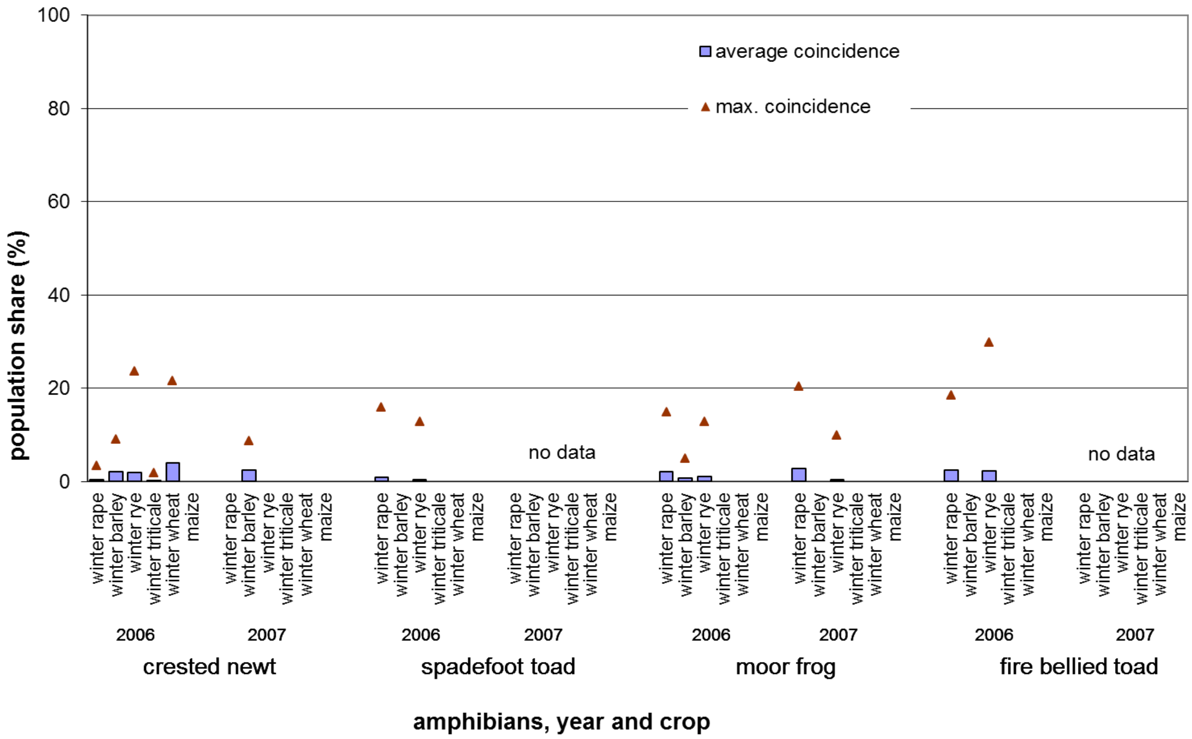


Figure 4.1-6: Population share of juvenile amphibians coincident with slurry application during migration from breeding ponds into terrestrial sites during late summer and autumn.

Discussion

Parts of amphibian populations coincide temporally with the application of slurry to field crops. The level of exposure of the amphibians depends on the slurry application type.

The liquid manure can be

a. spread onto the soil surface followed by incorporation into soil,
b. spread onto the soil surface without incorporation into soil,
c. directly incorporated into the soil during application using injection/drilling techniques (Figure 4.1-7).

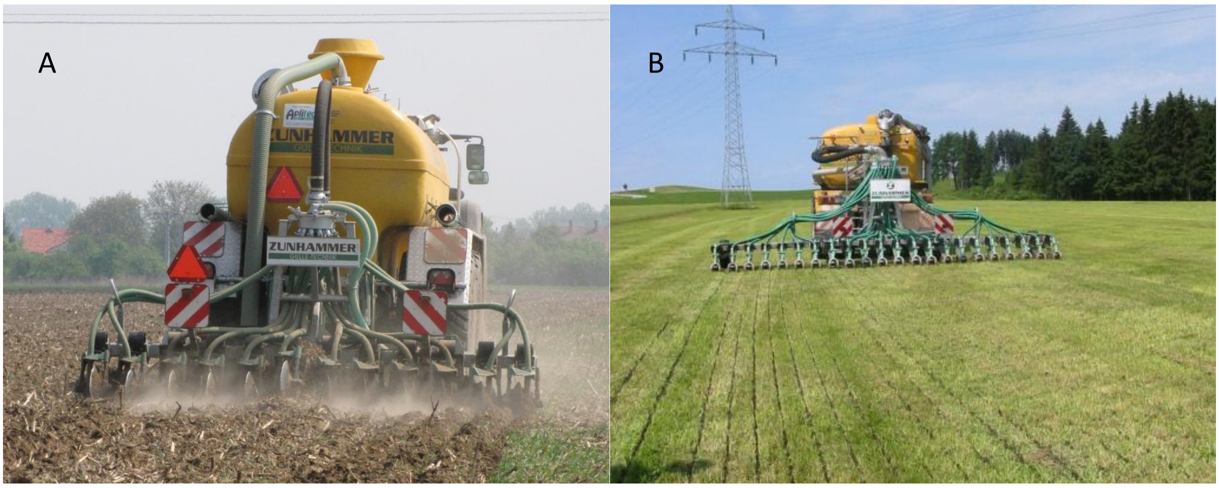


Figure 4.1-7: Slurry application directly into the soil using (A) cultivator shares and (B) cutting discs (images by Zunhammer GmbH, Traunstein)

Slurry not incorporated into soil is likely to entail the highest exposure for amphibians. Usually a large soil surface share is covered by slurry (Figure 4.1-8). Individuals crossing fields can be exposed to this liquid manure. We assume direct contamination of individuals resting in treated fields when slurry runs into the hiding places of amphibians (beneath plants, into soil caves etc.) (Figure 4.1-8).

Figure 4.1-8: Slurry applied in April prior to maize sowing (Eggersdorf 2010)

Farmers in Germany are obliged to incorporate slurry applied on uncropped land immediately after spreading (DüV 2006). Immediate incorporation is often regarded as soil cultivation up to four hours after application. Hence, the temporal coincidence as calculated in this study may be lower if this practice is followed.

Conclusions

Exposure of amphibians to slurry can be generally assumed. If biocides from livestock are applied the exposure of amphibians to these substances must be taken into account. Hence investigations regarding the toxicity of biocides to amphibians are recommended. This may help lowering the overall risk for amphibians in agricultural landscapes.

In short:

- Amphibian species living in agricultural landscapes are at risk of exposure to plant protection products both in fields and in neighbouring non-crop areas. They perform species specific migrations on crop fields which temporally coincide with the application of plant protection products. Depending on the vegetation cover of field crops and their related interception values, amphibians are at different exposure risk.
- Direct overspray of amphibians depends on the activity of individuals during daytime and availability of shelter. Because of amphibians being mostly nocturnal species, the risk of being oversprayed is likely to be low. Amphibians resting in fields are slightly buried in the soil surface (digging species), use sites beneath the plants or enter animal burrows. Resting in fields without any type of cover is very unlikely.
- However, higher exposure risk is caused by their movements on treated soil or vegetation, due to their potentially intense skin-soil or skin-vegetation contacts.
- Preferred habitats in crop fields are areas next to breeding ponds and wet spots. Under normal cultivation, there is for amphibians a rather high risk to be exposed to plant protection products because of their long sojourn in fields.
- Amphibians can be exposed to plant protection products outside crop fields by spray drift and run-off. This risk increases strongly with lower shares of non-arable land.
- Furthermore, manure applied to agricultural sites might contain biocides, especially insecticides (product type 18) and disinfectants (product type 3). There is a temporal coincidence of amphibian populations with slurry application and, hence, exposure of amphibians to slurry can be generally assumed.

4.2 Effects of other stressors than pesticides on potentially exposed amphibian species

4.2.1 Amphibian red list species: Evidences of decline due to agricultural practice

In general, agricultural land use and connected activities including agrochemical use is highly correlated with global amphibian population declines (Houlahan & Findlay 2003), often interacting with other environmental stressors (Blaustein et al. 2003; Beebee & Griffiths 2005; Pounds et al. 2006). Agricultural activities most detrimental to amphibians result in a) physical destruction of habitats and habitat fragmentation for gaining agricultural land, for instance, through deforestation, slash-and-burn agriculture, wetland drainage or by turning extensively into intensively cultivated land and in b) land degradation and overuse, for instance, due to soil tillage, plant protection product and fertiliser spraying, ground contamination, overgrazing, and water pollution (Millennium Ecosystem Assessment 2005; Berger et al. 2011a; BfN 2012). These activities do pose a threat not only to amphibians but also to most other non-target organisms.

Most amphibians don't have special preferences for agricultural habitats, however, if breeding ponds and terrestrial amphibian habitats neighbour farmland, they may be present on the arable fields during certain life stages. Most amphibian populations in Germany are present in agricultural landscapes (Gasc et al. 1997; Berger et al. 2011a). An overview of landscape types preferred and/or partly inhabited by amphibians is presented in Table 4.1-1. In general, many amphibians prefer rich structured landscapes and habitat complexes with arable fields, grassland, and forest elements. However, a few species such as spadefoot toad, fire-bellied toad, and mountain newt are also often found in open agricultural landscapes. From the introduction of agriculture onwards and prior to the green revolution most middle European landscapes due to large-scale pastoralism were richer in structural elements and thus more suitable for amphibians (Riecken et al. 2004). The today's open and poorly structured landscapes are often intensively cultivated and marginally suitable as habitats to any amphibian species, except for instance to the open land species spadefoot toad. Hence, agricultural intensification is well correlated to a decline in –and fragmentation of – terrestrial habitats, increasingly treated with agrochemicals and often with neighbouring aquatic biotopes polluted (Beebee 1996; Houlahan & Findlay 2003; Mann et al. 2009).

Twelve out of the 20 native amphibian species occurring in Germany are listed in Annex II and/or IV of the European Habitats Directive and all amphibians in Germany are specially protected under the German "Bundesartenschutzverordnung". In Germany, amphibians (35%) are among the most endangered Red List species among all vertebrates (Haupt et al. 2009). According to the German Red List (RL) categories, their populations are largely endangered, very vulnerable or vulnerable, while many species are near threatened (Appendix 5.19). For most amphibian species, a long term trend of declining populations is stated, while the large majority of both amphibians and reptiles have a short term (10-25 years) trend of decline (Haupt et al. 2009).

4.2.2 Other direct agricultural effects/stressors to amphibians

There are multiple agricultural stressors apart from the application of plant protection products directly affecting the different amphibian life stages (Berger et al. 2011a). These stressors to amphibians resulting from agricultural practice are mainly the implements for soil tillage, sowing, fertiliser application, harvesting, and the respective mechanical run over by tractor tyres. These activities can only be harming if they coincides with the presence of the amphibians on the arable field. An overview of the mechanical harm of different implements to amphibians is presented in Figure 4.2-1. It shows that the mechanical harm risk depends on the crop management measure, the implement used, and its working width. The stronger and more direct the soil contact is, the higher the risk of mechanical injury, with implements for soil tillage being the most harmful ones (30-100% injury rate) and agrochemical applications being the least harmful ones in terms of mechanical injuries. The lower the working width is, the more likely a run over by tractor tyres (Pfeffer et al. 2011). Tractor wheels hitting amphibians cause an 80-100% injury rate, while the likelihood of the tyres hitting amphibians increases with the tyre width. For some crop management measures and associated implements, the range of calculated injury rates is larger than for others. The higher uncertainty is due to variation in working width and implement types and lack of experimental knowledge. Depending on the crop type, soil type, regional climate, and various production inputs, the frequency and intensity of crop management measures varies. Implements, such as machinery and equipment for agrochemical and manure applications are applied more often than for sowing or harvesting.

There are many different implements for soil tillage differing in the intended purpose (Roth & Rosner 2011b). The most common gear used is a) the plough (turning over and breaking up of top soil layer, incorporation of plant residues, up to 30 cm working depth), the grubber (breaking up of top soil, mixing of plant residues, fertiliser and soil material, 10-20 cm working depth), c) the harrow (breaking up of soil surface crusting, shallow mixing of plant residues, fertiliser and soil material, removal of weeds, 5-10 cm working depth), and d) the roller (soil compaction, 0-10 cm working depth). Minimum tillage (without ploughing), no-tillage and/or direct sowing –in order to conserve soil structure, prevent soil erosion and preserve nutrient availability – is an option not often practiced in Germany.

The implements used for soil tillage and their timing depend on crop species, crop vegetation stage, soil humidity, soil type, and the farmer’s tillage preference (Roth & Rosner 2011b). Soil tillage of summer crops immediately prior sowing such as for maize is expected to coincide strongly with the early season amphibian migration (Berger et al. 2011a), whereas soil tillage of winter crops is carried out during late summer to autumn, with little amphibian migration activity on arable land.

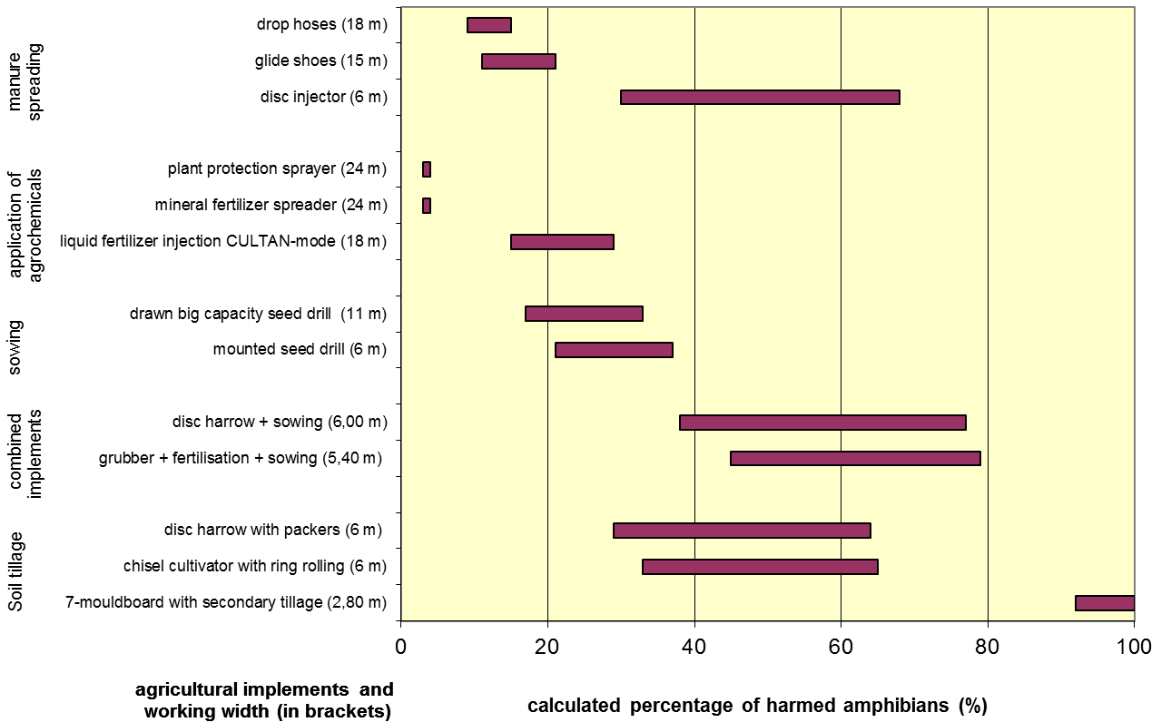


Figure 4.2-1: Estimated amphibian injury rates due to mechanical effects of different agricultural implements (Pfeffer et al. 2011, modified)

On the other side, crop fields may also function as summer habitat with a higher share of amphibian populations. This applies particularly to well covered stubble fields (like winter rape or winter cereals) providing food and shelter.

The impact of soil tillage on amphibians can be detrimental, but also differs depending on the implement. Pfeffer et al. (2011) found 80% frogs injured and/or buried by ploughing, compared to 40% for combined direct-sowing and to 40% for disc harrow, all three having even 12-20% more potentially injured individuals. For the roller, a 30-70% amphibian injury rate is expected. As mentioned earlier, sowing may be conducted a) either as a single work step after soil tillage, b) without any tillage (no-tillage farming), or it may be combined with a soil tillage device. The harm to amphibians thus varies largely ranging between estimated 17-37% for the sowing per se and between 38-79% for a combined implement (Figure 4.2-1).

Fertiliser applications in agriculture are known to harm and/or kill amphibians through a) mechanical tractor tyre impact during the application, b) physiological stress on the permeable amphibian skin due to the fertiliser chemical properties (Schneeweiss & Schneeweiss 1997; Dürr et al. 1999; Ortiz-Santaliestra et al. 2005), and c) the pollution of adjacent water bodies, impacting particularly the early development stages of amphibians (Orton et al. 2006; Mann et al. 2009). There is a wide range of different fertilisers differing largely in applied quantity, toxicity, solubility and application mode (Schneeweiss & Schneeweiss 1997; Berger et al. 2011a), the largest used quantities being calcium ammonium nitrate and calcium carbonate (Appendix 5.20). There are mineral and organic fertilisers and they may be applied as liquid, dung, or solid particles. Apart from reports on mortal effects (Lenuweit 2009), the toxic effects reported for amphibians exposed to mineral fertilisers are breathlessness, osmotic stress, paralysis, low food intake, increased breathing frequency, and reddening of the skin/irritation (Schneeweiß & Schneeweiß 1997; Dürr et al. 1999; Ortiz-Santaliestra et al. 2005). There is little knowledge about the toxicity to amphibians of organic fertilisers. Oldham et al. (1997) showed that humid dung and slurry increase breathing frequency.

Results in NE-Germany (Berger et al. 2011a and Berger et al. submitted) show that amphibian presence may coincide considerably with fertiliser treatments, however, for different species to a varying extent. Fertiliser management in farms differs highly, depending on the cultivation with winter or summer crop and also on the crop species. For winter barley and winter rape, Berger et al. (submitted) recorded two very early spring and temporally close fertiliser applications within 10 days indicating the highest coincidence for the early active amphibian species compared to the other fertilisation systems/farms/crops. With a single fertiliser application –or two fertiliser applications with a pause of about 4 weeks –, the temporal coincidences were considerably lower. Fertilisers for summer crops such as maize are applied later in the year, indicating low coincidence and only partial overlap with amphibian migration periods.

A maximum coincidence was demonstrated for the moor frog with over 60% of its population presence on fields coinciding temporally with fertilisation in a winter crop. Since the species generally differs in migration start, in animal activity, in the duration of the migration period, and in spawning habits (Timm et al. 2007), the level of coincidence was found to depend on crop species, farm fertilisation system, amphibian species, and year. For instance, species active early in the year such as the moor frog starting to migrate in late February coincide much more with crop fertilisation of winter cereals and winter rape, compared to species that are active later. Berger et al. (2011a) indicate also the importance of differing breeding characteristics. Moor frogs having only short breeding periods (Wells 1977), seem to have short migration periods into spawning habitats, whereas prolonged breeders such as fire-bellied toad or crested newt (Wells 1977) have a longer migration period.

Harvesting activities may physically harm amphibians sojourning on cultivated fields, especially with combine harvesters. Adelmann (2001) identified 11% injuries of all animals counted on a 500 m² cereal area after crossing with a combine harvester. Severe injuries (contusions) are caused by the tyres passing over and through the cutter (cuts). Also when harvesting forage (clover or lucerne grass), the technique used heavily damages amphibian populations with an average of 21% injured individuals as calculated by Oppermann (2007).

4.2.3 Indirect effects due to loss of food animals with plant protection product applications

The main prey for amphibians is moving arthropods. Various stressors impact the arthropod populations in arable fields (see chapter 3.1.1) as also shown for amphibians in chapter 4.2.2. These stressors result from agricultural practice and are mainly caused by plant protection product application, implements used for soil tillage, sowing, fertiliser application, harvesting, and by the associated mechanical run over by tractor tyres but may also be influenced by crop rotation, catch cropping or type and duration of vegetation cover (Figure 4.2-1). The abundance of arthropod prey for amphibians is significantly reduced after harvesting and/or soil tillage.

Insecticide applications drastically decrease the food availability to amphibians. Weber (2008) shows that arthropod populations are significantly reduced with insecticide application entailing a food shortage during 14 days after application.

Following herbicide applications, weed biomass is significantly reduced resulting in smaller forage availability to herbivore arthropods, an important prey of amphibians. It is also associated with a reduction in structural diversity of habitats required for arthropods (Haughton et al. 2003). The extent of biomass reduction and indirect effects on herbivores largely depends on the herbicide application regime, i.e. the timing, amount and mixture of the active ingredient (ai) and the frequency of herbicide applications but also depends on their capability to control the weeds. In conventional agriculture, usually three to four (up to six) herbicide applications are performed, with a broad-spectrum herbicide such as Glyphosate often applied at the pre-seeding or pre-emergent stage to clear fields and/or postharvest for volunteer and weed control, while other herbicides are applied during crop development (Champion et al. 2003).

A large scale comparison of different herbicide management regimes for different summer and winter crops and their effects on various field invertebrates was carried out during the Farm Scale Evaluations (FSE) in the UK (Firbank et al. 2003). The FSE showed that efficient weed suppression leads to less biomass, food, and flowers for field herbivores after spraying. This entails lower abundances of various herbivores, pollinators, and also predatory species (Heard et al. 2003; Bohan et al. 2005). Both minimum and/or no-till associated with only few broad-spectrum herbicide applications and conventional tillage associated with multiple herbicide applications lead to drastic temporary reduction in forage availability to amphibians both in arable fields and on field edges (Roy et al. 2003). After herbicide application, the weeds successively recover and the herbivores re-colonise the arable fields. However, in case of multiple herbicide applications during the cropping period, the level of forage to amphibians remains low.

3.4 Risk for amphibians on non-arable land due to spray drift input of plant protection products

Plant protection product spray drift to habitats neighbouring arable fields is common in agriculture (Roy et al. 2003). The risk of amphibians to be exposed to these plant protection products on non-arable land depends on both the traits of the amphibian species and the composition of the landscape.

Species specific properties of amphibians

As shown in chapter 4.1, both species specific and often also age dependent amphibian moving types influence the risk of stripping off herbicides from soil surface and vegetation.

With respect to direct overspray, the timing of land activity during day or night time is to be considered. Most amphibians are nocturnally active. During the main migration periods to breeding ponds and under specific conditions, e.g. a long lasting winter, species usually nocturnally active can be seen during daytime too. Behaviour in summer habitats is typically species specific with respect to the time of activity. Thus, during summer, the risk of direct overspray for most amphibian species is comparably low.

Specific places for resting and sun bathing may imply higher exposure towards direct overspray in terrestrial habitats. This applies particularly to tree frogs often sitting on sun exposed higher herbaceous plants next to field edges during sunny days. Non-arable land can be used as migration corridors with higher amphibian activity and as terrestrial summer habitats having a more or less stable amphibian presence (Berger et al. 2011a, p.150). We assume that the exposure risks by stripping off off-site deposited plant protection products from soil and vegetation is higher for the more active amphibians.

Some species are also present in dry conditions of normal “fresh“ soils. Some species, particularly brown frogs (moor frog etc.), prefer summer habitats with higher soil moisture usually covered by wet grassland. These sites – if present in the landscape - often have a width of more than a few meters implying a low probability of high exposure by spray drift from neighbouring crop fields.

The importance of the share of non-arable land in landscapes hosting amphibians

Spray drift from crop fields may reach 20 % of the applied plant protection product field rate and is mainly a matter within the first 5 m from the emitting field. But it can also be measured to lower extent beyond 20 m field distance (Donkersley & Nuyttens 2011). In the following, we present an analysis of the change of potentially “non-impacted” non-crop land depending on spray drift distances from 1 to 10 m and the share of crop land in a given agricultural landscape. About 30 % of all NATURA2000 areas of the German Lands Brandenburg and Mecklenburg-Vorpommern designated to support amphibian populations (Annex II species: fire-bellied toad and crested newt, and many other amphibians) have a share of arable land exceeding 40 % of the protected area. In both German provinces there are also some protected areas with over 80 % of arable fields implying small shares of terrestrial non-arable plots (Berger et al. 2011a, p. 17). Appendix 5.21 illustrate four NATURA2000 sites in the Land of Brandenburg with non-crop land ranging from less than 5 % to more than 50 %.

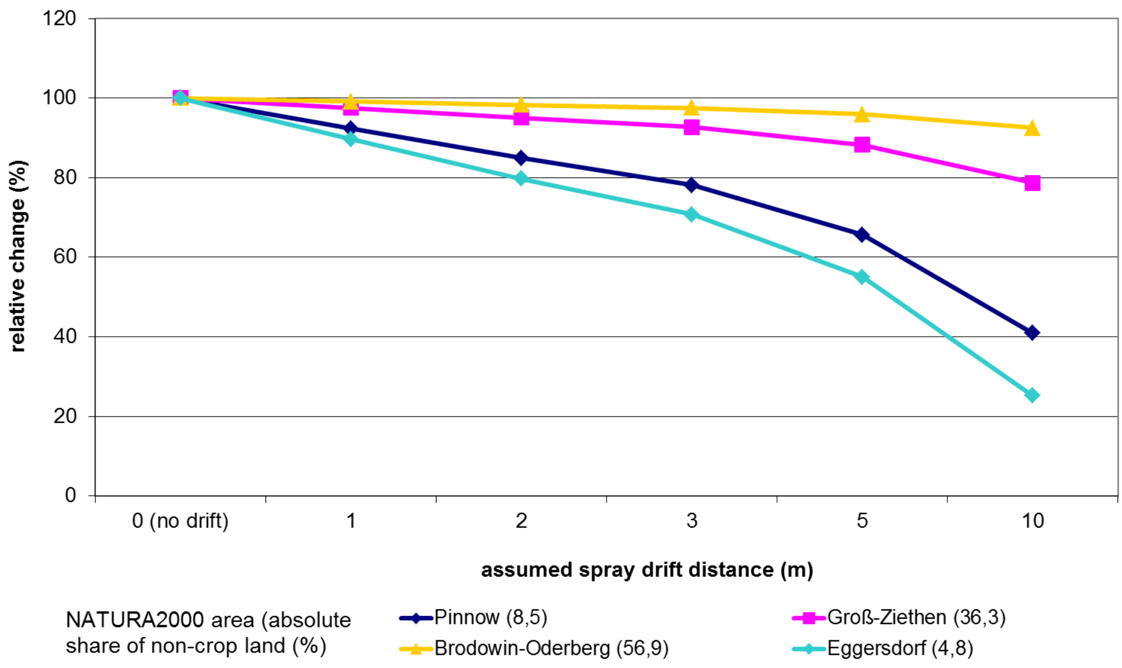


Figure 4.2-2: Relative change of non-crop land not impacted by spray drift depending on the assumed drift distance and the share of arable land in NATURA2000 areas (in brackets: relative share of non-arable land)

On sites with higher shares of non-arable land, even when considering a plant protection product input corresponding to 10 m drift, even less than 20% non-crop land could be considered as not impacted. For the other two areas the relative change was considerable (Figure 4.2-2). Even with 3 m drift distance the potentially "impacted" non-crop land amounted for up to 30 % of the total non-crop land of these areas. Assuming 10 m drift distance only 20 to 40 % of the former non-crop land is not affected which means that the major share would be impacted by plant protection product drift. In Müncheberg as well as in Pinnow, the small field edges (5 m width) that are often the ones located between arable fields are assumed to be completely "impacted" by a spray drift from 3 m. This leads to large and connected areas with potentially impacted land.

With respect to the exposure risk of amphibians to plant protection products, the increasing shares of arable land in the agricultural landscape show that a) the field area regularly treated with plant protection products increases and b) that the non-crop land not impacted by plant protection product drift decreases disproportionately. Thus, if assessing the risk of plant protection product applications to amphibians, the specific landscape situations need to be kept in mind.

4.2.4 Literature study on amphibian stressors: Main focus on non-agricultural stressors

Biodiversity in all terrestrial and aquatic habitats has been shown in the Millennium Ecosystem Assessment (2005) to be declining during the last century as a result of anthropogenic activities. Changes in land use and/or habitats are expected to be the major drivers of biodiversity decline, followed in importance by climate change, the accidental or deliberate introduction of species into ecosystems, overexploitation, and pollution (e.g. nitrogen deposition) (Sala et al. 2000, Appendix 5.22). However, the precise ranking of some drivers may vary depending on ecosystem type and species group. Thomas et al. (2004), for instance, suggested that climate change will be as important as land use change in enhancing biodiversity loss over the next 50 years. According to another ranking and listing of BfN (2012), the main threats to biodiversity are

a) habitat destruction, for instance through urbanisation and infrastructure building, deforestation, slash-and-burn agriculture, open-cast mining, draining, various fishing practices, and industrial farming;

b) over-exploitation and degradation, due, for instance, to overgrazing, soil erosion, habitat fragmentation, unsustainable harvesting of timber for firewood, spraying of plant protection products, ground contamination, water pollution, unsustainable tourism, and unsustainable farming;

c) land use change, for instance when ‘extensively’ cultivated land is abandoned or modified for a more intensive kind of farming;

d) deliberate or unintended invasive alien species introductions outside of their natural range, causing severe consequences for their new habitats (e.g. rabbits, rats, and camels in Australia);

e) climate change; if the change takes place faster than ecosystems can adapt, this may result in extinction of isolated populations and species.

The Millennium Ecosystem Assessment (2005) ranks freshwater taxa and particularly amphibians among the most vulnerable species at risk of extinction. 32% of the approximately 7,000 worldwide occurring amphibian species are threatened, but information is more limited compared, for instance, to birds or mammals, leading possibly to an underestimation. Apart from the agricultural stressors, many other causes have been identified to play possibly a role in the global amphibian decline. According to Sala et al. (2000) the five most important drivers of biodiversity loss in lakes and rivers are eutrophication, land use change, acidification, climate change, and water withdrawal. This is also partly because humans strongly inhabit riparian habitats, leading to a concentration of anthropogenic impacts near coastal and freshwater habitats. Most of the aforementioned general threats to biodiversity of all species (e.g. BfN 2012) also apply to amphibian species. An evaluation of the global amphibian decline carried out by Mann et al. (2003) considers pollution in general to be the most important threat to amphibian populations after habitat loss and/or fragmentation.

Collins & Storfer (2003) list six major drivers for amphibian decline that can be assigned to two classes. The first class refers to adverse effects on amphibians taking place since more than a century such as introduction of alien species, habitat over-exploitation and land use change. The second class refers to more recent causes, dating from the middle of the 20th century onwards such as global environmental change including UV radiation and climate change, contaminants, and emerging infectious diseases. All drivers –independently from classification– may strongly interact with each other (Blaustein et al. 2003).

Amphibians are generally more vulnerable to environmental changes and contamination than birds or mammals (Quaranta et al. 2009a). This is because most amphibian species spend their first life stages in aquatic environments and then migrate to terrestrial environments. Hence, if aquatic or terrestrial environments are altered and/or contaminated, amphibians will be affected in both cases. Furthermore, the amphibian skin is generally characterized by a high permeability because it is physiologically involved in respiration and in the regulation of internal concentration of water and ions (Quaranta et al. 2009a). It is therefore also highly susceptible to physico-chemical stressors such as UV radiation, pathogens, or xenobiotics, which may occur as co-stressors to, for instance, agricultural stressors.

Blaustein et al. (1994a) and Blaustein et al. (2003) analysed and reviewed the effects of UV and toxic chemicals on amphibian declines. They showed that many studies exhibited detrimental effects of UV radiation and different toxic chemicals on amphibians. A number of studies also investigated synergistic effects of multiple factors. Blaustein et al. (2003) suggest these interactions to be explored further because amphibians in many cases are exposed to several environmental stressors simultaneously.

Amphibian decline is also suggested to be the result of emerging pathogens and/or parasites. Tropical montane amphibian declines have been associated with chytridiomycosis, a disease caused by a fungal pathogen (*Batrachochytrium dendrobatidis)* leading to an infection of the amphibian skin. This pathogen was found to occur also in relatively remote habitats far off agricultural activity (Berger et al. 1998). Chytridiomycosis is now widely viewed as the leading cause of amphibian decline (Berger et al. 1998; Hero & Morrison 2004; Mann et al. 2009) in the six continents and its outbreak is associated to global warming (Pounds et al. 2006). The Chytridiomycosis effects are also often described to be associated to pollutants and/or pesticides. According to Mann et al. (2009), pesticide exposure may be an important cofactor facilitating the outbreaks of infectious diseases such as Chytridiomycosis.

Another major disease possibly causing amphibian decline and triggered by anthropogenic environmental change is Helminthiasis, a parasitic trematode infection (Johnson & Sutherland 2003). Rohr et al. (2008) assume that the sublethal exposure of amphibians to Glyphosate increases their susceptibility to trematode infections. The authors consider as a major driver for increased trematode infection a pesticide-induced immuno-supression in the tadpoles. Viruses are also considered important emerging pathogens of amphibians worldwide, for instance the *Ambystoma tigrinum* virus from Arizona (Collins & Storfer 2003). Enhanced viral spread may have been caused by human activity.

Numerous studies have demonstrated the role of alien species invasion in amphibian population declines (Kats & Ferrer 2003). Adverse effects recorded include predation by alien species on native species, competition between one or more life stages, introduction of pathogens by non-natives and hybridization (Collins & Storfer 2003). Major contributors to amphibian decline as proved in experimental and field studies are fish, bullfrogs, and crayfish, causing in many places of the world local extinction.

4.2.5 FFH (Habitats Directive) conservation activities

The Habitats Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora aims to protect approx. 220 EU habitats and approximately 1,000 EU species listed in the directive's Annexes. Monitoring of the conservation status is an obligation under the Habitats Directive for all habitats (as listed in the Directive’s Annex I) and species (as listed in the Directive’s Annex II, IV and V) of Community interest. Data needs to be collected both in and outside the Natura 2000 sites (EU 2006). The monitoring also relates to possible successful management measures to conserve or enhance the conservation status in managed Natura 2000 sites such as arable fields. Such management measures can be defined by the Member States and may imply reducing or avoiding plant protection product applications and/or their quantity in and close to the Natura 2000 sites (LUGV 2011). Minimizing or avoiding plant protection product applications may apply to arable fields, their edges, and grassland. Interviews with experts involved in Natura 2000 area management, however, show that implementation of management practices and their monitoring are not yet put into practice.

4.3 Risk assessment of plant protection products as related to amphibian species

4.3.1 Evaluation of the present risk assessment methodology for birds and mammals exposed to plant protection products

The risk of plant protection products to (human and) animal health is considered as a function of exposure, intrinsic toxicity and assessment uncertainty related to a given specific active substance (EFSA 2009a). The risk assessment approaches of plant protection products for birds/mammals and fish are based on a TER (toxicity/exposure ratio) assessment that may comprise several assessment steps (one worst case tier and possible higher tier; Appendix 5.23). The first step is a “screening” of so-called indicator species with worst-case assumptions regarding their exposure. This step is performed in order to highlight those substances that do not require further consideration due to low risk. The second step is a “first tier assessment” for acute and reproductive risks applying more realistic exposure estimates together with generic, not specified focal species. For this assessment, acute and/or reproductive toxicity endpoints may be used. If the acceptability criteria for the calculated toxicity to exposure ratios are not met, further refinement of the assessment can be performed. This third step according to the guidance document (EFSA 2009) is a “higher tier assessment”, with a greater degree of realism, using more realistic and diverse exposure estimates as well as real focal species to calculate the risk. Indicator and generic focal species are assumed to represent real species occurring in a particular environment at a certain time (EFSA 2009a). They are considered to have higher exposure and thus to be protective for all species they represent. The risk assessment approach for fish (EC 2002b) is less complex if compared to the one for birds and mammals. However, the ecotoxicological endpoints can be assessed in complex, higher tier mesocosms, giving a more realistic figure of the impact of ecological processes.

Current risk assessment practice

We reviewed the current risk assessment practice for plant protection products using the relevant EFSA guidance documents for birds and mammals (EFSA 2009a) and fish (EC 2002b) and compared it to the exposure scenarios for amphibians developed in this project. The current assessment practice was then evaluated for applicability to aquatic and terrestrial amphibian life stages.

More specifically, we checked the variety of potential exposure pathways and subsequently investigated the single exposure pathways for their relevance for amphibians. This was done both for lower as well as for higher-tier assessment. We evaluated the toxicity tests whether they cover the specific sensitivity of amphibians a) with respect to acute toxicity testing, reproduction testing, and long-term tests and b) with respect to their endpoint, exposure pathway, and duration of exposure. In case of bird, mammals or fish exposure and toxicity scenarios differing from those we developed and/or reviewed for amphibians (chapter 4.1 and 4.2), we give recommendations regarding the criteria relevant for the exposure and toxicity assessment for amphibian exposed to plant protection products.

Evaluation of the exposure assessment

Table 4.3-1 provides details of the exposure assessment approach for birds and mammals described in the EFSA guideline. The exposure is determined and, in case of low toxicity/exposure ratio, refined by increasing the level of detail among the different steps (screening, first tier, higher tier) (Appendix 5.23). The guidelines list exposure scenarios implying single exposure pathways. For the screening step and the first-tier scenarios, a total of 22 crop groups with similar growing patterns have been defined, covering most of the major crop species in order to account for the plant protection product interception at different BBCH stages. Higher tier assessments according to EFSA (2009a) include inter alia a) refined models of exposure for dietary routes, b) modelling non-dietary routes of exposure, c) field and semi-field studies on animal behaviour and/or residue decline, d) data on wildlife incidents, e) population modelling, f) refinements of phase specific reproductive assessment, g) additional toxicity studies, h) additional toxicity studies on the identified critical life stage, i) increasing scale for species moving in landscape with both treated and untreated areas.

The exposure pathways for mammals/birds covered by the guidelines of EFSA (2009a) are applicable to some extent also to amphibians (Table 4.3-1). In many cases, however, the methodological assessment approach is only partly applicable. Applicable pathways include a) dietary intake of food after spray application; b) food contaminated with granule plant protection product residues; c) drinking water; d) overspray; and e) plant surface contact.

The pathway best transferable to address amphibian exposure to plant protection products appears to be oral food uptake of invertebrates after spray application. Oral intake of treated grains and/or granules does not apply, since amphibians only ingest living organisms. We consider most methodological assessment factors to require adaptation to amphibian biology, for instance, the food intake rate of indicator species, the concentration of active compound in fresh diet, the fraction of diet obtained in treated area, the fraction of food items in mixed diet, and the daily dietary dose.

Direct dermal uptake apart from the plant surface contact and/or inhalation is missing among all pathways for birds and mammals, but constitutes an exposure pathway that has to be additionally evaluated for amphibians together with the pathway listed in the current guidance.

Acute endpoints such as LD_{50} values require being specifically determined for amphibian species –not to mention the missing information on plant protection product toxicity to male and/or female and/or eventually for terrestrial juvenile stages. Also the determination of chronic plant protection product toxicity needs reference to possible agreed endpoints and test settings for amphibians.

In general, there is a lack of information regarding relevant exposure pathways and assessment methodologies for amphibians.

Table 4.3-1: Evaluation of the EFSA 2009a guidelines for the assessment of the risk arising from plant protection product exposure of birds and mammals as for its applicability to amphibian species

exposure risk	**exposure pathway**	**step**	**methodological approach**	**relevance for amphibians**		**adjustments to EFSA (2009)**
				pathway	methodol. approach	
spray application	dietary intake, acute toxicity	screening	ETE = FIR/bw x PT x C *	+ *	+	specific indicator species; amph. migration / sojourn periods; amph. specific FIR, PT; dermal contact
		first tier	ETE = FIR/bw x PT x C	+	+	generic focal species; amph. migration / sojourn periods; amph. specific FIR, PT; dermal contact
		higher tier	residue levels; refinement of PT and PD*; avoidance of contaminated food; semi-field and field studies	+	+	focal species; amph. migration / sojourn periods; amph. specific FIR, PT, PD; dermal contact; residue levels; no food avoidance
	dietary intake; reproduction	screening	ETE = FIR/bw x PT x C; DDD=ARxSVxMAF xTWA*; short term exposure; long term exposure	+	±	specific indicator species; amph. migration / sojourn periods; amph. specific reproduction; dermal contact; amph. specific SV, MAF, TWA
		first tier	ETE = FIR/bw x PT x C DDD=ARxSVxMAF xTWA*;	+	±	generic focal species; dermal contact; amph. specific FIR, PT, SV, MAF, TWA; amph. specific reproduction;
		higher tier	residue levels; refinement of PT and PD*; avoidance of contaminated food; semi-field and field studies; different phases of reproduction; refine ecological parameters	+	±	focal species; amph. migration / sojourn periods; residue levels; no food avoidance; ecological parameters; dermal contact; amph. specific FIR, PT, PD, SV, MAF, TWA; amph. specific reproduction;
seed treatment	oral dietary intake by omnivores, granivores	first tier	ETE_{akut} = FIR/bw x NAR* $ETE_{longterm}$= FIR/bw x NAR x f_{TWA}*	-	-	(dermal contact in case of digging amphibians); amph. migration / sojourn periods;
		higher tier	focus species; refinement of PT, PD; availability of untreated grains; size of food habitat; quantity of ingested grains; seed preference and avoidance; field studies	-	-	(dermal contact in case of digging amphibians); amph. migration / sojourn periods;
granules	a) as food; b) granules as	first tier	amount of granules; amount of active	+	±	amph. migration / sojourn periods; amph. specific

exposure risk	exposure pathway	step	methodological approach	relevance for amphibians		adjustments to EFSA (2009)
granules (contin.)	grit; c) granules instead of small seed; d) food contaminated with soil; e) food contaminated with granule residues; <u>both:</u> acute toxicity and reproduction		substance / granule; amount of soil intake; e) earthworms, seedlings (no standards available); acute toxicity: per day intake; reproduction: f_{TWA}			prey (arthropods etc); no food avoidance; dermal contact; amph. specific reproduction;
		higher tier	avoidance of contaminated food; field studies	-	-	amph. migration / sojourn periods; amph. specific prey (arthropods etc); no food avoidance; dermal contact; amph. specific reproduction;
Drinking water	oral intake	first tier	a) intake from leaf whorls: PECpool = Cspray / 5*; b) intake from puddles: PECpuddle = (AR/10) / (1000 x (w + Koc x s))*; drinking water rates (DWR)	±	±	dermal intake for a) and b); guttation
		higher tier	a) intake from leaf whorls, b) intake from puddles: concentration; drinking water intake and inhalation; dermal exposure (high uncertainty)	±	±	dermal intake for a) and b); guttation
special topics						
bioaccumulation, food chain effects	food intake	first tier	a) food chain: earthworm→birds, mammals; soil moisture content; b) food chain: fish→birds, mammals; c) biomagnifcation	+ (a, c)	+	amph. specific prey (arthropods etc); amph. specific FIR; toxicokinetic studies
endocrine disruption (banned)	food intake, overspray	first tier	in vitro and in vivo screening tests; reproduction; behaviour,	+	±	amph. specific screening tests (whole life cycle);
		higher tier	2-generation test; life stage studies	+	±	amph. specific screening tests (whole life cycle); dermal uptake
metabolites	food, soil, plant surface	higher tier	metabolite levels in food, plants; toxicity test on rats, birds	+	±	amph. specific toxicity tests; dermal uptake

* **Legend**: + applicable; ± partly applicable; - not applicable;

Abbreviations: ETE = Estimated theoretical exposure; FIR = Food intake rate of indicator species [g fresh weight /d]; bw = Body weight [g]; C = Concentration of compound in fresh diet [mg/kg] or can be calculated using residue unit doses (RUD) for the relevant food items; PT = Fraction of diet obtained in treated area (number between 0 and 1); PD*i,dry* = fraction of food item [i] in mixed diet [related to dry weight]; DDD = daily dietary dose; AR = plant protection product application rate; SV = shortcut value; MAF = multiple plant protection product application factor; TWA = time-weighted average factor; NAR = Nominal loading/application rate of active substance in mg/kg seed; f_{TWA} = time weighted average factors; PECpool = Predicted environmental concentration in the pool of the leaf whorl; PECpuddle = plant protection product concentrations in puddles on a field after rainfall; Cspray = plant protection product concentration in spray; Koc = organic carbon adsorption coefficient of a substance; w = Vpw = 0.02 (pore water term); s = Vs × d × fracOC = 0.0015 (soil term);

The exposure assessment for fish (and other aquatic organisms) is detailed by EC (2002b). It includes how FOCUS (Forum for the Co-ordination of pesticide fate models and their USe) surface water exposure assessment methods can be used in aquatic risk assessment procedures, standard (first tier) risk assessments to identify potential issue areas for further assessment, and higher tier risk assessment approaches for toxicity testing, e.g. with fish. Exposure calculations are based on FOCUS surface water scenarios for drift, including drainage and runoff entry routes into surface water and include a tiered sequence of exposure assessment steps: Step 1 = Worst-case loadings; step 2 = Worst-case loadings based on sequential application patterns; step 3 = Realistic worst-case based on crop/climate scenarios (using realistic worst-case soils, topography, water bodies, climate, agronomy); step 4 = Localised/regionalised risk assessment, including potential mitigation measures.

Exposure pathways for aquatic organisms covered by EC (2002b) are generally applicable to amphibian early life stages, however the general limitations of the current FOCUS Surface Water approach (Knäbel et al. 2012) need to be acknowledged. However, the susceptibility of the different life stages of amphibians to plant protection product exposure (Greulich & Pflugmacher 2003), particularly the terrestrial life stages have to be considered.

To evaluate the current status on toxicity data for terrestrial life stages a literature study revealed that only very few data exist that can be used for comparisons with the toxicity data for the bird and mammal risk assessment (see Research Box 9). It was therefore clear, that a data gap regarding the assessment of plant protection product toxicity to amphibian exists and that toxicity data for some selected plant protection products were acutely needed to understand if the risk assessment currently performed for birds and mammals also covers the potential risk of amphibian exposed to plant protection products. These data should allow an evaluation of the effects of a plant protection product application on amphibian in the field through dermal exposure; therefore application rates were tested –in contrast to plant protection product doses or concentrations in the food needed for the assessment of dietary toxicity.

RESEARCH-BOX 9:

Amphibians at risk? - Susceptibility of terrestrial amphibian life stages to plant protection products

BACKGROUND: Current risk assessment of plant protection products does not specifically consider amphibians. Amphibians in the aquatic environment (i.e. aquatic life-stages or postmetamorphic aquatic amphibians) and terrestrial living juvenile or adult amphibians are assumed to be covered by the risk assessment for aquatic invertebrates and fish, or mammals and birds, respectively. This procedure has been evaluated as being sufficiently protective regarding the acute risk posed by a number of plant protection products to aquatic amphibian life stages (eggs, larvae). However, it is unknown whether the exposure and sensitivity of terrestrial living amphibians is comparable to mammalian or avian exposure and sensitivity.

We reviewed the literature on dermal plant protection product absorption and toxicity studies for terrestrial life stages of amphibians focusing on the dermal exposure pathway, i.e. via treated soil or direct overspray.

METHODS: The scope of this review was to evaluate if terrestrial amphibian life-stages (juveniles and adults) are at risk if exposed towards a plant protection product. We disregarded co-stressors and kept the focus on data related to dermal exposure via soil treated with plant protection products or direct overspray, since this is seen as the main pathway in the terrestrial habitat. The literature search revealed that - compared to studies simulating plant protection product exposure and effects in the aquatic habitat - the number of studies simulating and examining exposure and effects in the terrestrial amphibian habitat is low.

RESULTS: The results of the evaluated studies indicate that the transport of plant protection products across the skin is likely to be a significant route of exposure for amphibians and that plant protection products can diffuse one or two orders of magnitude faster into amphibians than into mammals.

Only 13 toxicity studies for terrestrial amphibian life-stages linking field relevant dermal or dietary exposure to terrestrial toxicity data could be evaluated. However, the few existing toxicity data suggest that amphibians can be sublethally or even lethally affected by field relevant terrestrial plant protection product application rates. The fungicide formulation Headline (active ingredient pyraclostrobin) caused the most severe effects, with >50% mortality in juvenile toads at the corn label application rate.

The paucity of published data on terrestrial amphibian life-stages is remarkable, especially with the variety of plant protection product formulations in use for crop protection, the countless possible combinations thereof, the numerous co-stressors like UV-B radiation, pathogens and parasites, as well as the differences in amphibian species sensitivity, indicating the need for further research. However, it should be kept in mind that examining single plant protection products at high concentrations and without addressing the effects of co-stressors may lead to an underestimation of the role of plant protection products in affecting amphibian populations. For the terrestrial life stages of amphibians, the verification of a sufficient protection from unacceptable risks by using the vertebrate data from bird and mammal studies in the risk assessment of plant protection products is imperative.

SOURCE: Carsten A. Brühl, Silvia Pieper and Brigitte Weber (2011) Amphibians at risk? - Susceptibility of terrestrial amphibian life stages to pesticides. *Environmental Toxicology and Chemistry*, Vol. 30, No. 11, 2465–2472. DOI: 10.1002/etc.650

Evaluation of the toxicity assessment

The toxic potential of plant protection products for e.g. mammals, birds or fish is determined for different endpoints in tests for acute toxicity, short-term toxicity and long-term toxicity (EC 2002b; EFSA 2009a; Table 4.3-1). Long-term (chronic) toxicity considers different life stages, health parameters and reproduction.

Mammals and birds: Toxicity testing is generally based on oral uptake of food, grains, granules, soil and water. There is no toxicity testing that specifically addresses the dermal pathway for birds. For mammals, endpoints for dermal toxicity are derived from tests that are employed in the toxicological risk assessment for worker and bystander. However, the characteristics of amphibian skin clearly diverge from the skins employed in the tests, since these always include a thick *stratum corneum*. Acute toxicity provides the LD_{50} values, the lethal threshold dose for oral ingestion, time courses of response and recovery and the no observed effect level (NOEL) for lethality, and must include relevant gross pathological findings (EFSA 2009a). LD_{50} values may be extrapolated from limited dose tests. Reproductive toxicity testing of birds, rats and/or mice can apply a) two-generation reproduction toxicity studies; b) prenatal developmental toxicity study; c) repeated dose (28 days) oral toxicity; and d) subchronic oral toxicity (90 days). Parameters observed include, for instance, adult body weight and food consumption; number of eggs laid per hen. Reproductive toxicity testing (usually rats and mice) of mammals address e.g. male and female reproductive performance including gonadal function, and oestrous cycling, (EFSA 2009a), and is somewhat applicable to amphibians.

Fish: Acute toxicity testing is always required for rainbow trout (*Oncorhynchus mykiss*) and a warm water fish species (EC 2002b). Long-term/chronic toxicity tests of fish have to be carried out if continued or repeated exposure is likely to occur and if the acute toxicity exposure ratio (TER) is assessed to not exceed 100. Testing should follow standard OECD guidelines, for instance, when a full lifecycle (FLC) –testing, extended mortality testing for 14 days, and juvenile growth testing is required. This might be the case if effects on reproduction arising from endocrine disruption are anticipated. In case of toxic metabolites assumed to reach –or to form in– surface waters, the assessment principles are the same as those for active substances.

The practice of testing the toxicity of plant protection products with regard to amphibians is not yet applicable, since currently no test does cover the full life cycle of amphibians as well as the amphibian specific reproduction biology.

Since the existence of this data gap emerged from a detailed literature study (see research box 9), the need for suitable data for ecotoxicological risk assessment regarding amphibian became evident. Experimental studies were performed with a focus on a native frog species and their possible risk through dermal exposure to plant protection products at relevant field rates.

4.3.2 Ecotoxicological studies on the dermal toxicity of plant protection products for amphibians (terrestrial phase): Impacts on juvenile survival of the common frog (*Rana temporaria*)

Studies on the potential exposure, the uptake and the toxicity of plant protection products for the terrestrial life stages of amphibians are scarce or not existent. In order to obtain data concerning the toxicity of plant protection products under realistic conditions, we conducted investigations in the laboratory with juveniles of the European Common Frog *(Rana temporaria*). The following three questions were addressed:

- Establishment and optimization of a test system (**study A**): Which effects has a plant protection product on juveniles of *R. temporaria* exposed under realistic test conditions (i.e. soil substrate planted with barley seedlings)?
- Validation and optimization of a test system according to available literature data using juvenile amphibians and simultaneous exposure to treated soil substrate and spray drift (**study B**). In an extensive literature study (Brühl et al. 2011), the study of Belden et al. (2010) was identified as a suitable reference study (**study B**): Which effects has a plant protection product, used in the reference study, on juveniles of *R. temporaria* exposed under worst-case test conditions (i.e. soil substrate without vegetation)?
- Screening tests using the modified test system from study B (**study C**): Which effects have different plant protection products on juveniles of *R. temporaria* exposed to recommended field rates?

4.3.2.1 Materials and methods

Juvenile *R. temporaria* were caught from the nature protection area "Tal" between CH-4467 Rothenfluh and CH-4469 Anwil (Appendix 5.24) with permission of the responsible nature conservation authority (Bau- und Umweltschutzdirektion/Kanton Basel-Landschaft, Amt für Raumplanung, Abteilung Natur und Landschaft, Rheinstrasse 29, 4410 Liestal, Switzerland). The Common Frog juveniles were sampled using spoon nets and transferred to plastic vessels (378 x 217 x 180 mm) filled with moist paper and grass. For study A, 30 juveniles were sampled on 01 September 2010, for study B and C 150 juveniles on 05 August 2011.

During keeping in the laboratory, the juveniles were transferred from the sampling containers to modified plastic containers Typ III H (378 x 217 x 180 mm; supplier: UNO Roestvaststaal BV, Nederlands). At one side of the containers, an area of 30 x 3 cm was replaced by a mesh (mesh size: 1 mm) to increase ventilation within the containers. The containers were covered with mesh lid (pore size: 1.5 mm), tight-fitted within wooden frames (study A) or frames made of high-quality steel (study B and C). The containers were filled with an approx. 3 cm layer of LUFA-soil 2.3 (LUFA Speyer, Obere Langgasse 40, 67346 Speyer, Germany) planted with barley seedlings (*Avena sativa*, sort: Eunova). Two pot sherds (diameter: 5-7 cm) provided shelter, a petri-dish (diameter: 9 cm) was filled with water. The containers were watered with tap water from above regularly. The juveniles were fed with fruitflies (*Drosophila hydei*), crickets (*Acheta domestica, Gryllus assimilis*) or white woodlouse

(*Trichorhina trementosa*) every two days (suppliers: Zoo Schaub, Rheinstrasse 89, 4410 Liestal, and Qualipet Center Pratteln, Rütliweg 9, 4133 Pratteln, both in Switzerland). The density of the test organisms was 10 per container in study A and 8 per container in study B and C.

During keeping and during the toxicity tests, the environmental conditions in the climate chamber were set at a temperature of 20 ± 2°C, a relative humidity of 75 ± 15% and a light regime of 16:8 hours (day:night).

Experimental set-up

Modified plastic containers Typ II (225 x 167 x 140 mm; supplier: UNO Roestvaststaal BV, Nederlands) were used as test containers (Figure 4.3-1). As already described for the keeping containers (see above), also these test containers were modified by adding an additional mesh area of 170 x 25 mm at one side to optimize ventilation. The containers were covered with a mesh lid (pore size: 1.5 mm), tight-fitted within wooden frames (study A) or frames made of high-quality steel (study B and C). The test containers were filled with an approx. 2 cm layer of LUFA-soil 2.3 (LUFA Speyer, Obere Langgasse 40, 67346 Speyer, Germany), in case of study A additionally planted with barley seedlings (*A. sativa*, sort: Eunova, height approx. 10 cm), in case of study B and C without vegetation.

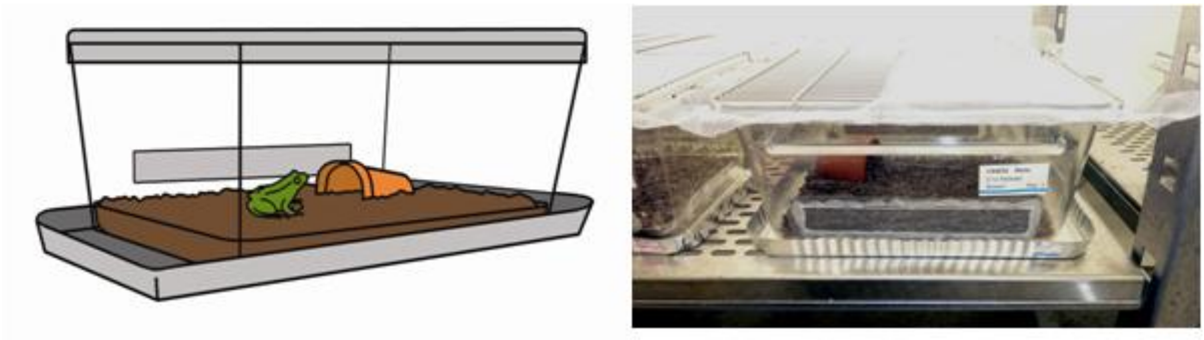


Figure 4.3-1: Test container with extra-ventilation area at one side and potsherd

Watering of the test containers was conducted either from above using a watering can or a spray bottle (study A) or from below (study B and C). In the latter case, test containers were located on aluminium shells (315 x 215 x 15 mm; supplier: LANDI, 4460 Gelterkinden, Switzerland). The substrate was regularly watered by filling the shells with adequate amounts of tape water (target soil moisture 30-50% for study A, near the water holding capacity for study B/C), which diffused to the substrate through the five holes (diameter: 3.2 mm) of the bottom parts of the test containers. Watering from below was advantageous since no forced leaching of the test item from the soil surface took place, and the test organisms were not disturbed by watering from above. Feeding was conducted regularly with the food mentioned above. One half of a potsherd served as shelter. Additional water was provided for the test organisms of study A by a water-filled petri-dish (diameter: 9 cm). The test organisms of study B and C did not get a petri-dish, since the soil substrate was sufficiently moist for water uptake.

For all studies, a method of separating the substrate from the remaining of the test container was introduced. This procedure had the advantage that the soil substrate could be sprayed without so called 'shadow effects' of the side walls which could affect unpredictably the actually applied amounts of the test substance on the substrate. In the case of study A, a plastic foil was located between the test container and the substrate and allowed the removal of the substrate for application. In the case of study B and C, it was not possible to use a plastic foil since the bare soil substrate was not inherently stable in comparison to the rooted substrate with plants of study A. Therefore, the test containers were further modified: the bottom part of the test containers (i.e. 225 x 167 x 15 mm) was cut from the remaining part of the container. Due to the special shape of the test containers (i.e. the area at the bottom (225 x 167 mm) increased continuously to the top (250 x 180 mm), the cut bottom part could be inserted custom-fit into the remaining part of the test container (Figure 4.3-2). Therefore, it was made possible to apply the test substance only to the substrate within the removable bottom part of the test containers.

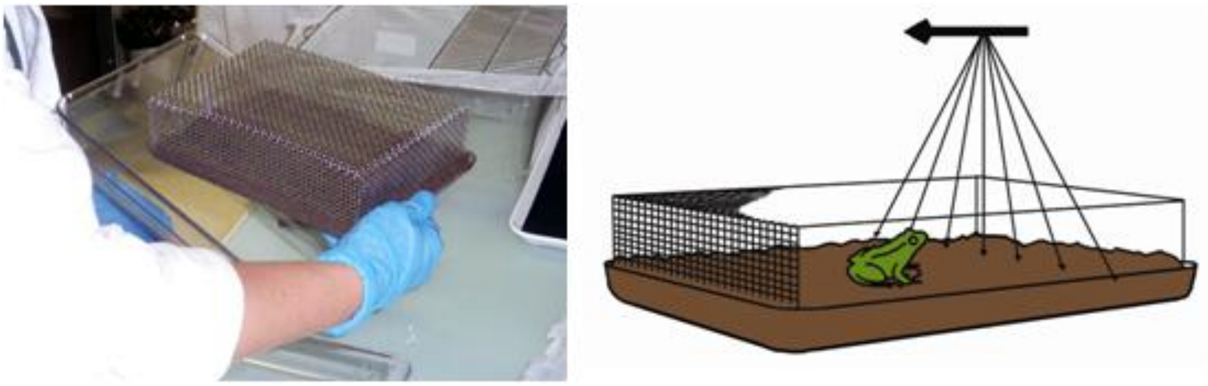


Figure 4.3-2: Transfer of the bottom part of the test container (filled with soil and covered with the application wire cage) into the upper part (left), application of the test organisms confined to the substrate (right).

The test organisms were weighed one day before application and then transferred individually into the test containers by using a spoon net. During application, the test organisms were confined to the substrate within a wire cage, a smaller one for study A (area 5 x 5 cm, height 2.5 cm with a mesh size of 2-4 mm and a wire thickness of approx. 1 mm) and a larger one for studies B and C (21 x 15 x 5 cm with the same mesh size and wire thickness as in study A, Figure 4.3-2). These procedures permitted the simultaneous application of the test substance to the substrate and test organism and avoided as well the escape of the test organisms.

Fungicides, herbicides and insecticide were tested using formulations instead of pure active ingredients (a.i.) since, under field conditions, amphibians come into contact with formulations (i.e. active ingredients and formulation by-products). Seven formulations of plant protection products were selected due to their widespread use in the agricultural practice and their toxic properties regarding fish toxicity, inhalation toxicity, potential for skin and/or eye irritation, potential for skin and/or eye sensitisation. The contents of a.i. and formulation by-products, as available in the public Material Safety Data Sheets, are summarized in the Table 4.3-2. (One of these compounds (Lambda-Cyhalothrin) is also used as biocide.)

Table 4.3-2: Products with contents and CAS-Numbers of active ingredients and by-products, used in studies A, B and C

Study	Product	Active ingredient (a.i.)/by-products	CAS-Nr.	(%)	Effect class	Supplier
A	Trafo WG	Lambda-Cyhalothrin (a.i.)	91465-08-6	5	insecti-cide	Syngenta Agro GmbH, D-63462 Maintal
		Naphthalinsulfonacid/formal-dehyde-condensated, Na-salt	9008-63-3	1-5		
		Citric acid	77-92-9	1-5		
		Talc ($Mg_3H_2(SiO_3)_4$)	14807-96-6	40-50		
B/C	Headline	Pyraclostrobin (a.i.)	175013-18-0	23.6	fungi-cide	BASF SE, D-67056 Ludwigshafen
		Naphthalene	91-20-3	< 8		
		1-Methyl-naphthalene	90-12-0	< 7		
		2-Methyl-naphthalene	91-57-6	<15		
		Solvent naphtha (heavy)	64742-94-5	67		
		Calciumdodecylbenzol-sulfonate in 2-ethylhexanol	n.a.	4-5		
C	BAS 500 18 F (only for internal use)	Pyraclostrobin (a.i.)	175013-18-0	23.6	fungi-cide	
		Solvent naphtha (heavy)	64742-94-5	< 25		
C	Curol B	Bromoxyniloctanoate (a.i.)	1689-99-2	31.7	herbi-cide	Spiess-Urania Chemicals GmbH, D-20097 Hamburg
		Dodecylbenzosulfonate, Ca-salt	68953-96-8	1-5		
		Isobutanol	78-83-1	1 - 5		
		Solvent naphtha (heavy)	64742-94-5	< 25		
C	Captan WDG Omya	Captan (a.i.)	133-06-2	80	fungi-cide	Omya AG AGRO, CH-4665 Oftringen
		Naphthalinsulfonate (condensed)	n.a.	8		
		Dialkylnaphthalinsulfonate	1322-93-6	3		
C	Dicomil ultra royal	Fenoxaprop-P-ethyl (a.i.)	71283-80-2	6.6	herbi-cide	
		Solvent naphtha (light)	64742-95-6	25 - 50		
		Fatalcohol-polyglycolether	9043-30-5	5 - 25		
		Mixture of 5-Chlor-2-methyl-3(2H)-isothiazolon and 2-Methyl-2H-isothiazol-3-on	55965-84-9	0.0015 – 0.1		
C	Prosper	Spiroxamine (a.i.)	118134-30-8	49.8	fungi-cide	Bayer Crop Science AG, D-40789 Monheim am Rhein
		Benzylalcohol	100-51-6	> 25		
		Dodecylbenzolsulfonate, MEA-salt	26836-07-7	5 - 10		
		Ethoxylierted Polyarlyphenol	99734-09-5	1 - 25		
C	Roxion	Dimethoate (a.i.)	60-51-5	40	insecti-cide	Stähler Suisse SA, CH-4800 Zofingen
		Cyclohexanone	108-94-1	40		
		Xylol	1330-20-7	15.2		

The spray applications were conducted with a laboratory track sprayer (Schachtner Fahrzeug- und Gerätetechnik, 71640 Ludwigsburg, Germany). The sprayer was calibrated for each application in order to reach adequately the spray volume of 400 L/ha for study A (standard volume for three-dimensional crops) and 200 L/ha (standard volume for two dimensional areas) (Appendix 5.25).

Since the target application rate referred to the amount of test substance applied on a specified area, it had to be considered that the wire cage would lead to a reduction of the amount of test substance (Figure 4.3-1). This reduction was taken in account in two different ways. For study A and B, the %-reduction of the spray volume by the wire cage was measured before calibration, and accordingly more test substance was measured in the application solution to meet the correct application rate within the wire cage. For study C, the procedure was optimized by conducting the calibration already under the wire cage. The used application rates and the results of calibration are presented in Appendix 5.25.

After calibration, the test organisms and the substrate were simultaneously sprayed (see section Introduction of test organisms). Directly after application, sprayed bottom parts were transferred to the test containers, the wire cages were removed and the mesh lids were drafted on the containers

Test design

In study A, the field rate and the drift rate (2.77% of field rate) of TRAFO WG were tested. Per treatment, 8-9 replicates were set up and treated in the order control (tap water), drift rate and field rate at the same day.

In study B, the field rate and 0.1x and 10x of the field rate of HEADLINE were tested in a design comparable to Belden et al. (2010). Per treatment, 6 replicates were set up and treated in the order control (tap water), 0.1x rate, field rate and 10x field rate at the same day.

In study C, the field rate and 0.1x and 10x of the field rate of six additional plant protection products were tested. Per treatment, 5 replicates were set up, but the test design was modified in order to reduce the number of test organisms as far as possible. First, three test organisms were treated individually with the 0.1x field rate and a time interval between the single treatments of 24 hours each. If these three individually treated test organisms showed no symptoms of toxicity, then the remaining two individuals designed to receive the same rate were treated simultaneously. In the same manner, the field rate and the 10x field rate were individually applied on three test organisms, and dependent on the outcome of these treatments, the remaining test organisms were tested. If in one treatment, the first three test organisms died, no further testing at this treatment was required, and no testing at higher treatment rates was conducted. Accordingly, mortality at these treatment rates was set 100%. A comparison of the different test designs is shown in Table 4.3-3

Table 4.3-3: Test Designs in Studies A, B and C in Comparison to Belden et al. (2010)

Study	A	B	C	Belden et al. 2010
Test species	*Rana temporaria*			*Bufo cognatus*
Developmental stage	juvenile			
Test container, area	375 cm^2			400 cm^2
Test substrate	LUFA-soil 2.3 planted with barley	LUFA-soil 2.3 with no vegetation		sterile soil
Maintenance of soil moisture	watering from above	watering from below		no data
Replicates per treatment	10	6	5	3
Number of test organisms per replicate	1			9
Test duration	14 days	7 days		4 days
Endpoints	mortality/body weight change			mortality
Procdeure of applications	all treatments on ony day		sequential application of individuals	all treatments on one day

Six control runs were performed: One control run with nine test organisms for study A, one run with six test organisms for study B and four runs with ten test organisms for study C (sequential applications of 0.1x field rate, field rate and 10x field rate; see Table 4.3-3). The different number of replicates in each of the three studies was caused by the different designs.

Endpoints and statistical evaluation

Mortality was determined one, two and four hours after application and afterwards daily until the end to the studies, the change of the fresh body weight after 14 (study A) and seven days of exposure (studies B and C). During study A, feeding behaviour and mobility of the test organisms were also recorded, but due to the high variability between individuals, no conclusions could be drawn on these endpoints (data not shown) and no additional assessments of behaviour were conducted during study B and C.

The LR50-values (the median application rate with 50% mortality of the test organisms) for each product were calculated with the software R (R-Development-Core-Team 2010) and the package drc (Ritz & Streibig 2005). Log-logistic and Weibull models were used for calculation of LT50 and to determine the lowest AIC-value (Akaike's Information Criterion) and the lowest variance of residues. For testing differences in the change of fresh weight between animals belonging to treatments and the respective controls, Dunnett t-tests were used after assessment of normal distribution and homogeneity of variances (package multcomp of software R, Hothorn et al. 2008).

The measured juvenile frog mortality values of the tested products (LR50-values standardized to unit kg/ha; Table 4.3-5) of study C were further analysed by two types of statistical calculations. Simple linear correlation analyses were conducted between the LR50-values caused by the products and substance specific parameters (log K_{ow} as a measure for lipophily, content of naphtha-compounds as one of the most frequently used formulation by-products

(Table 4.3-5) as well as with other toxicity endpoints, published in EFSA draft assessment reports (fish and inhalation toxicity, potential for skin and eye irritation and for skin sensitization; Table 4.3-4). The coefficient of determination R^2 indicated to the goodness of fit of the correlations. ANCOVA models were chosen in order to test simultaneously the influence on frog mortality by two variables: one continuous variable (fish and inhalation toxicity, log K_{ow}, content of naphtha-compounds) as independent variable and one categorical variable (potential for skin and eye irritation and for skin sensitization).

Table 4.3-4: Tested Plant Protection Products and Toxicty Data from Fish, Inhalation and Potentials for Skin and Eye Irritation and Skin Sensitisation (a: Classes 0-5 with increasing symptoms (0: no symptoms; 5: severe symptoms), n.a.: No data available.

Study	**Product**	**Active ingredient (a.i.)**	**Fish LC_{50} (mg/L)**		**Inhalation LC_{50} (mg/L)**		**Skin irrita-tion class[a]**		**Eye irrita-tion class [a]**		**Skin sensita-tion class [a]**	
			a.i.	product	a.i.	product	a.i.	product	a.i.	product	a.i.	product
A	Trafo WG	Lambda-Cyhalothrin	0.000002	<0.1	0.060	>1.030	2	4	2	4	1	1
B/C	Headline	Pyraclostrobin	0.006	n.a.	0.690	3.51	4	3	0	3	0	0
C	BAS 500 18 F	Pyraclostrobin	0.006	n.a.	0.690	n.a.	4	n.a.	0	n.a.	0	n.a.
C	Curol B	Bromoxyniloctanoat	0.041	0.127	0.720	2.82	0	4	0	5	1	1
C	Captan WDG Omya	Captan	0.186	0.073	0.670	0.76	0	2	5	4	1	1
C	Dicomil ultra royal	Fenoxaprop-P-ethyl	0.19	4.2	>1.224	10.7	2	1	2	0	1	0
C	Prosper	Spiroxamine	2'410	11.5	2.000	2.32	5	4	0	5	1	1
C	Roxion	Dimethoat	30.2	n.a.	1.680	n.a.	1	1	2	1	n.a.	0

4.3.2.2 Effects of plant protection products on amphibians in terrestrial systems

Mortality

The seven plant protection products, tested in study A, B and C, had different effects on the survival of the test organisms (Table 4.3-5 and Table 4.3-6).

Table 4.3-5: Mortality of Juvenile *Rana temporaria* and Substance-Specific Parameters (TER=Toxicty-Exposure Ratio)

Study	**Product**	**Active ingredient (a.i.)**	**Field rate**	**Density**	**Field rate**	**LR50**	**TER**	**Content of naphtha**	**log Kow**
			(L/ka)	(g/cm3)	(kg/ha)	(kg/ha)		%	(a.i.)
A	Trafo WG	Lambda-Cyhalothrin	---	---	0.150	>0.15	> 1	3	7.0
B/C	Headline	Pyraclostrobin	0.880	1.055	0.928	0.293	0.316	67	3.99
C	BAS 500 18 F	Pyraclostrobin	0.880	1.055	0.928	> 9.548	>10.3	25	3.99
C	Curol B	Bromoxyniloctanoat	1.500	1.034	1.551	1.480	0.954	25	5.9
C	Captan WDG Omya	Captan	---	---	3.200	0.342	0.107	11	2.57
C	Dicomil ultra royal	Fenoxaprop-P-ethyl	1.200	1.050	1.260	4.452	3.53	37.5	4.58
C	Prosper	Spiroxamine	1.500	1.000	1.500	1.431	0.954	0	2.8
C	Roxion	Dimethoat	1.000	1.090	1.090	0.931	0.854	0	0.704

Table 4.3-6: Cumulative Mortality of Juveniles of *Rana temporaria* Exposed to Seven Plant Protection Products

Study	Treatment	Nominal application rate		corre-spon-ding control[a]	No. of intro-duced test orga-nisms	% Mortality from 0 to 7 days after treatment (DAT)							
						0	1	2	3	4	5	6	7
A	control	---	---	---	9	Mortality 11% from DAT 1 to DAT 14							
	Trafo WG	4.155	g/ha		8	Mortality 0% from DAT 1 to DAT 14							
		150	g/ha		9	Mortality 11% from DAT 10 to DAT 14							
B	control	---	---	---	6	0	0	0	0	0	0	0	0
	Headline	131.54	mL/ha		6	0	17	17	0	0	0	17	17
		13144	mL/ha		6	100	100	100	100	100	100	100	100
		1315.4	mL/ha		6	100	100	100	100	100	100	100	100
C	control A	---	---	A	10	0	0	0	0	0	0	0	0
	control B	---	---	B	10	0	0	0	0	10	10	10	10
	control C	---	---	C	10	0	0	0	0	0	0	0	0
	control D	---	---	D	10	10	20	20	20	20	20	20	20
C	Headline[b]	88	mL/ha	D	5	0	0	0	0	0	0	0	0
		880	mL/ha	D	3	100	100	100	100	100	100	100	100
		8800	mL/ha	---	---	---	---	---	---	---	---	---	---
C	BAS 500 18 F	88	mL/ha	B	5	0	0	0	0	0	0	0	0
		880	mL/ha	C	5	0	0	0	0	0	20	20	20
		8800	mL/ha	D	5	0	0	0	0	0	0	20	20
C	Curol B	150	mL/ha	A	5	0	0	0	0	0	0	0	0
		1500	mL/ha	B	5	20	20	20	20	40	40	40	60
		15000	mL/ha	C	3	33	100	100	100	100	100	100	100
C	Captan WDG Omya[b]	320	g/ha	A	5	0	0	0	0	0	20	20	40
		3200	g/ha	B	3	0	100	100	100	100	100	100	100
		32000	g/ha	C	---	---	---	---	---	---	---	---	---
C	Dicomil ultra royal	120	mL/ha	A	5	0	0	0	0	0	0	20	40
		1200	mL/ha	B	5	0	0	20	40	40	40	40	40
		12000	mL/ha	C	5	40	40	40	40	40	40	40	60
C	Prosper	150	mL/ha	A	5	0	0	0	0	0	0	0	0
		1500	mL/ha	B	5	0	0	0	40	40	40	40	60
		15000	mL/ha	C	3	67	100	100	100	100	100	100	100
C	Roxion	100	mL/ha	A	5	0	0	0	0	20	20	20	40
		1000	mL/ha	B	5	0	0	0	0	0	20	40	40
		10000	mL/ha	C	3	0	67	100	100	100	100	100	100

a: In study C, different control runs were conducted for different treatment rates. Whereas the first three test organisms of the test substance rates were treated individually, all control individuals were treated together.

b: In study C, mortality of the first three test organisms in the field rate treatments of Headline and Captan WDG Omya was 100%. Therefore, no further testing was conducted; mortality at field rate and 10x field rate of these products were determined to be 100%.

Under extended laboratory conditions of study A (i.e. soil substrate planted with barley seedlings), mortality was 11, 0 and 11% in the control and in the treatments with the drift application rate and the field application rate of Trafo WG, respectively (Table 4.3-6). Therefore, Trafo WG did not increase mortality after 14 days of exposure at the used test conditions.

Under laboratory worst case conditions (i.e. soil substrate without vegetation), all plant protection products, tested at field rates, caused mortality from 20 to 100% (Table 4.3-6). The mortality ranged from 0 to 40% at 0.1x field rate. Mortality at 10x field rate was lower than 100% only in the treatments with Dicomil ultra royal (40%) and BAS 500 18 F (20%).

Mortality was observed in different time periods after application:

- Headline (a.i.: Pyraclostrobin): Within one hour after application of the field rate (both study B and C) and 10x field rate (study B), the test organisms showed uncoordinated behavior and reduced movement and died. Therefore, mortality was set 100% at field rate and no further testing of 10x field rate was conducted.
- BAS 500 18 F (a.i.: Pyraclostrobin): No abnormal behavior of the test organisms was observed the first days after application. Dead test organisms were recorded five and six days after application at the two higher rates.
- Curol B (a.i.: Bromoxyniloctanoate): Within one hour after application of the 10x field rate, the three test organisms showed uncoordinated behavior and reduced movement and died. No further testing at this test rate was conducted. At the field rate, test organisms died between the day of application and the seventh day of observation.
- Captan WDG Omya (a.i.: Captan): No unusual behavior was observed after the application of the field rate but all three test organisms were dead within 24 hours of exposure. Therefore, mortality was set 100% at field rate and no further testing of 10x field rate was conducted. At 0.1x field rate, mortality occurred between four and five and six and seven days after application.
- Dicomil ultra royal (a.i.: Fenoxaprop-P-ethyl): at 10x field rate, two test organisms showed reduced mobility and died within 24 hours after application, one additional test organism was found dead seven days after application. Mortality occurred earlier at the field rate (two to three days after application) than at 0.1x field rate (six to seven days after application).
- Prosper (a.i.: Spiroxamine): Within 24 hours after application of the 10x field rate, the test organisms showed uncoordinated behavior and died. No further testing at this test rate was conducted. At the field rate, dead test organisms were found three and seven days after application.
- Roxion (a.i.: Dimthoate): Within 24 hours after application of the 10x field rate, two test organisms died. One additional test organism was dead 48 hours after application. No further testing at this test rate was conducted. At the lower test rates, mortality occurred four and five days after application.

The sequential testing procedure in study C reduced the number of test organisms used in the tests. In the treatments with the field rate of Headline and Captan WDG Omya only three test organisms had to be tested and testing at 10x field rate could be avoided due to 100% mortality at the field rate. In the treatments with 10x field rate of Curol B and Prosper, only three test organisms were tested and further testing was ceased, since mortality at 10x field rate occurred after 24 hours of exposure. In the case of Roxion, testing was also stopped after mortality of three test organisms within 48 hours of exposure.

For the plant protection products tested in Study B and C, the calculated LR_{50}-values for amphibians ranged from 0.278 L/ha to >8.8 L/ha (Table 4.3-7) and were lower than the field application rates of five from seven plant protection products. Only BAS 50018 F and Dicomil ultra royal showed an opposite pattern. For Trafo WG of study A, no LR_{50} could be calculated since no significant mortality occurred.

Table 4.3-7: LR_{50}-Values for Amphibian exposed to plant protection products in terrestrial model systems. Overspray scenario; study period seven days.

Product	Field rate	LR50	SE [a]	95% conf. limits	AIC [b]	Residual variance	Model
Headline	0.88 L/ha	0.278 L/ha	10.000	n.a.	-40	n.a.	log-logistisch (LL.2)
BAS50018F	0.88 L/ha	> 8.8 L/ha	n.a.	n.a.	n.a.	n.a.	n.a.
CurolB	1.50 L/ha	1.431 L/ha	0.004	0.053	-51.9	7.20E-10	log-logistisch (LL.2)
Captan WDG Omya	3.21 kg/ha	0.342 kg/ha	0.165	n.a.	-29.1	n.a.	Weibull Typ 1 (W1.2)
Dicomil ultra royal	1.20 L/ha	4.240 L/ha	7.410	94.150	-1	0.017	Weibull Typ 2 (W2.2)
Prosper	1.50 L/ha	1.431 L/ha	0.004	0.053	-51.9	7.20E-10	log-logistisch (LL.2)
Roxion	1.00 L/ha	0.854 L/ha	1.280	16.259	3.64	0.080	Weibull Typ 2 (W2.2)

A: Standard error.
AIC: Akaike's Information Criterion.

Body weight change

The mean fresh body weight of all animals employed in the prospective treatments ranged from 820 to 901 mg (study A), from 294 to 345 mg (study B) and from 668 to 1405 mg (study C) before test start (Appendix 5.26) and did not differ statistically significantly between the prospective treatments ($p>0.05$, ANOVA with body weight as dependent variable and treatment as independent variable). The test organism were randomly assiged to the treatments

At test end, the mean body weight in all treatments ranged from 894 to 988 (study A), from 362 to 375 mg (study B) and from 703 to 1302 mg (study C). Accordingly, the mean body weight change in all treatments varied from -8.0 to +22.7%. In the six control runs, body weight change varied from -0.5 to 13.2%. Due to the high variability within treatments, no statistically significant differences in body weight between the test rates and the control could be detected at the end of the test for most comparisons. Only the mean values for body weight change of the treatments with 0.1x field rate of Dicomil royal ultra (-8.0%) and 0.1x field rate of Curol B (-5.8%) were statistically significantly increased compared to the respective control (+3.7%, Dunnett t-tests, one-sided smaller, $\alpha=0.05$). However, these statistical findings could not be cleary attributed to a treatment effect since no statistically significant effects on body weight change were found at the next higher test rates.

Relations between juvenile frog mortality, substance-specific parameters and additional toxicity data

The calculations of simple linear regressions between toxicity values of juvenile *R. temporaria* and different substance-specific parameters as well as published toxicity data revealed no statistical significant relationship for the majority of investigated parameters (Table 4.3-8). This was not unexpected, as the number of tested plant protection products was rather low (i.e. n=7 and lower, see Table 4.3-4 and Table 4.3-5). The only relationship that proved to be statistically significant was the one detected between values of product inhalation toxicity and the toxicity of the plant protection product to *R. temporaria* -expressed as LR50 as well as LR50 as % of the field rate.

Table 4.3-8: Simple linear regression between frog toxicity and substance-specific parameters

Simple linear regression: y = a * x					
Variable x		**Variable y1**		**Variable y2**	
Parameter	with reference to	LR50 (kg/ha)		LR50 as % of field rate	
		R^2	p-value	R^2	p-value
Fish toxicity	a.i.	0.032	0.736	0.012	0.837
	product	0.028	0.833	0.026	0.838
Inhalation toxicity	a.i.	0.277	0.362	0.055	0.656
	product	**0.857**	**0.024**	**0.915**	**0.011**
Potential for eye irirtation	a.i.	0.004	0.910	0.004	0.906
	product	0.315	0.247	0.419	0.165
Potential for skin irirtation	a.i.	<0.000	0.972	0.002	0.940
	product	0.124	0.494	0.18	0.401
% content naphtha	product	0.008	0.863	0.018	0.801
Log Kow	a.i.	0.143	0.459	0.105	0.531

Additionally, ANCOVA-models were calculated with LC50-values of *R. temporaria* and two variables, one continuous variable and one categorical variable (Table 4.3-9). These models did not show statistically significant results in the majority of combinations (data not shown), but in the case of inhalation toxicity, the results of the simple linear regression were corroborated. Furthermore, the inclusion of skin sensitization as categorical variable increased the statistical significance of the correlation. Since we do not know the physico-chemical parameters causing high inhalation toxicity and skin sensitization potential of the different products, the described correlations are insofar spurious as the 'true' variables are unknown.

Table 4.3-9: Relationships between toxicity of plant protection products to frogs (LR50 for *R. temporaria*) and other determined toxicity parameter as well as co-formulant contents in tested products

ANCOVA model: y = a * x + b						
y = LR_{50} of *R. temporaria*						
Variable x		**Variable b**		**Statistical overview**		
Parameter	with refe-rence to	Parameter	with refe-rence to	p-value for linear regression	p-value for ANCOVA model	R^2 for ANCOVA model
Inhalation toxicity	a.i.	Skin irritation	a.i./product	>0.05/>0.05	---	---
		Eye irritation	a.i./product	>0.05/>0.05	---	---
		Skin sensitisation	a.i./product	>0.05/>0.05	---	---
		% content of naphtha	a.i./product	>0.05/>0.05	---	---
	product	Skin irritation	a.i./product	>0.05/>0.05	---	---
		Eye irritation	a.i./product	>0.05/>0.05	---	---
		Skin sensitisation	**a.i.**	**0.038**	**0.011**	**0.989**
		Skin sensitisation	**product**	**0.010**	**0.003**	**0.997**
		% content of naphtha	a.i./product	>0.05/>0.05	---	---

4.3.2.3 Relevance of plant protection product toxicity findings for amphibians in the field

Suitability of the used test system

The conditions during keeping the juveniles before the start of the tests were favourable resulting to very low mortality. The test system with plastic test containers, modified by improved ventilation at one site, natural soil substrate with plants (study A) or unplanted (study B and C) and one individual per test container showed reliable results concerning survival of the test organisms. During the exposure of seven (study B and C) or 14 days (study A), mortality was low in the seven control runs, ranging from 0 to 20% (in sum 4 dead individuals out of 45).

The test containers allow testing of different conditions, e.g. worst case laboratory conditions (i.e. unplanted soil substrate) and extended laboratory conditions (i.e. soil substrate with plants at different densities). Therefore, studies with specific questions concerning different degrees of realistic exposure scenarios are possible.

This test design used a similar design as in former study (Belden et al. 2010) with a reduced number of test organisms and came to comparable results: Belden et al. tested juveniles of the Great Plains toad (*Bufo cognatus*) in three test containers per treatment with natural soil and a comparable size of test containers but with a density of 9-10 individuals per test container. They tested three treatment rates (0.1x, 1x and 10x field rate) of the fungicides Headline (a.i.: Pyraclostrobin), Stratego (a.i.s: Propiconazole and Trifloxystrobin) and Quilt (a.i.s:

Propiconazole and Azoxystrobin) as overspray and found the largest effects for Headline. Juvenile mortality of *B. cognatus* after three days of exposure was 8, 65 and 100% with increasing field rate. In study B and C, the effects of Headline on *R. temporaria* were even larger with 0, 100 and 100% with increasing field rate. Therefore, the results of these two amphibian species are comparable, the higher susceptibility of the frog species compared to the toad species may be caused by differences in skin properties. Additionally, also the timing of the effect between application and the observed mortality was rather comparable, within one hour in study B and C, within 24 hours in Belden et al. (2010).

Therefore, the test system is suitable to detect potential lethal effects of plant protection products, applied at agriculturally relevant rates. In the opposite to Belden et al. (2010), the density of one individual per test container should be preferred in order to reduce the number of test organisms per treatment. The sequential test procedure, as recommended in the OECD Guidelines 420 and 425 (OECD 2001; 2008b), is an additional important instrument mean to minimize the number of test organisms.

Exposure to plant protection products in a treated field

The relevance of the results of the presented studies C depends on the answer to the question whether amphibians do spend a relevant part of their lifetime in agricultural fields and are potentially exposed to plant protection products.

Amphibians can come into contact with plant protection products during or after applications of these products 1) during foraging on agricultural fields by direct overspray, 2) during foraging on field margins by exposure to drift, and 3) during crossing agricultural fields to or from breeding sites by exposure to overspray and drift (see chapter 4.1). The majority of amphibian species have no preference for agricultural fields (Sowig 2007), but the Great Crested Newt (*Triturus vulgaris*), the European Common Spadefoot (*Pelobates fuscus*), the Natterjack Toad (*Bufo calamitans*), European Green Toad (*Bufo viridis*) and the Moor Frog (*Rana arvalis*) are known to use agricultural fields as habitat (Günther 1996a; Laufer et al. 2007a). In a recent study in NO-Germany, 25-40% of the population of *P. fuscus*, *R. arvalis* and the Common Toad (*Bufo bufo*) were found within agricultural fields (Berger et al. 2011b). In the opposite, less than 15% of populations of *T. vulgaris* and the European Fire-Bellied Toad *(Bombina bombina*) used agricultural fields. Activity of juveniles of *T. vulgaris*, *R. arvalis* and *B. bufo* within agricultural fields was higher compared to the activity of the adults. Set-aside fields and field margins were preferred by *T. vulgaris*, *B. bombina*, *B. viridis* and the Common Newt (*Triturus vulgaris*) exemplified by up to 80% of captures.

During migrations to or from breeding sites, many species normally avoid agricultural fields do cross them, especially if the breeding sites are located within the agricultural fields (Berger et al. 2011c). On cereal and rape fields in NO-Germany, a synchronicity was identified between the immigration of the adults to the breeding sites as well as the emigration of the juveniles and adults from the breeding sites and the application of plant protection products, especially fungicides and herbicides (Berger et al. 2011c). During these periods, up to 80% of migrating individuals were counted on agricultural fields. Similar relationships were found for five out of eight amphibian species within a viniculture landscape in S-Germany (Lenhardt

2011). Therefore, the risk for amphibians to come into contact with plant protection products is rather high and a reality in differently cultivated agricultural fields. An exposure to biocides might occure via the application of manure or sewage sludge (for manure see chapter 4.1.6.).

Incorporation of plant protection products into amphibians

During the authorization procedures of plant protection products, the potential risk for terrestrial vertebrates exposed to plant protection products and biocides is assessed through the evaluation of the oral exposure, since this is considered to be the main exposure route for birds and mammals (Smith et al. 2007b). Amphibians can intake plant protection products with contaminated food (Schuytema et al. 1993; Albert et al. 2007), but, probably, the most severe route of contamination is through the adsorption by the skin (Smith et al. 2007b; Linder et al. 2010).

The outer skin of the amphibians, the *stratum corneum*, is composed of only one layer of flat cells and is 10x thinner than the *stratum corneum* of pigs (Quaranta et al. 2009b). The thickness, blood supply and permeability of the amphibian skin are variably distributed over the body surface. The ventral skin is thinner and more permeable than the dorsal skin, especially the skin in the pelvic region (pelvic patch) is permeable and well provided with blood vessels (Wells 2007).

In two *in vitro* studies, 46-83% of the insecticide Malathion was incorporated by the American Bullfrog (*Rana catesbeiana*) and the Cane Toad (*Bufo marinus*) through the skin (Willens et al. 2006). In another *in vitro* study, the skin permeability of pig ears was used as a skin model for mammals and compared to the ventral skin of the European Tree Frog (*Hyla arborea*) testing two chemicals (Mannitol and Antipyrin) and three herbicides (Atrazine, Paraquat, Glyphosate). The frog skin was up to 320x more permeable (in the case of atrazine) than the pig ear skin. The potential to permeate membranes correlates with the lipophily of the substances (log Kow). In an *in vivo* experiment, dehydrated individuals of the American Toad (*Bufo americanus*) were exposed to soil treated with atrazine and incorporated the herbicide in the gall bladder and gut (StorrsMendez et al. 2009). Individuals of the Tiger Salamander (*Ambystoma tigrinum*) absorbed Malathion from the treated soil and distributed the insecticide within the body (Henson-Ramsey et al. 2008).

Therefore, the results of these studies indicate that the dermal route can be considered as a far more important entry for plant protection products and biocides into the terrestrial life stage of amphibians than it is the case for birds and mammals. Moreover, the oral uptake route for plant protection products in amphibians is deemed to be less significant than in birds and mammals routinely addressed in the plant protection product risk assessment, since the food uptake rate is lower.

Toxicity of plant protection products in the performed studies

In study A, juveniles of *R. temporaria* were exposed to the overspray of drift and field rate of the insecticide Trafo WG (a.i.: Lambda-Cyhalothrin) on natural soil planted with barley seedlings and did not show any sign of treatment-related mortality after 14 days of exposure. This result may be caused by an intrinsic low toxicity of Lamda-Cyhalothrin to amphibians.

However, an interception of the test substance by the vegetation (i.e. proportion of sprayed plant protection product which does not reach the soil surface due to retention on plant surfaces) as well as the known rapid degradation of the active ingredient on soil and plant surfaces should not be disregarded in the interpretation of the studies. Clearly, it is important to select the suitable test system for detecting the risk of amphibian juveniles within the agriculturally modified landscape. The juveniles may be rather protected from overspray by plant protection products within a dense vegetation structure but may be at risk when they move on bare soil between rows of crop or on fields after harvest or before emergence of sown crop (Berger et al. 2011b).

Therefore, a worst case but realistic scenario with bare soil substrate was chosen for the subsequent studies (B and C). Seven plant protection products were tested and caused amphibian mortalities from 20 to 100% at the currently authorized field rate. Even at 0.1x field rate, amphibian mortality was 40% after application of three out of seven plant protection products. Therefore, juvenile frogs are not only at risk within the agricultural fields but also in field margins and field boundaries when exposed to drift during and after applications.

Herbicides are normally used for reducing weeds in fields with low vegetation cover and accordingly with low interception, either before or already after emergence of the crop (Roth & Rosner 2011a). Therefore, the study results for the herbicides Curol B (a.i.: Bromoxyniloctanoate) and Dicomil ultra royal (a.i.: Fenoxaprop-P-ethyl) showing amphibian mortalities between 40 and 60% at field rate point to a high risk for migrating amphibians through herbicides sprayed on turf grass and cereal fields (authorisation by Swiss Federal Office for Agriculture FOAG, status from January 2013).

Fungicides and insecticides are normally applied on full grown crops with high plant densities, resulting in interception values up to 90% (Roth & Rosner 2011a). Accordingly, only up to 10% of the applied field rate is expected to reach the soil surface and to come into contact with amphibians. It should be remembered, though, that a proportion of the plant protection products intercepted by the vegetation might be washed off by rain and reach later the soil as well. Whereas the insecticide Roxion (a.i. Dimethoate) caused 40% ampibian mortality at 0.1x field rate, no effects were observed for the fungicides Headline (a.i.: Pyraclostrobin) and Prosper (a.s. Spiroxamine) at these rates. The fungicide Captan Omya WG (a.i.: Captan) is widely used in orchards and its application in the test system resulted in amphibian mortalities of 40% at 0.1x field rate and 100% at field rate. Therefore, a high risk is expected for amphibians exposed to Captan at the intended uses, since even drift deposition rates cause significant mortality rates after seven days. Since the dead animals were recorded at the end of the test, it is not precluded that higher mortalities would have occurred after seven days.

The only plant protection product with low effects up to 10x field rate was the fungicide BASF 500 18 F which contains the same content of the active ingredient Pyraclostrobin as Headline but a lower amount of naphtha-compounds (Table 4.3-2). This suggests that the high mortality of *R. temporaria* treated with Headline is not caused by the active ingredient itself but by the sum of by-products or some of the constituents, e.g. the naphtha-compounds.

Possibly, the co-formulants do enhance the effects of the active ingredient by improving their uptake and ease the access of the active ingredients to target organs.

Naphtha-compounds as whole may have an impact but cannot be conclusively evaluated since the number of data points is only limited and no statistically significant relationship was detected between the LR50 values of the used plant protection products and the %-content of naphtha-compounds. In particular, the high toxicity of Captan, formulated as wettable powder did not support the hypothesis of a linear correlation between content of Naphtha and the LC/R50 values for amphibian. It should be kept in mind, though, that no test was performed with the aim of investigate the toxicity of co-formulants to amphibians. Therefore, it cannot be ruled out that one or several specific compounds within the mixture or a synergistic interaction between naphtha-compounds or other by-products with the active ingredient may be responsible for the high mortality of test organisms treated with Headline as compared to the treatments with BASF 500 18 F. These results highlight once more the frequent discrepancies in toxicity between active ingredients and formulations and to the scarce knowledge about potential effects of by-products in formulations. In particular, even if some acute tests are performed with the formulated product and evaluated during the authorization of plant protection products, usually even less data are available on the chronic toxicity of plant protection products to non-target organisms. An additional very important but disregarded problem is the influence of mixtures of different plant protection products on juveniles and adult amphibians, not to forget fertilizers that can also be detrimental (Schütz et al. 2011). (Note: The application of manure or sewage sludge might also be detrimental to amphibians since (1) it could contain biocides and (2) the overspray with manure or sewage sludge itself might harm the skin of amphibians (for manure see chapter 4.1.6).

The mortality of juveniles and adults amphibians due to the effects of plant protection products may have negative effects on the level of populations. In a modeling study, the Common Frog (*R. temporaria*), the Northern Red-Legged Frog (*R. aurora*) and the Western Toad (*Bufo boreas*) were more impacted by reduced survival of the juveniles and adults than by a reduction of survival of the other life-stages (Biek et al. 2002). Therefore, the long-term use of plant protection products at the same time as the migrations of juvenile and adult amphibians is a matter of concern.

4.3.3 Development of supplementary modules for amphibian specific risk assessments in the authorization of plant protection products

Adjusting and/or supplementing exposure assessments

Dermal exposure: In addition to oral ingestion of contaminated food, the dermal exposure pathway of amphibians to plant protection products should be considered for a realistic assessment of the risk arising for the use of plant protection products (Table 4.3-10).

On the one side, the amphibians may have direct contact to plant protection products both through overspray and to treated soils and plants, absorbing the chemicals through their skin (chapters 4.1 and 4.2; Research Box 9, Bruehl et al. 2011). During migration to spawning ponds, dermal absorption is to be primarily considered, because amphibians are oriented towards mating and feeding is very limited during this period (chapter 4.1). After migrating from the spawning ponds and the subsequent terrestrial sojourn, both an exposure to plant protection products via the food and the dermal pathway may occur. This applies to amphibianin in-field as well as off-field habitats adjacent to arable fields, and should be considered according to EC (2002c) for terrestrial organisms in general.

On the other side, amphibians may also have direct skin contact with treated seeds in the fields. At risk are particularly burying amphibians long-term exposed during dry weather periods or when searching for the winter habitats. Moreover, short-term exposure may happen daily during resting. In addition to treated seeds in field, an exposure may occur also to granules particles (contaminated food or residues in food), coming into contact with the amphibian skin.

In order to assess dermal exposure pathways, the guidance provided to users of plant protection products by EC (2004) can be consulted, but it requires adjustment to the amphibian biology.

Dietary intake: The consideration of the exposure pathway for dietary intake of food contaminated with plant protection products in risk assessment procedures is basically applicable to amphibians. For acute toxicity, however, it requires adjustments to amphibian specific physiology and behaviour. This applies particularly with regards to a) migration and field sojourn periods as amphibians may live and feed for long periods on and close to fields; b) the amphibian specific food intake rate; c) the composition of the animal’s daily diet, since amphibians are exclusively carnivorous; d) the proportion of diet from plant protection product treated area (food predominantly from treated areas); and e) residue levels (Table 4.3-10). Furthermore, amphibians are not known to avoid contaminated food, as suggested by EFSA (2009b). In order to reproduce, amphibians need both terrestrial and aquatic habitats. Hence, with regards to chronic toxicity and reproduction it requires correction of at least the following amphibian specific factors: b) the amphibian specific food intake rate; c) the proportion of animal’s daily diet from mixed diet; d) the proportion of diet from plant protection product treated area; e) time-weighted amphibian specific average factors; and f) multiple plant protection product application factor (Table 4.3-10).

Granule particles: Oral ingestion of granule particles is to be restricted to contaminated food or residues in the prey, such as earthworms.

Drinking water: Amphibians absorb water primarily through their skin. The two EFSA approaches for assessing drinking water toxicity should be amended or replaced by the dermal absorption of water to be applicable to amphibians (see above). Amphibian exposure in contaminated puddles usually representing suitable habitats is assumed to be considerable. Guttation droplets, formed by some crops and contaminated with systemic active ingredients, can be stripped off by amphibians moving by and should be considered as another relevant exposure pathway.

Bioaccumulation: Food chain parameters have to be adjusted to the amphibian biology. If specific differences are to be expected, amphibian toxicokinetic studies could be performed.

We suggest following specific methological adjustments to EFSA (2009a):

- crop and BBCH specific interception data require approximation to amphibian migration and sojourn habits (both juvenile/adults and different species groups) (chapter 4.1).

- indicator species, generic focal species, focal species should be specified for amphibians due to their specific physiology, morphology, and migration habits (e.g. energy requirements, body weight, absorption capacity, etc).

- the dermal absorption of plant protection products from contaminated soil requires a specific focus. The Guidance on dermal absorption (EC 2004) does not address the amphibian specific movement behaviour.

Table 4.3-10: Suggestions for methodological requirements as adjustment and supplement to the current exposure assessment for vertebrates in the authorization of plant protection products.

exposure pathway	requirements
diet	major methodological adjustments
other oral intake	
- granulate / treated seeds	major methodological adjustments
- soil	major methodological adjustments
- surface waters	major methodological adjustments
- puddles in fields	major methodological adjustments
dermal uptake	
- soil	new assessment methodology
- surface waters	new assessment methodology
- puddles in fields	new assessment methodology
- direct contact	new assessment methodology
- treated seeds	new assessment methodology
respiration	methodological adjustments

Adjusting and/or amending toxicity assessments

Acute toxicity: The methodology of oral intake as suggested to birds/mammals (EFSA 2009a) should be expanded to include acute dermal uptake of amphibians during terrestrial phases as detailed in the guidance provided to users of plant protection product products by EC 2004. Acute toxicity testing for fish with increased water plant protection product concentrations (EC 2002b) is considered partly applicable to the aquatic early phases of amphibians, but this should be verified periodically.

Short term toxicity: Short term toxicity assessments as suggested for birds/mammals (EFSA 2009a) should also include dermal uptake (EC 2004) by amphibians during their terrestrial phases. Apart from the pathological parameters previewed for birds/mammals it should encompass at minimum observations of amphibians' dermal irritations during terrestrial phases. We recommend, however, verifying the transferability of testing done for birds/mammals/fish to amphibians especially with regard to their biphasic behaviour and living.

Long term and/or reproductive toxicity: Long term toxicity assessments should include parameters for both dermal and dietary uptake of plant protection products by amphibians during their terrestrial phases (EC 2004; EFSA 2009a). Parameters observed should encompass inter alia body weight; food consumption habits; animal behaviour (hiding, reaction time after plant protection product exposure etc.); endocrine effects, for instance, spawning features and habits; proportion of fertile spawn; viability of embryos; larval development and survival, juvenile and adult survival rate under repeated dose (28 days) toxicity and subchronic toxicity (90 days); and dermal anomalies. Hence, toxicity parameters should relate to both aquatic and terrestrial phases.

In short:

- Amphibians have a particularly high exposure risk to plant protection products. Moreover, even the exposure to 10% of the field rate of several plant protection products causes lethal effects on juvenile amphibians in experimental set ups.
- The present risk assessment methodology for plant protection products is only partly appropriate to cover the amphibian specific risk exposed to plant protection products. Approaches for acute toxicity testing of food intake after spray application are applicable to amphibians.
- The dermal pathway of plant protection product exposure is presently not assessed. Our results point out that the skin-soil contact (and likely the skin-vegetation contact) can be considerable and that, treated soil provided, the exposure to plant protection products during migration may by far exceed the exposure to direct overspray with plant protection products.
- Thus, we recommend to broaden the present risk assessment methodology for plant protection products with regard to the dermal pathway of plant protection product exposure for amphibians.
- The application of manure or sewage sludge might also be detrimental to amphibians since (1) it could contain biocides and (2) the overspray with manure or sewage sludge itself might harm the skin of amphibians. Therefore, it should be checked whether also the environmental risk assessment for biocides needs to be adapted accordingly.

4.4 Risk management measures for plant protection products

Risk management concepts for plant protection products dedicated to the protection of amphibians should cover different approaches.

Apart from a wide range of rather specific measures, first of all an overall reduction of plant protection product use should be envisaged. We point to the organic farming as a consequent way to omit synthetic plant protection product application in agriculture and to avoid exposure of amphibians to these chemicals. Because of the broad knowledge on this type of agriculture and its dependency from politics and markets we do not particularly stress this topic here. We therefore exclusively refer to agricultural systems applying synthetic plant protection products. Integrated pest management (IPM) is an appropriate measure for achieving this (please refer to Table 4.4-2, point 1.1) and its application is fixed in the EU directive on the sustainable use of plant protection products (EC 2009). This framework suggests primarily applying appropriate non-chemical cultivation measure including crop rotation, adequate cultivation techniques (seed bed preparation, sowing dates etc.), balanced fertilisation and others. The usage of synthetic plant protection products is restricted to the minimum necessary extent and considered the ultimate measure for pest control. As of 2014, general principles of IPM shall be fixed via national legislation in cross-compliance and its application will be controlled. Additionally, (point 1.2), a voluntary implementation of sophisticated integrated pest management measures based on crop or sector-specific guidelines of IPM is strived for. Due to high labour efforts and some uncertainties on economic implications, the interest of farmers in applying this voluntarily may be rather low. Further instruments like stepwise increasing taxation of plant protection products may lead to higher prices and make non-chemical cultivation measures more attractive (point 1.3). This would strengthen farmers interest in IPM but may have unexpected and unbalanced impacts on agriculture and does not have any positive repercussion on policy. Increasing public awareness – the probably most powerful instrument – while forcing farmers to use less plant protection product is also not likely (1.4).

Measures specifically dedicated to avoid exposure of amphibians to plant protection products includes a wide range of options: a) the improved management of terrestrial hot spots of amphibian presence (Table 4.4-2, points 2.1-2.4), b) measures for reducing migration demands of amphibians on crop fields (points 2.5 and 2.6), c) controlling plant protection product application on fields by time shift of application dates (points 2.7 and 2.9), d) replacing plant protection products (2.8), and e) avoiding spray application by injecting plant protection products into soils (point 2.10).

However, some of these measures might be accompanied by side-effects deleterious for other organism groups.

With regard to the benefit of the suggested measures to amphibians and their possible short- or medium-term implementation (coloured boxes in Table 4.4-2), we primarily recommend

a) buffer strips around breeding ponds and suitable areas on wet spots as well as their pro-amphibian management (2.1 and 2.2),

b) providing terrestrial habitats next to breeding ponds (2.5),
c) establishing flowering strips and areas in fields to reduce migration distances and to provide terrestrial habitats (2.6), and
d) time shifting of plant protection product application (2.9).

The chances for establishing suitable habitats on cropland leading to reduced exposure risk of amphibians to plant protection products largely depends on the decision of the European Commission on the proposed obligation for providing ecological focus areas (EFA) on seven per cent of agricultural land in Europe from 2014 onward (Ciolos 2011). Furthermore, the pro-amphibian management of EFA needs funding possibly provided by agri-environmental measures of the German Lands as part of the second pillar of EU agricultural policy. The common action “Improvement of agrarian structures and coastal protection“ (GAK) of the German government provides further funding for establishing flowering strips and areas partially functioning as amphibian habitats, too (BMELV 2008). This programme covers the period from 2010 to 2013 and is already in place.

Shifting plant protection product application dates offers risk management opportunities particularly during the main migration periods of amphibians over crop fields. The pre-emerging herbicide application to summer crops highly coincides with amphibian migration into breeding ponds and entails a high exposure risk (see Appendix 5.16). Combining soil cultivation, catch cropping and drilling techniques should help shifting the pre-emerging application of herbicides from spring towards autumn of the preceding year thus reducing this risk (Table 4.4-1).

Applying direct drilling into the dead vegetation cover of catch crops in spring without soil disturbance (except for the cutting discs of the drill) allows omitting pre-emerging herbicide application to field pea (Table 4.4-1, please compare sowing and herbicide application in “standard” and “alternative”). This time shift may, however, lead to disadvantages for amphibians during other terrestrial activity periods. Thus, a profound knowledge and a careful analysis of possible trade-offs are necessary.

Omitting completely plant protection product applications to field crops (Table 4.4-2, 2.4) without adaptation of other cultivation measures in the sense of integrated pest management generally leads to low yield, poor product quality, technical problems, and implicates risks for high economic losses. Although partly applicable, it cannot be recommended in general. Risk management measures like applying forecast models predicting animal activity on fields to control timing of application (2.7), replacing highly toxic plant protection products by less toxic ones (2.8), and alternative plant protection product application techniques like plant protection product injection into soils (2.10) are considered useful prospectively but require further scientific investigation and development.

While specific hotspots of amphibian presence can be identified and managed easily by farmers, production integrated measures such as forecast models for controlling application or replacing products need more knowledge to be most effective in a given situation. Thus, the farmers producing in areas hosting populations of amphibians should be provided with the knowledge and appropriate decision support tools required. Furthermore, close confidence-based contacts to naturalists familiar with amphibians and short communication paths between farmer and advisers are essential. Mutual respect for both the specific needs of agriculture and the threat of amphibians in intensively used landscapes is most important.

In short:

- Risk Management Measures (RMM) should include a) an overall reduction of plant protection product use, b) specific measures on hot spots of amphibian presence in crop fields, and c) modifying the mode and/or timing of plant protection product application.
- While amphibian sound management on wet spots or pond edges (buffer areas) is easily to apply and can be easily implemented, other measures are more difficult to implement but may offer some future prospects: e.g. short-term time shifting of plant protection product application dates, replacing one plant protection product by other, alternative application techniques like plant protection product injection into soil instead of spraying.

Table 4.4-1: Scheme for time shifting of herbicide application from spring to autumn of last year to avoid higher risk for exposure of adult amphibians during spring migration into breeding ponds (herbicide applications in red coloured boxes). sbp: seed bed preparation; std: standard drill, applied in “conventional” seed bed; dd: direct drill in plant material using cutting discs; th: total herbicide (Glyphosate); be/ae: herbicide application before(pre-)/after(post) emerging of plants; (ae) not applied regularly

		July			August			September			October			November			December			January			February			March			April			May			June			July		
cultivation system/measures		**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
crop sequence		winter cereal				catch crop																					field pea													
standard	**soil cultivation**				sbp																				sbp															
	sowing					std																					std													
	herbicide																										be					(ae)								
alternative	**soil cultivation**				sbp																																			
	sowing					std																					dd													
	herbicide											th																				ae								

Table 4.4-2: Risk management measures for lowering exposure of amphibians to synthetic plant protection products in agriculture: Approaches, advantages, constraints and chances for implementation (grey boxes: measures already in action or with high chances for short- or medium-term implementation potentially leading to substantial improvements for amphibians)

objective	**mitigation measure**	**motivation**	**practical implementation**	**advantages**	**constraints**	**chances for implementation (good preposition are highlighted)**	**No.**
decreasing amount of plant protection product application / less plant protection product load in intensively used arable landscapes	applying integrated farming [IF] (incl. integrated pest management [IPM])	obliged by policy and legislation	cross compliance	because of this obligation an area wide application	initially cost intensive, huge demand for knowledge and labour, partly substantial changes of production necessary => rejection by farmers; labour intensive and difficult controlling of farms by administration => resistance in administration too?	applying the principle of IPM is requested by EU and German policy => there is a trend towards practical applying of IPM => medium-term implementation can be expected	1.1
		self interest/ personal commitment of farmers	voluntary participation supported by agro-environmental schemes	farmers are trained and practise IPM with interest	mostly the "best" farmers will take part, already working with "better" practices; lacking public money => just partial application; the general market situation impacts its actual application => no sustainable application	because of a lack in public funding, neither a substantial short-term nor medium-term implementation is expected; some good examples "lighthouses" will occur immediately	1.2
			additional taxes on plant protection products	plant protection products are used as last measure after applying all other appropriate and less expensive cultivation measures including crop rotations	unexpected market movement, poor adaptation in production and unbalanced effects on different farm types could lead to economic problems; plant protection industry fears less sales	there are no political signs indicating a short- or a medium-term implementation in Germany	1.3
			massive public pressure and/or changed consumer behaviour	plant protection products are used as ultimate measure after applying all other appropriate cultivation measures	there is only partly and/or temporarily awareness in German public with very little impact on the plant protection product use in agriculture (except organic farming)	although sometimes very enthusiastically and encouragingly discussed, there are no significant and sustainable medium-term changes in Germany to be expected	1.4

objective	mitigation measure	motivation	practical implementation	advantages	constraints	chances for implementation (good preposition are highlighted)	No.
avoiding exposure of amphibians to plant protection products in presence hot spots	buffer strips around breeding ponds, wet spots and wood edges partly used as terrestrial habitat	obliged by policy and legislation	obligation for establishing ecological focus areas (EFA) in EU agricultural policy	wide application in EU; optimal management as habitat for amphibians is likely possible. Needs specific funding by agri-environmental schemes and voluntary participation of farmers	partly deprivation of productive land and a respective loss of income => likely refusal by farmers if no funding available	High chance of implementation due to the present proposals for EU agricultural policy from 2014 onward to provide land for ecological purposes and its option for combining with 2nd pillar funding for management	2.1
		voluntary	supported by agro-environmental schemes and by funding in GAK[1]	provides terrestrial habitat optimised for amphibians	mostly the "best" farmers already working with "better" practices will take part; unflexible participation and no public funding => just partial application; the general market situation impacts its application => no sustainable application	medium to high chances of implementation due to the proposals for EFA (see 2.1) and the proposed measures for agri-environmental programmes in the German Lands; at present, application partly supported by GAK	2.2
	converting arable land into grassland	voluntary	supported by agro-environmental schemes	very low or even no plant protection product application, no soil disturbance	high expenses for funding; re-cultivation after 5 years; no market adaptation possible => low participation by farmers if no funding available	partly already in action, depending on specific funding of German Lands; but low participation	2.3
	local omission of plant protection product application	voluntary	technically possible, but considerable losses in productivity possible	no "loss" of arable land	under normal cultivation and on wet and often fertile soils: massive weed infestation, low yield with poor quality, technical problems (eventually separate harvest with extra effort for soil preparation) => very cost intensive; other cultivation risks remain for amphibians	little chances of implementation due to considerable disadvantages and constraints; less appropriate as EFA (see 2.1)	2.4
reducing field crossing by amphibians	providing (partly) uncontaminated terrestrial habitat next to breeding ponds to avoid migrations	voluntary	providing shelter by applying regional or local environmental	little arable land requirements, can be usually implemented on pond and ditch edges when	water body management by water management administration may hinder the realization	partly already in action, depending on specific funding of German Lands, partly co-financed by EU	2.5

objective	mitigation measure	motivation	practical implementation	advantages	constraints	chances for implementation (good preposition are highlighted)	No.
	(e.g. small woodlots and wood pile for resting and hibernation)		measures	large enough			
	establishing grass and flowering strips and areas in fields to reduce migration distances and to provide (partly) uncontaminated terrestrial habitats	voluntary (partly obliged)	supported by funding in GAK, partly supported by agro-environmental schemes	comparably high compensation	participation fixed for 5 years => no market reaction possible for farmers => not widely applied; GAK rules appear complicated	already in action (GAK), but not widely applied; probably more interest will arise in farmers when ecological focus areas are obliged in EU (see 2.1)	2.6
reducing coincidence and exposure risk of amphibians to plant protection products in fields	using models predicting animal activity on fields to control timing of application	provided by administration and used voluntarily	daily estimates of main population activity in fields => provides recommendations for controlling plant protection product application to avoid high coincidences	no omission of plant protection product application; small time shift hardly affecting productivity of crops	suitable data, valid quantification on amphibian activity drivers and reliable models are necessary; at present this is lacking; applying models mainly reduce the risk of direct overspray; exposure of amphibians by contact to soil and vegetation during migration is hardly to control	short- and medium-term application is not expected; research effort; lacking models presumably could be developed within 5 years (based on systematic field investigations)	2.7
	replacing plant protection products: persistent plant protection products with high DT50 values and higher exposure risk (e.g. soil acting herbicides) are replaced by products with less impact (e.g. leaf acting herbicides); highly toxic products are replaced by less toxic products	voluntary	causes higher management effort in farms, but principally applicable	less temporal coincidence with migrating amphibian populations; less toxic effects	replacement may lead to adverse "trade offs" due to higher toxicity and/or high DT50 values; an increasing number of applications might be necessary if plant protection product target effect is reduced. This may lead in the end to higher risk of exposure	further scientific proof on exposure, uptake rate and toxicity of plant protection products for terrestrial stages of amphibians is required	2.8

objective	mitigation measure	motivation	practical implementation	advantages	constraints	chances for implementation (good preposition are highlighted)	No.
	time shifting of plant protection product application	voluntary	often needs further management changes, e.g. adapted soil cultivation	less exposure of amphibians in fields during main migration periods	partly limited by available machinery in farms; time shift may benefit one population but may impact others too (trade-offs in protection goals")	medium-term implementation seems possible; but very specific knowledge on amphibians is to be provided to agriculture	2.9
	replacing plant protection product spray by injecting liquid plant protection product solutions into soil	voluntary	systemic plant protection products in plants are absorbed by plant roots after injection	no spray dependent exposure of amphibians above soil surface; mechanical equipment is available (at present already used for fertilisation)	possible risk for digging amphibian species and soil organism due tohigher exposure; mechanical disturbance during application may harm amphibians too	no short- or medium-term implementation desiderable; not enough valid results available on plant protection product injection; plant protection products are not authorized for this purpose	2.10

Legend: [1] … GAK: Improvement of agrarian structures and coastal protection (Gemeinschaftsaufgabe Agrarstruktur und Küstenschutz)

4.5 Reptile exposure to plant protection products

4.5.1 Introduction

Reptiles might be grouped according to their preferred habitat: one group is bound to the presence of open water such as the European pond turtle (*Emys orbicularis*) and grass snake (*Natrix natrix*), and a second group prefers more open and/or semi-open dry sites such as the wall lizard (*Podarcis muralis*) and the smooth snake (*Coronella austriaca*). Habitats for reptiles should have both sheltered and sun-exposed sites, mating and nesting places, hunting areas with adequate food supply, and cover and hiding places for overwintering (Blab & Vogel 1996). Unlike amphibians, reptiles inhabiting favourable habitats don't have particular seasonal migration habits, however, some of them can cover large distances when foraging or ovulating (Günther 1996b). A common feature of reptiles is their activity phase between March and October (±1 month depending on species).

Eight out of 14 native reptile species occurring in Germany (Table 4.5-1) are listed in Annex II and/or IV of the European Habitats Directive. In Germany, 61 % of the reptile species are listed among the Red List species (Haupt et al. 2009). Thus, they are the most endangered group among all vertebrates. According to the German Red List categories, their populations are largely endangered, very vulnerable or vulnerable, while some species are nearly threatened (Appendix 5.19) and all are protected under the German "Bundesartenschutzverordnung".

A list and description of the 14 reptiles species in Germany is presented in Table 4.5-1 with regards to habitat types preferred, habitat requirements, the food spectrum, and the activity radius. Most reptile species are facing a long term population decline, while the large share of reptiles show a short term (10-25 years) trend of decline (Haupt et al. 2009). As demonstrated for amphibian species, the decline of reptile species is caused by various stressors (chapter 4.1 and 4.2.2), inter alia, by agricultural land use and management activities (Millennium Ecosystem Assessment 2005). The poorly structured open landscapes with high share of arable fields, often intensively cultivated, are marginally suitable as habitats to reptile species. Agricultural practice (soil tillage, sowing, fertiliser and plant protection product application, harvesting, and the respective mechanical run over by tractor tyres) may directly and/or indirectly impair reptiles occurring in and close to arable fields (Figure 4.2-1).

There is little information on reptile exposure to plant protection products in arable fields. A review on plant protection product exposure pathways and their effects to reptiles was presented by Fryday & Thompson (2009), however, without providing real field data. They showed that the dermal permeability of reptiles is to be considered somewhat lower as compared to amphibians. We screened the Web of Science (Thomson Reuters 2012 as for 14.11.2011) for existing information, reviewed it, and analysed own survey data (Berger et al. 2011a). The results of the analyses are presented below.

4.5.2 Likelihood and relevance of reptile species exposure to plant protection products in Germany

Data availability

Data directly linking reptile decline to agricultural land use and activity are scarce. We conducted a topic search on reptile research in the agricultural context in the Web of Science (Thomson Reuters 2012 as for 14.11.2011). From altogether 10787 indexed articles on reptiles, only 52 related to the item “agriculture”, 60 related to the item “plant protection products”, and 19 related to the item “herbicides”. Looking in more detail, for “agriculture” we found 8, for “plant protection products” we found 23, and for “herbicides” we found only 6 articles to be relevant to our study.

Reptile habitats and their relevance in agricultural fields

We reviewed the available information on the reptile species’ habitats, particularly with regard to potential duration and frequency of sojourns in agricultural landscapes and fields. As mentioned above, for all reptile species the intensive agricultural area is not their preferred habitat. We distinguish in our evaluation three groups of reptiles:

- Group 1 represents those reptiles more or less regularly visiting crop fields and thus potentially most affected by agricultural measures (sand lizard, common lizard, blindworm, grass snake)
- Group 2 only occasionally visits arable fields and thus may be moderately affected (wall lizard, western green lizard, adder, smooth snake, aesculapian snake) and
- Group 3 is not expected to live in arable field but might be found in structured agricultural areas with permanent crops like vineyards (European terrapin, European green lizard, horvath's rock lizard, dice snake, common viper).

In the following, we present in more detail the characteristics of those reptiles more often occurring on agricultural fields (group 1): Agricultural land with cropped fields is only used by these species for seeking food or for traversing to get to other habitats. The grass snake often appears on agricultural fields. It prefers open habitats close to moving and/or still waters, such as shores, islands and the edges of forests, embankments, and agricultural land. Its prey primarily comprises amphibian species and fish, but occasionally it feeds on small rodents in arable fields and may even rest there for digesting, sun-bathing or hiding. The edges of agricultural land are also the preferred habitats of the slow worm especially with ditches occurring there. The high death rates in hay bales indicate their presence there. According to a study of Ekner et al. (2008), the common lizard can be often found in diversified landscapes, where agricultural fields are scattered in non-agricultural areas (152 individuals in study area). They inhabit the edges of the diversely structured habitats (Table 4.5-1). Ekner et al. (2008) found 123 sand lizards in an intensively used landscape in Poland. Our relevées also indicate their frequent visits to agricultural fields. Amat et al. (2003) demonstrated that the adults of sand lizards tend preferably to search for prey in fields. Our investigations showed that both grass snakes and sand lizards can be found within arable fields in more than hundred metres distance to non-crop biotopes, which are their likely habitats (Table 4.5-2).

Concluding, there are no systematic quantitative studies on reptile occurrences in cropped fields, but from existing information and observations it can be assumed that they are common visitors in agricultural land and thus their presence potentially coincide with plant protection product application.

Table 4.5-1: Reptile species, evaluation of their habitats and behaviour (after Günther (1996b) and Blab & Vogel (1996)); the marked reptiles are the ones most frequently found in agricultural land. Presence in agricultural areas: 0: hardly ever present, 1: sometimes present; 2: regular presence, WI: wine-growing regions; OR: orchards

Reptile species	**habitats**	**habitat requirements**	**food spectrum**	**activity radius**	**presence in agric. areas**		
					arable land	grass land	special crops
European Terrapin (*Emys orbicularis*)	lake and fen landscape, ponds and backwaters;	static and slowly moving water bodies, xerothermic sites	arthropods, gastropods, fishes, amphibian species, water plants	migration to ovulation sites (up to several km); if water bodies dry out they migrate over large distances	0	0	0
Sand lizard (*Lacerta agilis*)	dunal areas, heath land, semi-dry and dry grasslands, railway dams, spoil heaps, waste land and ruderal sites	sun places, loose soil substrate, uncovered micro-sites, small structures (stones, deadwood, etc.), moderate vegetation cover hibernation and shelter sites: rock and soil cracks and tree stumps	arthropods, (wasps, bees, ants, bugs, butterflies, etc.)	adults move over 100 m, during oviposition and birthing the females are place-scatters	1	1	0
Wall Lizard (*Podarcis muralis*)	*formerly*: sun-exposed rocks, course gravel, dropdown rims, dry grassland *presently*: vineyard walls, ruins, garden walls, railway dams, quarries	cracks of drywalls, free rocky areas, sun exposed rocks, loose sandy sites with little vegetation cover, (= oviposition sites)	arthropods and plants		0	0	WI; 2
Common lizard (*Lacerta vivipara*)	coastal areas, sand dunes, heath land, edges of fens and forests, forest clearings and lanes	structural elements: tree stumps, deadwood, bushes, stones hibernation sites: soil voids, rodent holes	spiders, chilopods, homopters, locusts, flies	up to 50 m (less distances for females and juveniles)	1	1	0
European green lizard (*Lacerta viridis*)	dry grassland, bush zones close to traffic ways, edges of rocks and gravel areas, edges of pine heath land	structural elements: tree stumps, deadwood, stones sandy areas with little vegetation cover for oviposition	insects, spiders, snails, woodlouse, small vertebrates, occasionally reptile eggs und berries	place-scatters with 30 – 50 m radius, up to 150 m,	0 to 1	0 to 1	0
Western green lizard (*Lacerta bilineata*)	edges of dry forests, grass vineyards, dry grassland, broom and open heath, orchards, railway and road dams		insects, spiders, snails, woodlouse, small vertebrates, occasionally reptile eggs und berries		0	0	WE: 2 ST: 2
Horvath's rock lizard	rocky areas	rocky, partial cover with grass, herbs and low bushes	snails, woodlouse, spiders, insects,		0	0	0

Reptile species	habitats	habitat requirements	food spectrum	activity radius	presence in agric. areas		
					arable land	grass land	special crops
(*Lacerta horvathi*)			diplopods				
Slow Worm (*Anguis fragilis*)	field edges, light forests, hedges, herbal edges, swamp forests, edges of fens, forests, and dikes, open grassland	close, covered vegetation, deadwood, old grass, stones and branches hibernation sites: dry, frost-free earth holes and rock cracks, compost piles	Snails and earthworms, butterfly caterpillars, larvae of wasps, and bugs, locusts, woodlouse, spiders		1 to 2	1 to 2	0
Adder (*Vipera berus*)	bogs and adjoining areas, heath land with bushes, seasonally humid forest edges and clearings, coastal zones, avoids open intensively used land	day shelters and hibernation sites: frost-free rodent holes, cracks and tree stumps	lizards, frogs, and small mammals •	usually place-scatters	0 to 1	0 to 1	0
Grass snake (*Natrix natrix*)	semi-open to open areas close to still or flowing water bodies, swamp meadows, shallow bogs, swamp forests, forest and their edges	structural elements: heterogeneous vegetation, reed piles, deadwood, stones shelters and hibernation sites: earth holes, rock cracks, compost piles, small mammal holes	amphibians, lizards, small mammals, eggs, fish		2	1	0
Smooth Snake (*Coronella austriaca*)	semi-open to open areas	heterogeneous vegetation, various biotopes, shelters and hibernation sites: earth and mammal holes, rock cracks, tree stumps, stone walls	small reptiles, rodents and birds, eggs, insects, earthworms		0	0 to 1	?
Aesculapian snake (*Elaphe longissima*)	meadow slopes, orchards, edges of roads and forests, shores, avoiding dense forrests, dry grassland and agricultural or forest monoculture•	oviposition sites: tree holes, compost and leaf piles, hibernation sites: earth and mammal holes, rock cracks	small rodents and birds, eggs		0	0 to 1	OR: 1 to 2
Common viper (Vipera aspis)	southern-exposed slopes, xerothermic areas	shelters and hibernation sites: rodent and root holes, gravels, rock cracks	small mammals, reptiles		0	0	0
Dice snake (*Natrix tesselata*)	areas close to water bodies	water body features: natural, open, shallow shores, rich in fisch structural elements: gravel areas, walls, dams and embankments, railway dams with cracks or voids	fish, amphibians, larvae		0	0	0

Table 4.5-2: Accidental finds of adult reptiles during census of amphibian trap fences located inside crop fields and distances to next non-crop biotopes (Eggersdorf site,own data 2006-2008)

species	**month**	**distance (m) to next**		
		grass edge	**woody element**	**pond/water body**
grass snake	5	38	366	421
	7	46	333	95
sand lizard	4	83	201	93
	5	44	404	140
	5	136	206	153
	5	46	333	95
	6	103	135	170
	7	44	404	140
	7	69	153	74

Table 4.5-3: Time of leaving hibernation sites (LW), mating (M), oviposition (O), the hatching (H) and the return in the hibernation sites (RW) for grass snake, slow worm, common lizard and sand lizard and coinciding applications of herbicides (H), insecticides (I) and fungicides (F) for winter oilseed rape and maize (based on Günther (1996b) and own calculations)

month/decade	2/1	2/2	2/3	3/1	3/2	3/3	4/1	4/2	4/3	5/1	5/2	5/3	6/1	6/2	6/3	7/1	7/2	7/3	8/1	8/2	8/3	9/1	9/2	9/3	10/1	10/2	10/3
grass snake (Natrix natrix)				LW	LW	LW	LW	LW	LW	M	M	M	M	M	M / O	O	O	O / H	O / H	H	H	H / RW	H / RW	H / RW	RW	RW	RW
slow worm (Anguis fragilis)							LW	LW	LW	M	M	M	M	M	M				AJ	AJ	AJ	AJ	AJ	AJ / RW	RW	RW	RW
common lizard (Lacerta vivipara)				LW	LW	LW		M	M	M	M							AJ	AJ	AJ	AJ		RW	RW	RW	RW	RW
sand lizard (Lacerta agilis)			LW	LW	LW	LW	LW	LW / M	LW / M	M	M	M	M / O	O	O	O		H	H	H	H	H / RW	H / RW	H / RW	RW	RW	
winter rape								I	I	I	I+F					I+F				H		(H)+(I)					
maize										H		(H)			(I)												

Likelihood of reptile exposure to plant protection products

We investigated the potential coincidence of reptiles presence with plant protection product applications based on agricultural census data and interception calculations presented in chapter 4.1.2 and 4.1.3 (see also Appendix 5.11). We narrowed our investigation down to those four reptile species most often occurring in arable landscapes and fields respectively (group 1). Table 4.5-3 gives an overview of the activity periods, the mating periods and the intervals for oviposition and hatching (grass snake and sand lizard) and birth of offspring (slow worm and common lizard). It also gives an overview of the application time of plant protection products in winter oilseed rape and maize crops.

At the time of herbicide and/or insecticide application in early spring (decades 3/3 to 5/1), grass snakes, slow worms and sand lizards are still leaving their hibernation sites and due to low temperatures are assumed to be less mobile, apart from the earlier active common lizard. Beginning with mid of April, insecticides applications in winter rape and herbicide and fungicide applications in maize are likely to coincide with all reptile species active in fields. However, spring and summer plant protection product applications in oilseed rape (Appendix 5.11: Table 5.11-2) and late summer application in maize (Appendix 5.11: Table 5.11-3) are likely to be intercepted by the dense crop canopy reducing the exposure risk. Female reptiles giving birth or laying eggs in summer tend to remain in their habitats and thus are considered having a minor exposure risk to plant protection product applications as compared to males. However, they may also be impaired from spray drift among field edges (chapter 4.1.4), since reptiles are known to sunbathe in exposed micro-sites within their habitats during day time.

Concluding, since there are hardly any quantitative data on reptile field occurrence, estimation on their plant protection product exposure remains rather vague and restricts to exposure likelihood considerations under different crop management and plant protection product interception scenarios. Nevertheless, based on their habits and their scarce presence in fields, it may be assumed that they are more likely to be exposed to spray drift and possibly also dermal soil-skin contact than to direct overspray in the field and thus in total are assumed to have a lower exposure risk to plant protection products than amphibians.

In short:

- Based on a very scarce body of literature and accidental own observations we conclude on reptiles having a lower risk of plant protection product exposure than amphibians. They usually do not migrate that extensively between different habitats and cross crop fields to a less extent. If present on fields, they are likely to be close to field edges. However, own accidental findings show that some reptiles, for instance sand lizard, also may be sporadically active within crop fields at places with more than 100 m distance from the field edge.
- Reptiles, contrary to most amphibian species, are also active during daytime. On sunny days lizards often do sunbathing in grass-herb edges adjacent to crop fields providing open sandy soils or rocks. Under this scenario, the exposure risk by spray drift of plant protection products applied on neighbouring fields is presumably high.

4.6 Outlook

Our analysis and results are based on field investigations, expert estimations and literature review. Some of our conclusions need further scientific affirmation and validation by appropriate field investigations. For instance, the moving types of individual amphibians should be investigated with respect to the relative skin-soil and skin-vegetation contact on their total migration track. We assume this to be one important input parameter for the risk assessment methodology specifically for the dermal pathway. Furthermore, the dermal uptake rate in general and in particular during brief skin-soil contacts should be determined too.

Models for forecasting the activity of amphibian populations on crop fields that aim at operating and/or timing plant protection product applications offer good options for reducing exposure risk. Models, however, do require better quantification of the drivers of migration activity. Input parameters should be data collected in systematic field investigations conducted in different regions of Germany during amphibian’s main migration periods, so to account for potential regional climate and amphibian variability. A concerted research action with a time span between three and five years is recommended.

Amphibian populations are affected by multiple direct and non-direct stressors. The impact of plant protection products on population decline still cannot be distinguished from other agricultural and non-agricultural stressors. Population models appropriate to estimate population effects may help to identify core stressors. Such models can also be important to evaluate enhanced plant protection measures and systems and for a technology assessment in general. We suggest supporting their development and/or improvement.

This project was mainly focused on the consideration of biodiversity in the risk assessment and risk management of plant protection products. Based on the results of the project it should be investigated in a follow-up if biodiversity is adequately considered in current risk assessment practice of biocides. Dependent on the results of this follow-up project it might be necessary to implement an improved basis of decision for the assessment and management strategy for biocides.

5. Appendix

5.1 Literature review non-target arthropods

Table 5.1-1: Evaluated literature and results for Hymenoptera: Wild bees

Index	Literature	Measurement
a	Gathmann et al. (1994)	species richness of trap-nesting Hymenoptera
	Tscharntke et al. (1998)	Species richness and abundance of bees
	Steffan-Dewenter & Tscharntke (2001)	species richness and abundance of bees
	Carvell (2002)	numbers of both long- and short-tongued bumblebee species, abundance of all bumblebees and species richness
	Croxton et al. (2002)	abundance of bumblebees
	Sjodin et al. (2008)	abundance of bees
	Ekroos et al. (2008)	abundance of bumblebees
	Ockinger & Smith (2007)	density and species number of bumblebees
	Brittain et al. (2010b)	bumblebee species richness (field scale)
b	Sjodin et al. (2008)	species richness of bees
c	Carvell (2002)	species number, abundance, species richness of bumblebees
d	Schuepp et al. (2011)	abundance, species richness of bees
e	Steffan-Dewenter (2002)	species number of trap-nesting bees
	Steffan-Dewenter et al. (2002)	Species richness and abundance of solitary wild bees
	Morandin et al. (2007)	number of bumblebees
	Holzschuh et al. (2010)	species richness of bees, the number of brood cells of total bees/ the red mason bee *O. rufa*/ other bees
	Le Feon et al. (2010)	species richness of wildbees and bumblebees
	Franzen & Nilsson (2008)	species richness and the number of red-listed solitary bee species
f	Westphal et al. (2003)	density of bumblebees
g	Meek et al. (2002)	number of bumblebees
	Marshall et al. (2006)	abundance of bees
	Carvell et al. (2007)	number, species richness of bees
	Lye et al. (2009)	density of nest site searching and foraging bumblebee queens
	Croxton et al. (2002)	abundance of bumblebees (comparison of green lanes and field margins)
h	Schuepp et al. (2011)	species richness and abundance of bees
i	Steffan-Dewenter & Tscharntke (1999)	abundance, species richness of bees
	Kosior et al. (2007)	not specified (Literature review)
	Ockinger & Smith (2007)	number of red-listed solitary bee
	Franzen & Nilsson (2008)	species richness, density of bumblebees
j	Westphal et al. (2003)	density of bumblebees
	Walther-Hellwig & Frankl (2000)	number of bumblebees
k	Walther-Hellwig & Frankl	diversity in the bumblebee forage community

	(2000)	
l	Kosior et al. (2007)	not specified (Literature review)
	Williams & Osborne (2009)	density, species richness of bumblebees
	Brittain et al. (2010b)	species richness of bumblebees (regional scale)
	Le Feon et al. (2010)	species richness, abundance and diversity of wild bees
m	Holzschuh et al. (2008)	species richness of bees, density of solitary bees, density of bumblebees
	Holzschuh et al. (2010)	species richness of bees, number of brood cells of *Osmia rufa*
	Ekroos et al. (2008)	diversity of bumblebees
n	Brittain et al. (2010a)	abundance of bees
o	Brittain et al. (2010b)	species richness of bumblebees (field and regional scale)
p	Kosior et al. (2007)	not specified (Literature review)
	Brittain et al. (2010b)	species richness of wildbees (field scale)
	Morandin et al. (2005)	foraging speed on artificial complex flower arrays (bumblebees)
	Williams & Osborne (2009)	not specified (Literature review)
q	Kosior et al. (2007)	not specified (Literature review)
r	Carvell (2002)	number of bumblebees, species number of bumblebees (in cattle grazed grasslands)
s	Sjodin et al. (2008)	species richness and abundance of bees
t	Williams & Osborne (2009)	bumblebee nests
u	Haaland & Gyllin (2010)	abundances and species numbers of bumblebees (sown wildflower strips vs. greenways)

Table 5.1-2: Evaluated literature and results for Coleoptera: Carabidae

Index	Literature	Measurement
a	Purtauf et al. (2005)	species richness, activity density (seminatural habitat: grassland)
b	Holland & Luff (2000)	not specified (literature review)
	Hof & Bright (2010)	abundance
	Andersen (1997)	abundance of most species
	Stachow (1988)	evenness, diversity
	Pfiffner & Luka (2000)	occurrence during overwintering (*Bembidion lampros, Agonum muelleri, Demetrias atricapillus*)
	Thomas et al. (2001)	occurence of e.g. *Amara spp., Harpalus rufipes, Nebria brevicollis*
	Welling et al. (1994)	species number
c	Marshall et al. (2006)	number of Carabidae
d	Holland & Luff (2000)	field boundaries may inhibit movement between fields resulting in populations becoming isolated (literature review)
	Marshall et al. (2006)	number (comparison: in-field and field boundary)
e	Irmler (2003)	composition of ground beetle assemblages
f	Holland & Luff (2000)	carabid assemblage
	Eyre et al. (2009)	species activity, species richness
g	Cole et al. (2002)	percentage of *Carabus* spp. and individuals
h	Irmler (2003)	activity density of *Carabus granulatus, Agonum muelleri, Pterostichus melanarius, Bembidion tetracolum*
	Döring & Kromp (2003)	species richness
	Döring & Kromp (2003)	benefits from organic management (especially for *Carabus auratus*, *Harpalus affinis* and some *Amara* species (*A. aenea, A. similata, A. familiaris*))
	Holland & Luff (2000)	abundance, diversity
	Pfiffner & Luka (2003)	species richness, activity density, abundance of threatenden species
	Purtauf et al. (2005)	abundance (7 species)
i	Purtauf et al. (2005)	species richness, activity density
	Holland & Luff (2000)	it is suggested that short-term variations in species' abundances more important than overall farming system (literature review)
	Pocock & Jennings (2008)	abundance of most Carabidae species
j	Pfiffner & Luka (2003)	abundance
	Purtauf et al. (2005)	abundance (8 species)
k	Holland & Luff (2000)	occurrence of xerophobic or euryhygric species (literature review)
	Minarro et al. (2009)	species richness, diversity
l	Holland & Luff (2000)	occurrence of hytophagous, xerophilic species (literature review)
	Haughton et al. (1999)	abundance
	Holland & Luff (2000)	not specified (literature review)
	Vickerman & Sunderland	abundance of predatory Carabidae

	(1977)	
	Jepson & Thacker (1990)	relative levels of recovery by populations
m	Holland & Luff (2000)	not specified (literature review)
n	Porhajasova et al. (2008)	assemblage structure
o	Holland & Reynolds (2003b)	abundance of *Notiophilus biguttatus*
p	Holland & Luff (2000)	occurrence of large beetles (e.g. *Carabus* species)
	Holland & Reynolds (2003b)	emergence (6 species), number of total Carabidae
q	Holland & Luff (2000)	carabid assemblage
r	Minarro et al. (2009)	Shannon – Wiener's diversity index
s	Minarro et al. (2009)	abundance
t	Welling et al. (1994)	species number
	de Snoo (1999)	activity density
	Hassall et al. (1992)	abundance, species number
	Felkl (1988)	abundance
u	Frampton & Dorne (2007)	effect size for several measurements (meta-analysis)
	Felkl (1988)	spectrum of species
v	Hassall et al. (1992)	abundance
	Holland & Luff (2000)	not specified (literature review)
w	Thomas et al. (1992)	proportions of boundary carabids

Table 5.1-3: Evaluated literature and results for Coleoptera: Staphylinidae

Index	Literature	Measurement
a	Clough et al. (2007)	activity-density of *Tachyporus hypnorum*
b	Clough et al. (2007)	total activity-density, total species richness
c	Andersen (1997)	abundance (overwintering) of most species
	Pfiffner & Luka (2000)	abundance (overwintering), species number
	Balog & Marko (2007)	occurrence of species with larger body size
	Balog et al. (2008)	occurrence of species with larger body size, pooled α-diversity (40m vs. 20m wide hedge), activity density in the field (40-vs. 20m wide hedge)
	Feber et al. (1995)	abundance (in field)
	Clough et al. (2007)	species number (in field)
d	Andersen (1997)	abundance (overwintering) of the genus *Lathrohium*
e	Bohac (1999)	dominance in communities (literature review)
	Balog et al. (2008)	species composition, species diversity
f	Clough et al. (2007)	activity-density and species richness of detritivores
g	Clough et al. (2007)	species richness, activity density and species richness of fungivores, species richness of predators
h	Clough et al. (2007)	activity-density of predators
i	Bohac (1999)	activity (literature review)
	Feber et al. (1995)	abundance (field margins)
j	Bohac (1999)	abundance, oviposition, activity (literature review)
k	Minarro et al. (2009)	species richness
	Bohac (1999)	abundance (literature review)
l	Bohac (1999)	species number (literature review)
m	Bohac (1999)	species number, abundance (literature review)
n	Holland & Reynolds (2003b)	emergence
	Bohac (1999)	activity (literature review)
o	Minarro et al. (2009)	abundance, species richness
p	Feber et al. (1995)	abundance
q	Frampton & Dorne (2007)	abundance (meta-analysis)
	Felkl (1988)	abundance
	Moreby & Southway (1999)	number
r	Frampton & Dorne (2007)	abundance (meta-analysis)
s	Hart et al. (1994)	abundance
t	Feber et al. (1995)	abundance

Table 5.1-4: Evaluated literature and results for Diptera: Syrphidae

Index	Literature	Measurement
a	Molthan & Ruppert (1988)	frequency, species diversity
	Cowgill et al. (1993)	number of *Episyrphus balteatus*
	MacLeod (1999)	abundance and dispersal rates of *Episyrphus balteatus*
b	Sjodin et al. (2008)	abundance
c	Sjodin et al. (2008)	species richness, abundance
d	Sjodin et al. (2008)	species composition
e	Sjodin et al. (2008)	species richness, abundance
f	Steffan-Dewenter & Tscharntke (1999)	abundance
g	Sjodin et al. (2008)	species richness, abundance
h	Moens et al. (2011)	number of viable *Episyrphus balteatus* eggs (1 of 5 insecticides)
i	Hautier et al. (2004)	mortality of *Episyrphus balteatus* larvae (19 of 19 fungicides, 5 of 11 insecticides, 4 of 4 herbicides)
	Moens et al. (2011)	mortality of *Episyrphus balteatus* larvae (3 of 5 insecticides)
j	Vickerman & Sunderland (1977)	mortality
	Hautier et al. (2004)	mortality of *Episyrphus balteatus* larvae (6 of 11 insecticides)
	Moens et al. (2011)	mortality of *Episyrphus balteatus* larvae (2 of 5 insecticides), number of viable *Episyrphus balteatus* eggs (3 of 5 insecticides)
k	Cowgill et al. (1993)	number of adult *Episyrphus balteatus*, foraging
	Kühner (1988)	abundance
	deSnoo & deLeeuw (1996)	number of adults
l	Felkl (1988)	oviposition
m	Felkl (1988)	number of larvae

Table 5.1-5: Evaluated literature and results for Arachnida: spiders

Index	Literature	Measurement
a	Asteraki et al. (2004)	abundance
b	Pfiffner & Luka (2003)	community composition
c	Hassall et al. (1992)	species richness
	Bogya & Marko (1999)	abundance, biovolume, numbers of nocturnal, agile hunters and ambushers
	Feber et al. (1998)	density
	Baines et al. (1998)	abundance, species number
	Jimenez-Valverde & Lobo (2007)	abundance, species richness
	Lemke et al. (2000)	species richness
d	Bogya & Marko (1999)	community structure
e	Marc et al. (1999)	spider community
	Woodcock et al. (2007)	species richness
f	Bedford & Usher (1994)	species richness, species diversity
g	Oberg (2009)	body condition of *Pardosa* spiders
h	Clough et al. (2005)	species number
	Schmidt-Entling & Dobeli (2009)	species richness, Simpson diversity, densities of all spiders, densities of Linyphiidae
i	Welling et al. (1994)	species number, diversity
	Meek et al. (2002)	abundance of lycosid spiders
	Marshall et al. (2006)	activity density of Linyphiidae and Lycosidae
j	Juen & Traugott (2004)	spider community
	Marshall et al. (2006)	total spider number
k	Marshall et al. (2006)	abundance of linyphiids
l	Pfiffner & Luka (2003)	abundance, abundance of some wolf-spiders species
	Birkhofer et al. (2008)	number of ground active spiders
	Feber et al. (1998)	abundance, species richness
	Asteraki et al. (2004)	abundance
m	Clough et al. (2005)	activity density, diversity
	Oberg (2009)	body condition and fecundity of *Pardosa* spiders
n	Frampton & Dorne (2007)	effect size: total counts (meta-analysis)
o	Feber et al. (1995)	linyphiid abundance
	Haughton et al. (1999)	abundance
	Baines et al. (1998)	abundance
	Marko et al. (2009)	abundance, proportion of females, (abundance of potential prey)
	Huusela-Veistola (1998)	abundance
	Marc et al. (1999)	diversity, density, spider communities
	Vickerman & Sunderland 1977	abundance
p	Minarro et al. (2009)	species richness, diversity
q	Minarro et al. (2009)	activity density
r	Holland & Reynolds (2003b)	emergence of Linyphiidae and Lycosidae
	Marc et al. (1999)	survival
s	Feber et al. (1995)	abundance of linyphiids

	Gibson et al. (1992)	species richness
	Baines et al. (1998)	abundance, species richness
t	Welling et al. (1994)	species number of ground spiders, abundance of *Erigone* spiders
	Hassall et al. (1992)	abundance, species diversity, species richness
	Felkl (1988)	abundance
	Moreby & Southway (1999)	abundance
u	Huusela-Veistola (1998)	abundance, abundance of spiders in the field
	Lemke & Poehling (2002)	density, activity density (overwintering)
v	Thomas et al. (1992)	distribution patterns for Linyphiidae
	Huusela-Veistola (1998)	community composition
w	Schmidt-Entling & Dobeli (2009)	densities of Thomisidae, Gnaphosidae, Lycosidae and young Araneidae in wheat fields
x	Schmidt-Entling & Dobeli (2009)	species richness

Table 5.1-6: Evaluated literature and results for Hemiptera: Heteroptera

Index	Literature	Measurement
a	Asteraki et al. (2004)	abundance
b	Di Giulio et al. (2001)	species number
c	Zurbrugg & Frank (2006)	community composition
d	Kruess & Tscharntke (2002)	abundance
	Zurbrugg & Frank (2006)	total number of bug species, species richness in trophic groups and overwintering strategies, total abundance of bugs, abundance in trophic groups
e	Woodcock et al. (2007)	occurrence
	Zurbrugg & Frank (2006)	community composition
f	Moreby et al. (1997)	species richness (12 species exclusively found in the field boundary)
	Moreby (1994)	abundance in headlands next to hedges vs. grass boundaries
g	Zabel & Tscharntke (1998)	species richness, abundance of populations
h	Di Giulio et al. (2001)	abundance (2 species)
i	Di Giulio et al. (2001)	abundance (16 species)
j	Di Giulio et al. (2001)	species diversity, number of individuals, abundance (6 species)
k	Moreby et al. (1997)	mortality (3 of 3 fungicides), number of predatory Heteroptera (5 of 5 insecticides), number of total Heteroptera (4 of 5 insecticides)
l	Haughton et al. (1999)	abundance
	Frampton & Dorne (2007)	abundance, taxonomic richness
m	Kruess & Tscharntke (2002)	percentage of herbivores
n	Gibson et al. (1992)	species richness, abundance of carnivorous species
o	Gibson et al. (1992)	abundance, faunal composition
p	deSnoo & deLeeuw (1996)	abundance
	Hassall et al. (1992)	abundance, species diversity, species richness
	Moreby et al. (1999)	abundance
q	Zurbrugg & Frank (2006)	abundance of bugs overwintering in the egg-stage, total species richness, species richness of phyophagous bugs, species richness of zoophagous bugs and bugs overwintering as eggs

Table 5.1-7: Evaluated literature and results for Hemiptera: Auchenorrhyncha

Index	Literature	Measurement
a	Asteraki et al. (2004)	abundance
b	Huusela-Veistola & Vasarainen (2000)	abundance, species richness
c	Asteraki et al. (2004)	community composition
d	Kruess & Tscharntke (2002)	abundance, species richness
	Morris (2000)	density, species richness
e	Hollier et al. (1994)	leafhopper assemblages
f	Zabel & Tscharntke (1998)	species richness, abundance of populations
	Biedermann et al. (2005)	risk of extinction
g	Nickel & Hildebrandt (2003)	species composition
h	Nickel & Hildebrandt (2003)	species number, proportion of specialists
	Nickel & Achtziger (1999)	species number, proportion of specialists
i	Haughton et al. (1999)	abundance
j	Morris (1992)	abundance of *Recilia coronifera*, *Adarrus ocellaris*, *Stenocranus minutus*, *Javasella pellucida*
k	Morris (1992)	species richness, abundance of *Euscelis incisus*, *Hyledelphax elegantulus*, *Deltocephalus pulicaris*
l	Kruess & Tscharntke (2002)	abundance of *Arthaldeus pascuellus*, *Streptanus sordidus*, *Steptanus aemulans*, *Errastunus ocellaris*, *Macrostelis sp.*
	Nickel & Hildebrandt (2003)	diversity
	Nickel & Achtziger (1999)	species richness
m	deSnoo & deLeeuw (1996)	abundance
	Moreby & Southway (1999)	number of Auchenorrhyncha
n	Frampton & Dorne (2007)	effects size: counts (meta-analysis)
o	Huusela-Veistola & Vasarainen (2000)	leafhopper abundance, species richness

Table 5.1-8: Evaluated literature and results for Lepidoptera: Macrolepidoptera

Index	Literature	Measurement
a	Ekroos et al. (2008)	Lepidoptera abundance (butterflies and diurnal moths), butterfly abundance, butterfly species richness
	Kuussaari et al. (2007)	species richness (butterflies and diurnal moths)
	Munguira & Thomas (1992)	species number and diversity of butterflies and burnets
	Ockinger & Smith (2007)	species number, density
	Saarinen (2002)	abundance (4 butterfly species)
	Sparks & Parish (1995)	species richness and abundance (butterflies)
	Winkler et al. (2009)	sugar content of *Plutella xylostella* in fields next to flowering vs. grass margins
	Brittain et al. (2010b)	butterfly species richness (at field scale)
	Franzen & Nilsson (2008)	species number of burnets
	Rundlof et al. (2008)	butterfly species richness and abundance
	Kirkham et al. (1999)	number and species diversity of butterflies
b	Munguira & Thomas (1992)	number of individuals
	Franzen & Nilsson (2008)	species richness of butterflies, number of red-listed burnets
c	Haaland & Bersier (2011)	butterfly abundance, butterfly species richness
d	Kuussaari et al. (2007)	butterfly species richness
	Ockinger & Smith (2007)	species richness (day active moths)
	Franzen & Nilsson (2008)	species number of burnets
e	Sjodin et al. (2008)	species richness and abundance of butterflies
f	Marini et al. (2009)	species richness of butterflies (field scale)
g	Sjodin et al. (2008)	species composition (butterflies)
	Sparks & Parish (1995)	butterfly populations
h	Haaland & Bersier (2011)	butterfly species richness
i	Franzen & Nilsson (2008)	species number of burnets
j	Franzen & Nilsson (2008)	species richness of butterflies, number of red-listed burnets
k	Fuentes-Montemayor et al. (2011)	macromoth abundance, macromoth species richness
l	Ekroos et al. (2008)	Lepidopteran diversity, abundance of lepidoptera, butterflies and meadow-preferring butterflies, butterfly species richness
	Feber et al. (1997)	abundance of non-pest butterflies
	Field et al. (2005)	abundance of butterflies, abundance of *Maniola jurtina*
	Field et al. (2007)	abundance of *Maniola jurtina*, *Thymelicus sylvestris and Thymelicus lineola*, butterfly abundance
	Hodgson et al. (2010)	butterfly density, butterfly species
	Meek et al. (2002)	abundance of butterflies
	Merckx et al. (2009)	abundance of moths
	Feber et al. (2007)	abundance of butterflies
m	Field et al. (2005)	butterfly species richness, abundance of *Pyronia tithonus, Thymelicus spp.*, and *Ochlodes venata*
n	Ockinger & Smith (2007)	species richness, density
o	Saarinen (2002)	abundance of *Aglais urticae* (1 out of 39 butterfly pecies)
p	Saarinen (2002)	species richness (butterflies), abundance (38 out of 39 butterfly species)

	Sjodin et al. (2008)	species richness and abundance (butterflies)
q	Marini et al. (2009)	diversity of butterflies (field scale)
	Saarinen (2002)	diversity (butterflies)
	Brittain et al. (2010b)	species richness of butterflies (regional scale)
	Ekroos et al. (2010)	α- and β-Diversity of butterflies and geometrid moths
r	Feber et al. (1997)	abundance of non-pest butterflies
	Hodgson et al. (2010)	butterfly density
	Jonason et al. (2011)	butterfly species richness, butterfly abundance
	Rundlof et al. (2008)	butterfly species richness, butterfly abundance, α-, γ-diversity (butterflies)
	Rundlof & Smith (2006)	butterfly species richness and abundance
	Wickramasinghe et al. (2004)	abundance of different moth families
	Feber et al. (2007)	abundance and species richness of butterflies
s	Ekroos et al. (2008)	Lepidopteran diversity
	Hodgson et al. (2010)	butterfly species richness
	Weibull et al. (2000)	butterfly diversity and number of species, number of observations (butterflies)
t	Brittain et al. (2010b)	species richness of butterflies (landscape scale)
	Frampton & Dorne (2007)	Lepidoptera larvae abundance (meta-analysis)
u	Longley et al. (1997)	mortality of *Pieris brassicae* larvae
	Longley & Sotherton (1997a)	mortality of *Spodoptera littoralis* larvae
	Russell & Schultz (2010)	survival, wing size and pupal weight of *Pieris rapae* (study from USA)
	Sparks & Parish (1995)	butterfly abundance
	Cilgi & Jepson (1995)	mortality of *Pieris rapae* larvae and *P. brassicae* larvae, weight of *P. brassicae* larvae, size of adults (*P. rapae, P. brassicae*)
	Feber et al. (1996)	butterfly abundance
	Frampton & Dorne (2007)	adult Lepidoptera abundance, species richness and total Lepidoptera catches
	Sinha et al. (1990)	mortality of *Pieris brassicae* (ranking of 8 insecticides)
	Tan (1981)	maximum larval and pupal weights of *Pieris brassicae*, duration of larval period (*P. brassicae*), consumed leaf area (*P. brassicae*)
v	de Snoo (1999)	butterfly abundance and species number
	Dover (1997)	foraging activity (butterflies)
	Dover et al. (1990)	abundance of butterflies
	Dover (1991)	number of butterfly species, abundance of butterflies
	Rands & Sotherton (1986)	butterfly abundance
w	Haaland & Bersier (2011)	butterfly abundance
	Haaland & Gyllin (2010)	butterfly abundance, butterfly species number (sown wildflower strips vs. greenways)
	Meek et al. (2002)	abundance of butterflies (especially *Meadow Brown* and *Aphantopus hyperantus*)
x	Haaland & Bersier (2011)	species richness

5.2 Calculation of the Margin Treatment Index

Table 5.2-1: Calculation of Margin Treatment Indices (MTI) used in chapter 3.2.1.2. Underlying Treatment indices (TI) are derived from Roßberg et al. (2002), Roßberg (2007a; 2009b) and are averaged for arable crops (cereals, vegetable, potatoes, maize, oil rape) and orchards (apple, pear, plum, cherry). Equations for the calculation of spray drift values are derived from Rautmann et al. (1999) and are also applied to distances smaller than 1 m resp. 3 m (not validated due to lack of data available), max.-value: 100%. *: plant protection product (ppp) input in relation to a distance of 0.75m (cereal fields).

	arable crops		vineyards		orchards	
Distance to field [m]	ppp input [% of application rate]	MTI (TI = 5.30)	ppp input [% of application rate]	MTI (TI = 14.20)	ppp input [% of application rate]	MTI (TI = 14.64)
0.2	50.00	2.65	100.00	14.20	100.00	14.64
0.4	50.00	2.65	100.00	14.20	100.00	14.64
0.6	50.00	2.65	98.71	14.02	97.90	14.33
0.8	*50.00	2.65	63.03	8.95	78.86	11.54
1	2.77	0.15	44.51	6.32	66.69	9.76
1.2	2.32	0.12	33.49	4.76	58.15	8.51
1.4	1.99	0.11	26.34	3.74	51.78	7.58
1.6	1.75	0.09	21.39	3.04	46.84	6.85
1.8	1.56	0.08	17.80	2.53	42.87	6.27
2	1.41	0.07	15.10	2.14	39.61	5.80
2.2	1.28	0.07	13.02	1.85	36.87	5.40
2.4	1.18	0.06	11.36	1.61	34.53	5.05
2.6	1.09	0.06	10.03	1.42	32.52	4.76
2.8	1.01	0.05	8.94	1.27	30.75	4.50
3	0.95	0.05	8.02	1.14	29.20	4.27
3.2	0.89	0.05	7.26	1.03	27.82	4.07
3.4	0.84	0.04	6.60	0.94	26.58	3.89
3.6	0.79	0.04	6.04	0.86	25.46	3.73
3.8	0.75	0.04	5.55	0.79	24.45	3.58
4	0.71	0.04	5.12	0.73	23.52	3.44
4.2	0.68	0.04	4.75	0.67	22.67	3.32
4.4	0.65	0.03	4.42	0.63	21.90	3.20
4.6	0.62	0.03	4.12	0.59	21.18	3.10
4.8	0.60	0.03	3.86	0.55	20.51	3.00
5	0.57	0.03	3.62	0.51	19.89	2.91
5.2	0.55	0.03	3.40	0.48	19.31	2.83
…	…	…	…	…	…	…
10	0.29	0.02	1.23	0.17	11.81	1.73

(mainly) overspray

spray drift

no spray drift data available

MTI ≥ 1

MTI ≥ 0.5

MTI ≥ 0.1

MTI < 0.1

5.3 Preferred habitats

Table 5.3-1: Butterfly species and their preferred habitats (based on abundances/ frequencies). Species can be listed several times if the results vary in different studies. Uncropped field margin/ conservation headland preferring species: statistically significant response or (if significance is not determined) species were at least twice more abundant in the margins/ headlands than in (normal) cropped areas. Field preferring species: statistically significant response or (if significance is not determined) species were at least twice more abundant in (normal) cropped areas than in the uncropped field margins/ conservation headlands. No preferrence: no statistically significant differences or abundances between the habitats differ less than the factor two. Used Literature: Rands & Sotherton (1986); de Snoo et al. (1998); Meek et al. (2002).

uncropped field margin / conservation headland preferring species	field preferring species	no preference (moving species)
Aglais urticae		*Aglais urticae*
Anthocaris cardamines		*Anthocharis cardamines*
Aphantopus hyperantus		*Aphantopus hyperantus*
Celastrina argiolus		
Coenonympha pamphilus		*Coenonympha pampilus*
		Colias croceus
Gonepteryx rhamni		
Inachis io		*Inachis io*
Lasiommata megera		*Lasiommata megera*
Maniola jurtina		*Maniola jurtina*
Melanargia galathea		*Melanargia galanthea*
Ochlodes venata		
		Pararge aegeria
		Pieris brassicae
Pieris napi		*Pieris napi*
Pieris rapae		*Pieris rapae*
Polyommatus icarus		
Pyronia tithonus		*Pyronia tithonus*
Thymelicus lineola		*Thymelicus lineola*
Thymelicus sylvestris		*Thymelicus sylvestris*
	Vanessa atalanta	*Vanessa atalanta*

Table 5.3-2: Coleoptera species and their preferred habitats (based on abundances/ frequencies). Detailed description is given in Table 5.3-1. Used Literature: Felkl (1988); Meek et al. (2002), own unpublished data.

uncropped field margin / conservation headland preferring species	field preferring species	no preference (moving species)
	Acupalpus meridianus	
		Agonum dorsale
		Agonum muelleri
Amara aenea		*Amara aenea*
Amara familiaris		*Amara familiaris*
Amara plebeja		*Amara plebeja*
Amara similata		*Amara similata*
	Anchomenus dorsalis	*Anchomenus dorsalis*
Asaphidion flavipes	*Asaphidion flavipes*	*Asaphidion flavipes*
		Bembidion aeneum
Bembidion lampros	*Bembidion lampros*	*Bembidion lampros*
	Bembidion obtusum	*Bembidion obtusum*
	Bembidion quadrimaculatum	
	Bembidion tetracolum	*Bembidion tetracolum*
		Cantharis nigricans
Clivinia fossor		*Clivinia fossor*
		Coccinella septempunctata
	Demetrias atricapillus	*Demetrias atricapillus*
Drusilla canaliculata		
Harpalus affinis		*Harpalus affinis*
		Harpalus rufipes
	Lathrobium castaneipenne	
		Lathrobium fulvipenne
Loricera pilicornis	*Loricera pilicornis*	
	Micropeplus porcatus	
Nebria brevicollis	*Nebria brevicollis*	*Nebria brevicollis*
Notiophilus biguttatus	*Notiophilus biguttatus*	*Notiophilus biguttatus*
		Notiophilus substriatus
Oxytelus rugosus		
	Philonthus cognatus	
		Philonthus fuscipennis
Philonthus varius		
		Platynus dorsalis
	Pterostichus melanarius	*Pterostichus melanarius*
Pterostichus niger		*Pterostichus niger*
Pterostichus strenuus		*Pterostichus strenuus*
Rhagonycha fulva		*Rhagonycha fulva*
	Stilicus subtilis	
	Synuchus nivalis	
Tachinus rufipes		
Tachyporus chrysomelinus	*Tachyporus chrysomelinus*	*Tachyporus chrysomelinus*
		Tachyporus hypnorum
		Tachyporus obtusus
Tachyporus nitidulus		
		Tachyporus solutus
		Trechus discus
Trechus quadristriatus		*Trechus quadristriatus*
Xantholinus semirufus		

Table 5.3-3: Spiders and their preferred habitats (based on abundances/ frequencies). Detailed description is given in Table 5.3-1. Used Literature: Felkl (1988); Meek et al. (2002), own unpublished data.

uncropped field margin / conservation headland preferring species	field preferring species	no preference (moving species)
		Alopecosa pulverentata
		Bathyphantes gracilis
Diplostyla concolor		*Diplostyla concolor*
		Enoplognatha ovata
Erigone atra		*Erigone atra*
Erigone dentipalpis		*Erigone dentipalpis*
		Lepthyphantes tenuis
Microlinyphia pusilla		*Microlinyphia pusilla*
		Oedothorax apicatus
		Oedothorax fuscus
Pachygnatha degeeri		
Pardosa amentata		*Pardosa amentata*
		Pardosa palustris
Pardosa pullata		*Pardosa pullata*
Pisaura mirabilis		

5.4 Red list species

Table 5.4-1: Spider species mentioned as (nearly) threatened in the national red list of Germany (Binot et al. 1998). 0: extinct in the wild (ausgestorben oder verschollen); 2: endangered (stark gefährdet); 3: vulnerable (gefährdet); G: potentially vulnerable (Gefährdung anzunehmen, aber Status unbekannt).

species	red list category
Agroeca cuprea	3
Allomengea vidua	3
Araneus alsine	3
Araneus triguttatus	G
Arctosa perita	3
Clubiona germanica	3
Clubiona subtilis	3
Cyclosa oculata	3
Drassyllus villicus	3
Enoplognatha tecta	3
Euophrys herbigrada	2
Hypsosinga sanguinea	3
Meioneta simplicitarsis	0
Micaria formicaria	3
Oxyptila brevipes	3
Ozyptila claveata	3
Ozyptila scabricula	3
Pardosa bifasciata	3
Pardosa nigriceps	3
Scotina celans	3
Silometopus elegans	3
Thanatus striatus	2
Trachyzelotes pedestris	3
Trochosa robusta	3
Walckenaeria incisa	G
Xysticus acerbus	3
Xysticus cf. lineatus	3
Xysticus luctuosus	3
Zelotes aeneus	3
Zelotes longipes	3
Zodarion germanicum	3

Table 5.4-2: Macrolepidoptera species mentioned as (nearly) threatened in the national red list of Germany (Binot et al. 1998). 3: vulnerable (gefährdet); V: near threatened (Arten der Vorwarnliste)

species	red list category
Abraxas grossulariata	V
Agriopis bajaria	3
Arctia caja	V
Catocala fraxini	V
Pasiphila chloerata	3
Eriogaster lanestris	V
Lasiocampa quercus	V
Pyronia tithonus	3
Rhagades pruni	3
Trichiura crataegi	3

5.5 Current test systems for arthropods

Table 5.5-1: Test systems for Tier 1 (test design white) and Higher-Tier (test design grey) tests. For detailed descriptions see Candolfi et al. (2000b).

Organism	Ecology	Test design	Endpoints
Aphidius rhopalosiphi (Hymenoptera: Braconidae)	Parasitoid	Test units consisting of glass plates are treated with the test item and – after glass plates are tried – adult wasps are added to each test unit. Treatment effects are assessed after 2, 24 and 48 hours.	mortality of adult wasps
		Surviving females are individually placed in cylinders which are put over pots of aphid-infested cereal plants. Females are removed after 24 h and, 10-12 days later, the numbers of aphid mummies on the plants are recorded.	fecundity of the surviving female wasps
Typhlodromus pyri (Acari: Phytoseiidae)	Predator	In each treated test unit 20 protonymphs are placed after spray residues have dried. 3 days (optional) and 7 days later, the mortality is recorded.	Cumulative juvenile mortality
		The reproduction of the surviving females (eggs and juveniles) is assessed three times during day 7 to 14.	Cumulative reproduction per female
Aleochara bilineata (Coleoptera: Staphylinidae)	Parasitoid on Diptera pupae	Adult beetles are added to treated substrate (quartz sand in the laboratory test, sandy soil in the extended laboratory test) which is carefully mixed up with host pupae afterwards. Four weeks later, adult beetles are removed. The emergence of A. bilineata is recorded.	overall reproductive capacity
Chrysoperla carnea (Neuroptera: Chrysopidae)	Predator (larvae), adults feed on honeydew, pollen/ nectar	Young C. carnea larvae (first instar) were put on glass plates treated with the test item. The surviving larvae stayed at the glass plates until they have pupated. Hatching of the adults is detected. The fecundity of the females as well as the fertility of the eggs can be assessed by sampling all eggs laid within 24 hours twice a week.	Mortality of larvae, reproductive performance of the emerging adults
Coccinella septempunctata (Coleoptera: Coccinelidae)	Predator	Young *C. septempunctata* larvae (3-5 days) are placed individually on dried glass plates which have been treated with the test item. After pupal stage the surviving ecdysis beetles are removed and taken in non-treated breeding cages. During a period of two weeks, the eggs laid are collected and observed for fertility.	Pre-imarginal mortality, reproductive performance of the ecdysed beetles
Orius laevigatus (Heteroptera: Anthocoridae)	Predator	The test units are treated with the test item. In each dried test unit 10 *O. laevigatus* are added for at least 9 days or until 80% of the bugs are adult. To assess the fecundity of surviving females, they are placed individually on oviposition substrate and their egg production is noted for two consecutive 2-day periods.	Mortality of juvenile bugs, fecundity of female bugs

Pardosa (Araneae: Lycosidae)	Predator	Field-collected spiders are introduced in the test units filled with moistened quartz sand (laboratory test) or standardized soil (extended laboratory test). The test units, each containing one spider, are treated. Spiders are monitored for at least 14 days in which mortality and behaviour is recorded. Furthermore, food consumption is assessed.	Mortality, behaviour, food uptake
Poecilus cupreus (Coleoptera: Carabidae)	Predator	6 *P. cupreus* (3 males, 3 females) are placed in test units filled with moistened quartz sand (laboratory test) or standardized soil (extended laboratory test). Afterwards, test units are treated. The test lasts 14 days (prolonged: 21 days) in which mortality and behaviour are assessed several times. The recording of the food consumption can provide further information.	Mortality, behavioural impacts
		Semi-field test: 10 *P. cupreus* (5 males and 5 females) are released in square metal frames. After the treatment of the test units (containing the beetles), mortality and behavior are recorded. The food consumption can also be assessed.	Mortality, behavioural impacts
Trichogramma cacoeciae (Hymenoptera: Trichogrammatidae)	Parasitic wasp	Female adults of *T. cacoeciae* are placed in each test unit, consisting of a frame and two treated glass plates (fresh dried). 24 hours after exposure, surviving wasps are recorded. To get information about the parasitisation capacity, 24, 48 and 96 hours after treatment host eggs are introduced which are analyzed at least 9 days after insertion.	Mortality, parasitisation capacity
Predatory mites (Acari: Phytoseiidae)	Predator	This is a test conducted in vineyards and orchards. As test organisms the naturally occurring mites are used. They can be exposed directly as well as indirectly in consequence of the spraying of the crop plants with the test item. Before and several times after the treatment leafs of the crops are collected and number of predatory mites is assessed.	Predatory mite population density with respect to the control

5.6 Soil-climate-regions (SCR)

Roßberg et al. (2007) established a soil-climate-region (SCR) classification of Germany based on soil, climate, and community border information. This classification is used in Germany for allocating the federal plant trial sites, classifying farms, and for aggregating administrative information in agriculture using coarser segmentations (Roßberg 2009a; Freier et al. 2010). It is also used for assessing the large scale spatio-temporal variability of plant protection product application in agriculture (Figure 5.6-1) under the National Action Plan on Sustainable Use of Plant protection products that was decided by federal and state governments in 2008. The aim of this national action plan is to reduce the risks associated with the use of chemical plant protection products and to limit their application on the necessary amount. It is based on a network of 66 to 86 reference farms selected for plant protection product surveys in the framework of the so-called NEPTUN-analyses and considered somewhat representative for the whole of Germany (Freier et al. 2011).

Thus, the SCR classification aims at the regional representation of plant protection product application and can be used for rough comparisons between regions. But it neither refers to the timing of application nor to the plant protection product used and the dose of its application. Thus, it can only provide little information on regionally differing exposure risk for amphibian and/or reptile populations. This information must be provided using more detailed classifications and/or surveys (BfN 2008) which do not yet exist at the level of detail required.

Figure 5.6-1: Distribution of reference agricultural farms in soil-climate-regions and greater regions (N, E, S, W) of Germany in 2009 (Roßberg et al. 2007 and Roßberg 2009a)

The SCR including reference farms were reclassified by Freier et al. (2010) into four greater regions, each encompassing sufficient numbers of farms for statistical evidence (Appendix 5.7). The so-called treatment indices (TI) were calculated for each region. It represents the number of plant protection product applications related to an operational area and a crop species in a farm unit, with tank mixtures of each plant protection product counting separately.

5.7 Treatment Indices for different crops

See next page.

Table 5.7-1: Treatment index for winter wheat, winter barley and winter oilseed rape in reference farms in Germany from 2007 to 2010 (average and standard deviations, Freier et al. 2011)

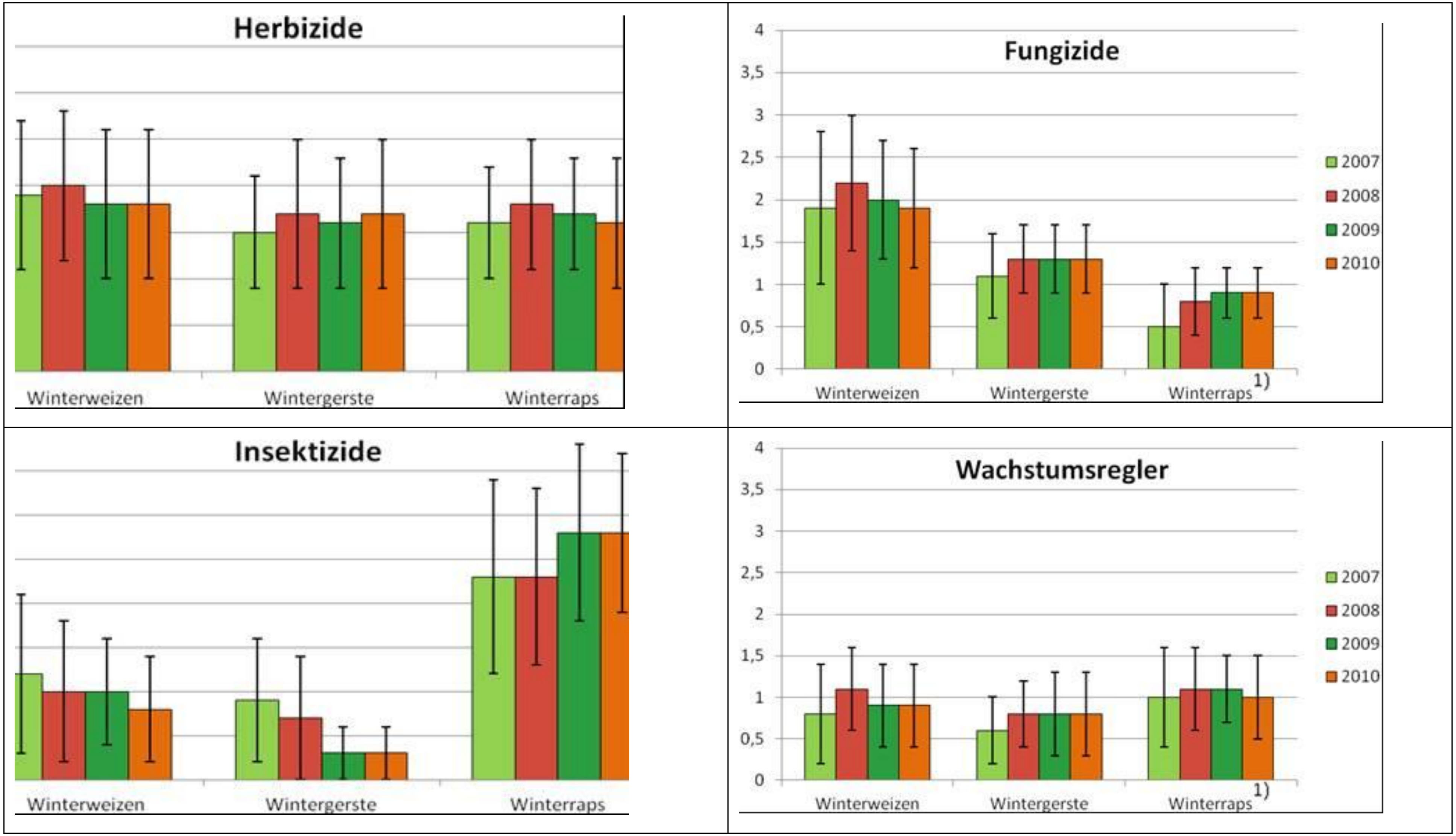


Table 5.7-2: Treatment index in winter wheat in the reference farms in Germany (DE) and the major regions (N, O, S, W) from 2007 to 2010 (without molluscicides, rodenticides, and seed treatments), mean (standard deviation) and statistical significant differences (Freier et al. 2011). Δ - Different letters represent significant differences (p <0.05) between the years (A and B) and between regions within the major plant protection product categories (a and b); comparisons tested with SAS 9.2.

Region			**Investigated years**											
	2007				**2008**			**2009**			**2010**			**2007-2010**
Region			**Δ**			**Δ**			**Δ**				**Δ**	**2007-2010**
					No. fields									∑
DE	**179**				**204**			**226**			**246**			**855**
N	60				60			63			68			251
O	41				41			44			47			173
S	15				23			25			47			110
W	63				80			94			84			321
					herbicides									□
DE	**1,9 (0,8)**				**2,0 (0,8)**			**1,8 (0,8)**			**1,8 (0,8)**			**1,9 (0,8)**
N	1,6 (0,8)	a			1,9 (1,0)			1,6 (0,8)			1,7 (1,0)			1,7 (0,9)
O	1,9 (0,8)				1,7 (0,6)			1,6 (0,7)			1,7 (0,7)			1,7 (0,7)
S	1,8 (0,8)				2,2 (0,9)			1,9 (0,8)			1,8 (0,6)			1,9 (0,8)
W	2,2 (0,9)	b			2,1 (0,7)			2,0 (0,8)			1,9 (0,8)			2,0 (0,8)
					fungicides									□
DE	**1,9 (0,9)**				**2,2 (0,8)**			**2,0 (0,7)**			**1,9 (0,7)**			**2,0 (0,8)**
N	2,4 (0,8)	a			2,5 (0,8)	a		2,2 (0,6)	a		2,4 (0,7)	a		2,4 (0,7)
O	1,3 (0,7)	b	a	A	1,6 (0,8)	b	a	1,6 (0,9)	b	B	1,8 (0,6)	b	B	1,6 (0,8)
S	1,5 (0,7)	b			1,6 (0,6)	b	a	1,9 (0,3)		a	1,7 (0,8)	b		1,7 (0,7)
W	1,8 (0,7)	b A	b		2,4 (0,7)	A	b B	2,1 (0,6)	B A	b B	1,8 (0,5)	b B	B	2,1 (0,7)
					insecticides									□
DE	**1,2 (0,9)**				**1,0 (0,8)**			**1,0 (0,6)**			**0,8 (0,6)**			**1,0 (0,7)**
N	1,5 (1,0)	a	A		1,4 (0,7)	a		1,3 (0,7)	a		1,0 (0,6)		B	1,3 (0,8)
O	0,6 (0,6)	b	a		0,7 (0,5)	b	a	0,9 (0,5)	b		0,8 (0,5)			0,7 (0,5)
S	0,5 (0,5)	b	a		0,3 (0,4)	b	a	0,4 (0,5)	b	a	0,4 (0,5)			0,4 (0,5)
W	1,4 (0,9)	A	b		1,2 (0,8)	B	b	1,1 (0,6)	B	b	0,9 (0,6)	B		1,1 (0,7)
					growth inhibitors									□
DE	**0,8 (0,6)**				**1,1 (0,5)**			**0,9 (0,5)**			**0,9 (0,5)**			**0,9 (0,5)**
N	1,0 (0,8)	a	A		1,4 (0,5)	a	B	1,2 (0,6)	a		1,2 (0,5)	a		1,2 (0,6)
O	0,7 (0,4)				0,9 (0,4)	b		0,8 (0,4)	b		0,9 (0,4)	b	a	0,8 (0,4)
S	0,4 (0,4)	b			0,5 (0,3)	b	a	0,7 (0,6)	b		0,6 (0,4)	b	b a	0,6 (0,4)
W	0,7 (0,4)	b	A		1,1 (0,4)	b A	b B	0,9 (0,4)	b B		0,9 (0,4)	b B	b	0,9 (0,4)
					total									□
DE	**5,7 (2,1)**	**A**			**6,2 (1,9)**	**B**		**5,8 (1,7)**	**A**		**5,4 (1,7)**	**A**		**5,8 (1,9)**
N	6,6 (2,2)	a			7,1 (2,0)	a		6,4 (1,8)	a		6,3 (1,7)	a		6,6 (1,9)
O	4,5 (1,8)	b		a	4,9 (1,5)	b	a	4,9 (1,6)	b	**a**	5,2 (1,6)	b		4,9 (1,6)
S	4,1 (1,5)	b			4,6 (1,4)	b	a	5,0 (1,1)			4,4 (1,6)	b		4,5 (1,5)
W	6,1 (1,9)			b	6,8 (1,6)	A	b	6,1 (1,5)	B	b	5,5 (1,4)	b	B	6,1 (1,6)

5.8 Changes in crop share over time

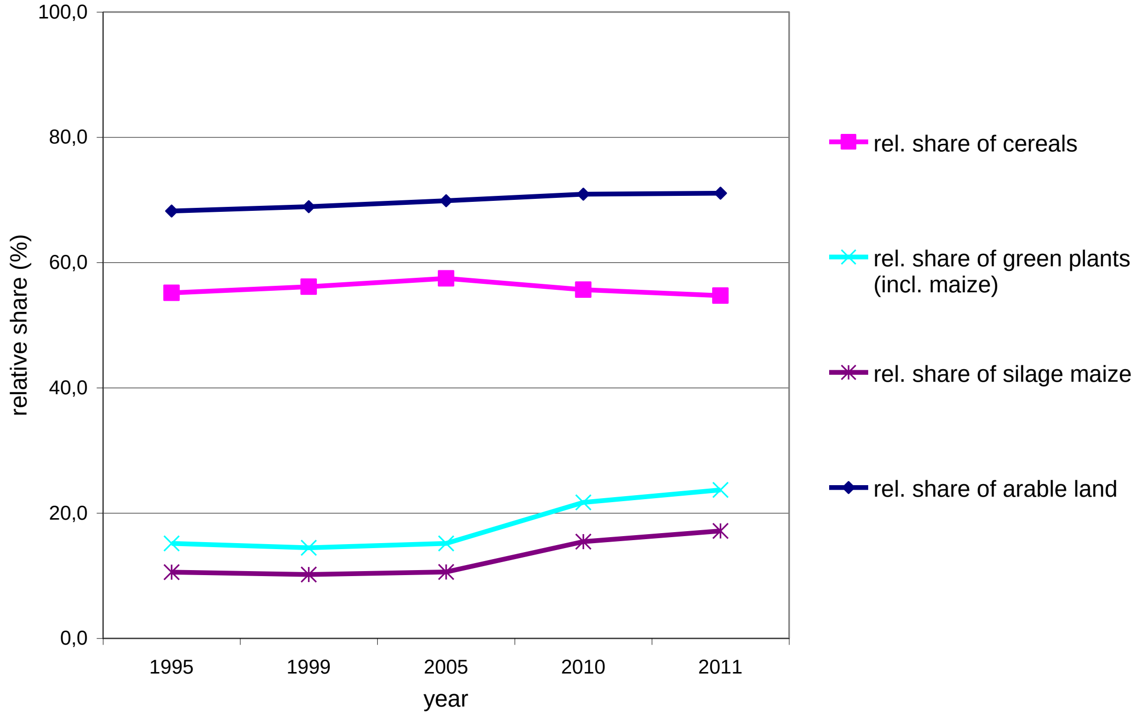


Figure 5.8-1: Relative share of arable land compared to total farm land in Germany and relative shares of crops in relation to arable land (Destatis (Statistisches Bundesamt) 2011).

5.9 Changes in plant protection product use over time

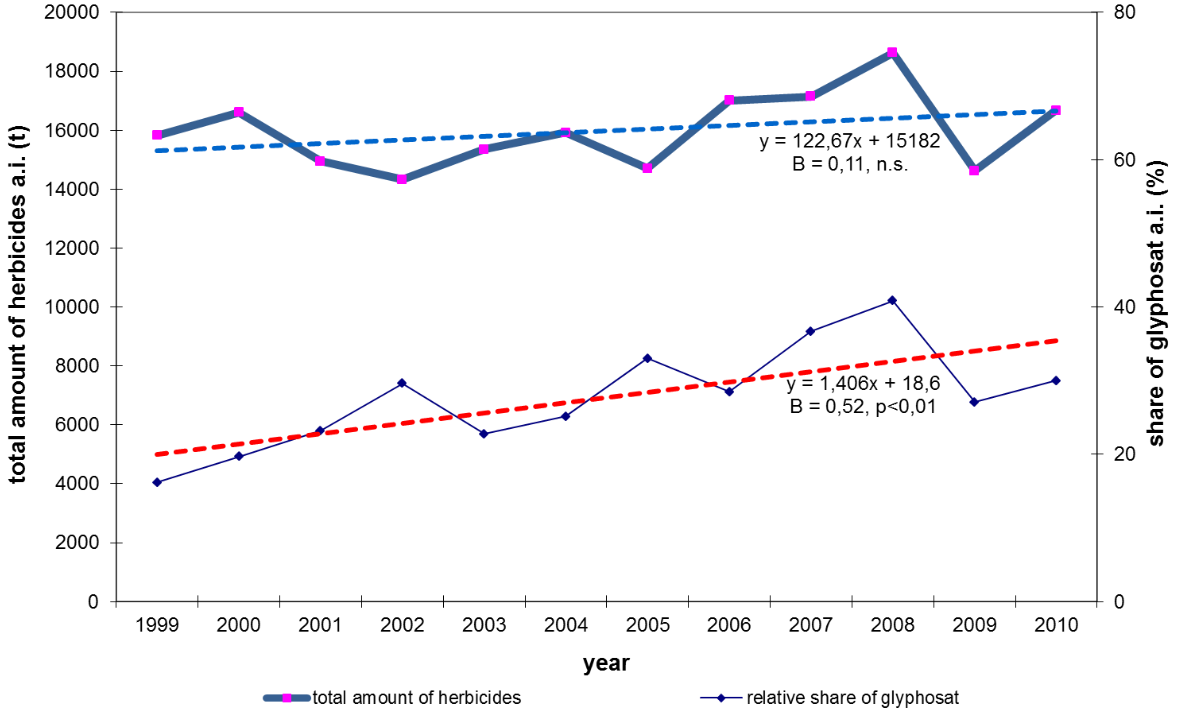


Figure 5.9-1: Domestic sale of active ingredients of herbicides in total and of the relative share of glyphosate and resulting trends (BVL 2011a).

Table 5.9-1: Domestic sales of active ingredients since 2001 in Germany (BVL 2011a)

	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
herbicides	15825	16610	14942	14328	15350	15923	14698	17015	17147	18626	14619	16675
fungicides	9702	9641	8246	10129	10033	8176	10184	10251	10942	11505	10922	10431
insecticides acaricides	6125	6111	6518	5889	6370	7328	6809	7780	9153	9665	9625	10360
others	3751	3232	3957	4332	4002	3704	3803	3740	3502	3624	3591	3378
sum	**35403**	**35594**	33663	34678	35755	35131	35494	38786	40744	43420	38757	40844

Table 5.9-2: Active ingredient quantities sold in 2010 in the domestic market in Germany (BVL 2011a, modified)

	tons sold	%
herbicides [1]	**16675**	**100**
organophosphorous herbicides	5060	30,3
amides, anilides	3641	21,8
other organic herbicides	2477	14,9
Fungicides [2]	**10431**	**100**
other organic fungicides	4368	41,9
carbamates, dithiocarbamates	1957	18,8
anorganic fungicides	1876	18
insecticides, acaricides [3]	**10360**	**100**
inert gases	9419	89,3
nicotinoids	256	2,4
organo-phosphates	241	2,3
other insecticides	133	3
other pesiticides [4]	**3378**	**100**

Most common active ingredients (bold letters in the list below > 1000 t / year):

[1] **glyphosate, isoproturon,** pethoxamid, prosulfocarb, metolachlor, terbuthylazine;

[2] **mancozeb, sulfur,** boscalid, chlorothalonil, fenpropimorph, propamocarb, prothioconazol, spiroxamine;

[3] methiocarp, thiacloprid, dimethoat;

[4] **chlormequate,** mepiquat

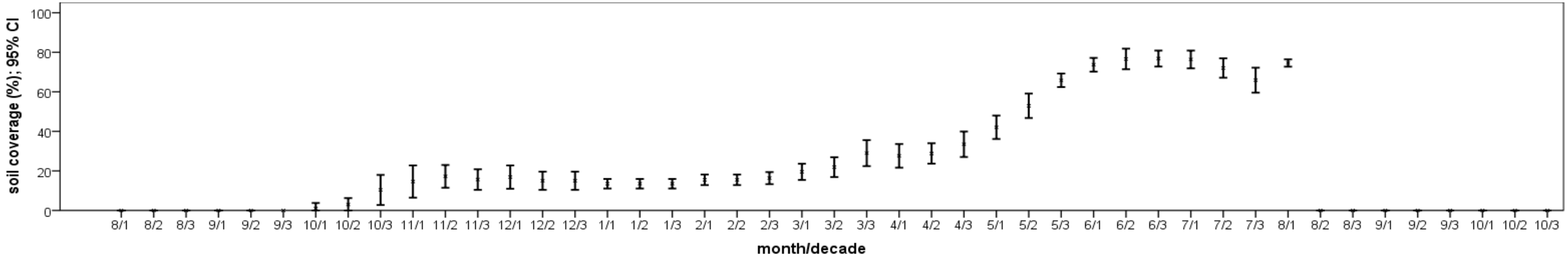
5.10 Analyzed fields per crop

Table 5.10-1: Number of analysed fields per crop for the two investigation periods (year split; data pooled for 2006 and 2007, investigation area Eggersdorf)

crop	abbreviation	crop overwintering	nr. of fields in the investigation period	
			01.01.-31.07.	01.08.-31.12.
winter rape	wra	yes	47	47
winter barley	wbl	yes	17	27
winter rye	wry	yes	57	58
triticale	trc	yes	16	16
winter wheat	wwt	yes	34	29
maize	mze	no	24	24
sunflower	sfl	no	5	5
blue lupine	blu	no	5	5
oat	oat	no	4	4
field pea	pea	no	1	1

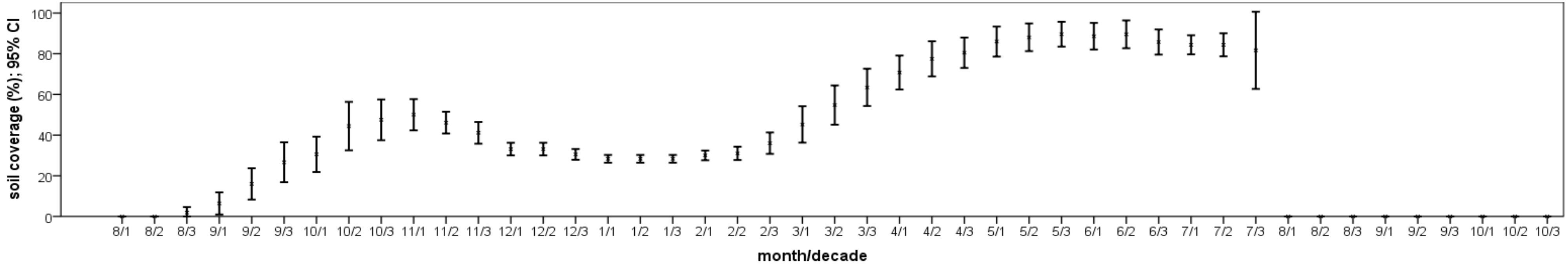
5.11 Soil coverage of different crops, crop specific application of plant protection products and habitat use of adult and juvenile amphibians

Table 5.11-1: Soil coverage of winter wheat in decades, crop specific application of plant protection products during growing period and habitat use of adult and juvenile amphibians for amphibians in general, crested newt and fire bellied toad (plots with average values and confidence intervals).



month/decade			8/1	8/2	8/3	9/1	9/2	9/3	10/1	10/2	10/3	11/1	11/2	11/3	12/1	12/2	12/3	1/1	1/2	1/3	2/1	2/2	2/3	3/1	3/2	3/3	4/1	4/2	4/3	5/1	5/2	5/3	6/1	6/2	6/3	7/1	7/2	7/3	8/1	8/2	8/3	9/1	9/2	9/3	10/1	10/2	10/3
growing period																																															
herbicides								H																H																							
fungicides																											F						F												
insecticides								(I)																						(I)						(I)									
growth regulators																									(GR)			GR																	
age	species	habitat period																																											
adults	all species	spring migration																																											
		water stay																																											
		terr. summer habitat																																											
		hibernation																																											
	crested newt	spring migration																																											
		water stay																																											
		terr. summer habitat																																											
		hibernation																																											
	fire bellied toad	spring migration																																											
		water stay																																											
		terr. summer habitat																																											
		hibernation																																											
juveniles	all species	water period																																											
		terr. summer habitat																																											
		hibernation																																											
	crested newt	water period																																											
		terr. summer habitat															no figures because of mistakable generation shift																													
		hibernation																																											
	fire bellied toad	water period																																											
		terr. summer habitat																																											
		hibernation																																											

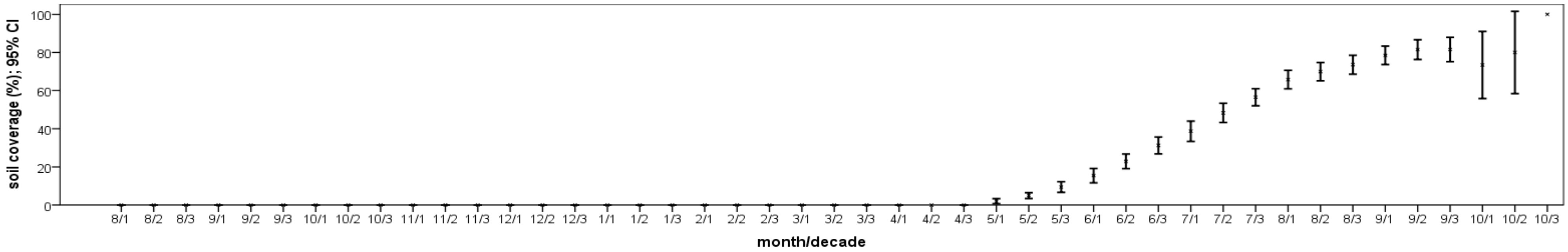
Table 5.11-2: Soil coverage of winter rape in decades, crop specific application of plant protection products during growing period and habitat use of adult and juvenile amphibians shown for amphibians in general, crested newt and fire bellied toad (plots with average values and confidence intervals).



month/decade	8/1	8/2	8/3	9/1	9/2	9/3	10/1	10/2	10/3	11/1	11/2	11/3	12/1	12/2	12/3	1/1	1/2	1/3	2/1	2/2	2/3	3/1	3/2	3/3	4/1	4/2	4/3	5/1	5/2	5/3	6/1	6/2	6/3	7/1	7/2	7/3	8/1	8/2	8/3	9/1	9/2	9/3	10/1	10/2	10/3
growing period																																													
herbicides		H			(H)																																								
fungicides																													F					F											
insecticides				(I)																						I	I	I	I					I											
growth regulators						GR																		GR																					

age	species	habitat period	8/1	8/2	8/3	9/1	9/2	9/3	10/1	10/2	10/3	11/1	11/2	11/3	12/1	12/2	12/3	1/1	1/2	1/3	2/1	2/2	2/3	3/1	3/2	3/3	4/1	4/2	4/3	5/1	5/2	5/3	6/1	6/2	6/3	7/1	7/2	7/3	8/1	8/2	8/3	9/1	9/2	9/3	10/1	10/2	10/3
adults	all species	spring migration																																											
		water stay																																											
		terr. summer habitat																																											
		hibernation																																											
	crested newt	spring migration																																											
		water stay																																											
		terr. summer habitat																																											
		hibernation																																											
	fire bellied toad	spring migration																																											
		water stay																																											
		terr. summer habitat																																											
		hibernation																																											
juveniles	all species	water period																																											
		terr. summer habitat																																											
		hibernation																																											
	crested newt	water period																																											
		terr. summer habitat															no figures because of mistakable generation shift																													
		hibernation																																											
	fire bellied	water period																																											
		terr. summer habitat																																											
		hibernation																																											

Table 5.11-3: Soil coverage of silage maize and corn in decades, crop specific application of plant protection products during growing period and habitat use of adult and juvenile amphibians shown for amphibians in general, crested newt and fire bellied toad (plots with average values and confidence intervals).



month/decade	8/1	8/2	8/3	9/1	9/2	9/3	10/1	10/2	10/3	11/1	11/2	11/3	12/1	12/2	12/3	1/1	1/2	1/3	2/1	2/2	2/3	3/1	3/2	3/3	4/1	4/2	4/3	5/1	5/2	5/3	6/1	6/2	6/3	7/1	7/2	7/3	8/1	8/2	8/3	9/1	9/2	9/3	10/1	10/2	10/3
growing period																																													
herbicides																												H		(H)															
fungicides																																													
insecticides																																				(I)									
growth regulators																																													
age / species / habitat period																																													
adults / all species / spring migration																																													
adults / all species / water stay																																													
adults / all species / terr. summer habitat																																													
adults / all species / hibernation																																													
adults / crested newt / spring migration																																													
adults / crested newt / water stay																																													
adults / crested newt / terr. summer habitat																																													
adults / crested newt / hibernation																																													
adults / fire bellied toad / spring migration																																													
adults / fire bellied toad / water stay																																													
adults / fire bellied toad / terr. summer habitat																																													
adults / fire bellied toad / hibernation																																													
juveniles / all species / water period																																													
juveniles / all species / terr. summer habitat																																													
juveniles / all species / hibernation																																													
juveniles / crested newt / water period																																													
juveniles / crested newt / terr. summer habitat																no figures because of mistakable generation shift																													
juveniles / crested newt / hibernation																																													
juveniles / fire bellied / water period																																													
juveniles / fire bellied / terr. summer habitat																																													
juveniles / fire bellied / hibernation																																													

5.12 Average soil coverage and interception values

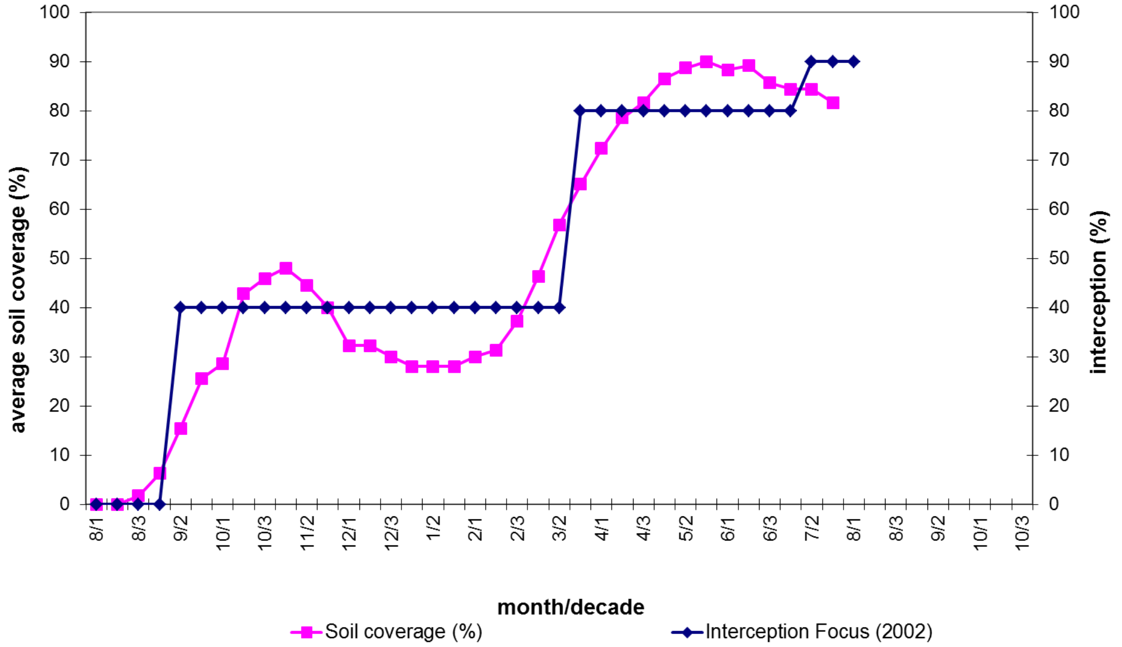


Figure 5.12-1: Average soil coverage of winter rape from field experiments and related standard interception values (FOCUS 2002)

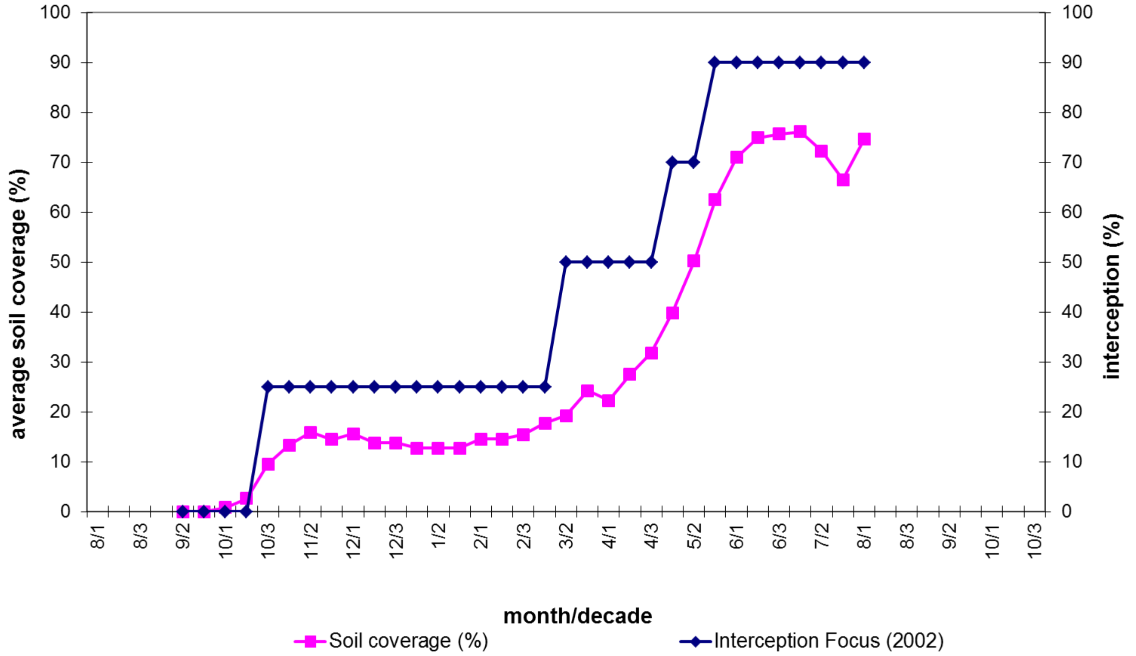


Figure 5.12-2: Average soil coverage of winter wheat from field experiments and related standard interception values (FOCUS 2002)

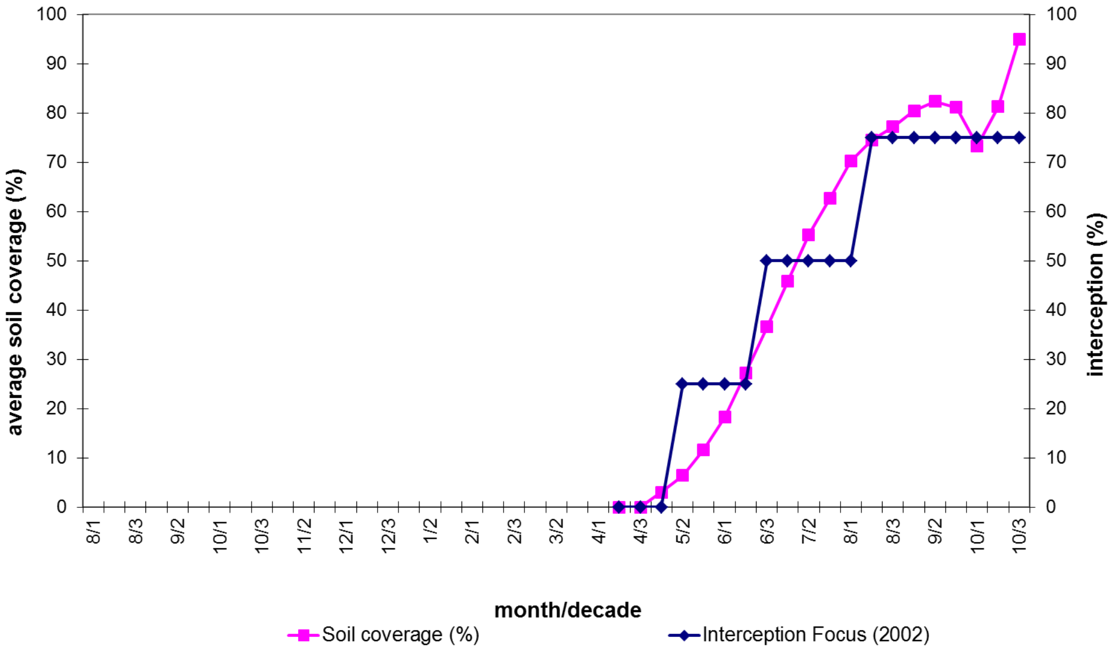


Figure 5.12-3: Soil coverage of maize revealed from field experiments and related interception values (FOCUS 2002)

5.13 Frequency of plant protection product applications

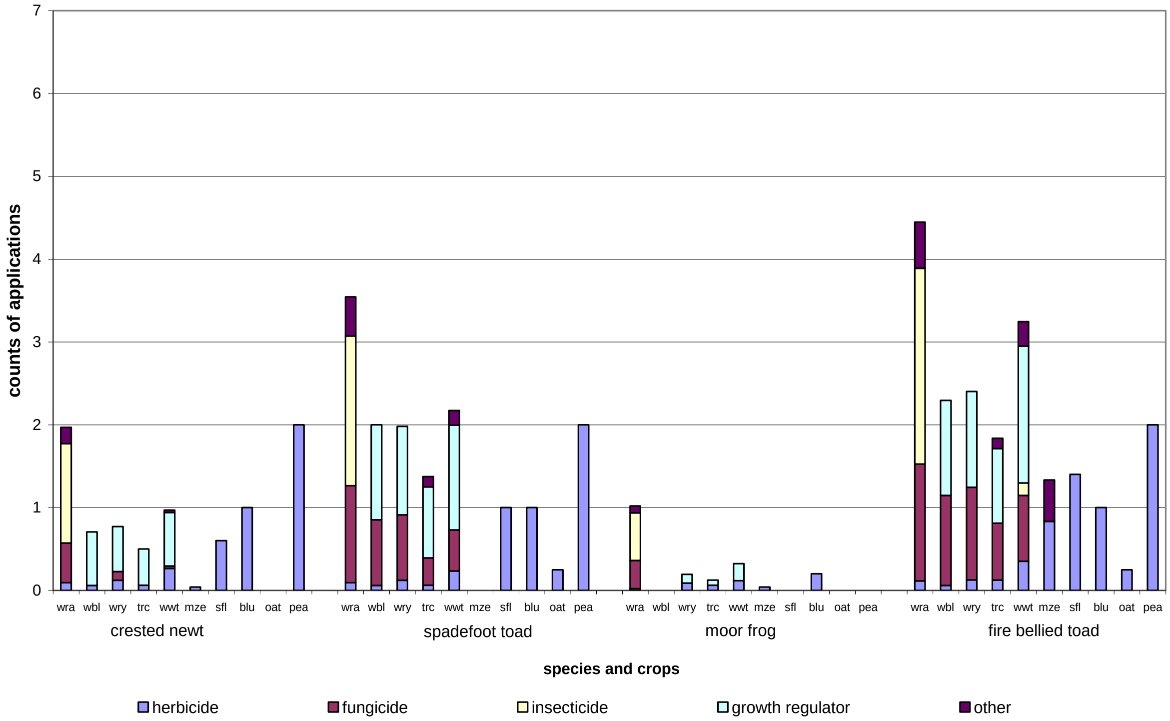


Figure 5.13-1: Frequency of plant protection product applications to field crops during spring migration period of adult amphibians from hibernation sites into breeding ponds (wra winter rape, wbl winter barley, wry winter rye, trc triticale, wwt winter wheat, mze maize, sfl sunflower, blu blue lupine).

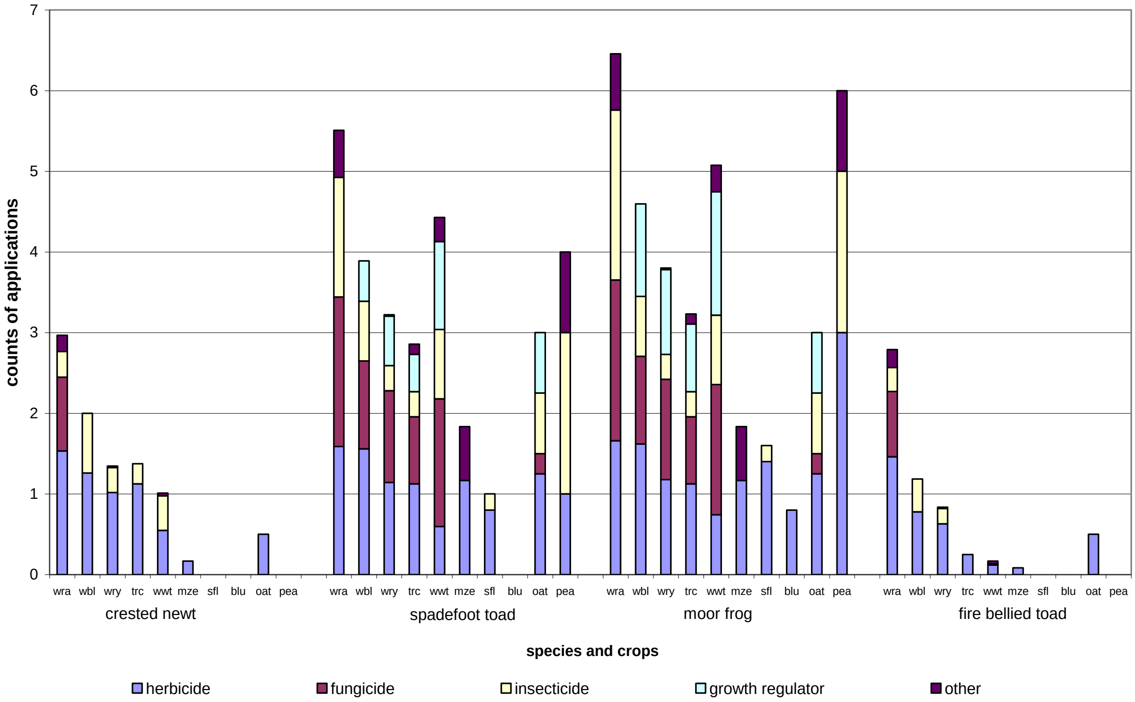


Figure 5.13-2: Frequency of plant protection product application to field crops during the phase of adult amphibians leaving breeding ponds until the end of vegetation/activity period (including migration to and sojourn in summer habitats and migration into hibernation sites; wra winter rape, wbl winter barley, wry winter rye, trc triticale, wwt winter wheat, mze maize, sfl sunflower, blu blue lupine)

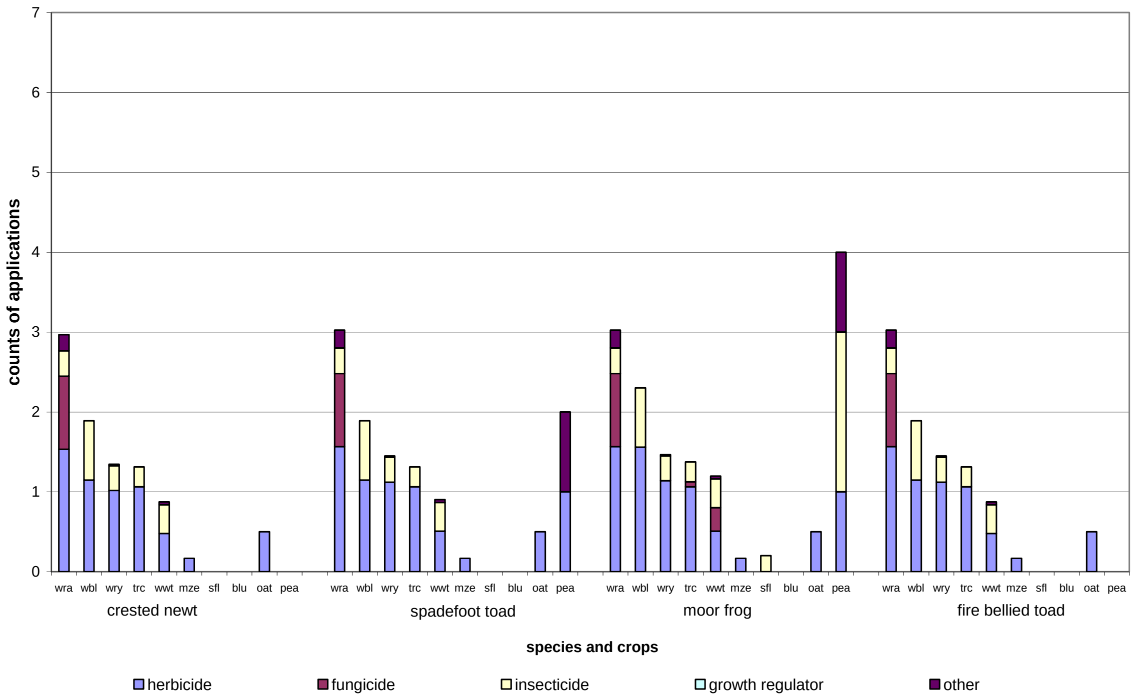


Figure 5.13-3: Plant protection product applications to field crops during the phase of juvenile amphibians leaving breeding ponds until the end of vegetation/activity period (including migration to and sojourn in summer habitats and migration into hibernation sites; (wra winter rape, wbl winter barley, wry winter rye, trc triticale, wwt winter wheat, mze maize, sfl sunflower, blu blue lupine)

5.14 Vegetation canopy of winter rape and winter wheat

Figure 5.14-1: Dense vegetation canopy of winter rape (above) and winter wheat (bottom left) next to winter barley (bottom right) at BBCH stages after flowering (BBCH > 40) and interception values of 90% (FOCUS 2002)

5.15 Application of herbicides

Figure 5.15-1: Application of herbicides in maize with soil coverage of less than 5 % and at the beginning of leaf development (BBCH about 10). The assigned interception value of 25% is overestimated (compare values in decade 5/2 and 5/3 in annex 11)

5.16 Population share

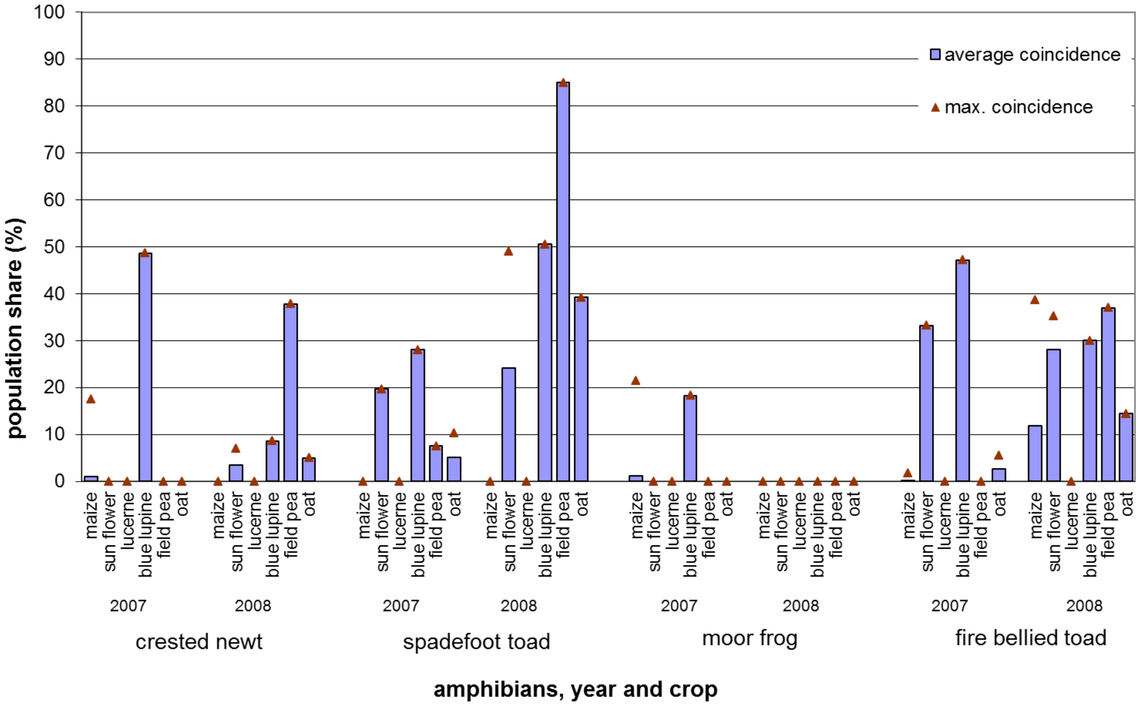


Figure 5.16-1: Population share of different amphibian species during spring migrating of adults into breeding ponds temporally coincident with application of herbicides to summer crops (Berger et al. 2011a, p. 172)

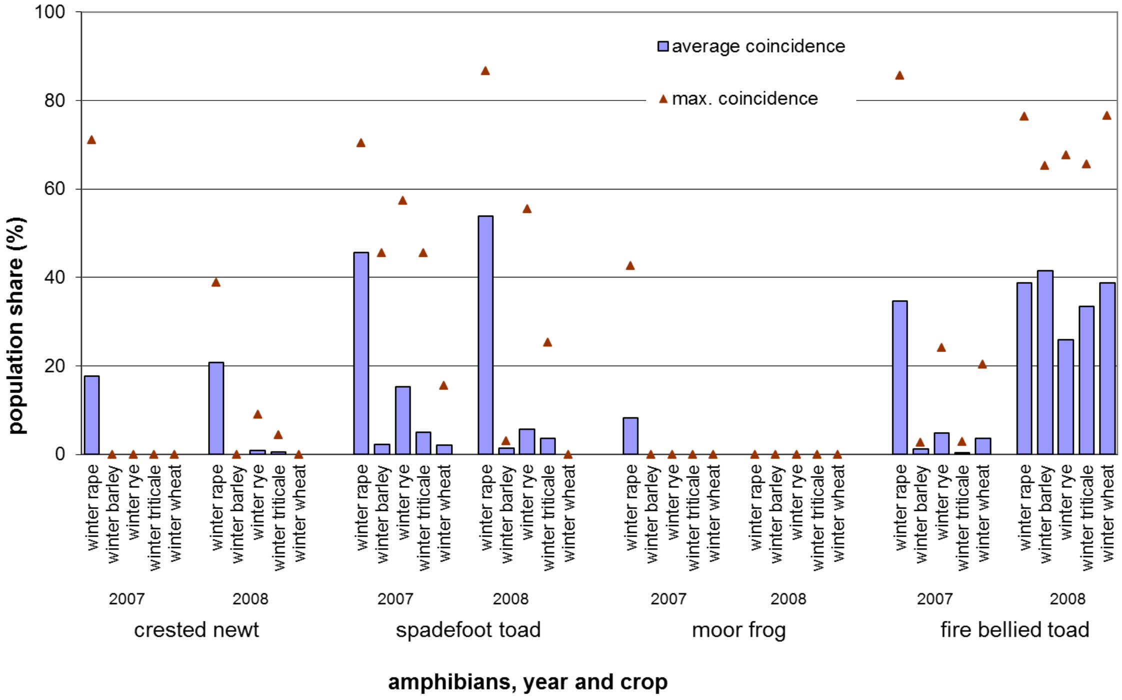


Figure 5.16-2: Population share of different amphibian species during spring migrating of adults into breeding ponds temporally coincident with application of fungicides to winter crops (Berger et al. 2011a, p. 173)

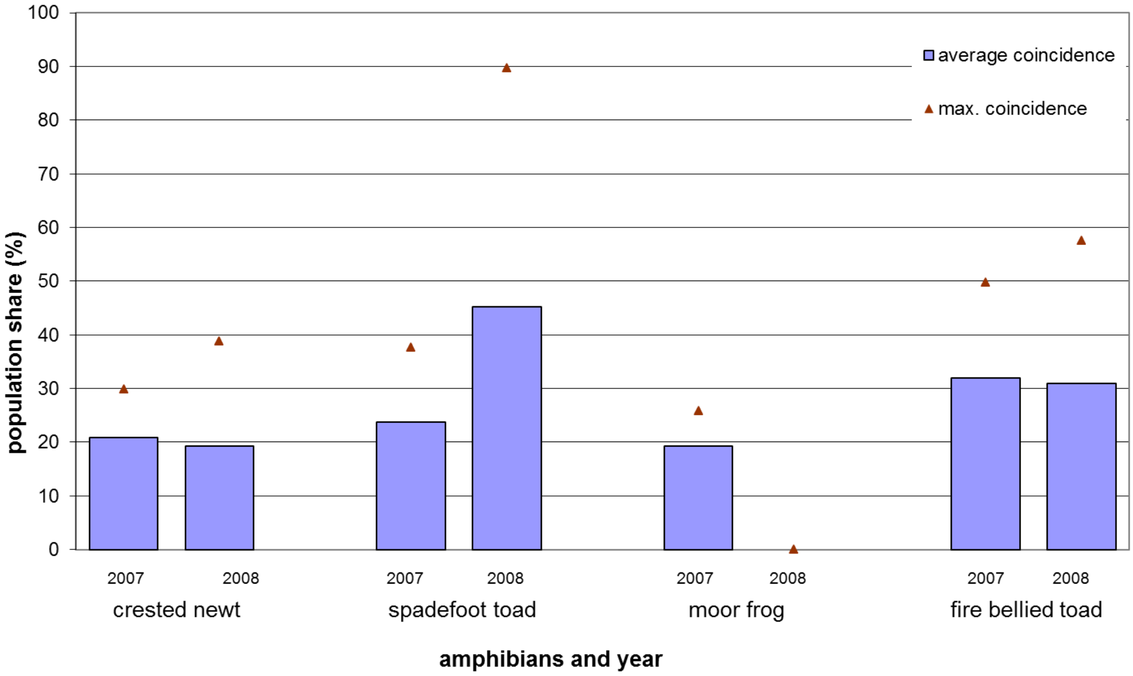


Figure 5.16-3: Population share of amphibian species during spring migrating of adults into breeding ponds temporally coincident with application of insecticides to winter rape (Berger et al. 2011a, p. 174)

5.17 Parameters to calculate skin area relevant for direct overspray and soil contact of adult amphibians

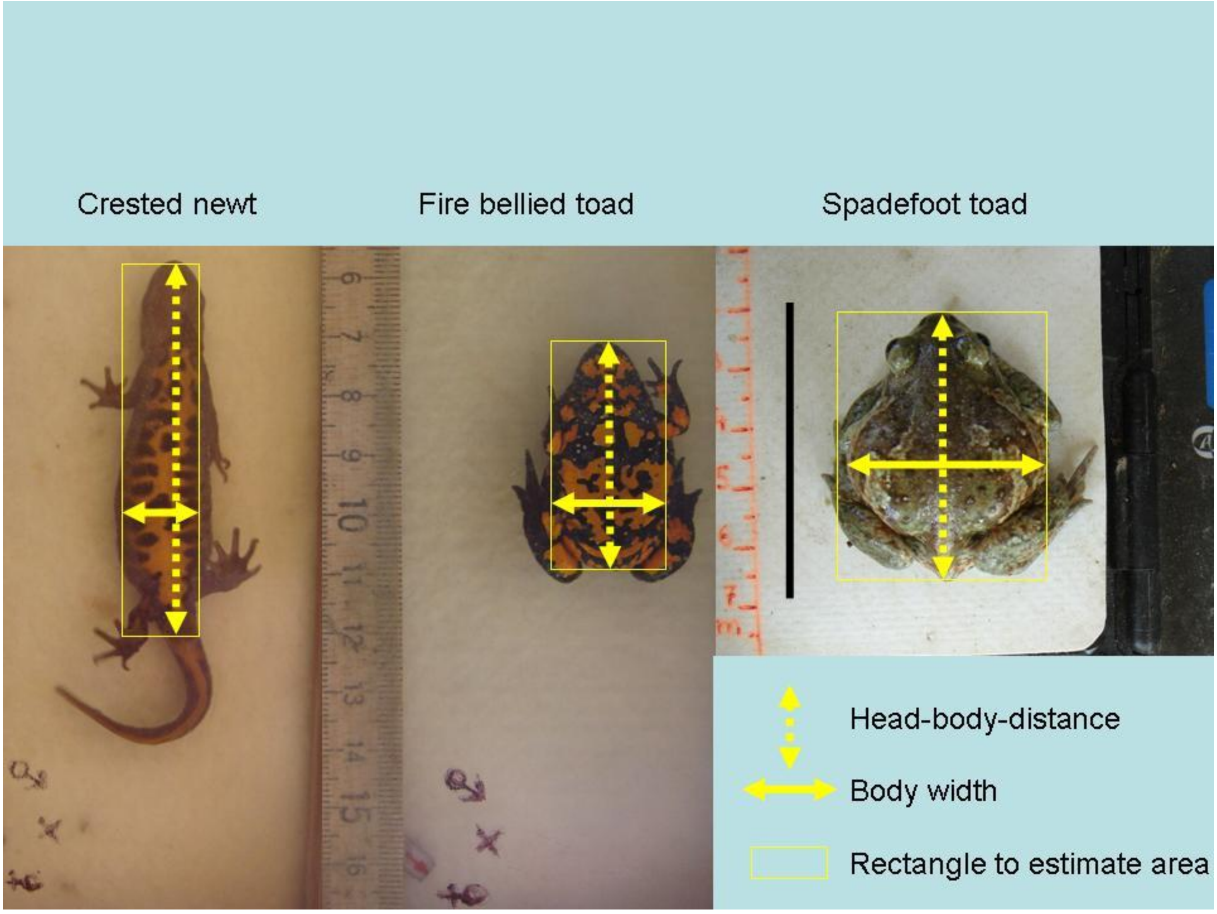


Figure 5.17-1: Parameters to calculate dorsal and ventral skin area relevant for direct overspray and soil contact of adult amphibians during migration (individuals are shown in the same image scale)

5.18 Field passage of amphibians

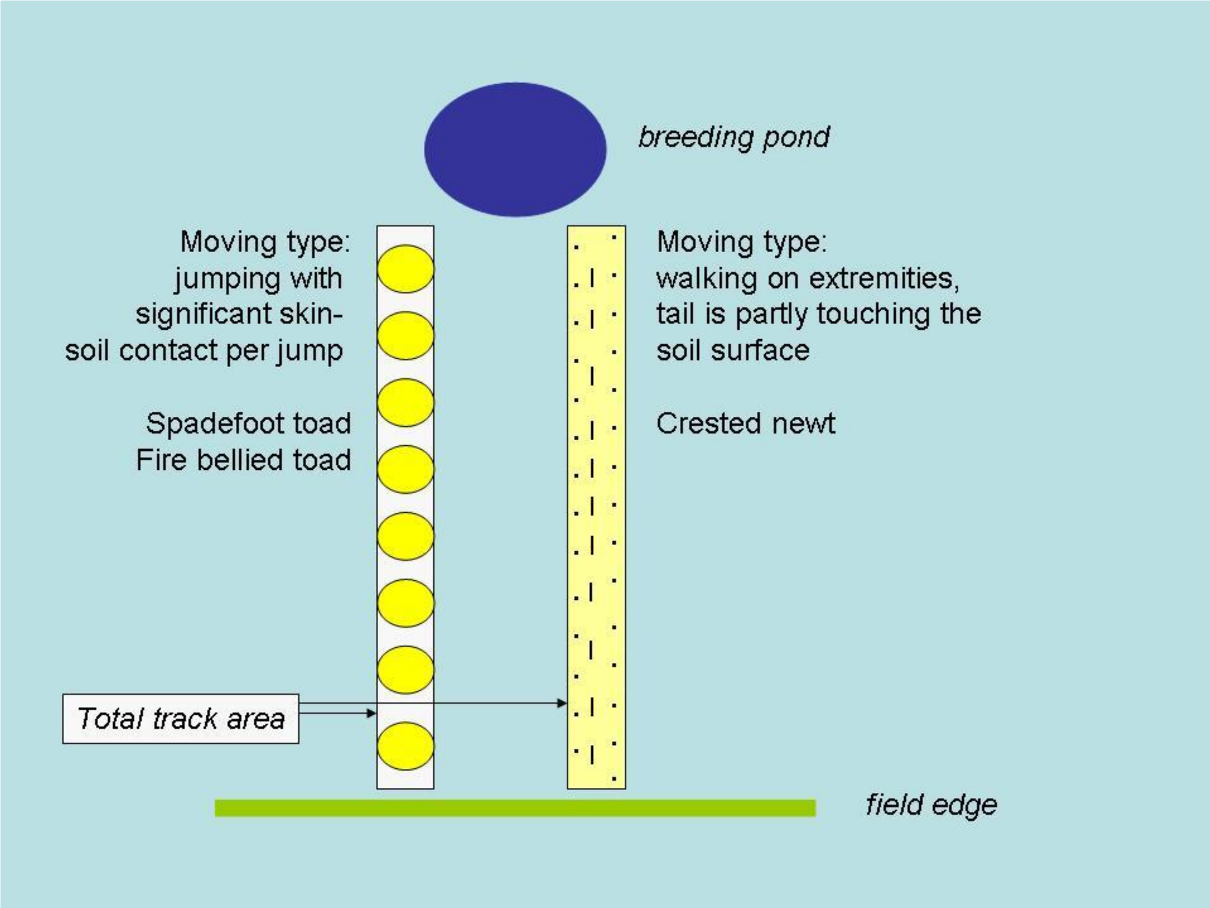


Figure 5.18-1: Schematic plot of field passage of adult amphibian species during spring migration into breeding ponds, moving type of amphibians and implications for skin-soil contact

5.19 Red List hazard categories of vertebrate groups

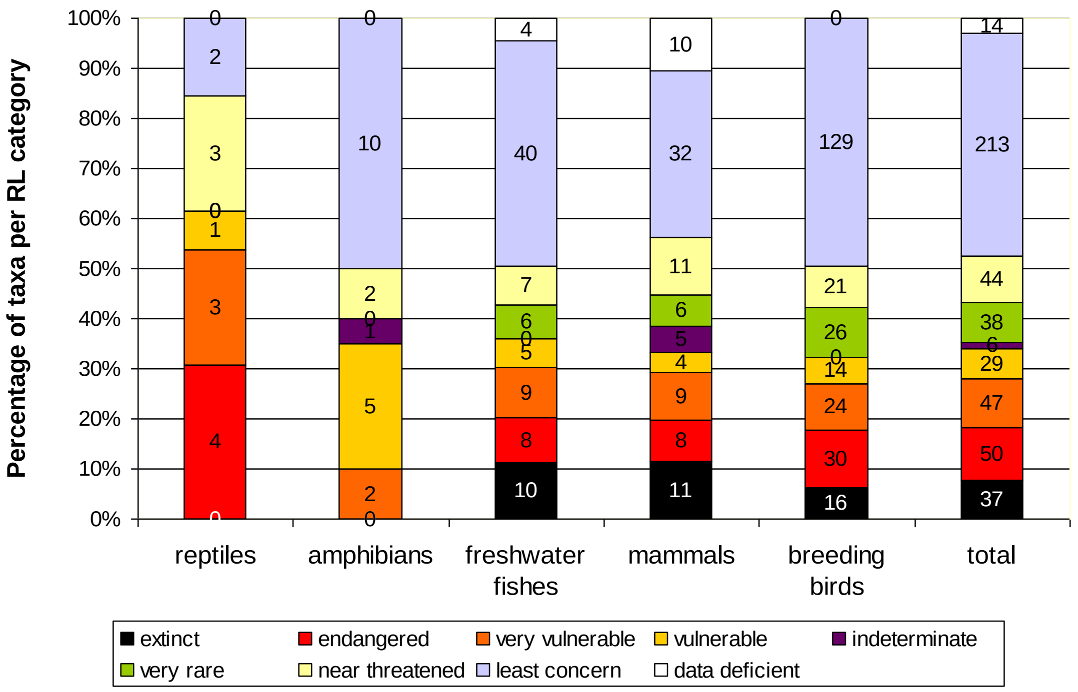


Figure 5.19-1: Red List hazard categories of vertebrate groups (n = 478) according to Haupt et al. (2009). The absolute species number is shown in the pillars. Neobiota (e.g. *Lithobates catesbeianus* for amphibians; *Lacerta horvathi* for reptiles) are not considered.

5.20 Supply of mineral fertilizer types to farmers

Table 5.20-1: Supply of mineral fertilizer types to farmers in 2008/2009 (Destatis 2009)

Mineral fertilizers	**Amount sold (thousand tons)**
Nitrogenous fertilizers (N)	
Calcium ammonium nitrate (KAS)	626
Urea	429
Urea ammonium nitrate solution (UAN)	152
NK and NPK	59
NP fertilizer	52
other	229
Potash, (K2O)	
Potassium chloride	82
NK and NPK	53
Potassium sulphate	21
PK fertilizer	16
crude potassium	3
Phosphate-containing fertilizers (P2O5)	
NP-fertilizer	119
NPK fertilizer	36
PK fertilizer	8
Superphosphate	6
other	2
Lime (CaO)	
Calcium carbonate	1.567
other	362
slag	234
quicklime	72

5.21 NATURA 2000 areas designed to amphibians

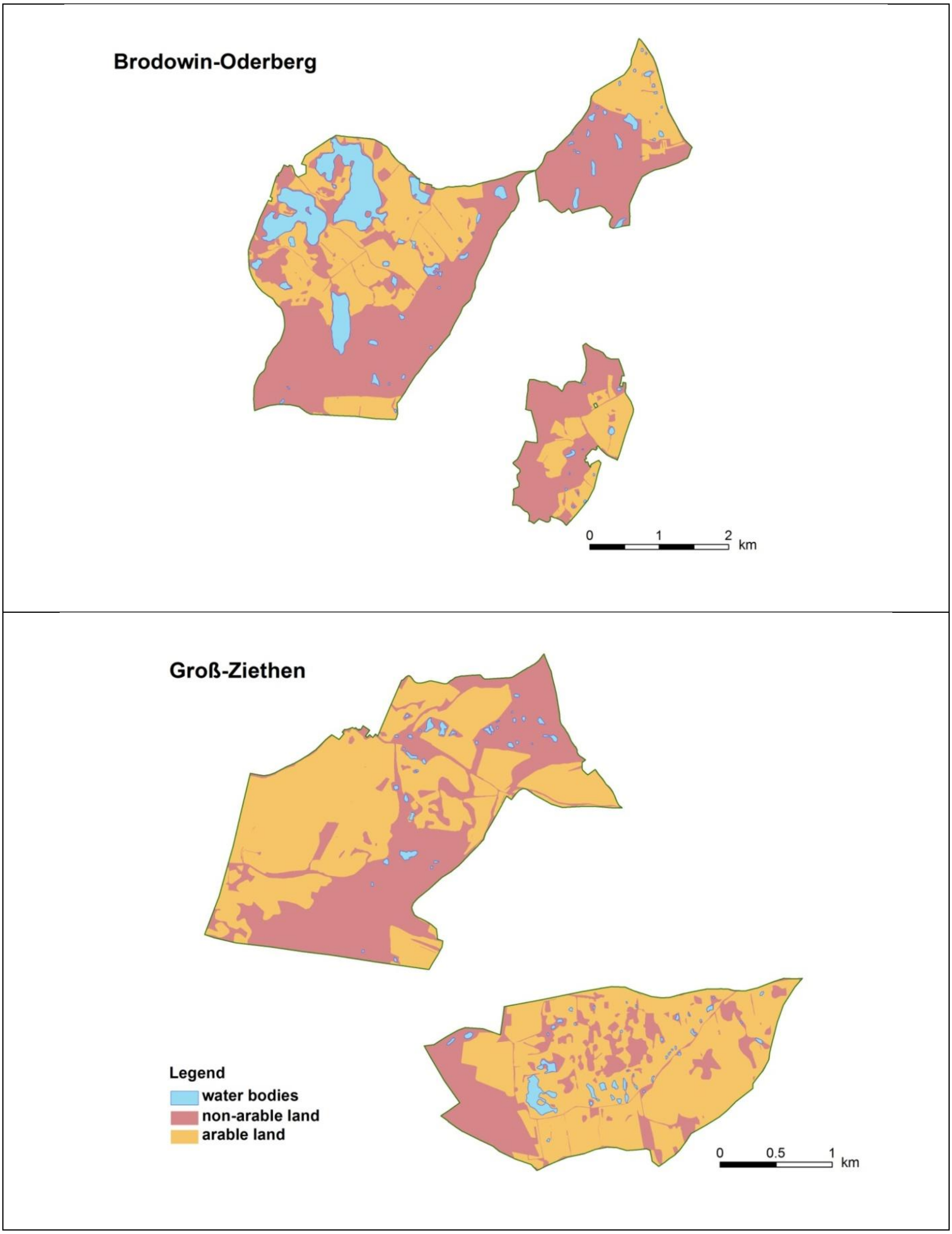


Figure 5.21-1: NATURA2000 areas designated to amphibians with shares of non-arable land of more than 35 %.

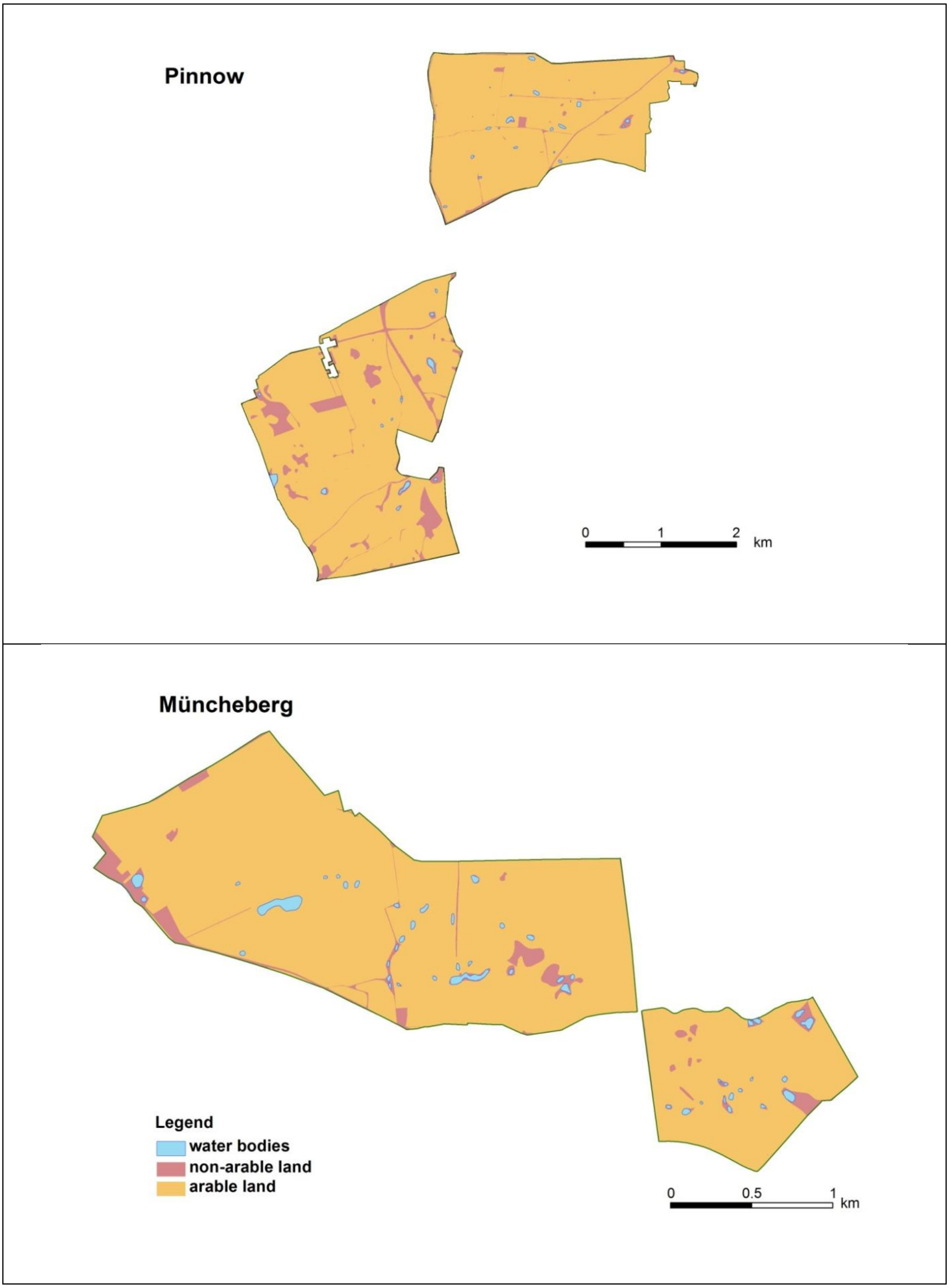


Figure 5.21-2: NATURA2000 areas designated to amphibians with shares of non-arable land of less than 10 %.

5.22 Direct drivers for biodiversity decline

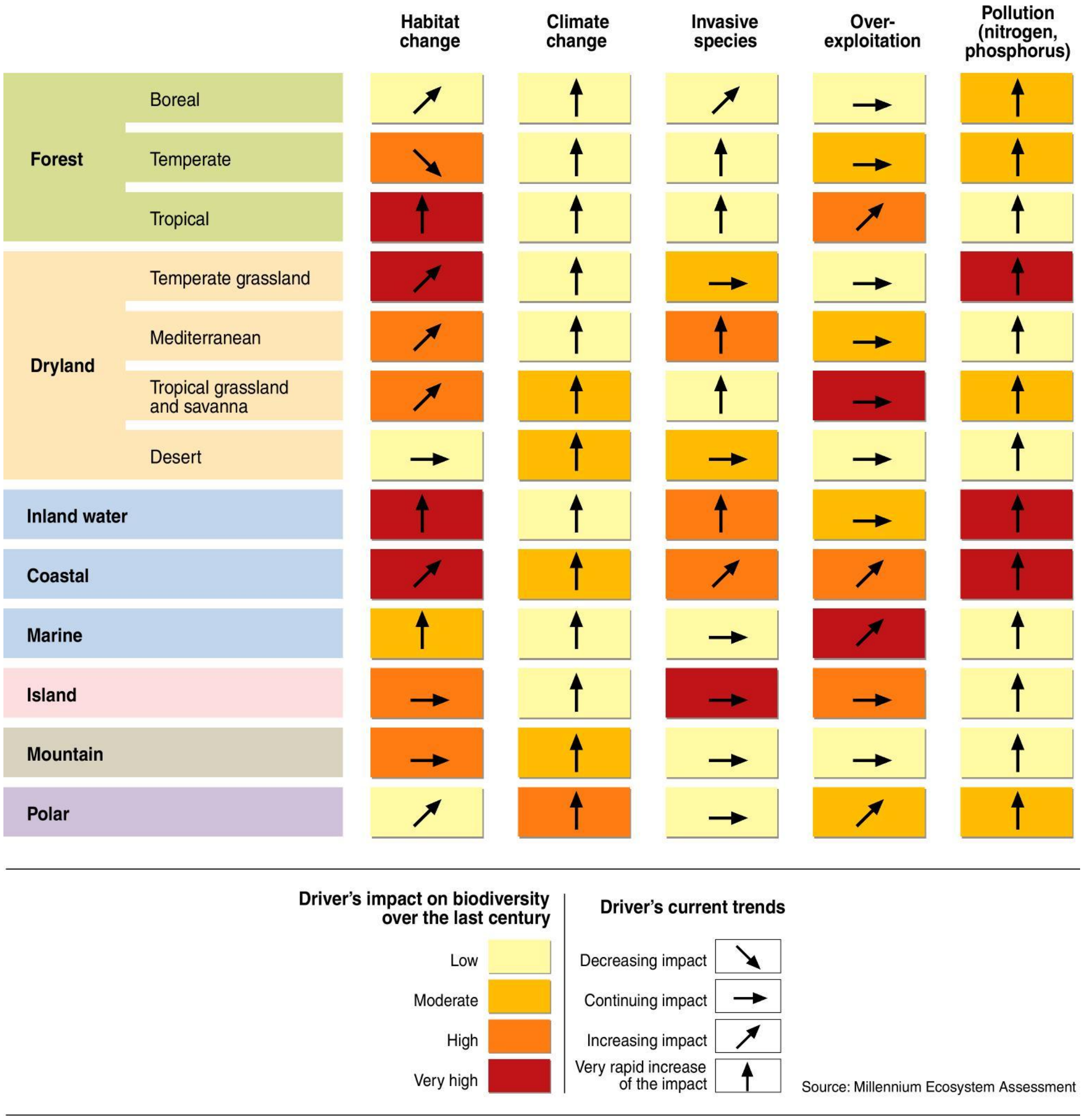


Figure 5.22-1: Direct drivers for biodiversity decline in different regions and ecosystems (Millennium Ecosystem Assessment 2005, Synthesis Report). Most drivers remain constant or are growing in intensity in most ecosystems.

5.23 Flowchart for the risk assessment of mammals and birds

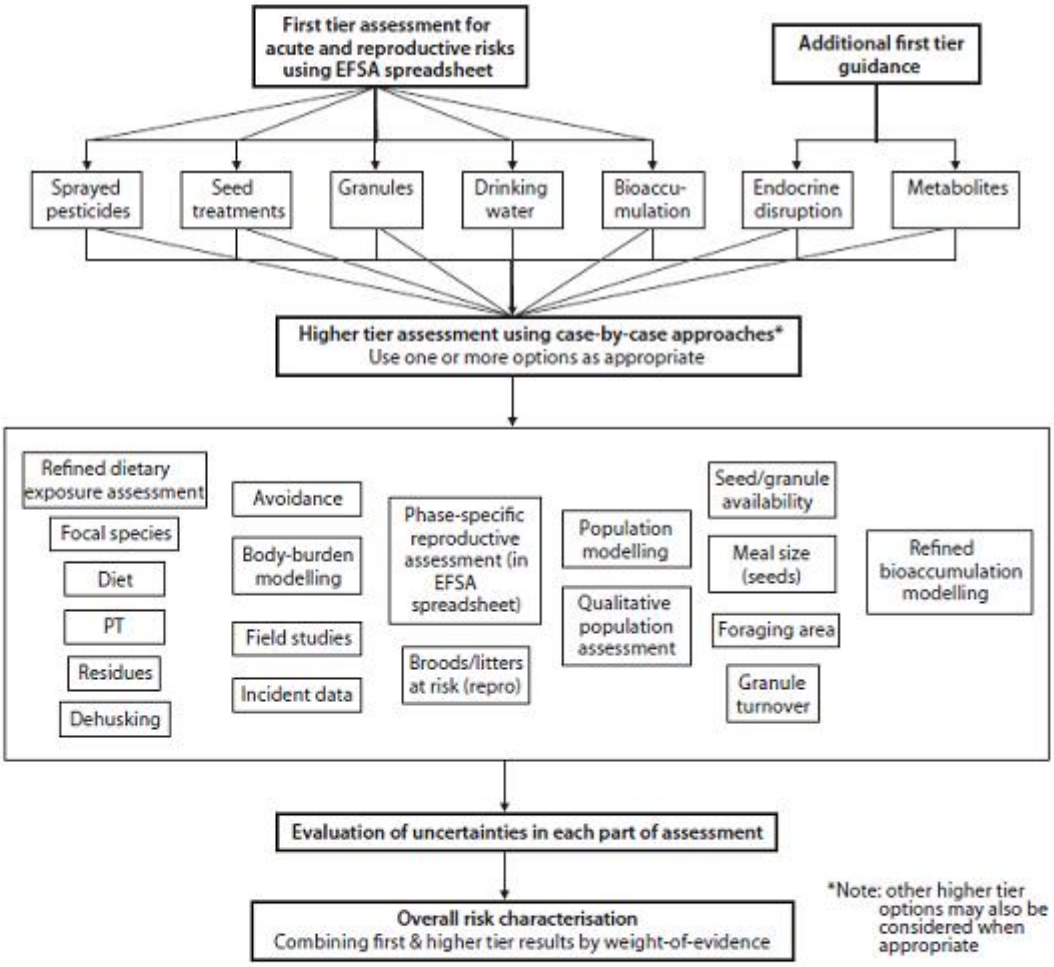


Figure 5.23-1: Flowchart for the risk assessment of mammals and birds (EFSA 2009a).

5.24 Sampling area of juveniles of the grass frog for the experimental part

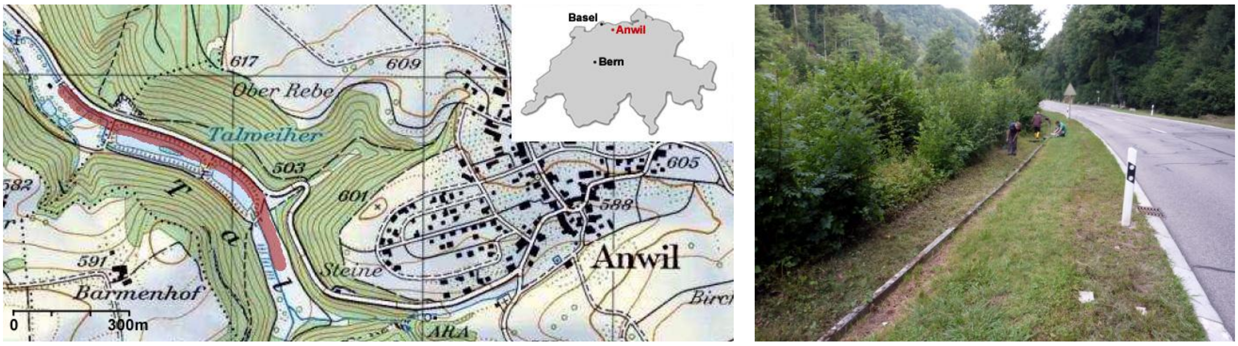


Figure 5.24-1: Nature protection area “Tal” (sampling area red marked, left) and “Krötenzaun” (right)

5.25 Application rates and calibration results for the juvenile frog experiments

Table 5.25-1: Target application rates and calibration results of the tack sprayer

Study	Product	Target application rate	Volume of application solution (mL)	Amount of test substance measured [a]	Spray volume (L/ha) target	Spray volume (L/ha) actual [b]	% Deviation	Actual application rate [a]
A	Trafo WG	4.155 g/ha	500	13.8 mL of A	400	379	-5.3	3.937 g/ha
		150 g/ha	500 (A)	0.188 g	400			142.1 g/ha
B	Headline	131.54 mL/ha	500	50 mL of B mL	200	200.2	+0.1	131.67154 mL/ha
		1315.4 mL/ha	500 (B)	50 mL of A mL	200			1316.7154 mL/ha
		13144 mL/ha	500 (A)	32.885 mL	200			13157.144 mL/ha
C	Headline	88 mL/ha	125	0.055 mL	200	201.0-206.2	+0.5 to+ 3.1	88.4-90.7 mL/ha
		880 mL/ha	125	0.550 mL	200	201.0-206.2	+0.5 to+ 3.1	884-907 mL/ha
		8800 mL/ha	---	--- ---	200	---	---	--- ---
C	BAS 500 18 F	88 mL/ha	250	0.119 mL	200	201.6-204.0	+0.8 to +2.0	88.7-89.8 mL/ha
		880 mL/ha	250	1.194 mL	200	199.4-206.3	-0.3 to +3.2	877.4-908.2 mL/ha
		8800 mL/ha	250	5.968 mL	200	201.0-206.2	+0.5 to+ 3.1	8844-9073 mL/ha
C	Curol B	150 mL/ha	250	0.188 mL	200	197.8-202.7	-1.1 to + 1.4	148.4-152.1 mL/ha
		1500 mL/ha	250	1.875 mL	200	201.6-204.0	+0.8 to +2.0	1512-1530 mL/ha
		15000 mL/ha	250	18.750 mL	200	199.4-206.3	-0.3 to +3.2	14955-15480 mL/ha
C	Captan WDG Omya	320 g/ha	250	0.400 g	200	197.8-202.7	-1.1 to + 1.4	316.5-324.5 g/ha
		3200 g/ha	250	4.000 g	200	201.6-204.0	+0.8 to +2.0	3226-3264 g/ha
		32000 g/ha	---	--- ---	200	---	---	--- ---
C	Dicomil ultra royal	120 mL/ha	250	0.158 mL	200	197.8-202.7	-1.1 to + 1.4	118.7-121.7 mL/ha
		1200 mL/ha	250	1.575 mL	200	201.6-204.0	+0.8 to +2.0	1210-1224 mL/ha
		12000 mL/ha	250	15.750 mL	200	199.4-206.3	-0.3 to +3.2	11964-12384 mL/ha
C	Prosper	150 mL/ha	250	0.188 mL	200	197.8-202.7	-1.1 to + 1.4	148.4-152.1 mL/ha
		1500 mL/ha	250	1.875 mL	200	201.6-204.0	+0.8 to +2.0	1512-1530 mL/ha
		15000 mL/ha	250	18.750 mL	200	199.4-206.3	-0.3 to +3.2	14955-15480 mL/ha
C	Roxion	100 mL/ha	250	0.125 mL	200	197.8-202.7	-1.1 to + 1.4	98.9-101.4 mL/ha
		1000 mL/ha	250	1.250 mL	200	201.6-204.0	+0.8 to +2.0	1008-1020 mL/ha
		10000 mL/ha	250	12.500 mL	200	199.4-206.3	-0.3 to +3.2	9970-10320 mL/ha

a: The nominal application rates were corrected according to the reduction caused by the cage used during application.

b: A range of actual application rates was used in study C, since individual test organisms of one treatment rate were treated on different days.

5.26 Body weight change of Juvenile *Rana temporaria*

Table 5.26-1: Body weight change of juveniles of *Rana temporaria* after seven (Study B and C) and 14 Days (Study A) of exposure to plant protection products.

Study	Treatment	Corre-spon-ding control	Fresh body weight (g)						Body weight change (g)	Body weight change (%)	Stat.
			Day -1		Day -1 (correct.[a])		Day 7 (Day 14 for Trafo WG)				
			mean ± s.d.	n	mean ± s.d.	n	mean ± s.d.	n	mean ± s.d.	mean ± s.d.	
	Trafo WG										
A	control	---	0.837 ± 0.278	9	0.818 ± 0.291	8	0.894 ± 0.267	8	0.076 ± 0.049	13.2 ± 15.8	---
	0.1x field rate		0.901 ± 0.192	8	0.931 ± 0.202	8	0.988 ± 0.170	8	0.057 ± 0.091	7.3 ± 9.3	n.s.
	1x field rate		0.820 ± 0.254	9	0.883 ± 0.179	8	0.931 ± 0.163	8	0.048 ± 0.102	6.3 ± 9.8	n.s.
	Headline										
B	control	---	0.345 ± 0.212	6	0.345 ± 0.212	6	0.375 ± 0.199	6	0.030 ± 0.027	12.0 ± 11.8	---
	0.1x field rate		0.315 ± 0.138	6	0.289 ± 0.137	5	0.362 ± 0.188	5	0.073 ± 0.054	22.7 ± 11.0	n.s.
	1x field rate		0.300 ± 0.125	6	--- ± ---	0	--- ± ---	0	--- ± ---	--- ± ---	n.s.
	10x field rate		0.294 ± 0.112	6	--- ± ---	0	--- ± ---	0	--- ± ---	--- ± ---	n.s.
C	**Control**										
	A	---	0.809 ± 0.242	10	0.809 ± 0.242	10	0.841 ± 0.260	10	0.032 ± 0.041	3.7 ± 4.3	---
	B		0.895 ± 0.306	10	0.924 ± 0.310	9	0.916 ± 0.290	9	-0.009 ± 0.052	-0.5 ± 5.5	---
	C		0.958 ± 0.265	10	0.958 ± 0.265	10	0.984 ± 0.278	10	0.026 ± 0.083	3.4 ± 9.6	---
	D		0.984 ± 0.278	10	1.040 ± 0.257	8	1.112 ± 0.194	8	0.072 ± 0.094	8.8 ± 12.1	---
	Headline										
	0.1x field rate	D	0.838 ± 0.161	5	0.838 ± 0.186	4	0.896 ± 0.235	4	0.058 ± 0.063	6.0 ± 7.8	n.s.
	1x field rate	D	1.021 ± 0.247	3	1.021 ± 0.247	3	--- ± ---	0	--- ± ---	--- ± ---	n.s.
	10x field rate	---	--- ± ---	---	--- ± ---	---	--- ± ---	---	--- ± ---	--- ± ---	n.s.
	BAS 500 18										
	0.1x field rate	B	0.783 ± 0.182	5	0.783 ± 0.182	5	0.796 ± 0.188	5	0.013 ± 0.035	1.7 ± 5.1	n.s.
	1x field rate	C	0.795 ± 0.218	5	0.763 ± 0.237	4	0.759 ± 0.291	4	-0.004 ± 0.066	-1.9 ± 8.6	n.s.
	10x field rate	D	1.405 ± 0.457	5	1.372 ± 0.520	4	1.302 ± 0.479	4	-0.070 ± 0.101	-5.0 ± 7.6	n.s.
	Curol										
	0.1x field rate	A	0.779 ± 0.233	5	0.779 ± 0.233	5	0.732 ± 0.214	5	-0.047 ± 0.039	-5.8 ± 4.1	*
	1x field rate	B	0.852 ± 0.163	5	0.831 ± 0.025	2	0.916 ± 0.245	4	0.085 ± 0.270	10.7 ± 32.8	n.s.
	10x field rate	C	0.702 ± 0.094	5	0.702 ± 0.094	3	--- ± ---	0	--- ± ---	--- ± ---	n.s.
	CaptanWDG Omya										
	0.1x field rate	A	0.738 ± 0.201	5	0.681 ± 0.224	5	0.703 ± 0.220	5	0.023 ± 0.032	3.7 ± 4.4	n.s.
	1x field rate	B	0.773 ± 0.167	3	0.773 ± 0.167	3	--- ± ---	0	--- ± ---	--- ± ---	n.s.
	10x field rate	---	--- ± ---	---	--- ± ---	---	--- ± ---	---	--- ± ---	--- ± ---	n.s.
	Dicomil										
	0.1x field rate	A	0.744 ± 0.322	5	0.900 ± 0.323	3	0.815 ± 0.245	3	-0.085 ± 0.080	-8.0 ± 6.1	*
	1x field rate	B	0.763 ± 0.522	5	1.000 ± 0.579	3	0.924 ± 0.414	3	-0.075 ± 0.176	-2.4 ± 16.9	n.s.
	10x field rate	C	0.742 ± 0.241	5	0.878 ± 0.337	2	0.881 ± 0.314	2	0.004 ± 0.023	1.0 ± 3.0	n.s.
	Prosper										
	0.1x field rate	A	0.766 ± 0.149	5	0.766 ± 0.149	5	0.778 ± 0.204	5	0.012 ± 0.061	0.5 ± 8.3	n.s.
	1x field rate	B	0.818 ± 0.370	5	0.832 ± 0.223	2	0.932 ± 0.256	2	0.101 ± 0.479	20.5 ± 63.1	n.s.
	10x field rate	C	1.084 ± 0.346	5	1.084 ± 0.346	3	--- ± ---	0	--- ± ---	--- ± ---	n.s.
	Roxion										
	0.1x field rate	A	0.824 ± 0.118	5	0.814 ± 0.091	3	0.826 ± 0.102	3	0.013 ± 0.022	1.5 ± 2.7	n.s.
	1x field rate	B	0.752 ± 0.282	5	0.739 ± 0.385	3	0.741 ± 0.454	3	0.002 ± 0.070	-2.7 ± 8.9	n.s.
	10x field rate	C	0.668 ± 0.289	5	0.668 ± 0.289	3	--- ± ---	0	--- ± ---	--- ± ---	n.s.

a: Corrected fresh weight refers to the fresh weight of survived individuals at test end.

s.d.: Standard deviation.

Stat.: Statistical evaluation of differences in body weight change between the control and the test rates (Dunnett t-test, one-sided smaller, α=0.05).

n.s.: Not statistically significantly different to the control.

*: Statistically significantly different to the control.

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