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Agricultural landscapes, their biodiversity and impacts of pesticides

Habilitationsschrift

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Mercy, mercy me
things ain't what they used to be.
Where did all the blue skies go?
Poison is the wind that blows from the north and south and east.

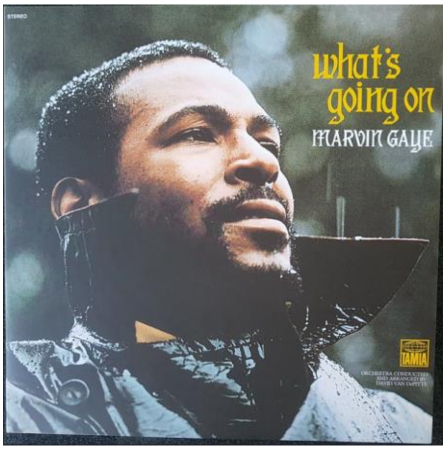
Mercy, mercy me
things ain't what they used to be.
Oil wasted on the oceans and upon our seas,
fish full of mercury.

Mercy, mercy me
things ain't what they used to be.
Radiation underground and in the sky,
animals and birds who live nearby are dying.

Mercy, mercy me
things ain't what they used to be.
What about this overcrowded land?
How much more abuse from man can she stand?

Marvin Gaye: Mercy, Mercy Me
(The Ecology)

1971
What's Going On



Dank

In dieser Habilitationsschrift sind die Ergebnisse meiner Forschung zu Pestiziden und ihren Umweltauswirkungen zusammengefasst, die im Laufe der letzten zehn Jahre an der Universität Koblenz-Landau am Campus Landau entstanden sind. In diesem Zeitraum entwickelte sich das Institut für Umweltwissenschaften von einer sehr kleinen Einheit mit drei Mitarbeitern zu einer echten wissenschaftlichen Einrichtung. Dabei kamen viele neue Kollegen hinzu und die bestehenden Studienkurse entwickelten sich zu erfolgreichen Curricula, die Studenten aus dem In- und Ausland anzogen. Diese Entwicklungen hatten einen direkten Einfluss auf meine Arbeit, da mehr und mehr Personen mit unterschiedlichem Hintergrund zu unserer Gruppe von Wissenschaftler hinzukamen, die eine große Vielfalt an Stimuli für meine Arbeit mitbrachten. Euch allen sei an dieser Stelle gedankt!

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Zusammenfassung

Der Verlust der Biodiversität wird sowohl auf einer globalen Skala als auch für die anthropogen geformten Landschaften, die heute fast 50% der terrestrischen Landfläche ausmachen, festgestellt. Auf den landwirtschaftlichen Anbauflächen werden Pestizide, biologisch aktive Chemikalien, ausgebracht um Schädlinge, Krankheiten und Unkräuter zu kontrollieren. Um die Auswirkung der Pestizide auf die Biodiversität zu verstehen ist die Quantifizierung der verbliebenen semi-natürlichen Strukturen wie Feldsäume und Hecken, die Organismen in Agrarlandschaften als Habitat dienen, eine Voraussetzung. Für eine Abschätzung ihrer potentiellen Pestizidexposition ist zudem die Anwesenheit der Organismen in diesen Habitaten und in den Feldkulturen notwendig. Im vorliegenden Text stelle ich Studien für Tiergruppen wie Amphibien, Fledermäuse und Motten vor, die bisher nicht in der Risikobewertung für Pestizide berücksichtigt worden sind. Für alle Gruppen wurde dargelegt, dass sie sowohl in der Agrarlandschaft leben als auch potentiell mit Pestiziden in Kontakt kommen und daher ein Risiko angezeigt ist. Für die Risikobetrachtung sind auch Informationen zur Empfindlichkeit der Organismen notwendig und hier werden neue Daten für Pflanzen, Amphibien und Bienen vorgestellt. Effekte die bis auf die Gemeinschaftsebene wirksam waren, wurden für die Auswirkungen von Herbizid, Insektizid und Dünger in einem natürlichen System betrachtet. Das Ergebnis nach drei Behandlungsjahren waren vereinfachte Pflanzengemeinschaften mit geringerer Artenzahl und einer reduzierten Anzahl von Blütenpflanzen. Die Abnahme an Blüten stellt ein Beispiel eines indirekten Effekts dar und war für die Effekte eines Herbizids auf den scharfen Hahnenfuß besonders auffällig. Subletale Herbizideffekte für Pflanzen hatten einen Einfluss auf daran fressende Raupen was durch eine Verminderung der Nahrungsqualität erklärbar ist. Für Feldsäume realistische Insektizidmengen reduzierten die Bestäubung der weißen Lichtnelke durch Motten um 30%. Diese indirekten Effekte durch Veränderungen im Nahrungsnetz spielen eine kritische Rolle für das

Verständnis des Rückgangs von verschiedenen Organismengruppen, allerdings werden sie bisher nicht in die Risikobewertung von Pestiziden mit einbezogen. Der aktuelle intensive Pestizideinsatz in der Landwirtschaft und ihre hohe Toxizität könnten zu einer chemisch fragmentierten Landschaft führen in der Population nicht mehr verbunden sind und damit deren Größe und genetische Struktur beeinflussen. Die Modellierung von möglichen Pestizideffekten als Kosten für die Anwanderung von Amphibien zu Fortpflanzungsgewässern in Weinbergen in Rheinland-Pfalz zeigte die Isolation der untersuchten Populationen an. Eine erste Validierung für den Grasfrosch bestätigte die Modellvorhersagen für einige Populationen. Für den terrestrischen Bereich der Risiko-bewertung ist eine Vielzahl von Richtlinien vorhanden oder wird aktuell entwickelt oder verbessert. Die Ergebnisse der vorliegenden Arbeiten zeigen, dass vor allem die reproduktiven Blütenstadien von Pflanzen sehr empfindlich sind und ihr Risiko unterschätz ist. Die Erholung von Arthropodenpopulationen nach Pestizideffekten muss auf Landschafts-ebene neu bemessen werden und eine Risikobewertung für Amphibien für die Zulassung wird vorgeschlagen. Die Etablierung und Anpassung von Risikobewertungssystemen ist allerdings ein zeitaufwändiger Prozess und daher stellt die Entwicklung von Risikomanagementmaßnahmen eine pragmatische Alternative mit unmittelbaren Auswirkungen dar. Künstliche Gewässer der Agrarlandschaft sind wichtige Nahrungsgebiete für Fledermäuse und ihre Anlage würde negative Auswirkungen des Pestizideinsatzes abschwächen. Die Einbindung von direkten und indirekten Effekten für alle Organismengruppen in eine Risikobewertung in der auch der Landschaftsmaßstab und Pestizidmischungen betrachtet werden wird viel Entwicklungszeit benötigen. Die Etablierung von Modellandschaften in der Managementmaßnahmen und integrierter Pflanzenschutz auf größerer Skala angewendet werden, würde es uns jedoch erlauben die Auswirkungen von Pestiziden in einem realistischen Szenario zu untersuchen und Ansätze für die Landwirtschaft der Zukunft zu entwickeln.

Summary

The loss of biodiversity is recognised on a global scale and also in the anthropogenic landscapes used for agriculture, now covering almost 50% of the global terrestrial land surface. In agriculture pesticides, biologically active chemicals are deliberately distributed to control pests, disease and weeds in the cropped areas. The quantification of remaining semi-naturals structures such as field margins and hedges is a prerequisite to understand the impact of pesticides on biodiversity, since these structures represent habitats for many organisms in agricultural landscapes. The presence of organisms in these habitats and crops is required to obtain an estimate of their potential pesticide exposure. In this text I provide studies on animal groups so far not addressed in risk assessment procedures for the regulation of pesticides such as amphibians, moths and bats. For all groups it becomes apparent that they are present in agricultural landscapes and potentially coincide with pesticide applications indicating a risk. Risk quantification also requires data on the sensitivity of organisms and here data for plants, amphibians and bees are presented. Effects translating to community level were studied for herbicide, insecticide and fertiliser effects in a natural system. After three years the treatments resulted in simplified plant communities with lower species numbers and a reduction in flowering plants. This reduction of flowers is used as an example for an indirect effect and was especially obvious for the effect of an herbicide on the common buttercup. Sublethal herbicide effects for a plant translated in an impact on feeding caterpillars, indicating a reduction in food quality. Insecticide inputs realistic for field margins also reduced moth pollination of white champion flowers by 30%. These indirect effects by distortions of food web characteristics are playing a critical role to understand declines in organism groups, however so far are not accounted for in pesticide risk assessment schemes. The current intense use of pesticides in agriculture and their inherent toxicity may lead to a chemical landscape fragmentation, where populations may not be connected anymore. Source-sink dynamics are important ecological processes and as a final result not only population size

but also genetic population structure might be affected. Including potential pesticide impacts as costs in a model for amphibians migrating to breeding ponds in vineyards in Rhineland-Palatinate indicated the isolation of investigated populations. A first validation by analyzing the population structure of the European common frog confirmed the model prediction for some sites. For the regulation of pesticides in Europe a risk assessment is required and for the organisms of the terrestrial habitat a multitude of guidance documents is in place or is recently developed or improved. The results of the presented research indicate that wild plants and especially their reproductive flower stage are highly sensitive and risks are underestimated. Population recovery of arthropods needs a reevaluation at landscape scale and the addition of amphibian risk assessment in regulation procedures is suggested. However, developing or adopting risk assessment procedures and test systems is a time consuming task and therefore the establishment of risk management options is a pragmatic alternative with immediate effects. Artificial wetlands in the agricultural landscape proved to be important foraging sites for bats and their creation could mitigate negative pesticide effects. The integration of direct and indirect effects in a risk assessment scheme for all organism groups addressing also landscape scale and pesticide mixtures requires a long developing time. The establishment of model landscapes where management options and integrated pest management are applied on a larger scale would allow us to study pesticide effects in a realistic scenario and to develop an approach for the agriculture of the future.

Content

1. Introduction

Biodiversity is declining on a global scale. This threat has been recognized by 196 countries that ratified the convention of Rio with the aim to protect and restore biodiversity (Heywood and Watson 1995). Global biodiversity is located in a network of protected areas and since many of them are situated in tropical areas we are inclined to assume the preservation of fauna and flora is guaranteed.

However, a recent survey of ecologists who have worked for at least two decades in the same tropical reserve revealed that the status of protected areas of tropical forest is not as good as intended. Many populations of vertebrates and plants in reserves are declining due to human pressures on their boundaries (Laurance et al. 2012). Additionally, land cover changes are pronounced in the tropics and the increase of agricultural monocultures reduces especially lowland rainforest distribution. In South-East Asia change has been very dramatic, since the deforestation of almost all the lowlands in Sumatra and Borneo over the last few decades, where now monocultures of oilpalm *(Elaeis guinenis)* are established on a landscape scale, threatens not only tropical biodiversity (Fitzherbert et al. 2008, Brühl and Eltz 2010) but also the climate (Danielsen et al. 2009). For tropical forests the conservation of global biodiversity is therefore not guaranteed by only focusing on the present network of conservation areas. The same might be true for temperate habitats and awaits evaluation.

1.1. Agriculture as land use

Currently humans use a large fraction of Earth's terrestrial surface for agriculture. Values on a global scale reach 40% (without Antarctica) leading to its identification as the largest terrestrial biome (Foley et al. 2005). For Europe about 45 % of its surface (in 2012, (Eurostat 2016) and in Germany about half of the countries area, 16.7 million ha, is used for agricultural production (Statistisches Bundesamt 2014). This area includes the land where crops are grown and grasslands for animal husbandry. In Germany more than 70% (12.1 Mio ha) is managed by 285.000 farmers for crop production, for the EU 27 the proportion of cropland is 25%. With this large proportion of terrestrial land cover used for agriculture biodiversity needs to be protected and restored here as well.

Agricultural landscapes on planet Earth have changed dramatically over the last century. Whereas in the 19th century the majority of agriculture was small scale, today agriculture has become an industry with totally different needs in respect of landscape. In these landscapes crops are grown on a large scale and therefore heavy machinery is used to plough soils, plant different crop varieties and fertilisers and pesticides are distributed on the fields to enhance their yield. Agricultural landscapes contain fields and meadows where humans grow crops and keep their domesticated animals but also areas that are not used intensively and therefore still contain some semi-natural features such as hedges or field margins, as well as natural or artificial waterbodies for irrigation or drainage of agricultural fields. Agricultural landscapes are not only defined on the basis of a visual assessment of land management but there is also an ecological component included since they contain habitats for organisms of many species. Although the primary purpose of these landscapes is food production, other objectives like conservation or recreation have a long history and are of increasing importance in wealthy and urbanized countries. Examples are the subsidies of the European Union Set-Aside Policy designed as a means of giving money to farmers to produce non-food environmental goods.

1.2. Modern crop varieties

The crops that we used to plant were local varieties adapted to environmental conditions at the site. There exist for example more than 40,000 different varieties of rice *(Oryza sativa)* worldwide that were domesticated over 10,000 years ago in the region south of the Himalayan mountain range (Choudhury et al. 2013). Over 1,000 apple varieties in Central Europe are adapted to local soil and climate conditions (Harris et al. 2002). Farming practices changed dramatically in the 1940s to 1960s. The development of new high yield crop varieties is also, together with the use of agrochemicals and machinery one of the main components of the "green revolution" shaping modern agricultural landscapes on a global scale (Dalrymple 1986).

Modern high yield crop varieties are not necessarily adapted to local conditions, especially soil type and water availability, since both factors can now be changed and ameliorated by humans through fertilization and mineralisation as well as drainage and irrigation respectively. Cereals, for example, are today planted at narrow row distances of only a few centimeters compared to former times. This is possible since the plants are provided with fertiliser with the result that crop development, even at high densities, is secured. High yield modern varieties of for example wheat produce a long ear with approximately 45-50 grains, if nitrogen supply is not limited (Austin et al. 1980). The resulting ear is comparatively heavy and therefore the length of the stalk in these varieties is reduced, so that the plant remains stable under wind and is not loping, since windfall ruins the crop entirely. The outcome is a short plant of 20-30 cm height, also called dwarfs with variety names such as "hobbit", with high nitrogen absorption potential and a high number of grains resulting in the disappearance of old varieties in the agricultural fields of today, which had reached more than a meter in height with only a few grains per plant. This development of high-yielding dwarf varieties started in the 1950 and 60s and one of the main researchers involved was Norman Ernest Borlaug, a laureate of the Noble Peace price in 1970 in recognition of his contributions to world peace through increasing food supply, especially in the developing world (Borlaug 2002). The new varieties also influence crop structure and the climate within the crop: in modern cereal fields the canopy is almost closed as a result of the high planting density and the low height which does not allow wind blowing through the field, exchanging the air below the canopy. Old varieties produced an open crop canopy with wind passing through, exchanging the air and especially reducing air moisture between plants. Air moisture and warm temperatures present a risk to modern crops since in these conditions fungal diseases can develop, destroying the crop. Therefore, as a result of the modern dwarf varieties and the sowing technology used today, it is necessary to apply fungicides to cereal fields to reduce fungal infections. However, the human caretakers are not only protecting the crops against fungal infestations. In today's agriculture men also ensures that yield is maximised by regulating any competing plant species in the field, termed "weeds", using herbicides and also guarantees that no insect pest is feeding on the crop plants and their fruits by applying insecticides.

1.3. Pesticides

The benefit of pesticides lies in their high biological activity. This activity is not specifically aimed for a target organism (the pest, weed or fungal disease) but affects basic biological pathways such as cell division (mitosis), protein synthesis, the photosystem I and II in photosynthesis, mitochondrial respiration, sterol synthesis or various processes in nerve conduction. Therefore, effects on other, especially related, organisms are expected that are not targeted by the applications (the so-called non-target organisms). In Germany more than 1,400 pesticide products are currently registered and more than 100,000 t of products or around 35,000 t of active chemical compounds (or active ingredients, a.i.) are used on an annual basis in the agricultural production. These numbers have been almost stable for the last 10 years with a slight increase (BVL 2016). The intensive use of pesticides is also shown in the annual crop specific pesticide applications where for 2014 on average 4 pesticide applications took place in wheat, 13 in potatoes, 20 in vine orchards and 34 in apple production orchards (JKI 2016).

Pesticide products are formulated to allow the active ingredient or molecule to pass membrane barriers for enhanced uptake into a target organism, to be miscible with water by the farmer, to be stable under ambient conditions or simply to improve activity or application of a pesticide. These enhancers of effectivity are called adjuvants and pesticide products can contain more than twenty adjuvants of a range of over 1000. Adjuvants themselves can be more toxic than the active molecule (Séralini 2015) and one group of adjuvants, the POEA (polyoxyethylene tallowamine) proved to cause toxicity in Glyphosate formulations towards amphibians (Relyea 2005).

In Germany mean pesticide application results in a volume of 8.8 kg formulation or 2.8 kg a. i. for every hectare of crop and every year, without taking the differences in application (see above) into account (based on 12.1 Mio ha cropland).

1.4. Agricultural landscapes

In addition to the cropped fields and orchards agricultural landscapes also contain other structural elements such as meadows, grassy field margins and hedges, as well as the vegetation along ditches and creeks. Whereas field margins exist as a consequence of the ploughing and sowing technology involved, hedges were established with the purpose to reduce wind erosion on fields or as enclosure for livestock on meadows. Additionally, the agricultural landscape is also structured by surface water bodies like ditches and creeks as well as natural and artificial rainwater retention ponds. Access roads to fields were until the 1960s unpaved dirt tracks but with the use of heavy machinery they were often transformed into cemented roads that allow continuous access to fields also under unfavourable weather conditions for tractors weighing 10 t and more.

Agricultural landscapes all have their own specific history. Agriculture has shaped European landscapes for thousands of years and not only the permanent structures like meadows and cropped fields were used for agriculture but also the rest of the landscape was influenced, permanently or from time to time, by for example grazing, peat cutting as well as timber and firewood extraction. Therefore, the history of agriculture is key to understanding the structure and components of the agricultural landscapes as present today (Grove and Rackham 2003).

1.5. Croplands

In recent times, especially in Europe, reallotment of agricultural land changed the structural landscape features profoundly by merging fields of different owners in more compact, and easier to manage, blocks of land. Reallotment is still carried out in some countries but the first profound structural changes were started as early as the end of the 18th century in Denmark (Levin et al. 2006). With the agricultural industrialisation field sizes have increased dramatically. In Germany fields that belonged to 50 small holders to grow their own crop of potatoes and vegetables on an area of 12 ha in the 1940s are now farmed by a single grower who manages 300 ha using heavy machinery, good infrastructure and modern satellite technology. The increase of field size on farms is a general feature in industrialised landscapes. In the UK in 1949 only 1% of farms covered 200 ha or more, by 1999 this had risen to over 6% and mean field size increased from 6.5 ha to 16 ha (Robinson and Sutherland 2002). Together with field size also farm size has increased, for example in Denmark from 1960 until 2000 mean farm size has more than tripled (Robinson & Sutherland 2002). A study in Sweden showed that although arable land remained constant from 1947-1978 mean field size increased from around 5 ha to almost 25 ha (Ihse 1995). On a larger scale there was obviously a higher structural diversity present since farms and fields were smaller and not all of them were planted with the same crop variety, but instead different crops were planted on smaller fields representing a heterogeneous habitat for many organisms in the cropped area. Since farms today also increasingly need to specialize in either arable crops or livestock, the result is a geographical separation between tillage and pastoral systems.

1.6. Grasslands

With the specialisation in agricultural production meadows for animal husbandry of e.g. cattle and sheep were transformed in agricultural fields and disappeared locally from the agricultural landscape or form clustered isolated patches. The losses of grasslands are not easy to assess since statistics often do not differentiate between semi-natural grasslands and modern meadows with high agricultural inputs. Data are available for Britain where 97% of semi-natural grasslands were converted in fields or high input meadows within 50 years from 1940 until 1990 (Walker et al. 2004). The same changes also occurred in Eastern Europe where regional comparisons for example in the Czech Republic reveal losses of over 50% from 1960 onwards (Lipsky 1995). In southern Sweden grasslands dominating the agricultural landscape until the middle of the last century were part of extended pastures established in the Middle Ages and were scattered all over the landscape (Ihse 1995). In the 1940s most of the farms had traditional mixed farming. Forty years later only a minority of 10% were growing cereals and keeping livestock and most of the farmers had specialized and thus no longer needed pasture land. The grassland meadows shrunk and became fragmented and almost 70% of the meadows disappeared and were ploughed and transformed into fields. The remaining meadows are fertilised and as a consequence the number of plant species has dropped by 50% and community composition has changed with typical grassland species decreasing and nitrophile species increasing (Ihse 1995).

1.7. Hedges

Apart from the cropped lands and meadows other structural elements such as hedges and field margins are recognized terrestrial landscape features. Hedges form a structural element especially in European agricultural landscapes but are also present in Canada, the USA or Australia. Mostly deciduous shrubs and tree species were planted in former times around pastures to fence in the animal livestock grazing on enclosed meadows or as a wind protection of crop fields. Hedges were clear boundaries of a famer's property and many hedges in Europe are remnants of valuable historical landscapes and were the result of planting in medieval times like the "bocage" landscapes of Brittany in France and Southern England or the ''pluzina'' in the Czech Republic (Sklenicka et al. 2009). The pattern of fields and meadows persisted through time due to the stabilizing network of hedgerows. In more recent times subsidies were paid from 1880 onwards in Denmark and other European countries to increase the planting of hedgerows.

Hedges generally form networks that provide connectivity between different locations and serve as corridors for some organisms. They consist of woody trees and shrubs and can comprise many different species. 38 species were recorded in hedges in one location in Spain (Schmitz et al. 2007). Additionally, they form an important habitat for many herbaceous plant species that grow in the provided microhabitat. An assessment of plant species richness in hedgerows in Canada recorded 46 woody but additionally 139 herbaceous plant species (Boutin and Jobin 1998). Furthermore, many insect species that are either specialised on the woody shrub species that form the hedge, the herbeacous species growing under the hedge or use their microhabitat are associated with hedges. An assessment of hedgerow diversity of arthropods over several years in Northern Germany in the 1940s detected more than 1,000 invertebrate species belonging to spiders, beetles, flies, bugs, butterflies, grasshoppers and bees and wasps (Tischler 1948) and more than 1,250 species were identified in a recent literature review (Brühl et al. 2015).

Hedgerow density and length in agricultural landscapes has declined in Europe since the 1950s. In the Czech Republic the total length of hedgerows decreased by over 70% between 1950 and 2005 (Sklenicka et al. 2009). In England and Wales farmers removed a quarter of the hedgerows between 1946 and 1974, about 200,000 kilometers in all, or 7,000 kilometers a year. The same was true for almost all European countries and agricultural intensification with its reallottment practice lead to field enlargement of arable land with a conversion of meadows and hedgerow removal.

1.8. Field margins

Agricultural fields can be separated by hedgerows but also by strips of herbaceous vegetation and the latter is here referred to as field margin. Field margins also occur along access roads or tracks to fields. Depending on land ownership field margins, as well as hedges, may belong to the farmer's property and therefore form a property boundary or they belong to the community that owns the access roads (then they are often referred to as road verges). Field margins are linear permanent vegetation strips consisting of grasses and herbaceous plant species adjoining to fields or meadows which are generally mown periodically. In recent decades flower strips as a result of agricultural subsidies become more and more present which are sown with a seed mixture containing flowering plants to improve nectar and pollen availability for honey and wild bees as well as other pollinating insects (Haaland et al. 2011). Both hold greater plant species richness and flower abundance and a more complex vegetation structure than the neighbouring crops. A large scale analysis conducted in field margins in three study regions in Germany in 2000, located in the federal states of North Rhine-Westphalia, Saxony, and Bavaria, identified 250 plant species and determined the typical plant community as ruderalised tall oat grass meadows, with the tall oat grass *Arrhenatherum elatius* being the characteristic plant species (Roß-Nickoll et al. 2004). The study also assessed the invertebrates in the grassy margins and recoded nearly 550 invertebrate species belonging to Araneae, Coleoptera, Collembola, Diptera, Hymenoptera, and Orthoptera. A literature review showed that about 650 species of invertebrates were observed in field margins in various studies (Brühl et al. 2015). This number is only a fraction of the present species since not all species groups are studied in detail and therefore information is incomplete.

In summary, grassy field margins and hedgerows have been documented as habitat for several hundred invertebrate species including phytophagous organisms like grasshoppers, butterflies and moths and predators like carabid beetles and spiders. Hence, the arthropod community of these semi-natural habitats can be highly diverse.

1.9. Biodiversity decline

Biodiversity in agricultural landscapes is present on differing levels in cropped areas and adjoining semi-natural features such as hedges or field margins. During the last decades, a decline of biodiversity associated with the agricultural landscape and its intensified management has been recognized (Robinson & Sutherland 2002). One of the best documented examples is the decline of farmland birds in Europe (Baillie et al. 1997, 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 steep 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 structural changes in the landscape and the use of pesticides and their negative influences (Rands 1985, Rands and Sotherton 1986, McLaughin and Mineau 1995, Boatman et al. 2004, Gibbs et al. 2009). An important factor influencing bird declines may be that management changes decrease the availability of insect food (Vickery et al. 2001, Vickery et al. 2009) since parental birds may need to forage more intensively to get the same or even only a reduced amount of food for their chicks (Brickle et al. 2000, Morris et al. 2005). A study in Scotland showed in a correlative approach a linked temporal decline of farmland birds, invertebrate numbers and agricultural practice (Benton et al. 2003).

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. 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). 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 (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 appreciated 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 has been realised on a global level (Attwood et al. 2008) and led to a discussion about the state of insects in the German Bundestag in 2016 (Bundestag 2016). Pollinators, and here especially bees, are well studied and declines in species richness on a national and global scale were reported (Biesmeijer et al. 2006, Kluser and Peduzzi 2007, Goulson et al. 2015). 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 population size in the past 30 years (Conrad et al. 2006, Shortall et al. 2009) 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 (Kromp 1999, Lee et al. 2001, Geiger et al. 2010) and bees (Alston et al. 2007). And, on a landscape scale, a negative impact of insecticide application on wild bee species was revealed in Italy (Brittain et al. 2010).

1.10. Pesticide regulation

All management procedures change the landscape and therefore also the biodiversity therein. As a result the need to protect biodiversity also in high intensity agricultural areas led to the implementation of specific protection goals. At EU level, the Regulation No 1107/2009 of the European Parliament and the Council was enforced in June 2011. This Regulation contains rules for the authorisation of pesticides in commercial form and for their placing on the market, use and control within the European Community. It aims to ensure a high level of protection of human and animal health as well as the environment. In this regulation, biodiversity is explicitly considered as a protection goal (Article 4.3e) which shall not be unacceptably affected by pesticides (here called 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 (European Commission 2009)).

The use of pesticides in agricultural landscapes is regulated by law on an European level and also for each member state separately. Authorities use a scheme of guidelines to evaluate pesticide effects in the terrestrial compartment for plants, soil dwelling organisms, arthropods, bees and bird and mammals. These guidelines are not fixed but evolve through discussions among industry, authorities and scientists and are evaluated on a regular basis. Therefore, the regulatory system itself is advancing by addressing open questions in a scientific approach and the resulting findings are then used for a refinement of the risk assessment scheme.

In my current field of research I study the effect of pesticides in agricultural systems focusing on areas where knowledge gaps exist that are recognized for refinement steps or where entire groups of animals or processes are currently not addressed in regulatory risk assessment procedures. In a first step it is necessary to quantify structural features in agricultural landscapes and also measure mere presence or community composition of organisms that use those habitats and the agricultural landscape in general. I studied the effects of pesticides on various levels of the food web in agricultural landscapes with projects on plants as primary producers, continuing with higher trophic levels such as insects and vertebrate groups like amphibians and bats. For the understanding of pesticide effects biodiversity it is not only important to evaluate the toxicity and direct effect on a specific organisms but also to address indirect effects on the next trophic level that result as a consequence of food depletion or changes in food quality. The results of my research can be used in the development of risk assessment and the identification of potential management tools.

This habilitation is structured in five chapters summarising the information of the appended publications:

- Terrestrial non-target habitats - Landscape features and presence of organisms
- Sensitivity of organisms towards pesticides
- Indirect effects of pesticides – food web
- Landscape effects on biodiversity – chemical fragmentation
- Development of pesticide risk assessment and management

For an easier reading flow I do not cite all references. The interested reader may refer to the original publications for further details and literature (see Appendix).

2. Terrestrial non-target habitats - Landscape features and presence of organisms

Many organisms of the biodiversity in agro-ecosystems rely on the availability of field margins as habitats (Kühne et al. 2000, Duelli and Obrist 2003, Roß-Nickoll et al. 2004, Pollard and Holland 2006, Holland et al. 2016) or use them as a corridor network between other semi-natural landscape elements (Holzschuh et al. 2009). Increasing mechanization and the resulting increase in field sizes led not only to the removal or size reduction of field margins, but also affect the quality of the margins because of exposure to inputs of pesticides applied in the adjoining field. To prevent or reduce negative effects of pesticides, product-specific risk mitigation measures can be defined during the registration of the pesticide such as field buffer zones to adjacent non-target areas like ditches, field margins, and/or the usage of low-drift-nozzles during the pesticide application. In Germany, risks are differentiated between aquatic habitats ("NW-Auflagen") and terrestrial non-target habitats ("NT-Auflagen), however risk mitigation is not required for terrestrial habitats less than 3m wide because they are not considered as non-target areas (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit 2013). It was argued by the authorities that, without such an exception, farmers would probably remove existing field margins completely. As a result, narrow field margins (< 3m) can receive pesticide inputs via spray drift and are also partly oversprayed. The overspraying of field margins can occur because the spray cones of neighboring nozzles on a spray arm have to overlap to apply the full 100% field rate of the pesticide (see Figure 1). As the last nozzle of the spray arm is placed over the field edge during the application, not only the field but also parts of the field margins receive an overspray.

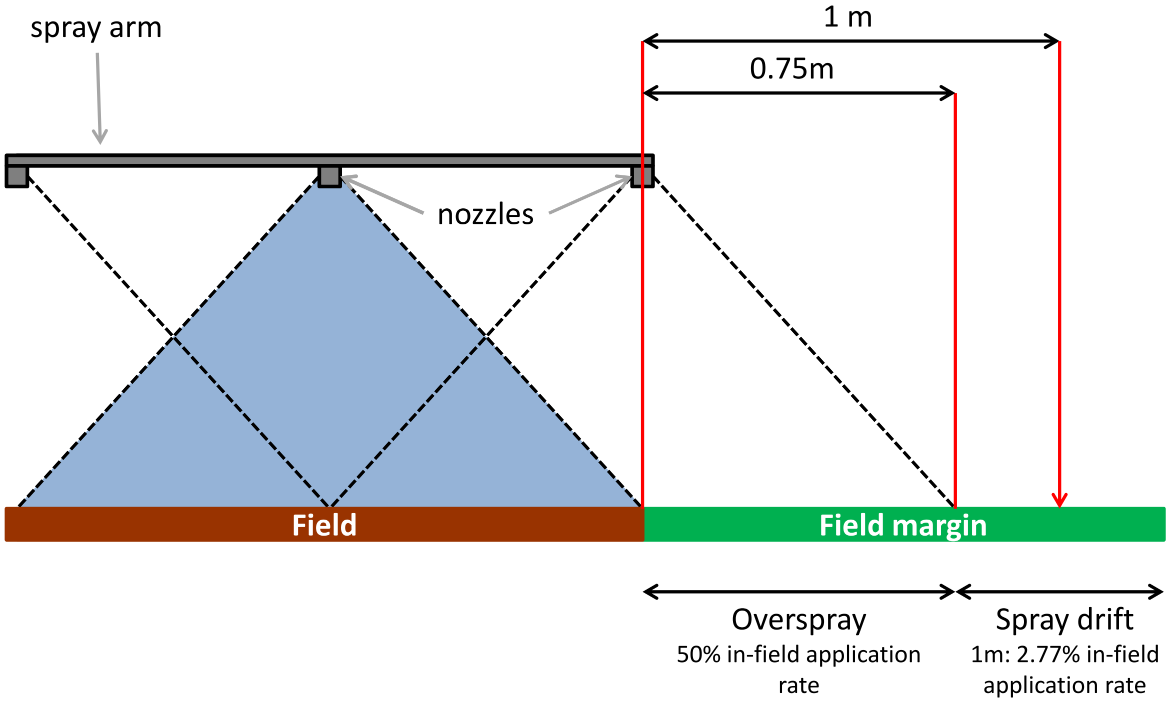

Figure 1 Scheme of the inputs of plant protection products via overspray and spray drift in cereal field margins. The blue coloured area illustrates the spray cone of one nozzle. (Based on a personal communication with Dirk Rautmann, after Brühl et al. 2015).

2.1. Landscape structure

Because quantitative data on structure, size, and width of field margins are scarce, we manually digitized field margins using digital orthophotos (4,000 ha) and analysed them in geographical information systems in two German agricultural landscapes: Rhineland-Palatinate (RLP) where vine growing is dominant and Brandenburg (BB) with high intensity cereal growing. In RLP, most of the field margins were less than 3 m wide (85% of margin length), whereas in BB wide margins were present along roads and narrow field margins accounted for almost 50% of the margin length. Hedgerows were only occasionally recorded. Hence, narrow grassy field margins can represent a large part of the available semi-natural habitats adjoining agricultural fields and therefore should be protected from pesticide inputs, at least in landscapes under intensive agricultural use. Because they are less than 3 m wide (narrow) the majority of margins is not considered relevant for risk management and the current practice for protecting the existing biodiversity from negative effects of pesticides seems questionable.

More data on field margin constitution in Germany and other European countries (Figure 2) is necessary to critically assess the current practice of pesticide risk assessment and management on a larger scale.

Figure 2 Agricultural landscapes in high intensity agricultural areas in Europe are dominated by cropped fields and exhibit only few semi-natural structures. Poland, near Krakow.

Publication 1: Melanie Hahn, Patrick P. Lenhardt & Carsten A. Brühl (2014) **Characterization of field margins in intensified agro-ecosystems – why narrow margins should matter in terrestrial pesticide risk assessment and management.** *Integrated Environmental Assessment and Management*, 207, 153-162. (See Appendix, A 3).

The presence of an organism group in agricultural landscapes is well established for some such as birds and bees. Others are, however not so well studied to immediately assume their occurrence. This is especially true for organisms that are not obvious to humans and thus may also be neglected in pesticide risk assessment approaches. Ideally the presence of these organisms is quantified not only in the agricultural landscape but also in different crops to understand the potential of pesticide exposure and associated risks.

2.2. Bats in agricultural landscapes

One of these neglected groups is bats, a group of mammals recognized as among the most endangered vertebrates in the world, with almost 50% of species considered as threatened and near threatened (Hutson et al. 2001). In Central Europe the 42 occurring bat species, comprising one-fifth of all European terrestrial mammals, have undergone serious population declines since the mid-20th century. Interestingly, bats are not even mentioned in the currently valid guidance document for the risk assessment of bird and mammals towards pesticides (EFSA 2009). There insectivorous mammals are only represented by a generic indicator "shrews". However, bats differ widely from other European mammals in their ecological traits as they hibernate, migrate, feed on aerial insects and have a single offspring per year. The reason for the missing implementation of bats in the risk assessment approach is related to the limited available knowledge about their occurrence and activity in agricultural landscapes and crops, which is necessary to evaluate uncertainties in pesticide risk assessment. This lack of information is astonishing, because bats are a well-studied group of mammals; however, habitat use is mainly examined in forested national parks and other pristine areas.

In order to assess bat activity in agriculture on a landscape scale it was first necessary to establish an acoustic survey method which fulfilled the needs of a standardized, quantitative recording to produce reliable, unbiased and comparable data sets of bat activity and community composition in different crops on landscape scale. Concerns regarding the methodological designs of many acoustic surveys have been expressed in the scientific literature. The reasons are the failing of addressing temporal and spatial variation in bat activity patterns using hand-held systems and the limitations of the suitability of the used acoustic detectors.

We therefore compared the efficiency of the available two different acoustic bat survey methods: the transect walk and the stationary measurement. We also tested the survey tool – the detectors themselves, which can be grouped into devices with two different methods of triggering the recording of ultrasonic signals: actively by a fieldworker or automatically by a built-in recording control algorithm of the detector.

By comparing different methods and detector systems we demonstrated that the set-up of several stationary calibrated detector systems, which automatically trigger the ultrasonic recording, has the highest potential to produce reliable, unbiased and comparable data sets on the relative activity of bats.

Publication 2: Peter Stahlschmidt & Carsten A. Brühl (2012 a) **Bats as bioindicator species – The need of a standardized method for acoustic bat activity surveys.** *Methods in Ecology and Evolution,* 3, 503-508. (See Appendix, A 13).

Bat diversity and activity was recorded in different crops and semi-natural habitats in southern Rhineland-Palatinate using the established survey method with newly developed batcorders®. In more than 500 sampling nights about 110,000 call sequences were acoustically recorded belonging to a total of 13 bat species (Table 1). Among them were the locally rare and critically endangered northern bat *(Eptesicus nilssonii)* and the barbastelle *(Barbastella barbastellum),* both of them also occurring over agricultural fields. In several agricultural crops, high abundances of suitable prey insects and high bat activity levels, comparable or even higher than in the nearby forests and meadows known to be used as foraging habitats, were recorded.

Table 1 Average number of bat call sequences per habitat. Average numbers of sequences per habitat were calculated as the mean of all sampling nights (n = 5 per site) and all sites per habitats (forest: n =6; forest edge: n = 2; meadow: n = 6; vineyard: n = 13; orchard: n = 5; vegetable: n = 19; cereal: n = 9). Highest average call sequences in **bold**.

		Habitat					
		natural		agriculture			
Bat species	*Latin name*	forest	meadow	vineyard	orchard	vegetable	cereal
Common pipistrelle	*Pipistrellus pipistrellus*	36.9	20.1	9.5	**140.4**	29.4	28.1
Nathusius' pipistrelle	*Pipistrellus nathusii*	0.2	0.5	0.3	**1.4**	0.9	0.9
Soprano pipistrelle	*Pipistrellus pygmaeus*	< 0.1	0.1	-	**0.5**	0.1	0.1
Serotine bat	*Eptesicus serotinus*	6.6	**12.4**	2.1	5.3	7.0	7.6
Northern bat	*Eptesicus nilssonii*	**1.8**	2.5	0.1	0.2	0.6	0.3
Common noctule	*Nyctalus noctula*	4.0	3.0	0.5	1.7	4.2	**5.0**
Lesser noctule	*Nyctalus leisleri*	**0.8**	0.6	0.3	0.6	0.3	**0.8**
Whiskered bat	*Myotis mystacinus*	**4.9**	0.5	0.3	1.3	1.0	0.5
Daubenton's bat	*Myotis daubentonii*	**3.2**	0.6	0.0	0.3	0.3	0.3
Bechstein's bat	*Myotis bechsteinii*	**3.7**	0.2	0.1	-	0.1	-
Natterer's bat	*Myotis nattereri*	**3.1**	0.3	0.4	1.2	0.3	0.1
Greater mouse-eared bat	*Myotis myotis*	0.3	0.1	0.1	-	**0.6**	0.1
Grey long-eared bat	*Plecotus austriacus*	0.1	< 0.1	**0.7**	-	0.1	0.1

Bat activity was particularly high over fruit orchards and vegetable fields where insects were also present. Both crops are known for high pesticide inputs, and, therefore, pesticide exposure through ingestion of contaminated insects cannot be excluded (see publication 11, below).

Publication 3: Peter Stahlschmidt, Melanie Hahn & Carsten A. Brühl (2017) **Nocturnal risks - High bat activity in the agricultural landscape indicates potential pesticide exposure**. *Frontiers in Environmental Science.* 5(62), 1-9 (doi: 10.3389/fenvs.2017.00062). (See Appendix, A 23).

2.3. *Amphibians in agricultural landscapes*

Another group of organisms that has been rarely studied in agricultural landscapes are amphibians. Although the public associates amphibians mostly with an aquatic environment, most temperate amphibians live outside the breeding season in terrestrial habitats for foraging and hibernation. These terrestrial habitats can be kilometers away from breeding ponds. In agricultural landscapes, breeding habitats (i.e. ponds and temporary wetlands) are often completely surrounded by arable land. Artificial rain water retention ponds frequently form the only available breeding water body for amphibians in agricultural landscapes. An evaluation in Rhineland Palatinate in 2007 documented the presence of 11 of the 18 occurring amphibian species in water bodies in intensively used vineyards with 66 of 75 being occupied by amphibians (Bischoff 2008). Amphibians have to reach breeding ponds (Figure 3) and therefore, they regularly have to cross agricultural land during migration from terrestrial to aquatic habitats for reproduction (Berger et al. 2011, Fryday and Thompson 2012). Exposure to agrochemicals, such as fertilizers and pesticides, is likely during migrations over arable land at field rates. To understand if agrochemicals pose a risk it is therefore not only necessary to understand whether amphibians occur in the landscape by monitoring breeding ponds, but also to measure any activity in fields during the migration period.

Pesticide management differs between crops and farms, particularly with regard to type, number, amount and date of application of pesticides. Such variations in pesticide application strategies result in different temporal coincidence with amphibian species migrating through or remaining in agricultural fields. We evaluated the data of a large scale field study that was performed over two years in Brandenburg by ZALF (Leibniz-Zentrum für Agrarlandschaftsforschung).

Figure 3 Temperate amphibians migrate to breeding ponds, often entirely surrounded by crops. Adults and emerging juveniles use the semi-natural structures and agricultural crops for foraging and pass them during migration to hibernation sites. Rainwater retention pond in Rheinland-Palatinate next to vineyards.

The study was designed to investigate the spring migration of adult amphibians and its temporal overlap with pesticide applications in different arable crops. Population proportions of four amphibian species migrating just before, during and directly after pesticide applications were quantified. Individuals of the fire-bellied toad *(Bombina bombina)*, moor frog *(Rana arvalis)*, spadefoot toad *(Pelobates fuscus)* and crested newt *(Triturus cristatus)* covering a wide range of life cycles and migration types for temperate amphibians were included in the analysis. Amphibians were captured by fence trapping in a 700 ha size study area where more than 300 pesticide applications were recorded in parallel. Across all pesticide types, crop growth stages and species the average population proportion coincident with applications varied between 0.8 and 74.6%. On average, more than 20% of the trapped amphibians coincided with each pesticide application in winter cereals and rape. The study showed that the extent of overlap of amphibian presence and potential pesticide exposure varies between years, crops and amphibian species; however, exposure of significant fractions of the occurring populations could be demonstrated.

As exposure of amphibians to pesticides seems inevitable, certain risk for amphibian populations must be anticipated since mortality can be high at 10% of the field application rate as demonstrated in laboratory experiments (see below, publication 6).

Publication 4: Patrick P. Lenhardt, Carsten A. Brühl & Gert Berger (2015) **Temporal coincidence of adult amphibians and pesticide applications on arable fields during spring migration.** *Basic and Applied Ecology,* 16(1), 54–63. (See Appendix, A 33).

2.4. *Moths in agricultural landscapes*

Apart from the mere presence in agricultural landscapes it can also be interesting to study a specific organism group and the services they provide for humanity, identified as "ecosystem services" (Power 2010). Crop pollination is of fundamental interest for the stability of food security and declines in pollinators have raised questions over the stability of ecosystem functions as well. Furthermore, these declines have raised awareness that species other than honey bees *(Apis mellifera),* which are considered the main pollinators within many agricultural systems, may also play an important role in the pollination of crops but also wild plants. Pollination of crops takes place in the fields or orchards where pesticides are directly applied at regular intervals and therefore an exposure risk for pollinators exists. So far only the risk of pesticides to honey bees has been assessed in a specific guidance document in the EU regulation, but no other pollinators are addressed so far.

Approximately 180,000 butterfly and moth species (Lepidoptera) have been described, however research has predominantly focused on butterflies which attract the attention of collectors and hobbyists. Nonetheless, butterflies account for approximately 10 % of the Lepidoptera of which the majority can be classified as moths with predominantly crepuscular or nocturnal lifestyles similar as for the bats mentioned above.

A literature review identified more than 120 moth - plant interactions where moths are true pollinators of plants in 7 families and 61 plant species in 14 families for which moths may play a role in pollination. In agricultural landscapes, studies of pollinators have focused on crop pollination. While there are a few exceptions where moths might act as co-pollinators (such as in blueberry), crops cultivated in Europe and North America do not appear to rely on moth pollination. However, agro-ecosystems do not exclusively consist of crop plants; field margins, hedgerows, meadows, and other semi-natural elements occur along with cropped fields, all of which are habitats for numerous non-crop plants. Approximately 40 % of the plant species pollinated by moths potentially occur in agricultural landscape habitats, such as meadows, pastures, old fields, field margins, and road sides. Hence, the importance of moths in agricultural landscapes is most likely related to their pollination of non-crop, wild plant species, which maintains biodiversity in agro-ecosystems, instead of their pollination of crops, which is commonly valued as an ecosystem service. Pesticide exposure of wild plants is caused by drift and overspray of field margins and may affect different pollinator life stages such as caterpillars and adult moths.

Moths are declining in agro-ecosystems in Great Britain and other European countries (Fox 2012). An analysis of a 35-year data set (1968–2002) for 337 widespread and common macro-moth species revealed significant decreases in abundance. 66% of the studied species had negative population trends with decline rates > 30 % in 10 years for more than 20% of the species. So far the loss of semi-natural structures was identified as a major factor for the observed reduction; however, the impact of pesticides has not been evaluated so far.

The decline of moths in agricultural landscapes is not only of concern because of their pollination of wild plants but also because they are important primary consumers and prey items for a wide range of other taxa such as amphibians, birds and mammals (see below).

Publication 5: Melanie Hahn & Carsten A. Brühl (2016) **The secret pollinators – an overview of moth pollination in natural and agricultural habitats with a focus on Europe and North America.** *Arthropod-Plant Interactions,* 10(1), 21–28. (See Appendix, A 45).

3. Sensitivity of organisms towards pesticides

To identify a risk towards pesticides, organisms must be exposed to the substances (see chapter 1) and the organism must be sensitive enough to cause an effect. Pesticides can cause effects on various levels: The most obvious is acute, immediate toxicity affecting organisms directly, resulting in mortality. Effects of chronic exposure at lower dosages, on for example reproductive capacity, are more difficult to assess since a longer study time frame is required to actually measure the endpoint offspring. However, changes in reproduction as well as mortality of organisms affect population development of a species and may result in shifts in community composition and variations in competition or predation. Additionally, other stressors or interactions of pesticides with these stressors come into play and the role of a single pesticide might be therefore difficult to be evaluated. Therefore, the sensitivity of organisms causing mortality is still a primary indicator of a potential risk, although some substances are recognized to affect populations at extremely low dosages by exerting effects on the hormone system, altering behavior and fertility. These endocrine disruptors are posing other threats on populations and are not easily identified. It therefore should be kept in mind that examining single pesticides at high concentrations and without addressing the effects of co-stressors may lead to an underestimation of the role of pesticides (Hayes et al. 2006).

3.1. Sensitivity of organisms – direct acute effects

Amphibians

Amphibians are recognized by the International Union for Conservation of Nature (IUCN) as the most threatened vertebrate group with more than 32.5% of the total number of species, compared to 12% for birds and 23% of for mammals. Amphibians are more sensitive to environmental changes and contamination than birds or mammals primarily for two reasons. First, most species spend the first part of their life in aquatic environments and the second part in terrestrial environments: As a result they may face alteration and contamination of both (Mann et al. 2009). Second, amphibian skin is highly permeable and is physiologically involved in gas, water, and electrolyte exchange with the environment; therefore, it is highly susceptible to physicochemical stressors such as ultraviolet B radiation, pathogens, or xenobiotics. In an evaluation of the global amphibian decline, pollution has been recognised as the most important threat to amphibian populations after habitat loss (Blaustein et al. 2003, De Lange et al. 2009, Mann et al. 2009). However current pesticide risk assessment does not specifically consider amphibians. Amphibians in aquatic environments (aquatic life stages or post metamorphic 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. It is however unknown whether the exposure and sensitivity of terrestrial living amphibians are comparable to mammalian and avian exposure and sensitivity. A literature review concentrated on available data for dermal pesticide absorption and toxicity studies for terrestrial life stages of amphibians, focusing on the dermal exposure pathway, which is, through treated soil or direct overspray. In vitro studies demonstrated that cutaneous absorption of chemicals is significant and that chemical percutaneous passage (P (cm/h)) is higher in amphibians than in mammals. In vivo, the rapid and substantial uptake of the herbicide atrazine from treated soil by toads *(Bufo americanus)* has been described. At the time of our literature analysis (2010) only nine studies reported toxicological data for juvenile or adult amphibians exposed dermally to pesticides. Unfortunately, exposure scenarios varied substantially from paper towels soaked in concentrations relevant for aquatic exposure to overspray of individuals. Oral studies were also available but used galvage and injection. It is questionable whether the toxicity data related to the injected doses are representative for field situations, because differences in processes such as absorption, distribution in the body, and transport to organs between amphibian and mammal species may result in other toxicity responses compared with a direct injection of the pesticides.

Our review concluded that dermal exposure was identified in most publications as the most important exposure pathway for terrestrial amphibian life stages. The paucity of published data on terrestrial amphibian life stages is remarkable, especially with the variety of pesticide formulations in use for crop protection, the countless possible combinations thereof, the numerous co-stressors such as ultraviolet B radiation, pathogens and parasites, and the differences in amphibian species sensitivity, indicating the need for further research.

Publication 6: Carsten A. Brühl, Silvia Pieper & Brigitte Weber (2011) **Amphibians at risk? – Susceptibility of terrestrial amphibian life stages to pesticides.** *Environmental Toxicology and Chemistry*, 30(11), 2465–2472. (See Appendix, A 55).

Based on the results of the literature review (see above) it became obvious that no direct acute toxicity data of realistic field rates of pesticide applications with commercial formulations for European amphibians were available. In a research and development project with the Federal Environment Agency Germany (R&D Project "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" (Brühl et al. 2015)) we therefore studied the effects of dermal exposure of seven pesticide products on juvenile European common frogs *(Rana temporaria)* in an agricultural overspray scenario. Mortality ranged from 100% after one hour to 40% after seven days at the recommended label rate of currently registered products. Three products showed a mortality of 40% after seven days at the lowest rate tested (10% of the label rate). The study also included two different formulations with the same content of the active substance Pyraclostrobin. Whereas the commercially available "Headline" formulation caused 100% mortality just after 1 h at the label rate, the formulation with the lower content of the main formulation additive of solvent naphta (67% versus <25%) revealed 20% mortality.

The demonstrated toxicity is alarming and a large-scale negative effect of terrestrial pesticide exposure on amphibian populations seems likely. Terrestrial pesticide exposure might be underestimated as a driver of amphibian decline calling for more attention in conservation efforts and the risk assessment procedures in place do not protect this vanishing animal group. The role of formulation additives seems especially crucial in this respect.

Publication 7: Carsten A. Brühl, Thomas Schmidt, Silvia Pieper & Annika Alscher (2013) **Terrestrial pesticide exposure of amphibians: An underestimated cause of global decline?** *Scientific Reports*, 3, 1135. DOI: 10.1038/srep01135. (See Appendix, A 69).

Pesticide products are formulated to allow the active ingredient or molecule to pass membrane barriers for enhanced uptake into a target organism, to be miscible with water by the farmer, to be stable under ambient conditions or simply to improve activity or application of a pesticide. These enhancers of effectivity are called adjuvants and pesticide products can contain up to 20 different molecules (Frische et al. 2016). A specific group of adjuvants (activator adjuvants) are surfactants, "surface active agents". Surfactants are molecules that lower the surface tension and their primary purpose is to allow for more contact between the pesticide spray droplet and the plant

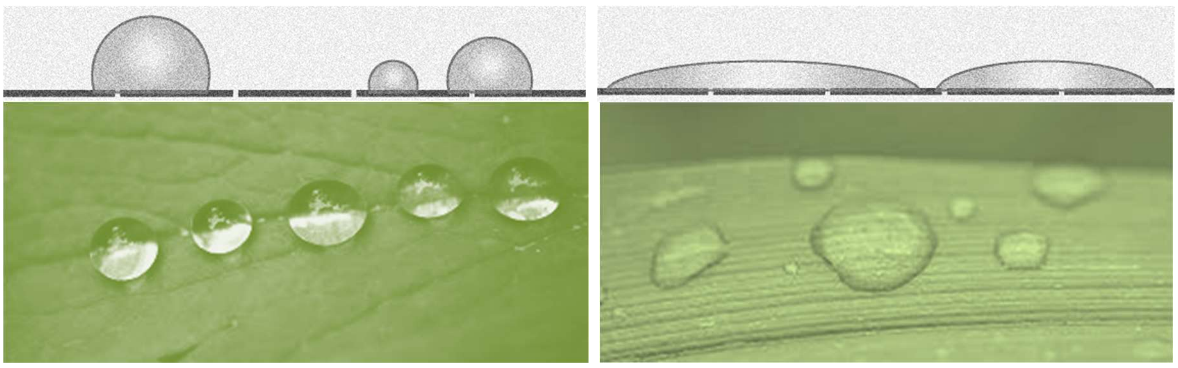

Figure 4 Water droplet without (left) and with surfactant (right). Agricultural surfactants can produce a phenomenon known as "super spreading" leading to "stomatal flooding" to increase pesticide performances. The aim is that the pesticide spray droplet must be able to wet the foliage and spread out evenly over a leaf, even when it is waxy or hairy.

(Figure 4). Adjuvants themselves can be more toxic than the active molecule (Séralini 2015) and one group of adjuvants, the POEA (polyoxyethylene tallowamine) proved to cause toxicity to amphibians in Glyphosate formulations (Relyea 2009). Another common adjuvant in pesticides is solvent naphtha, a petroleum distillate, which is toxic to aquatic organisms. Still adjuvants are most of the times declared inert. The main problem for scientists working with pesticide formulation is that the identity and quantity of adjuvants in the pesticide product are kept confidential. Although this problem has already been highlighted in the 1960s (Lüdemann and Neumann 1962), even today only the manufacturing industry and the authorities know the exact chemical composition of the formulations. Risk assessment for primary pesticide registration in the EU requires studies based on the active pesticide ingredient, but not the mixture which might underestimate the risk. Products are then assessed by member states for a zonal registration, however again data for formulations are not always available.

As assumed in a study with a toad species (Belden et al. 2010) and shown in the above study the adjuvant mix was the explanatory variable in two Pyraclostrobin formulations (Brühl et al. 2013). Unfortunately, even in this case the adjuvants and exact composition of the pesticide mixture is unknown, except for the solvent naphta content (67% versus <25%). However, other non-declared adjuvants might be responsible for the observed effect and not the naphta. To understand a formulations toxicity, it is vital to know the toxicity of adjuvants and the composition of pesticide formulations. It might be possible that only a few of the currently used adjuvants are responsible for the observed toxicity. A screening of adjuvant toxicity is urgently suggested not only for amphibian but also for human risk assessment (Séralini 2015).

Non-target arthropods

Pesticides are named insecticides when they are used to control insect pests in agriculture. The aim of integrated pest management is to protect the so called "beneficial" arthropod species that control pests such as specific species of carabid beetles, spiders or parasitoid wasps. Those "beneficals" are not the target of pesticide effects and are termed "non-target arthropods". Honey bees are important for crop pollination increasing yield and producing honey for human consumption and are therefore addressed in a specific risk assessment. Basic requirements for risk assessments are acute toxicity endpoints obtained in toxicity studies for a reduced set of standard test species. Although insects are specifically addressed in risk assessments in the EU now for decades, only a few species were ever studied thoroughly and a protection of others was only assumed resulting in huge knowledge gaps.

A decline in biodiversity is not only observed in birds and plants but also in many insect taxa such as moths, butterflies, carabids, and wild bees. Currently the protection of wild pollinators from pesticides is hotly debated and the non-target arthropod risk assessment is in revision (EFSA 2015). The function of insects as food for higher trophic levels is getting more attention and the loss of insects on a landscape scale has even been debated in the German Bundestag in 2016 (Bundestag 2016).

A basic requirement for the development of a science based risk assessment in both areas is an endpoint reflecting species specific sensitivity towards pesticides.

Bees

Wild and domesticated bees pollinate crops and wild plants and are affected by multiple environmental factors. The US and Europe have experienced substantial losses of domesticated honey bee *(Apis mellifera)* colonies and simultaneous decline in wild bee diversity reaching 52% of wild bee species identified at threat in the German Red List. Wild bee species such as bumble bees and solitary bees differ substantially from the honey bee in their ecological properties, e.g. sociality, life cycle, behaviour, which might affect their population responses. Pesticide effects on solitary bee populations and bumble bee colonies might be more pronounced than on honey bees since effects on individuals cannot be buffered by sheer numbers as in the hive of a superorganism. However, the honey bee is the only pollinator species required to be evaluated in the EU pesticide risk assessment scheme. In the current lower tier testing scheme, interspecific differences in bee sensitivity are accounted for by applying a safety factor of 10 to the toxic endpoint of the surrogate species, the honey bee.

Only one review addressed the sensitivity of different wild bee species towards pesticides; however, of the 19 wild bee species for which toxicity endpoints were available 9 belonged to the tropical bee family Meliponinae. In order to adequately assess the risk pesticides pose to European wild bees a comprehensive database is needed. Sensitivity data for European wild bee species are scarce, covering only a few species that are bred for pollination services so far.

Bee species occurring in the European agricultural landscape which may forage on crops and are therefore potentially exposed to insecticides in the field were selected and exposed towards a dimethoate formulation as used in honey bee acute toxicity studies. The goal was to collect sufficient data from dose-response experiments to generate a Species Sensitivity Distribution (SSD) and deduce the effect of dimethoate on wild bee species. The underlying idea of a SSD is that interspecific sensitivity follows a statistical distribution (Newman et al. 2000). By fitting a suitable distribution to the data the dose at which 5% of species in a community are affected by a pesticide (HD5) can be derived. To ensure a proper level of safety, i.e. reduce uncertainty, it was recommended to use the lower 95% confidence limit of the HD5 (lower limit HD5). To establish a SSD ecologically representative and comparable toxicity data are needed, as well as an appropriate statistical analysis method.

Toxicity endpoints were obtained for five wild bee species, and together with literature data for dimethoate for two other wild bees a SSD was calculated. The relatively large honey bee was more sensitive than all tested smaller wild bee species. The derived HD5 lower 95% confidence limit was equal to honey bee mean LD50 when applying a safety factor of 10, as suggested by European risk assessment, and covered the sensitivity data of all wild bees, even extrapolations for minute species. Body weight proved to be a predictor of interspecific wild bee sensitivity but did not explain the pattern completely and therefore factors such as metabolism and cuticular physiology might also be relevant. Using acute toxicity values from honey bees and a safety factor of 10 therefore seems to cover the interspecific sensitivity range of bees in the case of dimethoate. In this case acute endpoints of proposed additional test species, the buff-tailed bumblebee *Bombus terrestris* and the red mason bee *Osmia bicornis*, do not improve the risk assessment for wild bees. However, more comparative datasets are necessary for other pesticide groups to fully understand wild bee sensitivity towards pesticides and to develop a sound, science based risk assessment.

Publication 8: Philipp Uhl, Lea Franke, Christina Rehberg, Claudia Wollmann, Peter Stahlschmidt, Lukas Jeker & Carsten A. Brühl (2016) **Interspecific sensitivity of bees towards dimethoate and implications for environmental risk assessment.** *Scientific Reports,* 6, 34439, DOI: 10.1038/srep34439. (See Appendix, A 83).

Herbivorous insects - grass hoppers

Current terrestrial risk assessments for insecticides only consider a small set of non-target arthropod species, primarily beneficial organisms, with a focus on an aphid parasitoid wasp *(Aphidius rhopalosiphi)* and a predatory mite (*Typhlodromus pyri),* whereas herbivorous insects, such as grasshoppers, are ignored. However, grasshoppers living in field margins or meadows adjacent to crops may potentially be exposed to insecticides due to contact with or ingestion of contaminated food (Schmitz and Brühl 2008). Furthermore, only a contact exposure scenario (glass-plate tests) is assessed in risk assessment, which in turn excludes any risk of exposure via food ingestion by herbivorous arthropods. Food ingestion of herbivorous insects, however, is high because of the low nutritious value of the vegetation, compared with predatory arthropods that feed on a diet rich in proteins. Therefore, oral exposure to insecticide residues on the vegetation is presumably higher in herbivorous insects compared with the standard beneficial arthropods.

Five insecticide formulations (active ingredients: dimethoate, pirimicarb, imidacloprid, lambda-cyhalothrin, and deltamethrin) were selected to study the survival of *Chorthippus* sp. grasshopper nymphs by considering two routes of exposure (contact and oral). Grasshopper nymphs were exposed to pesticide rates applied with a custom made spraying device producing a realistic spray application. An LR_{50} after 48 h exposure was calculated and compared to data available for the standard test species. Toxicity was highest for the contact exposure scenario; and oral exposure scenario always showed the lowest toxicity. With regard to the insecticides tested in the present study the laboratory toxicity tests revealed a sensitivity of grasshopper nymphs similar to that of the standard test species used in arthropod risk assessments.

With a similar sensitivity as the non-target, beneficial standard test species we could assume that the risk assessment scheme in place is protecting also grasshoppers. To verify this hypothesis grassy field margins were monitored in parallel. Many herbivorous arthropods such as grasshoppers inhabit field margins and hence may be exposed to insecticides due to surface contact and/or ingestion of plant material containing insecticide residues. Grasshopper density at 110 sampling sites (15 m^2) in field margins of various widths located next to cereals, vineyards, and orchards was assessed in a 3 month monitoring phase using a catch cage and sifting through the enclosed vegetation (Figure 5). Grasshopper densities within field margins less than 9m wide were significantly reduced irrespective of the type of crop (cereals, vineyards, or orchards) grown next to the sampling site compared to grasslands.

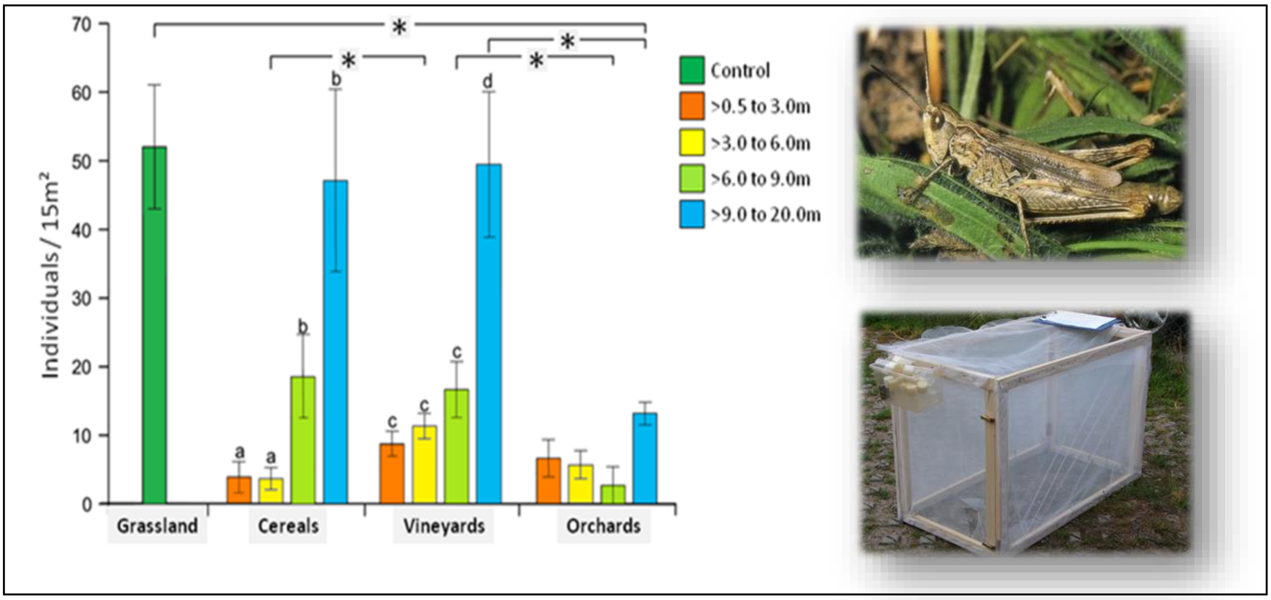

Figure 5 Monitoring of grass hoppers in field margins using a catch cage. Densities are reported for different crops and field margin widths (modified graph from Bundschuh et al. 2012).

The absence from even wider field margins next to apple orchards may be explained by the high number of insecticide applications (up to 8 / season) and the high drift rate caused by the use of air blast sprayers (summer orchard applications result in a drift at 3 m of more than 15% of application rate (JKI 2006)). The common narrow field margins (< 3 m, see above, publication 1) next to arable crops also showed a low density of grass hoppers because they receive a drift of at least 2.77% of the field application rate at a distance of 1 m from the field edge and are even partially oversprayed. The results of the monitoring indicate that current risk assessment procedures are insufficiently protective for grasshoppers in field margins. This might be related to the fact that each product is regulated on its own, implying that only this one product has an impact on the organisms in focus. However, dependent on the crop type, many pesticides can be used in a season (see above). The presented monitoring study shows expected differences between apple orchard field margins, where up to 8 insecticide drift events affect grasshopper populations compared to cereals, where usually only one insecticide is applied and drift is also lower.

Publication 9: Rebecca Bundschuh, Juliane Schmitz, Mirco Bundschuh & Carsten A. Brühl (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. (See Appendix, A 91).

Herbivorous insects – moth and butterfly caterpillars

In agricultural landscapes, field margins are potential habitats for moths and butterflies (Lepidoptera). However, because of their proximity to agricultural sites, field margins can be affected by inputs of pesticides and fertilizers. The presence of caterpillars in field margins was assessed in a first monitoring step. Furthermore, the effects of realistic field margin input rates of various agrochemicals on moths, especially on their caterpillar stages, were studied in field, semi-field, and laboratory experiments.

Caterpillars were surveyed in cereal field margins and meadows in the area surrounding Landau, Germany, using sweep nets during two sampling periods. The monitoring indicated that, although caterpillars were found in field margins, their mean abundance was 35–60% lower compared to meadows. This patter is influenced by three factors: First, the abundance of caterpillars could be affected by differences in habitat patch size (meadows > field margin). Second, a linear shape of a habitat can be associated with a reduced number of individuals, because linear elements have a higher ratio of edge to interior and, hence, pressure from edge-related stressors might be more important than in non-linear habitats. Third, field margins are exposed to inputs of agrochemicals which might affect caterpillar abundances either directly (sensitivity towards pesticide) or indirectly. Possible indirect effects include changes in abundance, diversity and quality of host plants (see below).

In a three year randomized field experiment with the aim of identifying the individual and combined effects of repeated agrochemical applications on fauna and flora (for details on the design see below and Schmitz et al. 2013), caterpillars were sampled twice during one growing season using sweep nets in 64 plots. The insecticide treatment (a pyrethroid formulation of lambda-cyhalothrin) significantly reduced the number of caterpillars and only 15% of the sampled caterpillars occurred in the insecticide-treated plots. Furthermore, the insecticide affected the community composition of the caterpillars, whereas the fertilizer treatment slightly increased caterpillar abundance.

In laboratory experiments, 14 d *Mamestra brassicae* caterpillars were exposed to different rates of the same insecticide formulation in a toxicity test by dipping plantain leaves into relevant solutions and mortality was assessed for 144 h. The caterpillars were shown to be very sensitive when exposed to insecticide-treated leaves with an LR_{50} (rate that kills 50% of the test caterpillars) after 48 h corresponding to 0.78% of the recommended field rate. This rate matches the arable spray drift input in field margins at a distance of 3 – 4 m from the crop. It therefore seems likely that the observations of the monitoring and field experiment are mainly driven by the high sensitivity of young caterpillars towards the pyrethroid insecticide.

But not only mortality of caterpillars might be a reason for observations of lower numbers in field margins. It might also be possible that pesticides act as repellence and caterpillars avoid feeding on them.

To test for repellent effects 20 caterpillars could choose between untreated plants and plants treated with 1% of the recommended field application rate. The caterpillars primarily fed either on the control leaves or their diet consisted of a mixture of both treated and untreated leaves. The caterpillars in the feeding experiment did not completely avoid the insecticide-treated leaves, but they appeared to prefer insecticide-free leaves, which indicates minor antifeedant effects.

Overall, these studies illustrate that moths use field margins as habitats and that they can be affected by realistic input rates of agrochemicals. Acute toxicity studies of caterpillars produced a low LR_{50} value for the tested pyrethroid and effects in narrow field margins are likely, if other species are similarly sensitive to insecticides. The low caterpillar numbers in the insecticide-treated plots of the field experiment might result not only from the toxic effects of the insecticide, but also from the repellent effects on the adults. As caterpillars are important prey organisms and adult moths can act as pollinators, inputs of agrochemicals in field margins should be reduced to maintain biodiversity in agricultural landscapes.

Publication 10: Melanie Hahn, Annalena Schotthöfer, Juliane Schmitz, Lea A. Franke & Carsten A. Brühl (2015) **The effects of agrochemicals on Lepidoptera, with a focus on moths, and their pollination service in field margin habitats.** *Agriculture, Ecosystems & Environment.* 207(1), 153–162. (See Appendix, A 99).

3.2. Effects on reproduction – sublethal effects

Bats

Based on the high bat activity levels recorded in apple orchards in the course of the conducted survey (see above), a field study was performed to record bat activity in detail and measure pesticide residues on bat specific prey items to perform a risk assessment using the toxicity-exposure ratio approach of the current European guidance document.

We could verify that bats generally used the study apple orchard for foraging during the time period of insecticide applications. The highest activity levels were measured for the common pipistrelle *(Pipistrellus pipistrellus)* and Natterer's bat *(Myotis natteri)* in the night following the dusk application of the insecticide. Activity levels of *P. pipistrellus* recorded at the orchard sites were more than 20 times higher than those of adjacent forest and meadow sites. This indicates that bats were not disturbed by the agricultural activity (e.g. tractor application).

Nocturnal arthropods were sampled and grouped according to the feeding preferences of the different bat guilds. A residue analysis of the two used compounds (formulations: Reldan and Insegar, active molecules: chlorpyrifosmethyl (organophosphate) and fenoxycarb (carbamate)) followed. Considering that most arthropod groups revealed peak residue values the night following insecticide application, avoidance of food items with pesticide residues seems unlikely.

The highest initial residue values were measured on foliage-dwelling arthropods, which was also four times higher than the default "generic" value that is provided in the ESFA guidance document for the risk assessment of bird and mammals. Following the toxicity-exposure ratio approaches of the current pesticide risk assessment, no acute dietary risk was found for all recorded bat species. Though, for a chronic exposure a potential reproductive risk for bat species that include foliage-dwelling arthropods in their diet, was indicated. But all these risk estimations are based on toxicity values generated for the rat and a safety factor for chronic exposure of 5 which is assumed to cover interspecific variability in sensitivity. So far the sensitivity of bats has been evaluated in a restricted study using one bat species and an insecticide. No data exists for any European bat species and current use pesticides. Additionally, pesticide exposure might not only be restricted to the currently assessed dietary pathway but also by uptake through the skin and inhalation. Compared to day-active mammals, a higher risk with regard to direct inhalation and dermal exposure may exist for bats as it is common practice to apply pesticides at dusk to avoid, for example, effects on honeybees.

A sublethal effect is not always easy to evaluate especially in mammal species that cannot easily be kept under laboratory conditions for a longer time-span and do not allow observation because of their nocturnal life.

Publication 11: Peter Stahlschmidt & Carsten A. Brühl (2012 b) **Bats at risk? – Bat activity and residue analysis of food items following insecticide applications in an apple orchard.** *Environmental Toxicology and Chemistry* 31(7), 1556-1563. (See Appendix, A 111).

Non-target plants

Field margins are important habitats for various wild plant species in agro-ecosystems and they are also exposed to agrochemicals. Plants growing outside the cropped field are not the target of herbicide applications and are therefore termed "non-target plants", although they could comprise the weed species. The effects of herbicide, insecticide, and fertilizer inputs on the plant community in field margins were studied in an experimental field study. The study was established on an extensively managed meadow with no fertilizer inputs within the last 10 years (Figure 6). The meadow represented a surrogate field margin adjacent to winter wheat fields since this crop constitutes the majority of farmed fields in Germany. The test design consisted of three single-application treatments: one fertilizer, one herbicide, and one insecticide with similar timings as performed in regional wheat growing. The applied fertilizer rates (25% of the field rate) and pesticide rates (30% of the field rate) were consistent with their average input rates (drift + overspray) in the first meter of a field margin directly adjacent to the field. A randomized block design was chosen to take into account potential underlying environmental gradients. Each treatment was replicated eight times in plots of 8 x 8 m with a 2-m distance to each plot (in total 64 plots). The test design allowed us to investigate the single and combined effects of repeated herbicide, insecticide, and fertilizer applications in successive growing seasons.

Figure 6 Extensively managed hay meadow of the Molinio-Arrhenatheretea type before (above) and during the study conduct (below). 64 Plots with 8x8 m were established with single fertilizer, herbicide and insecticide treatments and their combinations.

In the first year we already observed an obvious difference in flowering in the common buttercup *Ranunculus acris.* To assess the effects of the agrochemical applications on *R. acris*, plant community assessments were carried out by following a recording procedure for each plot. A photo documentation of the flowering intensity was performed over two years and combined with image analysis. In addition, we conducted a standardized monitoring survey of *R. acris* in field margins in the proximity of the study site with more than 1,100 monitoring points.

In the field experiment, *R. acris* plant density decreased significantly between 20-40% in the three study years with treatments including fertilizer. Plant density was not affected in these first years of the experiment by the herbicide. The herbicide however caused an immediate sublethal effect by reducing flower intensity by 85% two weeks after application. It is important to understand that sensitive flowerbuds of *R. acris* are present above the grass canopy at times when herbicide applications in wheat fields are conducted in reality.

In the long run, both effects will result in a decline of *R. acris* and lead to shifts in plant communities in field margins. The experimental result was confirmed by the monitoring survey, where *R. acris* could be observed in 2% of the monitoring points in field margins directly adjacent to cereal fields, whereas in margins next to meadows the species was recorded frequently (85%).

The results indicate that the current risk assessment for non-target plants is insufficiently protective for wild plant species in field margins and that consideration of sublethal effects is crucial to preserve biodiversity in agricultural landscapes.

Publication 12: Juliane Schmitz, Karoline Schäfer & Carsten A. Brühl (2013) **Agrochemicals in field margins – Assessing the impacts of herbicides and fertilizer on the common buttercup *Ranunculus acris*.** *Environmental Toxicology and Chemistry*, 32(5), 1124-1131. (See Appendix, A 121).

After the observations for the common buttercup flowers we extended the set of plants to four species *(Ranunculus acris, Lathyrus pratensis, Vicia sepium, Rumex acetosa)*. We recorded plant community composition and collected seeds of the four species at maturity to detect effects on plant reproduction. For each species, the fruit collection was conducted six times per plot and three different reproduction parameters per treatment were assessed: the number of fruits per species, the mean number of seeds per fruit, and the mean weight of one seed.

Plant frequencies of the four species were significantly reduced in all herbicide and fertilizer treatments in the third year. Furthermore, herbicide treatments suppressed the formation of flowers and led to a significantly reduced seed production of *R. acris*, *L. pratensis*, and *V. sepium*. In some herbicide treated plots, even no fruits were formed. The results of the present study showed also that the mean 1-seed weight of *R. acris* was reduced in the herbicide treated plots, which could reduce germination rates of the seeds.

Because field margins are exposed to repeated agrochemical applications over several years, and without an annual delivery of seeds, the soil seed bank is eventually depleted, and this possibly leads to shifts in plant community compositions and causes the disappearance of the affected plants in the long run. In the current EU non-target plant risk assessment for herbicide reproduction effects are not considered. However, the presented study revealed that reproductive endpoints are probably a more sensitive endpoint than currently evaluated biomass measurements and therefore herbicide effects on wild plant species are potentially underestimated.

Publication 13: Juliane Schmitz, Karoline Schäfer & Carsten A. Brühl (2014) **Agrochemicals in field margins – Field evaluation of plant reproduction effects.** *Agriculture, Ecosystems and Environment* 189, 82-91. (See Appendix, A 131).

3.3. Effects on community composition

Non-target plants

The composition of the entire plant community of the above mentioned field study was monitored over three years using a standardized sampling scheme. Here direct acute effects and sublethal effects on reproduction as well as the alteration of competitive interaction between species play together.

Fertilizer and herbicide applications resulted in significant reduction of plant species after 3 years. Both treatments also significantly reduced frequencies of several plant species. One obvious example was the great yellow rattle *(Rhinanthus alectorolophus)* which was already absent from the herbicide treated plots after the first application. The fertilizer promoted plants with a high nutrient uptake and decreased the frequencies of small and subordinate species. In addition to the disappearance of a few species, the herbicide caused predominantly sublethal effects, which gradually reduced the frequencies of certain species. This reproductive effect would most likely become more prominent in the following years. Significant herbicide–fertilizer interaction effects were also observed and could not be extrapolated from individual effects. The impacts of both agrochemicals became stronger over time and led to shifts in plant community compositions. Distinct communities could be identified after 3 years, revealing the separate effects of fertilizer and herbicide treatment and its combination (Figure 7).

The loss of species and effect on frequencies caused a significantly lower species diversity in these treatments than in the control plots. Species diversity was highest in the control and insecticide treated plots during all three years of the study. The insecticide application significantly affected the frequencies of only two plant species.

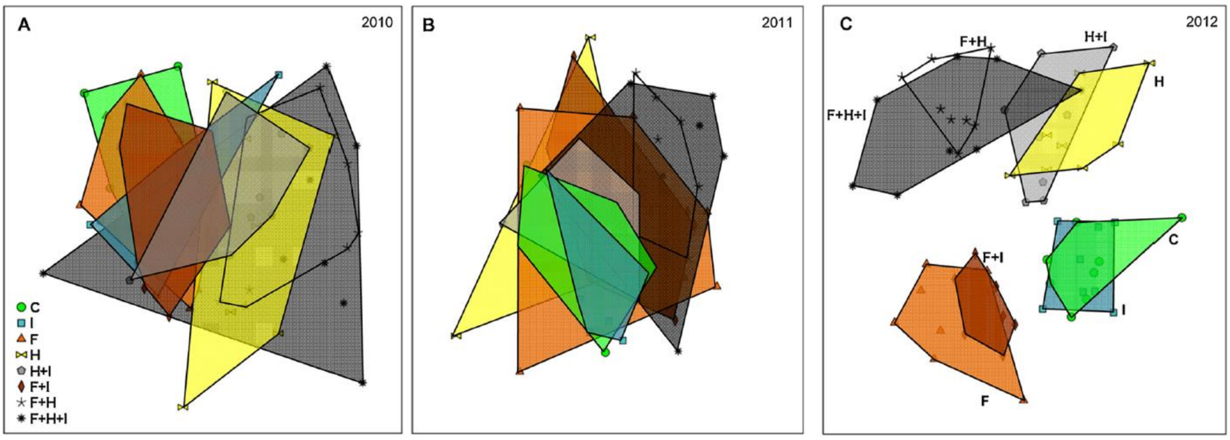

Figure 7 Two-dimensional NMDS graph of the 64 plots in June of 2010 (A), 2011 (B), and 2012 (C). Plots are labeled to their treatments. Different symbols represent different treatments and polygons enclose all plots of the same treatment. C = control, I = insecticide, F = fertilizer, H = herbicide (stress-values ranged from 0.20 to 0.25) (modified after Schmitz et al. 2014).

The results suggest that a continuous annual application of agrochemicals would cause further plant community shifts. The plant community composition changes were obvious to any observer visiting the field site after three years in June (Figure 8).

Figure 8 Flowering aspect in control (front) and herbicide (back) plots in June 2012. Each plot is 8 x 8 m.

The treatments with fertilizer and herbicide are representing a real world scenario and all showed a very low presence of flowering plants and a domination of a few grass species. Hence, to preserve biodiversity in agricultural landscapes, it is recommended to protect the vegetation in field margins from any agrochemical input. The establishment of buffer zones might be one possibility in this respect.

Publication 14: Juliane Schmitz, Melanie Hahn & Carsten A. Brühl (2014) **Agrochemicals in field margins – An experimental field study to assess the impacts of herbicides, insecticides, and fertilizer on the plant community.** *Agriculture, Ecosystems and Environment* 193, 60-69. (See Appendix, A 145).

4. Indirect pesticide effects – food web

Risk assessment of pesticides for their regulation currently only considers direct effects of pesticides, acute or sublethal. Direct effects are, for example, the mortality in bee species exposed to a pesticide, plants affected by herbicides or amphibians sensitive to fungicides (see above). In all cases, the chemical has a direct effect on an exposed organism (Figure 9). Indirect effects are effects where the food source of an organism is affected which might lead to lower food availability and effects on nutrition. Examples are the effects of herbicides on food plants of insects, leading to a reduced development of adult life stages and therefore affecting population size. This could even translate to the next higher trophic level in a food web when, e.g. birds do not find enough caterpillars to feed all their young.

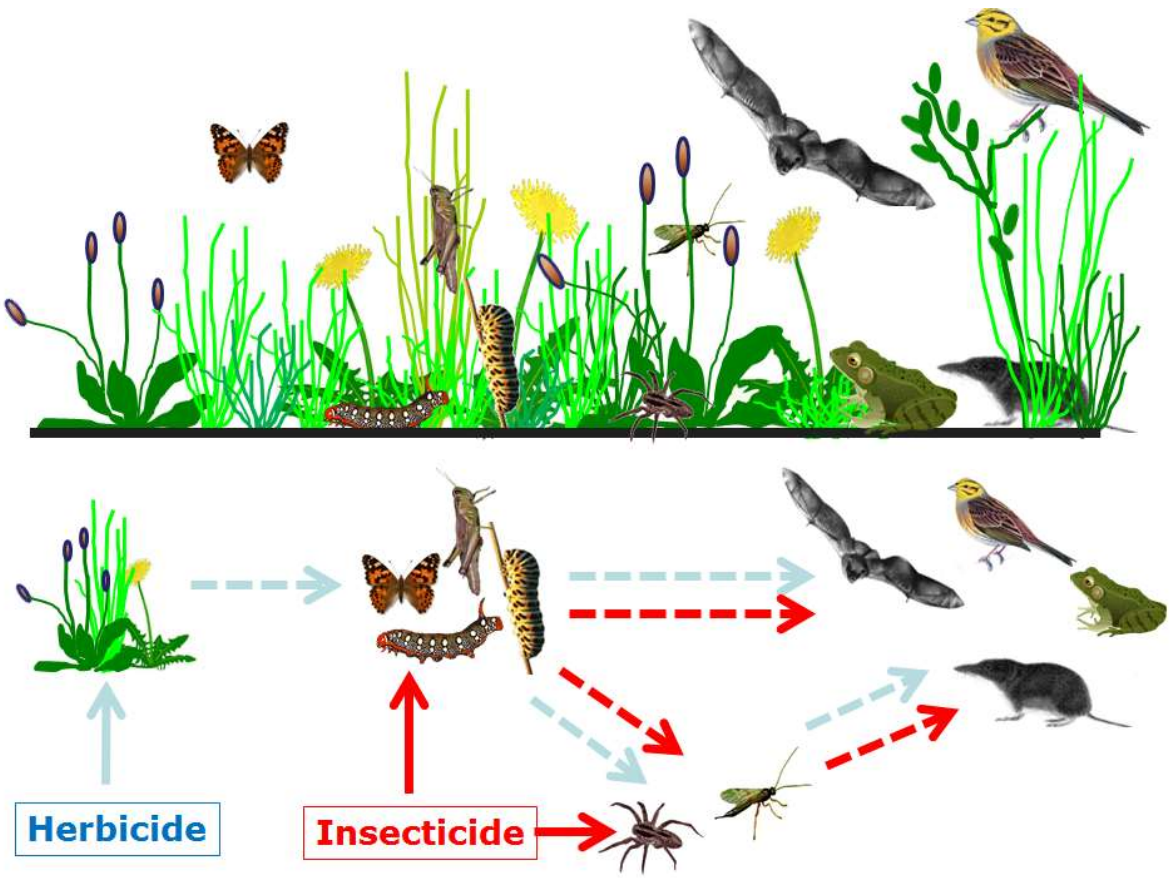

Figure 9 Above: Presence of non-target organisms. Below: Direct (solid arrows) and indirect effects (dashed arrows) of pesticides, blue: herbicide, red: insecticide.

The field experiment where we studied the effect of agrochemicals on plant community composition (see above) showed that flower intensity in the common buttercup *Ranunculus acris* was reduced by 85% two weeks after application of the herbicide and that *R. acris* was only present in 2% of the monitoring points in field margins bordering a wheat field in Germany. Although plants might still persist at lower abundances, flower production is reduced or the size of the flowers is affected. This herbicide effect might translate to insects that forage for pollen. This food source decrease might be especially severe for specialist pollinators such as the solitary bee *Osmia (Chelostoma) florisomnis*, which depends entirely on *Ranunculus* pollen. However, the pollen of *R. acris* is consumed by many insects, and a total of 117 flower-visiting insects were recorded on this plant species alone (Weiner et al. 2011).

Another obvious herbicide effect was the disappearance of the great yellow rattle *(Rhinanthus alectorolophus)*, a hemiparasitic plant, which did not occur anymore in the herbicide plots even after the first application. A closely related species, *Rhinantus minor*, had a density related effect over four trophic levels in a grassland system (Hartley et al. 2015). Manipulated enhanced densities almost doubled the abundance of invertebrates across all trophic levels. The authors suggest that the hemiparasite, despite being a subdominant and transient component within plant communities that it inhabits should therefore be recognized as a keystone species in grassland communities. It seems plausible that herbicide related reductions result in indirect effects over many trophic levels.

Publication 12: Juliane Schmitz, Karoline Schäfer & Carsten A. Brühl (2013) **Agrochemicals in field margins – Assessing the impacts of herbicides and fertilizer on the common buttercup *Ranunculus acris*.** *Environmental Toxicology and Chemistry*, 32(5), 1124-1131. (See Appendix, A 121).

Herbicides are widely used pesticides that affect and kill plants by changing their chemistry. They affect basic biochemical processes in plants, such as electron transport or amino acid synthesis. For example, glyphosate inhibits an enzyme of the shikimate pathway, and sulfonylurea herbicides inhibit acetolactate synthase. Both enzymes are necessary for the synthesis of some amino acids in plants. Amino acids and proteins are crucial to the development of herbivores (and other organisms), and therefore herbicide-treated plants may not meet their dietary requirements. In addition, herbicides cause stress in plants and can induce genes involved in plant defense, triggering the release of an entire battery of toxic compounds. Many plants can synthesize secondary metabolites, such as glucosinolates, as defense mechanisms which may deter or be toxic to herbivores. Accordingly, some plant secondary metabolites, such as pyrethrum, are also used as insecticides. Therefore, even if a plant is not killed by a herbicide, the chemical might trigger its defense mechanisms and the plant might become unsuitable or less nutritious to herbivores.

To study the effects of herbicides on host plant quality, three plant species, English plantain *(Plantago lanceolate)*, greater plantain *(Plantago major)* and common buttercup *(Ranunculus acris),* were treated with sublethal rates of either a sulfonylurea (Atlantis WG, Bayer CropScience) or a glyphosate (Roundup LB Plus, Monsanto) herbicide, and the development of caterpillars of the cabbage moth *(Mamestra brassicae*) feeding on these plants was observed. Of the six tested plant–herbicide combinations, one combination (*R. acris* and sulfonylurea herbicide) resulted in significant effects. Caterpillars revealed a significantly lower weight after three weeks (Figure 10, about one third of the control), increased time to pupation (approximately one third longer) and increased overall development time (six days longer) compared to larvae that were fed unsprayed plants.

Figure 10 Caterpillars of the cabbage moth *(Mamestra brassicae*) three weeks after feeding on control common buttercup *(R. acris)* plants and plants treated with 10% of the field rate of the herbicide Atlantis WG (sulfonylurea).

These observed effects may have been caused by a lower nutritional value of these host plants or increased concentrations of secondary metabolites that are involved in plant defense. Currently we cannot differentiate between the two. The results suggest potential risks to herbivores feeding on host plants treated with sublethal rates of herbicides. It is difficult to evaluate the impact of this effect in nature, because the effects of herbicides on host plant quality appear to be species-specific. For example, in Germany, there are approximately 3,500 Lepidoptera species, 4,200 flowering plant and fern species, and more than 580 registered herbicide products. This situation results in a vast number of possible combinations, making it difficult to estimate the overall risks of one or more herbicides to herbivorous insects.

Therefore, the protection of non-target habitats, such as field margins, should be considered to minimize the potential risks to herbivorous insects and wildlife.

Publication 15: Melanie Hahn, Martin Geisthardt & Carsten A. Brühl (2014) **Effects of herbicide-treated host plants on the development of *Marmestra brassicae* L. caterpillars.** *Environmental Toxicology and Chemistry*, 33(11), 2633–2638. (See Appendix, A 159).

We also studied the indirect effects of insecticides on adult moths and the pollination services provided for plants. We therefore established a semi-field study with potted test plants and free ranging moths. The test plant, the white champion *(Silene latifolia),* is specialized for nocturnal moth pollination and the lychnis moth *(Hadena bicruris),* its main pollinator, lays its eggs on the plant. *Silene latifolia* is a dioecious plant species; hence, self-pollination cannot occur because the male and female flowers are developed on different plant individuals. Moths could pollinate treated (Karate, Lambda-cyhalothrin, a pyrethroid insecticide; 30% of the field rate, corresponding to plant community field experiment, see above) or untreated female flowers in a choice study (Figure 11).

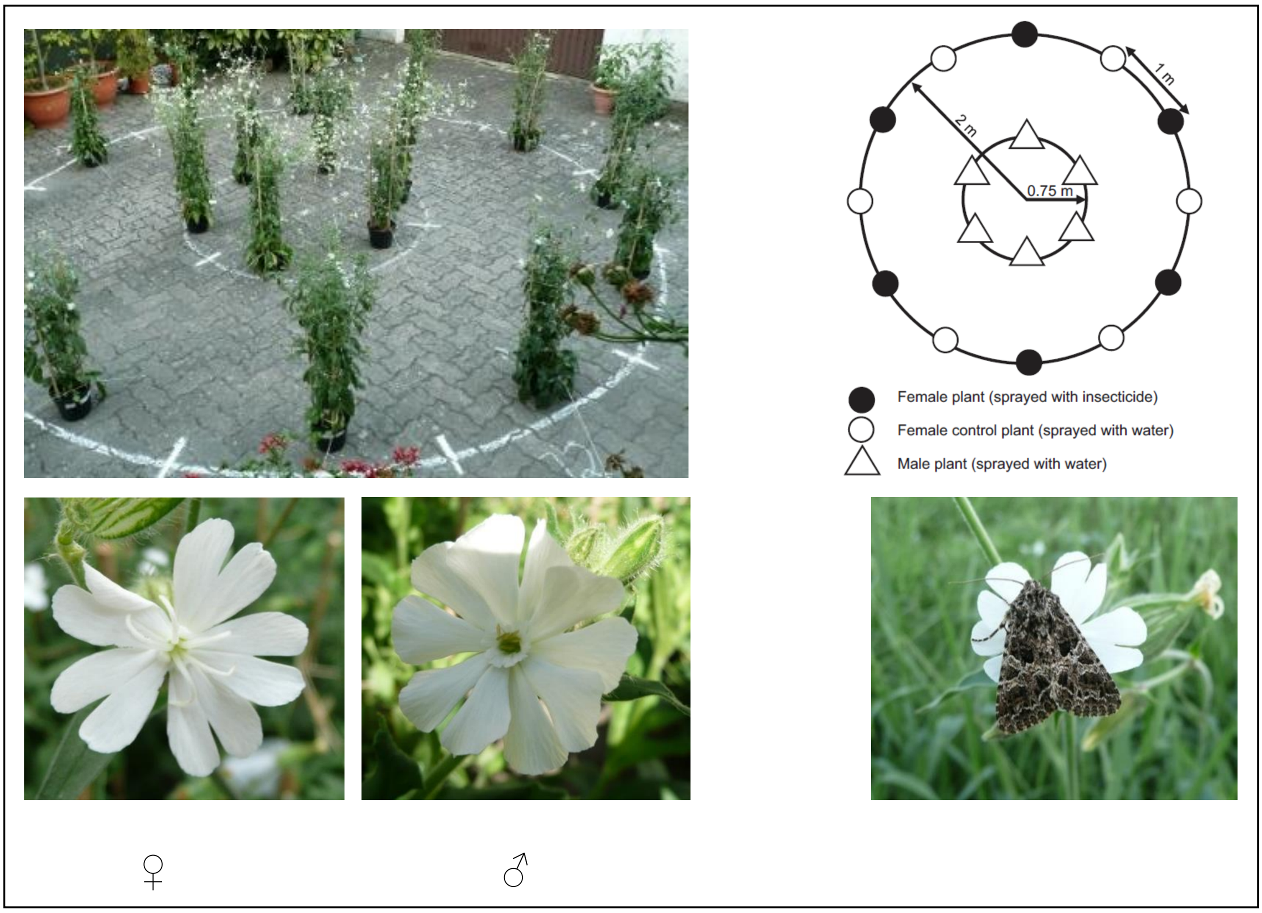

Figure 11 Semi-field choice experiment with central male and outer female flowers of the White Campion (*Silene latifolia* subsp. *alba*) and its main pollinator, the moth *Hadena bicruris* (Noctuidae).

The insecticide treatment resulted in a significant reduction in the number of pollinated flowers per plant. Approximately 30% of the insecticide-treated flowers were not pollinated and, hence, developed no seeds, whereas all of the control flowers produced seeds. There were approximately 40% fewer *H. bicruris* eggs recorded on the insecticide-treated flowers, indicating that adult moths avoided oviposition on insecticide-treated flowers.

In this study we observed the reverse case, where an insecticide has an effect on the plant species by reducing pollination of flowers.

Publication 10: Melanie Hahn, Annalena Schotthöfer, Juliane Schmitz, Lea A. Franke & Carsten A. Brühl (2015) **The effects of agrochemicals on Lepidoptera, with a focus on moths, and their pollination service in field margin habitats.** *Agriculture, Ecosystems & Environment*. 207 (1), 153–162. (See Appendix, A 99).

5. Landscape effects on biodiversity – chemical fragmentation

Current conservation efforts focus on structural features and their impact on biodiversity: how to improve connectivity between fragments, or quality of fragments and structures. Among all management impacts pesticides are often not recognized by biologists and only addressed by ecotoxicologists, and the majority studies aquatic compartments and does not necessarily infer pesticide impacts on entire ecological systems (Schulz and Brühl 2006). The current intense use of pesticides in agriculture and their inherent toxicity may lead to a chemical landscape fragmentation, where populations may not be connected anymore, source-sink dynamics become important ecological mechanisms and as a final result not only population size but also genetic population structure might be affected.

Besides the crop fields agricultural landscapes include other structural features such as different freshwater bodies like ditches, creeks or ponds and terrestrial elements such as hedges, field margins and smaller islands of vegetation. These structural features are easily recognizable and were intensively studied in the field of agroecology in the framework of island theory (MacArthur and Wilson 1967) over the last decades to understand population dynamics of different organisms within this "biome" (e.g. Bickel et al. 2006). Populations of many of the species inhabiting agricultural landscapes are thought to prefer those semi-natural structures as habitats (e.g. bees visit the flowers in a field margin, birds nest in hedges) and therefore size and connectivity of these structures are determining factors of population size. The idea is also used in conservation where new habitats are created as so called "stepping-stones" to facilitate connectivity and interchange of populations or within a meta population.

However, a European study in many countries revealed that the main explaining factors for lower abundance of carabid beetles, "weed" plants and birds in wheat fields is not size or proximity of structural elements but pesticide use in the crop (Geiger et al. 2010). In the growing season the cropped fields are treated with pesticides on a regular basis and these chemicals are highly toxic not only to target-organisms as they are affecting basic biological processes and pathways (e.g. cellular respiration, nerve conduction, see above) that might lead to direct acute or chronic effects or cause indirect effects in non-target organisms. Different cultivated crops receive a different number of applications and a suite of various products depending on their management. In Germany the applications of plant-protection products are recorded regularly in selected farms (JKI 2016). Total application frequency including herbicide, fungicide and insecticide applications are ranging from 2-9 for arable crops (maize 1.5, winter wheat 4.4, (**but**: initial systemic seed treatment for cereals is not included!) sugar beet 5.0, oilseed rape 6.1, potatoes 9.0; to vine (9.9) and apples (21) (data for 2015)).

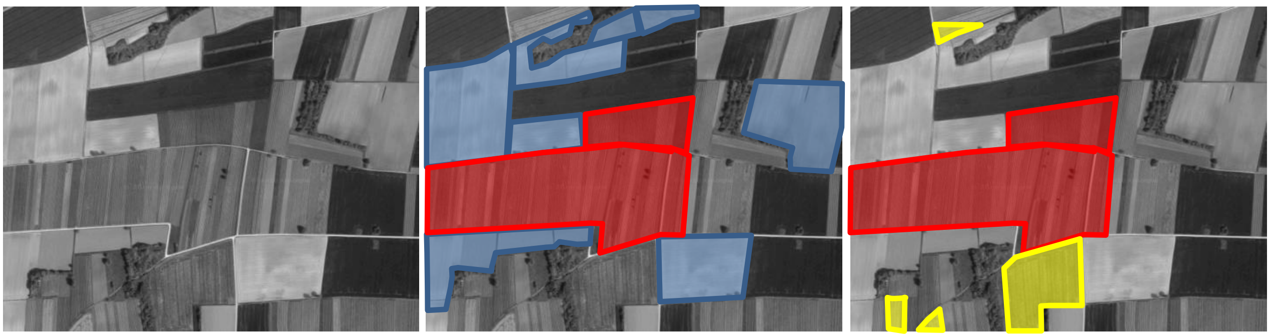

Figure 12 Crops in agricultural landscapes are treated with pesticides at specific times leading to a chemical landscape fragmentation. **Left**: A landscape with vineyards, cereal and oilseed rape fields in Southern Germany. **Center**: Treatment of maize fields with herbicides before seed drilling (blue) and first fungicide applications in vineyards (red) in May. **Right**: Insecticide treatment of oilseed rape fields (yellow) and fungicides in vineyards (red) in June.

The applications in the crops also affect the structural semi-natural elements by overspray the first 50 cm of a narrow field margin next to an arable crop (see above) and spray drift. Spray drift varies from about 3% of the field rate reaching the first meter next to an arable field to about 30 % in 3 m distance next to an apple orchard during a spring application (Rautmann et al. 2001). On the landscape level these applications in crops show a spatial-temporal pattern and could isolate habitats further. This “chemical landscape fragmentation” has so far not received much attention by agroecologists and conservation biologists, who do not account for the effect of pesticides in their analysis and management planning.

We study the aspect of a chemically fragmented landscape and its effects on amphibian populations in vineyards in the region around Landau. Amphibians in this agricultural landscape mostly breed in artificial water bodies such as rainwater retention ponds and therefore migrate through a matrix of vineyards and semi-natural structural elements in spring and then reside therein after breeding. The persistence of their populations is influenced on different spatial scales ranging from the individual breeding pond to surrounding habitat patches to landscape clusters of breeding pond populations. The connectivity among breeding ponds as well as the availability of suitable terrestrial habitats surrounding the ponds plays a major role in long-term viability of amphibian species. Beside road traffic and urban structures agricultural management can disrupt landscape connectivity by the use of pesticides and fertilisers and physical activity such as tillage. We developed an expert-based model to assess the impact of agricultural management measures on the migration area and terrestrial habitat availability for seven amphibian species. The model is based on a Habitat Suitability Index to identify suitable terrestrial habitats for each species and includes a landscape permeability approach. Size of migration areas, connectivity of breeding ponds and number of reachable terrestrial habitats were modeled considering species-specific migration ranges and habitat preferences. We wanted to get an idea how pesticide application might lead to fragmentation and isolation of amphibian breeding pond populations. Therefore, the potential disrupting impact of pesticides was simulated by inflating landscape resistance to medium and high migration costs. Medium migration costs were similar to crossing major roads whereas the high costs assume even worse conditions than highways. Including potential pesticide impacts as migration costs showed that migration areas were in decrease and some of the investigated populations were isolated at breeding pond level or restricted to pond clusters. Although the impacts of pesticides on the permeability of the landscape are unknown, a negative influence might be assumed due to the high mortality of amphibians at field application rates of pesticides revealed in laboratory experiments.

Publication 16: Patrick Lenhardt, Kathrin Theissinger, Ralf Schäfer & Carsten A. Brühl (2013) **An expert-based landscape permeability model for assessing the impact of agricultural management on amphibian migration.** *Basic and Applied Ecology* 14, 442–451. (See Appendix, A 167).

Together with Dr. Kathrin Theissinger we evaluated the published model predictions using population genetics of the European common frog *(Rana temporaria)*. We analysed microsatellite data of ten loci from ten breeding pond populations located within the viniculture landscape and in the adjacent forest block. We tested for significant correlation of genetic population differentiation and landscape elements, including land use as well as roads and their associated traffic intensity, to explain the genetic structure in the study area. Genetic differentiation among forest populations was significantly lower than between viniculture populations. Our analyses rejected isolation by distance based on roads and associated traffic intensity as the sole explanation of the genetic differentiation and suggest that the viniculture landscape itself has to be considered as a limiting barrier for *R. temporaria* migration, partially confirming the isolation of breeding ponds predicted by the landscape permeability model. Therefore, arable land may act as a sink habitat, inhibiting genetic exchange and causing genetic differentiation of pond populations in agricultural areas. In viniculture, pesticides could be a driving factor for the observed genetic impoverishment, since pesticides are more frequently applied than any other management measure and can be highly toxic for terrestrial life stages of amphibians. This study reveals the potential effects of pesticides on genetic diversity in amphibians.

Publication 17: Patrick P. Lenhardt, Carsten A. Brühl, Christoph Leeb & Kathrin Theissinger (2017) **Amphibian population genetics in agricultural landscapes: Do non-visible barriers in viniculture drive the population structuring of the European common frog *(Rana temporaria)*?** PeerJ. 3520, 1-20. (See Appendix, A 179).

In a continuing collaboration with Dr. Kathrin Theissinger this approach is extended to additional sites and amphibian species. The situation in this landscape is especially beneficial, because plowing, which is considered to be a mortality factor for amphibians in arable fields, is not included in the management practices in vineyards and fertiliser is also only applied to fractions of the arable area (root stock) and therefore pesticides are a major management factor, reaching up to 10 applications per season, consisting mostly of fungicides.

6. Development of pesticide risk assessment and management

For the regulation of pesticides in Europe a risk assessment is performed for the aquatic habitat (water bodies next to fields) and the terrestrial habitat, where specific guidance documents address the risk to plants, arthropods, bees and birds and mammals as well as the soil. The separation of aquatic, soil and terrestrial (meaning above ground) allows the specialization of staff dealing with the evaluation of submitted complex data, however it does not take into account the interactions between the different recognized habitats and therefore might underestimate effects of pesticides that translate from one into the other system. One example are larvae of beetles that live in the soil (e.g. carabid beetles or certain fly groups) and their adult life forms that live above ground. Pesticide exposure in agricultural soils might therefore have an indirect effect on food availability at higher trophic levels. The same is true for the interaction between aquatic and terrestrial habitats where aquatic habitats can provide a substantial part of the food resource of terrestrial organisms. The interaction of systems and the implication for risk assessment warrants further research. Currently only very few studies consider indirect effects in the receiving terrestrial system, e.g. via food web responses, as a result of anthropogenic alterations in aquatic ecosystems (Schulz et al. 2015). Overall, the current state of knowledge calls for an integrated assessment of interactions between systems to understand how one is affected by changes and effects caused by pesticides in the other. For the terrestrial risk assessment different organism groups are addressed separately (see above).

6.1. Non-target plants

In our studies with plants we could detect a higher sensitivity of the reproductive parts of a plant than for the leaves and therefore consider this as an important endpoint to be considered in the pesticide risk assessment. In a project with the Federal Environment Agency Germany

(Umweltbundesamt UBA) we additionally evaluated the sensitivity of the tested plant species compared to plants present in natural communities bordering crops using data provided by the agency. The analyzed toxicity data revealed that in all evaluated non-target plant studies for registration purposes only crop plants were tested. Wild plant species tested in research studies showed a hundredfold higher sensitivity in data sets for glyphosate and dicamba, the only two data sets available for a comparison. Many plant families occurring in natural field margins are not included in tests, although some of them are present with many species and also revealed high herbicide sensitivity (e.g. Lamiaceae). Some crop plants often assessed in non-target plant studies for regulatory purposes always showed low sensitivities for a range of herbicides. Among them were oats, onion and soybean and we suggest that these species should be excluded from testing and replaced by wild plants. So far only higher plants are tested and the sensitivity of terrestrial algae, mosses, ferns and lichen is assessed in only a few studies.

Publication 18: Juliane Schmitz, Peter Stahlschmidt & Carsten A. Brühl (2015) **Protection of terrestrial non-target plant species in the regulation of environmental risks of pesticides.** Federal Environment Agency (Germany), TEXTE 20/2015. ISSN 1862-4804. 142 pp. (See Appendix, A 201).

Risk assessment for non-target plants is based on single species phytotoxicity tests. This approach may not reflect relevant ecological processes in terrestrial ecosystems such as intra- and interspecific competition. The current risk assessment scheme is based on endpoints measured at the species level and the assessment of ecological effects relies on the extrapolation from one species to another or from a single species to a community. This extrapolation contains many uncertainties which may be reduced by adopting more realistic testing approaches. However, currently higher-tier plant studies are not obligatory in herbicide risk assessment.

We reviewed the published literature and found that potential higher-tier approaches for terrestrial non-target plants are limited. Sixteen studies could be identified that assess the effects of herbicides on non-target plants by performing microcosms, mesocosms, or field studies. These studies show that microcosms may provide useful data and help reduce uncertainties associated with single-species tests. However, due to the limited number of available studies, a lot of work is required to develop appropriate testing methods for regulatory processes. In addition, field experiments are necessary to establish baseline knowledge concerning the effects of herbicides on natural plant communities and to compare data generated in tiered testing approaches with data obtained from natural systems.

Publication 19: Juliane Schmitz, Peter Stahlschmidt & Carsten A. Brühl (2015) **Assessing the risk of herbicides to terrestrial non-target plants using higher-tier studies.** *Human and Ecological Risk Assessment.* 21 (8), 2137-2154. (See Appendix, A 205).

From our work with pesticides and plants we see many uncertainties in the currently implemented risk assessment requirements. Only a few crop plant species are tested and those endpoints are used to estimate a risk for wild plant communities. A new approach will require the development of standardized higher tier studies in parallel that address field level effects which will be very time consuming taken our current knowledge into account. We therefore propose to consider the development of risk mitigation options in parallel to refinements of the current risk assessment scheme. An in-field buffer where no herbicide is applied will reduce drift to low percentages and therefore reduce exposure and effects in plant communities of field margins. The development and implementation of this risk management option is a complicated issue and requires further applied research and the integration of socioeconomic considerations. Nonetheless, this approach has the potential to restore biodiversity in agricultural ecosystems, reestablish dwindling ecosystem services such as pollination and biological pest control leading to a sustainable agricultural system.

6.2. Non-target arthropods

In the pesticide risk assessment for arthropods the current legislation uses the results of a tiered testing scheme to estimate the risk for non-target arthropods. Many aspects of the regulation have been critically discussed in a current scientific opinion expressed by a working group of EFSA (EFSA 2015), including the test species and indirect effects. In the tiered approach field studies need to be conducted, when the worst case toxicity data obtained by standardized laboratory test indicate a risk at the field situation. Field studies are realistic scenarios and environmental factors such as rain and sunlight facilitate the break-down of a product. Non-target arthropod studies are generally conducted over the course of a year and the presence of a species at the end of a growing season or at the beginning of the next have been interpreted as "recovery". Return of population densities after disturbance (e.g., the application of a pesticide) to levels similar to those in undisturbed controls can be observed under field conditions (e.g., one-hectare field experimental plots). The observed pattern is in the majority of field studies highly influenced by the control plots and the surrounding and is therefore more correctly termed "recolonisation". This process only works if a source is in close proximity. However, especially for mobile taxa, the observed return to the control levels or its absence is not a robust predictive indicator for the likelihood of recovery with pesticide use at landscape scale: it does not consider, for example, applications of different products or different ecological conditions such as the size and distribution of refugia and reservoirs or life cycle parameters of species. This change in acceptance of field generated endpoints will affect the non-target arthropod risk assessment and new approaches are currently discussed. One possibility is to study the effect of a pesticide on field margin communities for only a short time period (e.g. six weeks) to estimate a "magnitude of effect". This endpoint could include the number of affected species and also the effect size in the populations compared to controls.

Publication 20: Carsten A. Brühl, Paul Neumann, Saskia Aldershof, Dave Bohan, Kevin Brown, Marco Candolfi, Flavia Geiger, Natalie Kovalkovičová, Christine Kula, Karin Nienstedt, Susana Pestanudo, Jörg Roembke, Thomas Schmidt, Chris Topping and Peter van Vliet (2012) **Recovery.** pages 41-44. **In**: *Anne Alix, Frank Bakker, Katie Barrett, Carsten A. Brühl, Mike Coulson, Simon Hoy, Jean Pierre Jansen, Paul Jepson, Gavin Lewis, Paul Neumann, Dirk Süßenbach & Peter van Vliet (Eds.)* ***ESCORT 3 - Linking Non-Target Arthropod Testing and Risk Assessment with Protection Goals****.* Proceedings of the European Standard Characteristics Of non-target arthropod Regulatory Testing workshop ESCORT3. *CRC SETAC Press. 160 Pages, ISBN 9781466511811*. (See Appendix, A 225).

6.3. Mammals

Cereal crops are grown over large areas in Europe and around 90% of the cereal seeds used in agriculture today have been treated with specific formulations that encapsulate the seed and protect the germinating plants from diseases. Seed treatments are applied on the outside of the seed, the husk, to target fungi (fungicides) and disease vectors (insecticides) or both. As for all pesticides, the risk of seed treatments to the ecosystem has to be evaluated in the registration process. The recently published scientific opinion and guidance document on the risk assessment of pesticides for birds and mammals (EFSA 2008, 2009) detail many specific aspects of exposure that should be considered. Beside birds, especially granivorous mice living in or near cereal fields are potentially exposed to seed treatments when feeding on the drilled seeds or occurring spillages. However, these granivorous mice dehusk seeds before consumption and, therefore, lower the potential exposure through this behaviour. Although the behaviour as such is known and its results have been observed in the field, quantitative data on the efficiency of dehusking by small mammals are so far not published and dehusking is also not included as a refinement option in the risk assessment in Europe.

Therefore, we aimed at providing a first quantitative estimate of this behaviour-related exposure reduction for the wood mouse *(Apodemus sylvaticus)* with four different seed types (wheat, barley, maize and sunflower). Residues of a fungicide and a pigment blank formulation were measured after seed consumption. We could demonstrate exposure reductions ranging from around 60% for cereals to almost 100% for sunflower seeds as a result of the dehusking behaviour. Since exposure reduction was similar in both approaches, working with pigments would be a generic way to estimate the impact of dehusking behaviour on seed treatment exposure. In the laboratory this behaviour can result in a substantial exposure reduction and could, after an evaluation in the field, be considered in a seed-type specific way in the risk assessment of pesticide seed treatments for granivorous mice. The approach of accounting for a dehusking-related exposure reduction by field relevant wild mammal species seems a more promising way to advance the risk assessment instead of using generic species and neglecting behavioural traits.

Publication 21: Carsten A. Brühl, Bernd Guckenmus, Markus Ebeling & Ralf Barfknecht (2011) **Exposure reduction of seed treatments through dehusking behaviour of the wood mouse *(Apodemus sylvaticus)*.** *Environmental Science and Pollution Research.* 18, 31–37. (See Appendix, A 231).

6.4. Amphibians

At present amphibians are not considered specifically in pesticide risk assessment. It is assumed that amphibians are covered either by the aquatic risk assessment for the tadpole live stage or by the birds and mammals risk assessment for the terrestrial life stage. In 2010, the European Union explicitly called for amphibian toxicity data to be considered when authorizing pesticides for the market (EFSA 2007, 2010). To date however, no concrete suggestions or guidelines for the regulatory risk assessment pesticides to amphibians. Currently a working group evaluates the scientific data and prepares a scientific opinion. I am involved in this process as an external expert and by delivering a literature review on the state of the art in an EFSA project (Ortiz Santaliestra et al. 2017). The topic was also discussed in a stakeholder workshop in Switzerland with the aim to identify research needs in conjunction with the sustainable use directive for pesticides of the EU (Commission 2009). The participants discussed current scientific studies and observations and came to the conclusion that pesticides pose a risk, especially in agricultural landscapes, although its extent is currently unclear. Research is lacking especially regarding the toxicity of pesticides for terrestrial life stages of amphibians, including co-formulants, mixtures, multiple stressors and interactive effects. The lack of knowledge in this area makes it impossible to develop a science based risk assessment framework.

A pragmatic way forward is the implementation of risk management strategies with the aim to reduce pesticide effects. One mitigation measure could be based on the prediction of amphibian migration so that pesticides are not used on farmland when amphibians are present in large numbers. Untreated corridors set up in farmland where amphibians are present or to which they migrate represent another possibility. However, these management options also need to be developed and tested.

Publication 22: Annette Aldrich, Marion Junghans, Caroline Aeberli, Carsten A. Brühl, Franz Streissl & Benedikt R. Schmidt (2016) **Amphibians and plant-protection products – what research and action is needed?** *Environmental Science Europe*, 28, 17. DOI 10.1186/s12302-016-0085-6. (See Appendix, A 241).

6.5. Management

In our project with bats we also studied the habitat use of different bat species in the vineyards of the region around Landau. Bats are known to use aquatic habitats for foraging and agricultural intensification has been recognized as the main reason for the loss of wetlands. However, artificial wetland creation (i.e. the construction of retention-ponds) in the agricultural landscape aiming at water or nutrient retention has recently gained importance. We evaluated to what extent bats use these artificial wetlands as foraging habitats in an agricultural landscape.

Bat activity and prey density were compared in matched pairs at seven retention-ponds and neighbouring vineyard sites using stationary bat-detectors (see above) and sticky-traps, respectively. To examine if bat activity is related to the number of bat individuals, a thermal infrared imaging camera was used. *Pipistrellus pipistrellus*, the dominant species, served as an example to assess habitat selection between retention-ponds and vineyards. This was performed by relating foraging activity to the available area available within the potential home-range.

Total bat activity and nocturnal prey density were significantly higher above the retention-ponds than above vineyards. High differences of activity levels between the ponds and the respective vineyard sites were found for *Pipistrellus* spp. (*P. pipistrellus* and *P. nathusii*) and *Myotis* spp. (*M. daubentonii* and *M. mystacinus*), being about 180 times and 50 times higher above the retention-ponds, respectively. A significant correlation was found between recorded bat activity and the maximum number of bat individuals observed with a thermal infrared imaging camera. When relating foraging activity to habitat availability within the assumed home-range of *P. pipistrellus*, retention-ponds had on average a higher importance as a foraging habitat than the complete vineyard area although they covered less than 0.1% of its area.

The creation of artificial wetlands would be a possibility to increase important foraging habitats for bats and mitigate negative effects of management practice in the agricultural landscape. This example also shows how subsidies from aquatic water bodies constitute a major food source for terrestrial organisms (see above).

Publication 23: Peter Stahlschmidt, Achim Pätzold, Lisa Ressl, Ralf Schulz & Carsten A. Brühl (2012) **Constructed wetlands support bats in agricultural landscapes.** *Basic and Applied Ecology,* 13, 196–203. (See Appendix, A 251).

7. Conclusion

Pesticides are the only group of organic chemicals that are released not accidentally but deliberately in the environment. Their high biological activity can cause toxic effects not only in the target-organisms but also in related taxa. The effects of those chemicals on terrestrial organisms and communities within agricultural landscapes have been the focus of the research projects that I conducted with my team of master and Ph.D. students as well as post-docs in collaboration with many colleagues during the last 10 years.

The composition of biodiversity in agricultural landscapes is understudied despite the large proportion of terrestrial habitat they provide today. Agricultural landscapes are dominated by crops and semi-natural structures such as field margins which are only covering a small proportion. The majority of these field margins are narrow and therefore also subject to overspraying during pesticide applications.

Knowledge about the presence of organisms in agricultural systems is important to fully acknowledge pesticide exposure that may occur. Together with available sensitivity data for relevant test species we can assess the risk that pesticides pose to these organisms and regulate or manage it accordingly. Nocturnal organisms such as bats and moths are not in the focus of current research or risk assessment approaches since we do not know too much about their use of European agricultural landscapes. In the last few years we demonstrated that the majority of bat and amphibian species uses the agricultural landscape to forage for food and is also present in fields and orchards. It also seems that moths are underestimated pollinators of wild plants in agricultural landscapes and therefore require specific protection because of the pollination service they provide and their role in the food web.

Semi-natural structures in landscapes in Germany are present as narrow field margins which are receiving a high pesticide load, since parts are even oversprayed. Agricultural landscapes are used by many organisms and we could show the presence of many bat species in crops and also the coincidence of amphibians with pesticide applications. Both groups are potentially exposed and should therefore be addressed in risk assessment. Direct pesticide effects were evaluated for non-target insects and are especially alarming for amphibians. The mortality endpoint we used is the crudest measure for an effect, but surprisingly so far no information on direct exposure with terrestrial amphibians was ever conducted, although pesticides have been applied in our landscapes for more than 60 years. Direct sublethal effects are more difficult to study, but they can translate, as in the case of the reproduction parameters in plants, to changes in population size and shifts in community composition over the years. Indirect effects are even more difficult to characterise and can be complex. The translation of an effect of herbicides on food plant biochemistry on caterpillar development was detected in our laboratory experiment, we can, however not yet quantify this effect on a landscape level. This might be an important factor for the risk assessment, leading together with the previously described effects to a chemically fragmented landscape.

The assessment of a landscape scale risk is sometimes discussed as a futuristic approach in regulation. Today we evaluate the risk for a specific pesticide molecule and its formulations on specific organisms and sum those up but. The landscape approach instead evaluates the sum of all pesticide applications and their effects on biodiversity in a landscape over the growing season. Therefore, we need to address direct acute and sublethal as well as indirect effects of multiple applications of numerous products composed of active molecules and several additives on a multitude of species. As mentioned before we currently only use an arbitrary safety factor, in most cases of 10, to account for inter-species variability in sensitivity. However, the majority of species, and this is even true for vertebrate species, have never been studied in a comparable design and we therefore do not know if this safety factor is actually protective.

Understanding and characterising the risk of pesticide application on a landscape level will be a challenging task. With time lines for guideline development on a European level of more than 10 years from the first meeting of a working group until implementation it may take a long time until we develop and accomplish a protective framework to conserve biodiversity in agricultural landscapes. A more pragmatic and faster approach would be to concentrate on the remaining semi-natural structures, reduce or stop all pesticide inputs and even consider enlarging these areas. Additionally, the percentage of organic agriculture, with zero use of organic chemical pesticides should be increased. The EU has already set the target for organic management of 20% of agricultural area by 2020 (BMEL 2013).

We could start this development of a new management approach with a model landscape where we employ many mitigation measures, such as edge of field drift reduction, buffer strips along waterbodies and no-spray areas along field margins to evaluate their impact on biodiversity. The idea will need a landscape, where agriculture uses less or even no pesticides at all and instead applied integrated pest management for example using beneficial insects for control in new approaches (e.g. *Trichogramma* distribution via drones). This model landscape could be a first experiment towards a new approach in agriculture which also encompasses the biodiversity of its landscape. By working together in an integrative project comprising chemistry, physics, biology, ecotoxicology, modelling and socio-economy as well as medicine the project could develop and evaluate the impact and costs of new tools in risk mitigation and pesticide reductions on plants and animals, but also human health and thereby contribute to advancing a sustainable concept for the agriculture of the future.

8. Literature

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9. Author contributions

In the following table the author contributions for each publication mentioned in this text are provided.

Publication	Title	Year	Co-authors (initials) following citataion order	Idea	Experimental design	Data gathering	Data analysis	Paper writing	Paper review
1	Characterization of field margins in intensified agro-ecosystems – why narrow margins should matter in terrestrial pesticide risk assessment and management.	2014	MH PPL CAB	CAB MH	CAB MH	MH PPL	MH	MH	CAB
2	Bats as bioindicator species – The need of a standardized method for acoustic bat activity surveys	2012	PS CAB	CAB PS	CAB PS	PS	PS	PS	CAB
3	Nocturnal risks - High bat activity in the agricultural landscape indicates potential pesticide exposure.	2017	PS MH CAB	CAB	CAB PS	PS MH	PS CAB	PS CAB	MH
4	Temporal coincidence of adult amphibians and pesticide applications on arable fields during spring migration.	2015	PPL CAB GB	CAB, GB	GB	GB	PPL	PPL CAB	GB
5	The secret pollinators – an overview of moth pollination in natural and agricultural habitats with a focus on Europe and North America.	2016	MH CAB	MH CAB	-	MH	MH	MH	CAB
6	Amphibians at risk? – Susceptibility of terrestrial amphibian life stages to pesticides	2011	CAB SP BW	CAB	-	BW	BW CAB	CAB BW	SP
7	Terrestrial pesticide exposure of amphibians: An underestimated cause of global decline?	2013	CAB TS SP AA	CAB	CAB AA TS	AA TS	AA CAB	CAB	TS SP AA
8	Interspecific sensitivity of bees towards dimethoate and implications for environmental risk assessment	2016	PU, LAF CR, CW PS, LJ CAB	CAB	CAB PU LJ	PU, LF CR, CW PS	PU LAF CW	PU	CAB LJ PS

Publication	Title	Year	Co-authors (initials) following citataion order	Idea	Experimental design	Data gathering	Data analysis	Paper writing	Paper review
9	Does drift of insecticide adversely affect grasshoppers (Orthoptera: Saltatoria) in field margins? A case study combining laboratory acute toxicity testing with field monitoring data.	2012	RB JS MB CAB	CAB RB	CAB RB	RB JS	RB JS	RB MB	CAB JS
10	The effects of agrochemicals on Lepidoptera, with a focus on moths, and their pollination service in field margin habitats	2015	MH, AS JS, LAF CAB	MH CAB	MH JS CAB	MH AS LAF	MH	MH	JS CAB
11	Bats at risk? – Bat activity and residue analysis of food items following insecticide applications in an apple orchard	2012	PS CAB	CAB PS	CAB PS	PS	PS	PS	CAB
12	Agrochemicals in field margins – Assessing the impacts of herbicides and fertilizer on the common buttercup *Ranunculus acris*.	2013	JS KS CAB	CAB JS	CAB JS	JS KS	JS	JS	CAB
13	Agrochemicals in field margins – Field evaluation of plant reproduction effects.	2014	JS KS CAB	CAB JS	CAB JS	JS KS	JS	JS	CAB
14	Agrochemicals in field margins – An experimental field study to assess the impacts of herbicides, insecticides, and fertilizer on the plant community	2014	JS MH CAB	CAB JS	CAB JS	JS MH	JS	JS	CAB MH
15	Effects of herbicide-treated host plants on the development of *Marmestra brassicae* L. caterpillars	2014	MH MG CAB	CAB MH	CAB MH	MH MG	MH	MH	CAB
16	An expert-based landscape permeability model for assessing the impact of agricultural management on amphibian migration.	2013	PPL KT RBS CAB	CAB PPL	PPL CAB	PPL	PPl RBS	PPL CAB	KT RBS
17	Amphibian population genetics in agricultural landscapes: Do non-visible barriers in viniculture drive the population structuring of the European common frog *(Rana temporaria)*?	2017	PPL CAB CL KT	CAB PPL KT	CAB PPL KT	PPL	PPL CL KT	PPL KT	CAB CL

Publication	Title	Year	Co-authors (initials) following citataion order	Idea	Experimental design	Data gathering	Data analysis	Paper writing	Paper review
18	Protection of terrestrial non-target plant species in the regulation of environmental risks of pesticides	2015	JS PS CAB	CAB JS	JS PS CAB	JS PS CAB	JS PS CAB	JS PS CAB	JS PS CAB
19	Assessing the risk of herbicides to terrestrial non-target plants using higher-tier studies.	2015	JS PS CAB	CAB JS	JS PS CAB	JS PS CAB	JS PS CAB	JS PS CAB	JS PS CAB
20	Recovery	2012	CAB WP	CAB	-	CAB WP	-	CAB	WP
21	Exposure reduction of seed treatments through dehusking behaviour of the wood mouse *(Apodemus sylvaticus)*.	2011	CAB BG ME RBa	RBa ME	RBa ME CAB	BG	BG CAB	CAB	BG ME RBa
22	Amphibians and plant-protection products – what research and action is needed?	2016	AAld, MJ, CA, CAB, FS, BRS	AAld MJ	-	-	-	AAld, MJ, CA, CAB, FS, BRS	-
23	Constructed wetlands support bats in agricultural landscapes.	2012	PS, AP, LR, RS, CAB	CAB PS	PS CAB AP	PS AP LR CAB	PS	PS	PS AP RS CAB

Abbreviations of authors:

Initials	Name	Initials	Name	Initials	Name
AA	Annika Alscher	**JS**	Juliane Schmitz	**PU**	Philipp Uhl
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BRS	Benedikt R. Schmidt	**LR**	Lisa Ressl	**SP**	Silvia Pieper
BW	Brigitte Weber	**MB**	Mirco Bundschuh	**TS**	Thomas Schmidt
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Paul Neumann, Saskia Aldershof, Dave Bohan, Kevin Brown, Marco Candolfi, Flavia Geiger, Natalie Kovalkovičová, Christine Kula, Karin Nienstedt, Susana Pestanudo, Jörg Roembke, Thomas Schmidt, Chris Topping, Peter van Vliet

10. Appendix

The appendix contains links to the original publications in the order as mentioned in the main text. Selected publications are available via https://www.researchgate.net/profile/Carsten_Bruehl .

Scientific papers and documents by authorities citing the publications are listed afterwards to document the progress in the respective research area in October 2017.

Publication 1:

Melanie Hahn, Patrick P. Lenhardt & Carsten A. Brühl

(2014)

Characterization of field margins in intensified agro-ecosystems – why narrow margins should matter in terrestrial pesticide risk assessment and management.

Integrated Environmental Assessment and Management, 207, 153-162.

https://setac.onlinelibrary.wiley.com/doi/abs/10.1002/ieam.1535

This publication was cited in:

1. Tschumi, M., Albrecht, M., Bärtschi, C., Collatz, J., Entling, M. H., & Jacot, K. (2016). **Perennial, species-rich wildflower strips enhance pest control and crop yield**. *Agriculture, Ecosystems & Environment*, 220, 97-103.

2. Holland, J. M., Bianchi, F. J., Entling, M. H., Moonen, A. C., Smith, B. M., & Jeanneret, P. (2016). **Structure, function and management of semi-natural habitats for conservation biological control: A review of European studies**. *Pest management science*. DOI 10.1002/ps.4318.

3. Mania, E., Isocrono, D., Pedullà, M. L., & Guidoni, S. (2015). **Plant diversity in an intensively cultivated vineyard agro-ecosystem (Langhe, North-West Italy).** *South African Journal of Enology and Viticulture*, 36(3), 378-388.

Publication 2:

Peter Stahlschmidt & Carsten A. Brühl

(2012)

Bats as bioindicator species – The need of a standardized method for acoustic bat activity surveys.

Methods in Ecology and Evolution, 3, 503-508.

https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/j.2041-210X.2012.00188.x

This publication was cited in:

1. Monadjem, A., Shapiro, J. T., Mtsetfwa, F., Reside, A. E., & McCleery, R. A. (2017). **Acoustic call library and detection distances for bats of Swaziland**. *Acta Chiropterologica*, 19(1), 175-187.

2. Froidevaux, J. S., Louboutin, B., & Jones, G. (2017). **Does organic farming enhance biodiversity in Mediterranean vineyards**? A case study with bats and arachnids. *Agriculture, Ecosystems & Environment*, 249, 112-122.

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6. Crisol-Martínez, E., Ford, G., Horgan, F. G., Brown, P. H., & Wormington, K. R. (2017). **Ecology and conservation of insectivorous bats in fragmented areas of macadamia production in eastern Australia**. *Austral Ecology*, 42(5), 597-610.

7. Lisón, F., Altamirano, A., Field, R., & Jones, G. (2017). **Conservation on the blink: deficient technical reports threaten conservation in the Natura 2000 network**. *Biological Conservation*, 209, 11-16.

8. Bruckner, A. (2016) **Recording at water bodies increases the efficiency of a survey of temperate bats with stationary, automated detectors.** *Mammalia*. DOI: 10.1515/mammalia-2014-0067.

9. Adams, A. M., McGuire, L. P., Hooton, L. A., & Fenton, M. B. (2015). **How high is high? Using percentile thresholds to identify peak bat activity.** *Canadian Journal of Zoology*, 93(4), 307-313.

10. Newson, S. E., Evans, H. E., & Gillings, S. (2015). **A novel citizen science approach for large-scale standardised monitoring of bat activity and distribution, evaluated in eastern England.** *Biological Conservation*, 191, 38-49.

11. Goodenough, A. E., Deans, L., Whiteley, L., & Pickering, S. (2015). **Later is better: optimal timing for walked activity surveys for a European bat guild.** *Wildlife Biology*, 21(6), 323-328.

12. Millon, L., Julien, J. F., Julliard, R., & Kerbiriou, C. (2015). **Bat activity in intensively farmed landscapes with wind turbines and offset measures.** *Ecological Engineering*, 75, 250-257.

13. Azam, C., Kerbiriou, C., Vernet, A., Julien, J. F., Bas, Y., Plichard, L., ... & Le Viol, I. (2015)**. Is part-night lighting an effective measure to limit the impacts of artificial lighting on bats?** *Global Change biology*, 21(12), 4333-4341.

14. Lacoeuilhe, A., Machon, N., Julien, J. F., Le Bocq, A., & Kerbiriou, C. (2014). **The influence of low intensities of light pollution on bat communities in a semi-natural context.** *PloS one*, 9(10), e103042.

15. Tonos, J. M., Pauli, B. P., Zollner, P. A., & Haulton, G. S. (2014). **A comparison of the efficiency of mobile and stationary acoustic bat surveys.** *Proceedings of the Indiana Academy of Science*, 123(2).

16. Lisón, F., & Calvo, J. F. (2014). **Bat activity over small ponds in dry Mediterranean forests: implications for conservation.** *Acta Chiropterologica*, 16(1), 95-101.

17. Andreassen, T., Surlykke, A., & Hallam, J. (2014). **Semi-automatic long-term acoustic surveying: A case study with bats.** *Ecological Infor*matics, 21, 13-24.

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19. Lacoeuilhe, A., Machon, N., Julien, J. F., Le Bocq, A., & Kerbiriou, C. (2014). **The influence of low intensities of light pollution on bat communities in a semi-natural context.** *PloS one*, 9(10), e103042.

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21. Müller, J., Brandl, R., Buchner, J., Pretzsch, H., Seifert, S., Strätz, C., ... & Fenton, B. (2013). **From ground to above canopy - Bat activity in mature forests is driven by vegetation density and height**. *Forest Ecology and Management*, 306, 179-184.

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23. Marques, J. T., Pereira, M. J. R., Marques, T. A., Santos, C. D., Santana, J., Beja, P., & Palmeirim, J. M. (2013). **Optimizing sampling design to deal with mist-net avoidance in Amazonian birds and bats.** *PloS one*, 8(9), e74505.

Publication 3:

Peter Stahlschmidt, Melanie Hahn & Carsten A. Brühl

(2017)

Nocturnal risks - High bat activity in the agricultural landscape indicates potential pesticide exposure.

Frontiers in Environmental Science. 5, 62, 1-9

(doi: 10.3389/fenvs.2017.00062).

https://www.frontiersin.org/articles/10.3389/fenvs.2017.00062/full

Publication 4:

Patrick P. Lenhardt, Carsten A. Brühl & Gert Berger

(2015)

Temporal coincidence of adult amphibians and pesticide applications on arable fields during spring migration.

Basic and Applied Ecology, 16(1), 54–63.

https://www.sciencedirect.com/science/article/pii/S1439179114001455

This publication was cited in:

1. Cusaac, J. P. W., Mimbs, W. H., Belden, J. B., Smith, L. M., & McMurry, S. T. (2017). **Factors influencing the toxicity of Headline® fungicides to terrestrial stage toads**. *Environmental Toxicology and Chemistry.* 22-25.

2. Mikó, Z., Ujszegi, J., Gál, Z., & Hettyey, A. (2017). **Standardize or diversify experimental conditions in Ecotoxicology? A case study on herbicide toxicity to larvae of two anuran amphibians.** *Archives of Environmental Contamination and Toxicology*, 1-8.

3. Aldrich, A., Junghans, M., Aeberli, C., Brühl, C. A., Streissl, F., & Schmidt, B. R. (2016). **Amphibians and plant-protection products: what research and action is needed?** *Environmental Sciences Europe*, 28(1), 1.

4. Van Meter, R. J., Glinski, D. A., Henderson, W. M., & Purucker, S. T. (2016). **Soil organic matter content effects on dermal pesticide bioconcentration in American toads *(Bufo americanus)*.** *Environmental Toxicology and Chemistry*. DOI: 10.1002/etc.3439.

5. Cusaac, J. P. W., Morrison, S. A., Belden, J. B., Smith, L. M., & McMurry, S. T. (2016). **Acute toxicity of Headline® fungicide to Blanchard's cricket frogs *(Acris blanchardi)*.** *Ecotoxicology*, 25(3), 447-455.

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8. Van Meter, R. J., Glinski, D. A., Henderson, W. M., Garrison, A. W., Cyterski, M., & Purucker, S. T. (2015). **Pesticide uptake across the amphibian dermis through soil and overspray exposures.** *Archives of environmental contamination and toxicology*, 69(4), 545-556.

Publication 5:

Melanie Hahn & Carsten A. Brühl

(2016)

The secret pollinators – an overview of moth pollination in natural and agricultural habitats with a focus on Europe and North America.

Arthropod-Plant Interactions, 10(1), 21–28.

https://link.springer.com/article/10.1007/s11829-016-9414-3

This publication was cited in:

1. Tierney, S. M., Friedrich, M., Humphreys, W. F., Jones, T. M., Warrant, E. J., & Wcislo, W. T. (2017). **Consequences of evolutionary transitions in changing photic environments**. *Austral Entomology*.

2. Ollerton, J. (2017). **Pollinator diversity: distribution, ecological function, and conservation.** *Annual Review of Ecology, Evolution, and Systematics*, 48(1).

3. Weller, S. G., Sakai, A. K., Campbell, D. R., Powers, J. M., Peña, S. R., Keir, M. J., ... & Weisenberger, L. (2017). **An enigmatic Hawaiian moth is a missing link in the adaptive radiation of Schiedea**. *New Phytologist*, 213(3), 1533-1542.

4. Funamoto, D., & Ohashi, K. (2017). **Hidden floral adaptation to nocturnal moths in an apparently bee-pollinated flower, *Adenophora triphylla* var. *japonica* (Campanulaceae).** *Plant Biology*.

5. Alonso-Rodríguez, A. M., Finegan, B., & Fiedler, K. (2017). **Neotropical moth assemblages degrade due to oil palm expansion**. *Biodiversity and Conservation*, 26(10), 2295-2326.

6. Wester, P., & Lunau, K. (2017). **Chapter nine - Plant–Pollinator communication**. *Advances in Botanical Research*, 82, 225-257.

7. Alonso, C., Arceo-Gómez, G., Meindl, G. A., Abdala-Roberts, L., Parra-Tabla, V., & Ashman, T. L. (2017). **Delimiting plant diversity that is functionally related via interactions with diurnal pollinators: An expanded use of rarefaction curves**. *Flora*, 232, 56-62.

8. Chakraborty, P., Smith, B., & Basu, P. (2017) **Pollen transport in the dark: Hawkmoths prefer non crop plants to crop plants in an agricultural landscape**. In *Proceedings of the Zoological Society* (pp. 1-5). Springer India.

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10. Funamoto, D., & Sugiura, S. (2016). **Settling moths as potential pollinators of *Uncaria rhynchophylla* (Rubiaceae)**. *European Journal of Entomology*, 113, 497.

Publication 6:

Carsten A. Brühl, Silvia Pieper & Brigitte Weber

(2011)

Amphibians at risk? – Susceptibility of terrestrial amphibian life stages to pesticides.

Environmental Toxicology and Chemistry, 30(11), 2465–2472.

https://setac.onlinelibrary.wiley.com/doi/full/10.1002/etc.650

This publication was cited in:

1. Holzer, K. A., Bayers, R. P., Nguyen, T. T., & Lawler, S. P. (2017). **Habitat value of cities and rice paddies for amphibians in rapidly urbanizing Vietnam**. *Journal of Urban Ecology*, 3(1), juw007.

2. Strong, R., Martin, F. L., Jones, K. C., Shore, R. F., & Halsall, C. J. (2017). **Subtle effects of environmental stress observed in the early life stages of the Common frog, *Rana temporaria***. *Scientific Reports*, 7.

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6. Wagner, Norman, et al. (2017) **Population and life-stage–specific effects of two herbicide formulations on the aquatic development of European common frogs *(Rana temporaria)***. *Environmental Toxicology and Chemistry* 36(1), 190-200.

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8. Marlatt, V. L., & Martyniuk, C. J. (2017). **Biological responses to phenylurea herbicides in fish and amphibians: New directions for characterizing mechanisms of toxicity**. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 194, 9-21.

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11. Aldrich, A., Junghans, M., Aeberli, C., Brühl, C. A., Streissl, F., & Schmidt, B. R. (2016). **Amphibians and plant-protection products: what research and action is needed?** *Environmental Sciences Europe*, 28(1), 1.

12. Jordan, M. A., Castañeda, A. J., Smiley, P. C., Gillespie, R. B., Smith, D. R., & King, K. W. (2016). **Influence of instream habitat and water chemistry on amphibians in channelized agricultural headwater streams.** *Agriculture, Ecosystems & Environment*, 230, 87-97.

13. Li, M., Li, S., Yao, T., Zhao, R., Wang, Q., & Zhu, G. (2016). **Waterborne exposure to triadimefon causes thyroid endocrine disruption and developmental delay in *Xenopus laevis* tadpoles.** *Aquatic Toxicology*. 177, 190-197.

14. Soloneski, S., de Arcaute, C. R., & Larramendy, M. L. (2016). **Genotoxic effect of a binary mixture of dicamba-and glyphosate-based commercial herbicide formulations on *Rhinella arenarum* (Hensel, 1867)(Anura, Bufonidae) late-stage larvae.** *Environmental Science and Pollution Research*, 1-11.

15. Li, M., Cao, C., Li, S., Gui, W., & Zhu, G. (2016). **Thyroid endocrine disruption of azocyclotin to *Xenopus laevis* during metamorphosis**. *Environmental toxicology and pharmacology*, 43, 61-67.

16. Onadeko, A. B. (2016). **Effect of Cocoa *(Theobroma cacao)* plantation types on the distribution of anuran communities in Ore, Ondo State, Nigeria.** *Journal of Applied Sciences and Environmental Management*, 20(1), 13-22.

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20. Wagner, N., Veith, M., Lötters, S., & Viertel, B. (2016). **Population and life‐stage–specific effects of two herbicide formulations on the aquatic development of European common frogs *(Rana temporaria)*.** *Environmental Toxicology and Chemistry*. DOI: 10.1002/etc.3525.

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22. Lajmanovich, R. C., Attademo, A. M., Simoniello, M. F., Poletta, G. L., Junges, C. M., Peltzer, P. M., & Cabagna-Zenklusen, M. C. (2015). **Harmful effects of the dermal intake of commercial formulations containing Chlorpyrifos, 2, 4-D, and Glyphosate on the Common toad *Rhinella arenarum* (Anura: Bufonidae).** *Water, Air, & Soil Pollution*, 226(12), 1-12.

23. Van Meter, R. J., Glinski, D. A., Henderson, W. M., Garrison, A. W., Cyterski, M., & Purucker, S. T. (2015). **Pesticide uptake across the amphibian dermis through soil and overspray exposures.** *Archives of environmental contamination and toxicology*, 69(4), 545-556.

24. Ujszegi, J., Gál, Z., Mikó, Z., & Hettyey, A. (2015). **No observable effect of a glyphosate‐based herbicide on two top predators of temporal water bodies.** *Environmental Toxicology and Chemistry*, 34(2), 307-313.

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26. Van Meter, R. J., Glinski, D. A., Hong, T., Cyterski, M., Henderson, W. M., & Purucker, S. T. (2014). **Estimating terrestrial amphibian pesticide body burden through dermal exposure.** *Environmental Pollution*, 193, 262-268.

27. De Arcaute, C. R., Pérez-Iglesias, J. M., Nikoloff, N., Natale, G. S., Soloneski, S., & Larramendy, M. L. (2014). **Genotoxicity evaluation of the insecticide imidacloprid on circulating blood cells of Montevideo tree frog *Hypsiboas pulchellus* tadpoles (Anura, Hylidae) by comet and micronucleus bioassays.** *Ecological Indicators*, 45, 632-639.

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30. Farabaugh, N. F., & Nowakowski, A. J. (2014). **Behavioral responses of the Strawberry Poison Frog *(Oophaga pumilio)* to herbicide olfactory cues: possible implications for habitat selection and movement in altered landscapes.** *Canadian Journal of Zoology*, 92(11), 979-984.

31. Nikoloff, N., Natale, G. S., Marino, D., Soloneski, S., & Larramendy, M. L. (2014). **Flurochloridone-based herbicides induced genotoxicity effects on *Rhinella arenarum* tadpoles (Anura: Bufonidae).** *Ecotoxicology and environmental safety*, 100, 275-281.

32. Wagner, N., Rödder, D., Brühl, C. A., Veith, M., Lenhardt, P. P., & Lötters, S. (2014). **Evaluating the risk of pesticide exposure for amphibian species listed in Annex II of the European Union Habitats Directive.** *Biological Conservation*, 176, 64-70.

33. Wagner, N., Züghart, W., Mingo, V., & Lötters, S. (2014). **Are deformation rates of anuran developmental stages suitable indicators for environmental pollution? Possibilities and limitations.** *Ecological Indicators*, 45, 394-401.

34. Böll, S., Schmidt, B., Veith, M., Wagner, N., Rödder, D., Weinmann, C., ... & Loetters, S. (2013). **Amphibians as indicators of changes in aquatic and terrestrial**

ecosystems following GM crop cultivation: a monitoring guideline. *BioRisk*, 8, 39.

35. Bernabò, I., Guardia, A., La Russa, D., Madeo, G., Tripepi, S., & Brunelli, E. (2013). **Exposure and post-exposure effects of endosulfan on *Bufo bufo* tadpoles: Morpho-histological and ultrastructural study on epidermis and iNOS localization.** *Aquatic toxicology*, 142, 164-175.

36. Berger, G., Graef, F., & Pfeffer, H. (2013). **Glyphosate applications on arable fields considerably coincide with migrating amphibians.** *Scientific reports*, 3, 2622.

37. Wagner, N., Reichenbecher, W., Teichmann, H., Tappeser, B., & Lötters, S. (2013). **Questions concerning the potential impact of glyphosate-based herbicides on amphibians.** *Environmental Toxicology and Chemistry*, 32(8), 1688-1700.

38. Andreu, V., & Picó, Y. (2012). **Determination of currently used pesticides in biota.** *Analytical and bioanalytical chemistry*, 404(9), 2659-2681.

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Publication 7:

Carsten A. Brühl, Thomas Schmidt, Silvia Pieper & Annika Alscher

(2013)

Terrestrial pesticide exposure of amphibians: An underestimated cause of global decline?

Scientific Reports, 3, 1135. DOI: 10.1038/srep01135.

https://www.nature.com/articles/srep01135

This publication was cited in:

1. Pochini, K. M., & Hoverman, J. T. (2017). **Immediate and lag effects of pesticide exposure on parasite resistance in larval amphibians**. *Parasitology*, 144(6), 817-822.

2. Pochini, K. M., & Hoverman, J. T. (2017). **Reciprocal effects of pesticides and pathogens on amphibian hosts: The importance of exposure order and timing**. *Environmental Pollution*, 221, 359-366.

3. Cusaac, J. P. W., Mimbs, W. H., Belden, J. B., Smith, L. M., & McMurry, S. T. (2017). **Factors influencing the toxicity of Headline® fungicides to terrestrial stage toads**. *Environmental Toxicology and Chemistry*.

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10. Wagner, N., Müller, H., & Viertel, B. (2017). **Effects of a commonly used glyphosate-based herbicide formulation on early developmental stages of two anuran species**. *Environmental Science and Pollution Research*, 24(2), 1495-1508.

11. Johnson, M. S., Aubee, C., Salice, C. J., Leigh, K. B., Liu, E., Pott, U., & Pillard, D. (2017). **A review of ecological risk assessment methods for amphibians: Comparative assessment of testing methodologies and available data**. *Integrated environmental assessment and management.*

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13. Mikó, Z., Ujszegi, J., Gál, Z., & Hettyey, A. (2017). **Standardize or diversify experimental conditions in Ecotoxicology? A case study on herbicide toxicity to larvae of two anuran amphibians.** *Archives of Environmental Contamination and Toxicology*, 1-8.

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