TEXTE
20/2015

Protection of terrestrial non-target plant species in the regulation of environmental risks of pesticides

For our Environment

Umwelt Bundesamt

TEXTE 20/2015

Project No. (FKZ) 360 03 053

Report No. (UBA-FB) 001962/E

Protection of terrestrial non-target plant species in the regulation of environmental risks of pesticides

by

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On behalf of the Federal Environment Agency (Germany)

Imprint

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Study completed in:
July 2013

Edited by:
Section IV 1.3 Plant Protection Products
Carmen Schweikert

Publication as pdf:
http://www.umweltbundesamt.de/publikationen/protection-of-terrestrial-non-target-plant-species

ISSN 1862-4804

Dessau-Roßlau, March 2015

The Project underlying this report was supported with funding from the Federal Ministry for the Environment, Nature Conservation, Building and Nuclear safety under project number FKZ 360 003 053. The responsibility for the content of this publication lies with the author(s).

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Research Boxes

Abstract

Before a new herbicide is approved for placement on the market, it needs to be evaluated in accordance to current risk assessment schemes that require the performance of non-target plant studies. These studies are generally conducted with crop plants under laboratory conditions following specific guidelines (Tier II studies according to OECD protocols). The aim of this report was to evaluate the sensitivity of the tested plant species compared to plants present in natural communities bordering crops. Additionally, more complex higher tier studies were assessed for their realism and possibilities for extrapolation to natural communities. Furthermore, we evaluated the few present studies that consider herbicide effects on natural communities and the protectivity of the current risk assessment scheme. Therefore, we searched the current literature in various databases and assessed draft assessment reports for herbicides published by EFSA and internal data of UBA to establish a database on endpoints obtained in standard Tier II studies.

The analyzed toxicity data revealed that in all evaluated non-target plant studies only crop plants were tested. Wild plant species tested in research studies showed a hundredfold higher sensitivity in data sets for glyphosate and dicamba. These two data sets are the only ones that allow this kind of comparison and the influence of cultivar variety of crop plants, differences in herbicide formulations and varying test conditions are discussed as confusing factors in this aspect and especially the latter call for an improvement of current test protocols. 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 showed low sensitivities for a few herbicides assessed. Among them were oats, onion and soybean and these species should be excluded from testing and replaced by wild plants. The question about how many plant species need to be tested to account for the sensitivity of the plant community could not be finally answered since only two data sets are available that were generated from different studies. So far only higher plants are tested and the sensitivity of terrestrial algae, mosses, ferns and lichen is only in very few studies assessed.

Only 19 studies with herbicide higher tier studies and field assessments of natural communities were located. Realistic drift studies performed in the field represent the field application perfectly and also address breakdown of the

product in the field under natural conditions. Exposure might therefore be reduced compared to Tier II standard laboratory studies. However, weather conditions have a big influence on spray drift exposure and therefore studies should be conducted under a realistic worst case scenario. Microcosm studies and field studies used a design where more species (between six and nine) were planted together in one container. Exposure was conducted either in greenhouses or in the field. It is assumed that these studies include interaction effects such as intra- or interspecific competition or shielding (lowering of exposure). Compared to natural plant communities, plant densities are low; plants are usually grown under optimum conditions (no resource limitation, no other stressors) and are exposed at young life stages. The experiments performed in natural plant communities showed that interactions between plant species cannot be predicted from Tier II standard studies. Since plants are exposed to herbicides at older life stages in the field (e.g. in spring when budding) especially reproductive endpoints are very sensitive. These endpoints are the relevant measures to address the persistence of a population of plants in the field. In the few available published field studies sublethal effects due to phytotoxicity were noticed in natural plant communities at experimental rates below the regulatory acceptable rates. Hence, it seems possible that the current risk assessment does not provide sufficient protection of non-target plant species and their habitats.

Plants provide the energy and form the basis of the food chain in any ecosystem. In agricultural landscapes birds and mammals depend on invertebrates that include herbivorous insects. This group of insects depends on often highly specific food plants and many different species are consuming various parts of a plant leading to high biodiversity. Herbicides have the potential to interrupt the food web in agricultural landscapes not only by killing or reducing the density of specific plants but also by reducing the food quality for herbivorous insects. It is therefore of great significance to protect natural plant communities in non-target habitats (such as field margins or hedges) from adverse effects of herbicide applications in fields.

In Germany most field margins are narrow (below 3 m wide) and are not considered a non-target habitat. This has the consequence that in the first meter even overspray occurs and impacts on plant community composition are expected. A current monitoring study revealed that the common buttercup (*Ranunculus acris*), a common species of meadows, almost vanished from field margins. The same plant species also revealed an 85% reduction of flowering after herbicide applications. In some studies an in-field buffer of 8-10 m is suggested as an option to avoid negative herbicide effects on plant communities bordering crops. Since our knowledge of herbicide effects in natural plant communities is poor and no studies are present that provide results to bridge from standard Tier II studies to natural communities we propose to consider the development of risk mitigation options in parallel to refinement of current risk assessment schemes. An in-field buffer where no herbicide is applied will reduce drift to low percentages and therefore reduce exposure and effects. The development and implementation of this risk management option is a complicated task and requires further applied research and the inclusion of socio-economic considerations. This approach has the potential to restore biodiversity in agricultural ecosystems and reestablish dwindling ecosystem services such as pollination and biological pest control.

Zusammenfassung

Bevor ein neues Herbizid auf dem Markt zugelassen wird, muss es nach den aktuellen Risikobewertungsrichtlinien bewertet werden, die die Durchführung von Nicht-Ziel-Pflanzenstudien erfordern. Diese Studien werden in der Regel mit Kulturpflanzen nach bestimmten Richtlinien (Tier-II-Studien nach OECD-Protokollen) unter Laborbedingungen durchgeführt. Das Ziel dieses Gutachtens war es, die Empfindlichkeit der getesteten Pflanzenarten im Vergleich zu Pflanzenarten, die an landwirtschaftliche Kulturen angrenzen, zu bewerten. Zusätzlich wurden komplexere, höherstufige Pflanzentests auf ihre Realitätsnähe und der Möglichkeit zur Extrapolation auf natürliche Pflanzengemeinschafen beurteilt. Darüber hinaus haben wir die wenigen vorliegenden Studien, die Herbizideffekte auf natürliche Gemeinschaften untersuchen, ausgewertet um die Protektivität des aktuellen Risikobewertungsschemas zu beurteilen. Eine Literaturrecherche wurde unter Verwendung verschiedener Datenbanken durchgeführt, ebenso wurden veröffentlichte „Draft Assessment Reports“ der EFSA und interne Daten des UBA hinzugezogen, um eine Datenbank mit Endpunkten aus Standard-Tier-II-Studien für verschiedene Herbizide zu etablieren.

Die analysierten Toxizitätsdaten zeigten, dass in allen untersuchten Nicht-Ziel-Pflanzenstudien nur Nutzpflanzen getestet wurden. Allerdings zeigten Wildpflanzenarten in Forschungsprojekten eine hundertfach höhere Sensitivität in Datensätze für Glyphosat und Dicamba. Diese zwei Datensätze sind die einzigen die diese Art des Vergleichs erlauben, wobei der Einfluss der Sorte der verschiedenen Kulturpflanzen, Unterschiede in Herbizidformulierungen und variierende Testbedingungen als verwirrend Faktoren diskutiert werden, und insbesondere letztere erfordern eine Verbesserung der aktuellen Test-Protokolle. Die meisten der natürlich in Feldrändern vorkommenden Pflanzenfamilien werden nicht in Tests mit einbezogen, obwohl einige von ihnen mit vielen Arten auftreten und auch eine hohe Herbizid-Empfindlichkeit zeigen (z.B. Lamiaceae). Einige der häufig in Nicht-Ziel-Pflanzenstudien für regulatorische Zwecke verwendete Nutzpflanzen zeigten eine geringe Empfindlichkeit gegenüber Herbiziden, darunter Hafer, Zwiebel und Soja. Diese Arten sollten daher von der Prüfung ausgeschlossen werden und stattdessen Wildpflanzen verwendet werden. Die Frage, wie viele Pflanzenarten zur Abdeckung der Empfindlichkeit der Pflanzengemeinschaft geprüft werden müssen, konnte nicht endgültig beantwortet werden, da nur zwei Datensätze zur Verfügung stehen, die zudem aus verschiedenen Studien generiert wurden. Bisher sind nur höhere Pflanzen

getestet worden und die Empfindlichkeit der terrestrischen Algen, Moose, Farne und Flechten ist nur in sehr wenigen Studien untersucht.

Es wurden nur 19 Studien mit höherstufigen Untersuchungen und Freilandbetrachtungen zu Herbizideffekten auf natürliche Gemeinschaften gefunden. Realistische, im Freiland durchgeführte Abdrift-Studien repräsentieren die Anwendung im Feld perfekt und stellen auch den Abbau des Produkts unter natürlichen Bedingungen dar. Die Exposition könnte daher im Vergleich zum Tier-II-Standard Laborstudien verringert sein. Allerdings haben Witterungsbedingungen einen großen Einfluss auf die abdriftrelevante Exposition und daher sollten diese Studien unter einem realistischen Worst-Case-Szenario durchgeführt werden. Mikrokosmos Studien und Feldstudien verwendeten einen Studienaufbau in dem mehrere Arten (zwischen sechs und neun) zusammen in einem Behälter gepflanzt wurden. Die Exposition wurde entweder in Gewächshäusern oder im Feld durchgeführt. Es wird davon ausgegangen, dass diese Studien Wechselwirkungen, wie intra-oder interspezifische Konkurrenz oder Abschirmung (Senkung der Exposition), darstellen. Im Vergleich zu natürlichen Pflanzengemeinschaften, sind die Bestandsdichten allerdings niedrig, die Pflanzen wachsen in der Regel unter optimalen Bedingungen heran (keine Begrenzung der Ressourcen, keine anderen Stressoren) und die Exposition erfolgt in jungen Lebensphasen. Die Experimente die in natürlichen Pflanzengemeinschaften durchgeführt wurden, zeigten, dass Interaktionen zwischen Pflanzenarten nicht von Tier-II-Standard Untersuchungen vorhergesagt werden können. Da Pflanzen oft in älteren Lebensphasen Herbizidbehandlungen ausgesetzt sind (z. B. im Frühjahr, bei Blatt-/Blütenaustrieb), sind besonders reproduktive Endpunkte sehr empfindlich. Diese Endpunkte sind die relevanten Messwerte um das Fortbestehen einer Population von Pflanzen in der Natur einschätzen zu können. In den wenigen vorhandenen veröffentlichten Felduntersuchungen wurden subletale Effekte auf Grund von Phytotoxizität in natürlichen Pflanzengemeinschaften unterhalb der Regulatorisch Akzeptierten Rate festgestellt. Es scheint daher möglich, dass die aktuelle Risikobewertung eine nicht ausreichende Protektivität für Nicht-Ziel Pflanzen und ihr Habitat bietet.

Pflanzen liefern die Energie und bilden die Grundlage der Nahrungskette in einem Ökosystem. In Agrarlandschaften sind Vögel und Säugetiere abhängig von Wirbellosen, zu denen auch pflanzenfressenden Insekten gehören. Diese Gruppe von Insekten hängt oft von sehr spezifischen Futterpflanzen ab und viele verschiedene Arten verbrauchen unterschiedliche Teile einer Pflanze, was zu einer hohen Biodiversität des Systems führt. Herbizide haben das Potenzial das

Nahrungsnetz in der Agrarlandschaft zu unterbrechen, nicht nur durch die Dichteverringerung oder komplette Verdrängung von spezifischen Pflanzen, sondern auch durch eine Verringerung der Nahrungsqualität für pflanzenfressende Insekten. Es ist daher von großer Bedeutung natürliche Pflanzengemeinschaften in Nicht-Ziel-Lebensräume (z. B. Feldsäumen oder Hecken) vor den nachteiligen Auswirkungen von Herbizid-Anwendungen auf Feldern zu schützen.

In Deutschland sind die meisten Feldsäume schmal (unter 3 m breit) und werden nicht als Lebensraum für Nicht-Zielorganismen betrachtet. Dies hat zur Folge, dass der erste angrenzende Meter eines Feldsaums sogar überspritzt wird und Auswirkungen auf die Zusammensetzung der Pflanzengemeinschaft zu erwarten sind. Eine aktuelle Monitoring-Studie ergab, dass der scharfe Hahnenfuß *(Ranunculus acris)*, eine auf Wiesen häufige Art, fast vollständig von Feldrändern verschwunden ist. Die gleiche Pflanzenart zeigte auch eine 85%ige Reduktion der Blütenbildung nach den Herbizid-Anwendungen. In einigen Studien wurde ein im Feld gelegener nicht gespritzter Pufferbereich als Option zur Minimierung von negativen Herbizideffekten auf die Pflanzengemeinschaften, die an Kulturen angrenzen, vorgeschlagen

Da unser Wissen bezüglich Herbizideffekte auf natürlichen Pflanzengemeinschaften sehr gering ist und keine Studien vorhanden sind, die die nötigen Ergebnisse liefern, um die Brücke von Standard-Tier-II-Studien zu natürlichen Gemeinschaften zu schlagen, empfehlen wir Risikominderungsoptionen in Betracht zu ziehen. Ein im Feld gelegener Applikationspuffer, wird die Abdrift in Feldsäume auf geringe Prozentsätze reduzieren und damit die Exposition und resultierende Effekte vermindern.

Die Entwicklung und Umsetzung dieses Risikomanagementansatzes ist eine komplizierte Aufgabe und erfordert weitere angewandte Forschung und die Einbeziehung von sozio-ökonomischen Überlegungen. Dieser Ansatz hat das Potenzial, die biologische Vielfalt in den landwirtschaftlich geprägten Ökosystemen wieder herzustellen und die schwindenden Ökosystemleistungen, wie Bestäubung und biologische Schädlingsbekämpfung, wieder aufzubauen.

1. Background

1.1. Introduction

In Europe, agriculture is the dominating land-use, covering nearly half of the EU member states' surface area (Stoate et al. 2009). In Germany 53% of the area is under agricultural use, whereof 70% are planted with crops (Statistisches Bundesamt 2004). The control of the proliferation of harmful pest organisms in the widespread cultivation of monocultures requires a high pesticide input (Robinson et al. 2002). The significant role of herbicides among pesticides is demonstrated by the fact that they are the most commonly used pesticides worldwide (Sánchez-Bayo 2011). The quantity applied, the large number of different herbicides used and the geographical extent of their use on different crops might result in side-effects on non-target plants in habitats adjacent to cultivated fields, vineyards or orchards. A reduction of the plant diversity in agricultural landscapes was detected in different parts of Europe (e.g. Andreasen 1996). Many different factors such as changes in crop rotation, fertilization, and mechanization have contributed to the observed changes and it is not possible to quantify unambiguously the impact of any single factor however, herbicides are considered a major driving force (Andreasen & Streibig 2011).

Field margins, known for their high ecological significance (De Snoo & van der Poll 1999), are by far the most common habitat types remaining for wild plants in farmlands. Field margins adjacent to cropped areas (fields, vineyards and orchards) are mainly affected by spray drift of pesticide applications. Moreover, field margins below 3 m wide, the typical margins left in the agricultural landscape, are not considered a non-target terrestrial habitat in Germany, and consequently, no minimum distance or drift reducing technology is required (BVL 2011). In a large-scale monitoring study the decreasing diversity of the flora of field margins was demonstrated for several regions in Germany (Roß-Nickoll et al. 2004).

Since plants form the energetic basis of terrestrial ecosystems, all other non-target organisms of agricultural ecosystems are very likely to be affected by changes in plant diversity, community composition and abundance. For instance, the reduction of plant biomass might affect herbivorous insects as well (Marshall et al. 2003). Insects, in turn, are substantial food sources for species of higher trophic levels such as birds and bats. Well known examples of farmland birds whose breeding success is influenced by the availability of insect chick food include the Grey Partridge *(Perdix perdix)* and the skylark *(Alauda arvensis)* (Wilson et al. 1999; Boatman et al. 2004).

The conservation of biodiversity is a key objective of the Convention on Biological Diversity (United Nations Conference in Rio de Janeiro in 1992), sanctioned by more than 190 countries, among them Germany. The protection of terrestrial non-target plants from adverse effects of pesticides is based on international (EU Directive 91/414/EEC) and national (Plant Protection Act) regulations. Standardized test methods for toxicity assessments of herbicides to non-target plants are conducted in accordance with existing OECD guidelines (OECD 208 and 227).

1.2. Risk assessment of herbicides

Before a new herbicide is approved for placement on the market, it needs to be evaluated in accordance with the Plant Protection Product Directive (Council Directive 91/414/EEC). Annex II and III of the Directive 91/414/EEC set out the data requirements for the inclusion of an active substance to Annex I of the Directive and for authorization of a plant protection product at Member State level. This directive requires that all unwanted effects are reported and that further studies have to be carried out when effects are indicated. Data requirements and testing of effects on non-target plants are described in the Guidance Document on Terrestrial Ecotoxicology, which serves as background for the Council Directive (European Commission 2002). According to this Guidance Document, the risk assessment of herbicides follows a tiered testing approach with three different steps (Tier I, Tier II and Tier III; European Commission 2002). The first tier (Tier I) is a preliminary assessment, also described as an "initial screening", which should be conducted with at least six plant species grown individually in pots. These species should be exposed to the highest nominal application rate and if the results show more than 50% effect for one plant species, data requirements and assessments move to the next tier. However, this initial step is always unprofitable for herbicides since these tests inevitably will end up in the second step.

The second tier (Tier II) is a quantitative risk assessment. In this step the risk for terrestrial plants should be assessed using a TER (toxicity exposure ratio) approach of the most susceptible plant species. The TER is calculated by using the determined PEC (predicted environmental concentration) and the estimated ER (effect rate) values. The Guidance Document on Terrestrial Ecotoxicology provides the possibility to assess the risk of herbicides on non-target plants with two methods depending on the data set obtained from the tests: a deterministic and a probabilistic approach.

In the deterministic approach, rate-response experiments with at least six plant species have to be conducted. These tests are generally performed separately for annual crop plant species in young development stages (2-4 leaf stage) in greenhouse experiments. The effects on the plants in the greenhouse experiments are expressed in terms of application rate (g or mL/ha) and the effect data are represented by ER_{50} values (= application rate causing 50% effect). If the calculated TER-value based on the most sensitive plant species is greater than 5 effects on non-target plants are considered acceptable. Otherwise, the risk for terrestrial plants is assumed to be not acceptable.

In the probabilistic approach a species sensitivity distribution (SSD) with six to ten plant species should be determined. Species-sensitivity distribution methods assemble single-species toxicity data (e.g. ER_{50} vales) to predict hazardous concentrations (HC) affecting a certain percentage of species in a community (Newman et al. 1999). Therefore, a log-normal or another defined type of distribution of the data should be presented. Generally, this approach is considered as more suitable then the deterministic calculation since the variability in species sensitivity can be characterized. If the HC5 (hazard concentration for 5% of the species) is below the highest predicted exposure value, the risk for terrestrial plants is assumed to be not acceptable. In this case a Tier III study, a semi-field or field study, using realistic exposure have to be conducted, if no options for the refinement of exposure and effects (e.g. with risk mitigation strategies) can or will be undertaken.

For Tier I and Tier II, it is recommended to follow the test guidelines developed by OECD (OECD 208 and 227; OECD 2006 or the OPPTS test guidelines developed by the US EPA (European Commission 2002). No guideline exists for the conduct of Tier III studies. However, currently the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC is under revision and therefore, also the data requirements and testing of effects on non-target plants. Thus, there is a need to investigate to what extent non-target plants, as well as non-target plant communities, are protected by the current risk assessment.

1.3. Aim

The aim of this project was to assemble the data that address the effects of herbicides on non-target plants growing in semi-natural habitats adjacent to agricultural fields and to evaluate whether the current risk assessment provides a sufficient protection of natural plant communities. Therefore, a detailed literature search was performed to identify scientific studies that could enhance our understanding of herbicide effects on natural plant communities. Moreover, an evaluation of existing and available toxicity data of herbicides for non-target plants was performed to understand whether the sensitivity of the test species (mainly crop species) used in standard risk assessment tests represents an adequate safety for wild plant species growing in semi-natural habitats. In accordance with these findings the current risk assessment for terrestrial non-target plants was evaluated.

The project focused on three main topics:

1. Species sensitivity of plants towards herbicides (see chapter 3)
2. State of knowledge of higher tier studies (see chapter 4)
3. Evaluation of the current risk assessment approach (see chapter 5)

2. Methodological approach

The aim of this review was the development of recommendations for an enhancement of the current risk assessment for terrestrial non-target plants. Therefore, a comprehensive literature search was conducted to provide an overview of studies evaluating the effects of herbicides on non-target plants in greenhouse, microcosm and field studies and to identify the current knowledge of phytotoxicity testing (tiered testing approach, Tier I, II and III).

The literature was searched mainly within the database “ISI Web of Knowledge”, in which the current published literature in “peer-reviewed” journals can be found comprising the database “Medline” (1950-now), “Web of Science” (1990-now) and “Science Citation Index Expanded” (1990-now)). Furthermore, the databases “OvidSP” and “Google scholar” were used where older literature, as well as books and the so called “grey literature” are listed. In addition, former research and development reports (F+E Berichte) of the German Federal Environment Agency (Umweltbundesamt UBA) were also integrated in the analysis. For the literature search in the databases multiple search terms in English and German were used, e.g. non-target plant, field margin, herbicide drift, ecotoxicology, risk assessment, exposure, phytotoxicity test, greenhouse experiment, microcosm, field study AND/OR e.g. plant community, margin, boundary, pesticide, herbicide, fertilizer, vegetation and agriculture. The resulting hits were carefully screened and the cited sources, as well as the articles in which this literature had been cited were also analysed. In addition and to support the own literature search, two reference databases (Endnote databases) on the subject of herbicide effects on non-target plants created by the Environmental Food and Safety Authority (EFSA) were provided by UBA. The literature searches conducted by EFSA were also performed with the database “ISI Web of Knowledge” (EFSA subscription comprised Web of Science (1990-now), “CABI: CAB Abstracts” (1910-now), “FSTA” (1969-now) and “Medline” (1959-now)). The two provided reference databases were carefully screened and the relevant literature was considered in the analysis.

With these search methods we tried to thoroughly cover the existing literature on the subject of herbicide effects on terrestrial non-target plants. The retrieved relevant literature (in total 159 references) was transferred in a reference management software (Endnote Library X2) and a literature database was created including the citation information, keywords and abstract to ensure a quick orientation.

Overall, 65 studies evaluating the effects of herbicides on non-target plants were found. In the following and for a better overview, the retrieved publications were sorted and classified according to the tasks of this report (Table 1). Therefore the literature was attributed to study types such as Tier I and II studies (= greenhouse experiments with individual plant species), higher tier studies (= realistic drift studies in the field, microcosm and field studies) and studies evaluating the effects of herbicides on natural plant communities (Table 1). Additionally, review articles and summaries were integrated for the discussion.

Table 1 Summary of literature evaluating the effects of herbicides on non-target plants. The references are listed and presented in the corresponding chapters.

Study Type	Number of Studies	Classification	Number of Studies	Chapter
Tier II studies:	37			3: species sensitivity
		dose-response studies (ER values generated)	24	
		dose-response studies (ER values not generated or presented)	13	
higher tier studies	15			4: higher tier studies
		realistic drift studies - species in pots placed at different distances from the treated field	4	
		microcosm studies	5	
		higher tier field studies (simulated drift studies)	6	
effects on plant communities	4			5: evaluation of the RA
		simulated drift studies of herbicide on natural communities	1	
		simulated drift studies of herbicide and fertilizer on natural communities	3	
reviews and summaries	9			
		reviews of plant toxicity testing	6	
		reviews of field experiments with plants	2	
		proposal to update NTP toxicity testing	1	
Total:	65			

3. Species sensitivity of plants towards herbicide exposure

To evaluate the effects of herbicides on non-target plants, the guidance document of the European Union (European Commission 2002) recommends testing six to ten plant species representing as many taxonomic groups as possible. The question as to whether or not the recommended number and assessed plant species represent an adequate safeguard for environmental protection against the impact of herbicides is debatable (e.g. Boutin et al. 2004, Strandberg 2012).

The objectives presented in this chapter were:

1. to examine the available data on the sensitivity of different plant species to a range of herbicides,
2. to identify plant species that should preferably be tested to assess the risk of herbicides, and
3. to estimate the optimal number of plant species that should be tested.

3.1. Differences in species sensitivity of plants towards herbicide exposure

In the pesticide risk assessment for non-target plants it is assumed that the sensitivity of the selected test species is representative for all plant species found in habitats surrounding cropped areas such as field margins – the so called non-target area. However, the narrow taxonomic and life form (e.g. ecological traits) range of six to ten standard test species (mainly crops) raises the question whether the majority of plant species, including native wild plants, will be protected.

To obtain an understanding for the range of species sensitivities we searched published literature, draft assessment reports (as provided by EFSA) and data provided by the German Federal Environment Agency (Umweltbundesamt, UBA) for toxicological endpoints (ER_{50} values) of herbicide plant tests (Tier II studies). The literature search was performed as described in chapter 2 and all publications are provided in Appendix 1. The aim was to perform species sensitivities distributions (SSD) with toxicological endpoints (ER_{50}) to compare the sensitivity of the different species and to examine differences between crops and non-crops. The ER_{50} is the effective application rate (measured in weight or volume herbicide / area) that results in 50% reduction of the tested endpoint, in this case biomass, being measured relative to a control.

To realize a comparable dataset we only considered absolute (no “>”) ER_{50} values of vegetative vigor and seedling emergence tests performed by following protocols for standard plant tests according to OECD guidelines (OECD 2006). Moreover, only herbicides were used for which suitable data for at least four crops and four non-crops were found. However, only for two vegetative vigor tests for two herbicides, glyphosate and dicamba, suitable data were located (Boutin et al. 2004; Strandberg et al. 2012; Vielhauer, 2010 (Research Box 1); Geisthardt, 2012 (Research Box 2), Draft Assessment Reports for dicamba, and UBA data for glyphosate; see Appendix 2). The ER_{50} values for the assessed plant species were fitted to obtain lognormal species sensitivities distributions (SSD). The SSDs were performed with the ETX program developed by Aldenberg and Slob (1993).

In the case of seedling emergence tests, only for Chlortoluron ER_{50} values for two non-crops were available. One non-crop, *Phacelia tanacetifolia*, was the most sensitive species of the performed test, while the second non-crop (*Trifolium pratense*) was not more sensitive than half of the tested crop species. However, no conclusion could be drawn on the basis of that limited dataset. More research is needed to understand the sensitivity of non-crop seedling emergence towards herbicides.

RESEARCH BOX 1: Effects of an herbicide treatment on terrestrial non-target plants: A comparison of the sensitivity to glyphosate between closely related wild and crop plants

BACKGROUND: Environmental risk assessment of herbicides requires careful consideration of the influence of herbicides on the growth and survival of non-target plants within the ecosystems. Therefore during the authorization of plant protection products the phytotoxicity of herbicides to non-target plants has to be investigated. The risk assessment of herbicides is often performed with single and annual plant species in young development stages (2-4 leaf stage) in greenhouse experiments. Generally crops plants are used for phytotoxicity testing since these species can easily be handled and cultivated (e.g. high germination rate, fast growth). However, the sensitivity of wild plant species, which should be protected, is largely unknown.
The objectives of this study were to compare the sensitivity towards herbicides of crop plants that are standard species in non-target plant tests with their wild relatives that can be found in field margins in the agricultural landscape of southern Germany. Additionally, the leaf structure of the plant species was considered.

METHODS: For the greenhouse tests 6 species were selected: Lettuce (*Lactuca sativa,* Asteraceae) and its wild relative prickly lettuce (*Lactuca serriola,* Asteraceae), and catsear (*Hypochoeris radicata,* Asteraceae) that has a leaf surface with more hairs. The second group was carrot (*Daucus carota, ssp. Sativus,* Apiaceae), wild carrot (*Daucus carota, ssp. Carota,* Apiaceae) and yarrow (*Achillea millefolium,* Asteraceae), with a similar leaf structure as carrot (Fig. 1).

Based on a vegetative growth test according to OECD guideline 227 effects of an herbicide on the test plants in single pots were assessed. Plants were treated at the 2-4 leaf growth stage and test duration after treatment was 21 days. The broadband herbicide "Roundup Ultra Max" by Monsanto, containing 450 g/l of its active ingredient glyphosate, was used. In this study the tested application rates ranged between one and 30% of the actual application rate in viticulture (0, 16, 29, 51, 93, 167, 300 and 540 g a.i./ha of the maximum field application rate of 1800 g glyphosate/ha (= 4 L roundup ultra max/ ha). Each treatment was replicated 6 times.

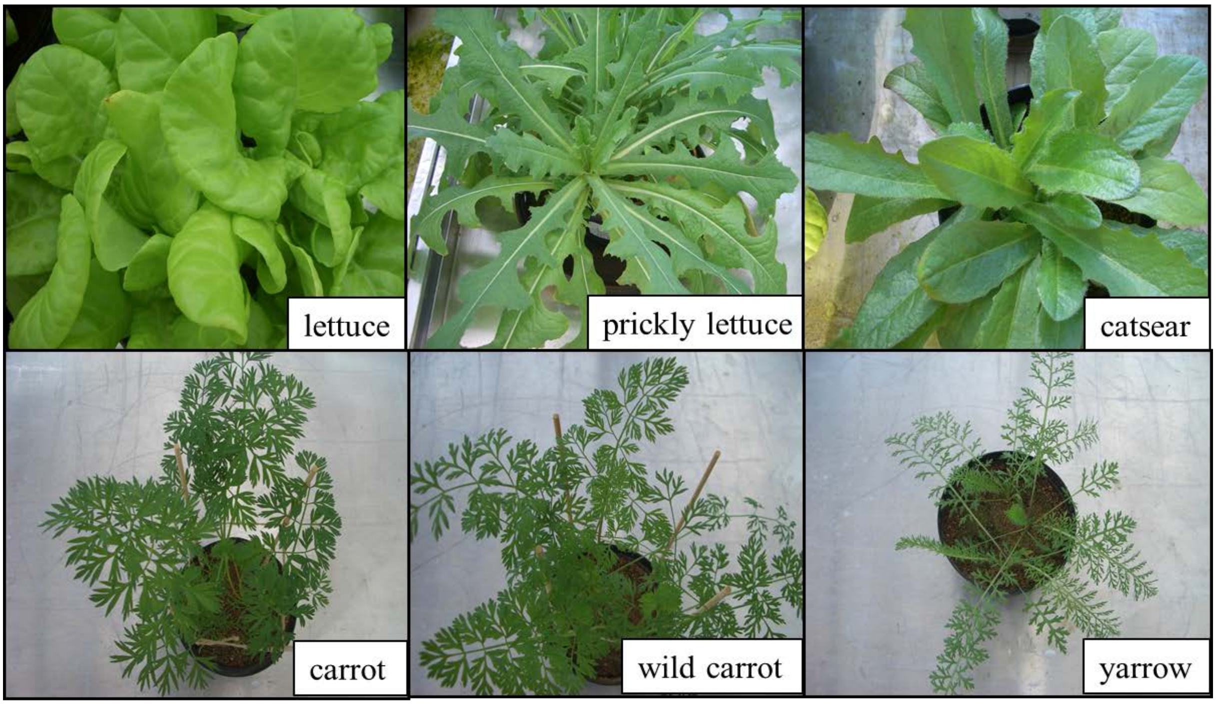

Figure 1: Selected test species of crop plants and wild relatives.

RESULTS: The intensity of herbicide effects varied between crop species and their wild relatives. The estimated ER_{50} values based on biomass are shown in Table 1. The plant species prickly lettuce showed the highest ER_{50} value compared to the other plant species. However, the confidence intervals show that the crop plant lettuce has a similar sensitivity. Catsear had a two times higher sensitivity than the crop plant lettuce possibly due to its hairy leaves and surface structure where droplets of herbicide stay longer than on the crop plants with a smooth surface that enhances runoff.

The ER_{50} values of the other test plants indicate a higher sensitivity of the wild plant species wild carrot and yarrow towards roundup than the crop species carrot. Especially yarrow revealed a more than four times higher sensitivity than the crop plant carrot although it that has a very similar leaf structure. However, yarrow belongs to another plant family than carrots.

A biomass reduction of 50% occurs for yarrow at rates that compares to a drift of 4% of the maximum field rate whereas for the crop plants lettuce and carrot this effect rate was reached at 14% of the recommended field application rate.

Table 1: Results of the vegetative vigour test. ER_{50}-values with 95% CIs (fresh weight determined 21 days after treatment) and the corresponding drift of the maximum field rate of 1800g ai./ha in % (n = 5-6).

species	common name	Family		ER_{50} (with 95% CI) [g ai/ha]	ER_{50} = drift rate in %	Factor compared to crop
L. sativa	lettuce	Asteraceae	crop	249.8 (203.9 to 295.8)	14	
L. serriola	prickly lettuce	Asteraceae	non-crop	311.9 (274.9 to 348.8)	17	0
H. radicata	catsear	Asteraceae	non-crop	113.3 (95.3 to 131.2)	6	2
D. carota, ssp. sativus	carrot	Apiaceae	crop	247.0 (210.0 to 283.8)	14	
D. carota, ssp. carota	wild carrot	Apiaceae	non-crop	150.7 (122.7 to 178.8)	8	1,6
A. millefolium	yarrow	Asteraceae	non-crop	62.8 (50.2 to 75.5)	4	4

CONCLUSION: The present study reveals a higher sensitivity of wild than crop plants in the same family. Leave morphological structures such as hairs may play a major part to explain the observed differences, although parameters as cuticula thickness and structure seem important as well. In plants with structurally similar leaves differences were observed between plant families.
Therefore, it can be concluded that sensitivity of plant species is not related to family or leaf structure. Consequently any conclusions on the sensitivity drawn from any tested plant species to others are speculative and should be covered in a risk assessment approach with an appropriate safety factor. This is especially true if mainly crop plants are tested as surrogate species for wild plants. As a consequence, it is suggested to further consider the use of wild plant species in standard tests assessing toxicological effects of pesticides.

SOURCE:

Bianca Vielhauer (2010): Auswirkungen von Pflanzenschutzmitteln auf terrestrische Nicht-Ziel-Pflanzen Sensitivitätsvergleich von nah verwandten Kultur- und Wildpflanzenarten gegenüber einem Breitbandherbizid. *Diplomarbeit*, Institut für Umweltwissenschaften. Universität Koblenz-Landau. Campus Landau.

RESEARCH BOX 2: Effects of herbicides on non-target plants

BACKGROUND: Herbicides are widely used pesticides which can affect wild plant species in non-target areas (e.g. field margins). While in the risk assessment mainly crop plants are used for phytotoxicity testing, less is known about the sensitivity of wild plant species towards herbicides. Hence, this research project focused on herbicide effects on wild plant species.

METHODS: Phytotoxicity tests (dose-response experiments) based on the OECD guideline 227 were performed. Five plant species, which can be found in field margins, were selected: *Ranunculus acris, Rumex acetosa, Plantago lanceolata, Plantago major* and *Barbarea vulgaris.* The plants were grown individually in pots and treated at the 4-6 leaf growth stage. For the treatments of the test plants two herbicides were used: Atlantis WG (field rate: 400 g/ha, a.i.: 30 g/kg Mesosulfuron-methyl; 6 g/kg Iodosulfuron-methyl-natrium) and Roundup LB Plus (field rate 5L, a.i.: 360 g/L Glyphosate). The tested application rates were 1, 3, 10, 30 and 100% of the field rate. Each treatment was replicated six times and effects were assessed at 7-day intervals with a total test duration of 28 days. Additionally, the results (estimated ER_{50} values) were compared with the sensitivity of a crop species (*Lactuca sativa*) used in non-target plant tests (ER_{50} Glyphosate value for *L. sativa* from Vielhauer 2010).

RESULTS: The estimated ER_{50} values based on the biomass (fresh weight) 28 days after treatment are presented in table 1. The plant species *R. acetosa* showed the highest sensitivity towards the herbicide Atlantis WG followed by *B. vulgaris* and *P. major.* The highest sensitivity to the herbicide Roundup LB Plus showed the two species *P. major* and *P. lanceolata*. The biomass reduction of 50% occurs for all tested wild species, expect for *R. acris*, at drift rates below 10%. *P. major* and *P. lanceolata* are already affected at drift rates of 2% of the field rate. The lowest sensitivity was revealed by the crop species *L. sativa.*

Table 1: Results of the vegetative vigor test. ER_{50} biomass-values with 95% CI (fresh weight 28 days after treatment) and the corresponding drift of the field rate, n.d. = data not determined since the highest test concentration produced not a 100% mortality of all test plants; n.d. = data not determined since the test had to be terminated prematurely due to herbivores infestation. ( ):95% CI g a.i./ha*

			Atlantis WG		**Roundup**	
species	**common name**	**family**	**Mesosulfuron + Iodosulfuron [g a.i./ha]**	**% field rate**	**Glyphosate [g a.i/ha]**	**% field rate**
R. acetosa	common sorrel	Polygonaceae	0.60 (0.36-0.72) + 0.12 (0.07-	5	54 (36-72)	3
B.vulgaris	bittercress	Brassicaceae	0.72 (0.48-0.84) + 0.14 (0.10-	6	n.d*	n.d.*
P. major	broadleaf plantain	Plantaginaceae	2.88 (1.44-4.44) + 0.58 (0.29-	24	36 (18-54)	2
P. lanceolata	buckhorn plantain	Plantaginaceae	n.d.	n.d.	36 (18-54)	2
R. acris	common buttercup	Ranunculaceae	n.d.	n.d.	108 (54-180)	6
L. sativa	lettuce	Asteraceae	n.d	n.d.	250 (204-296)	14

CONCLUSION: The herbicides Atlantis WG and Roundup LB Plus affected the growth of the tested plant species. The biomass was used as endpoint for the comparison of the sensitivity of the species to herbicides. However, sublethal effects such as leaf discoloration occurred at much lower application rates, which might influence the performance and fitness of the species in natural communities. The comparison of the sensitivity between the wild species and the crop species showed that the wild species used in this study (especially *P. major* and *P. lanceolata*) showed a 7 times higher sensitivity than *L. sativa.*

SOURCE:

Geisthardt, M. (2012): Effekte von Herbiziden auf phytophage Insekten am Beispiel der Kohleule *Mamestra brassicae*. *Diplomarbeit*, Institut für Umweltwissenschaften. Universität Koblenz-Landau. Campus Landau.

In the following figures (Figure 1 & 2) the SSDs for crop and wild non-crop plant species are shown (ER_{50} values taken from Boutin et al. 2004; Strandberg et al. 2012; Vielhauer, 2010 (Research Box 1), Geisthardt 2012 (Research Box 2), Draft Assessment Reports for dicamba, and UBA data for glyphosate; see Appendix 2). Species are depicted by using different symbols. The SSD for glyphosate was calculated on a basis of 41 plant species (13 crop and 28 non-crop species) (Figure 1). With the exception of *Lolium perenne* (considered a crop by most authors) and *Lactuca serriola*, all non-crop species were more sensitive compared to the crop species. *Allium cepa* was the least sensitive plant species and *Lactuca serriola* the least sensitive non-crop species.

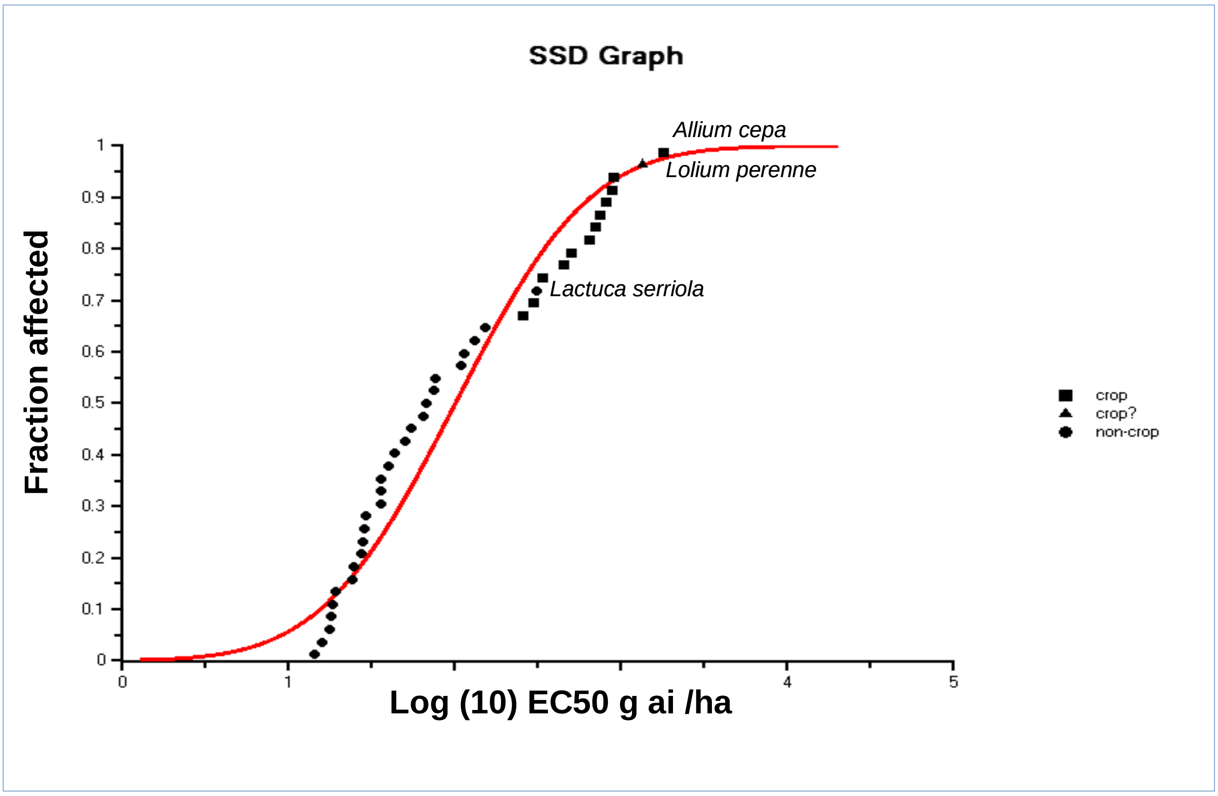

Figure 1 Species sensitivity distribution of 12 crop and 28 non-crop plant species tested with glyphosate. Since *Lolium perenne* is occasionally considered a crop, it is marked as "crop?". The SSD is based on ER_{50} values (shoot biomass 21 days after application) of vegetative vigor tests after post emergence exposure to Glyphosate following the OECD guideline 227. The positions of *Allium cepa* and *Lactuca serriola*, the least sensitive crop species and the least sensitive non-crop species, respectively, are indicated.

Figure 2 shows the SSD for 19 plant species (4 crop and 15 non crop species) tested with dicamba. *Glycine max* was the least sensitive plant species. With the exception of *Solidago canadensis* all non-crop plants were less sensitive compared to the tested crops.

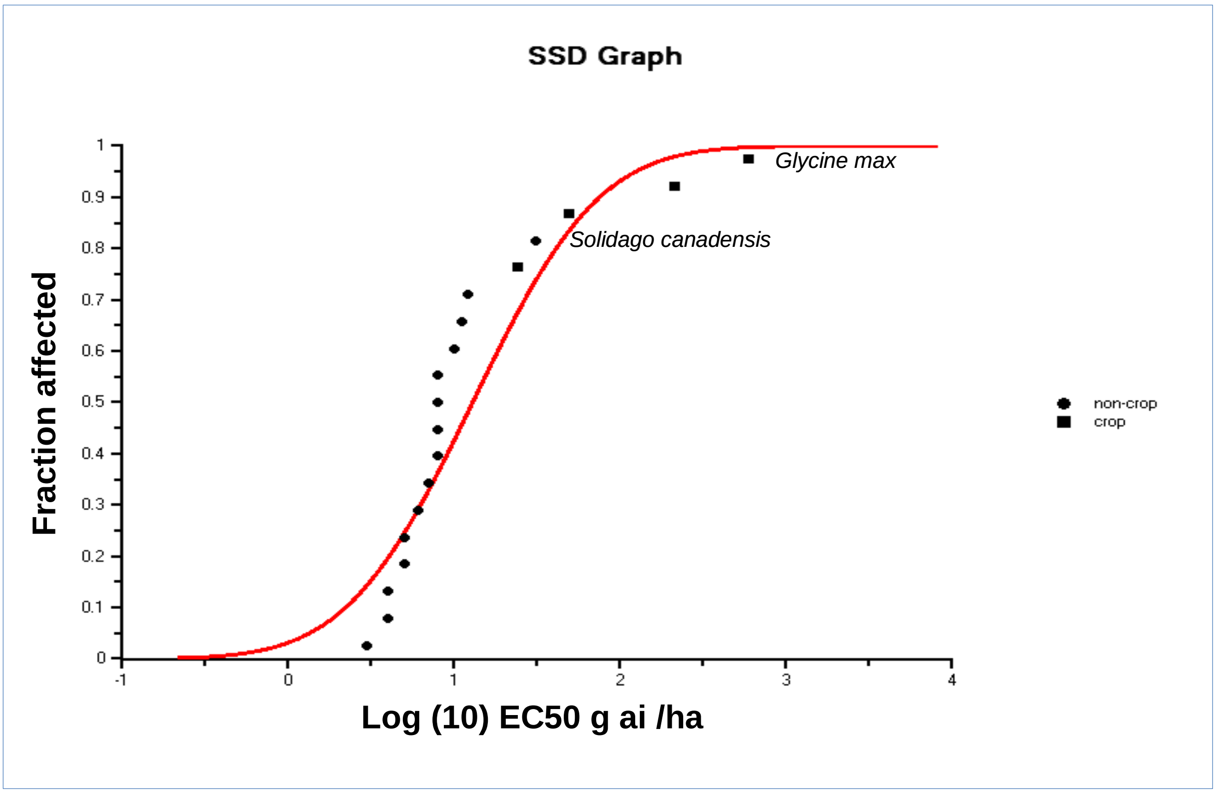

Figure 2 Species sensitivity distribution of 4 crop and 15 non-crop plant species tested with dicamba. The SSD is based on ER_{50} values (shoot biomass) for vegetative vigor test after post emergence exposure to Dicamba following the OECD guideline 227. The positions of *Glycine max* and *Solidago canadensis*, the least sensitive crop species and the least sensitive non-crop species, respectively, are indicated.

The differences in magnitude between the ER_{50} values of the most and least sensitive species were 125 for glyphosate and 178 for dicamba (species-specific ER_{50} data are given in Appendix 2).

Since crop plants are typically selected as test plants for assessing the risk of herbicides for non-target plant species, we calculated SSDs for crop and non-crop species separately and determined and compared the HC5 values (hazard concentration that will protect 95% of the species) for both herbicides using the ETX program (Table 2).

Our analysis demonstrated that the HC5 values for crop plants normally used in standard test for risk assessment of herbicides are higher than for wild plants, 21 times in the case of glyphosate and three times in the case of dicamba (Table 2).

Table 2 HC5 values for vegetative vigor test after post emergence exposure to glyphosate and dicamba, respectively, based on ER_{50} values (shoot biomass) for crop and non-crop species.

	glyphosate	dicamba
HC5 crop (90% confidence interval)	243.3 (138.5-344.2)	7.9 (0.1 -37.8)
HC5 non-crop (90% confidence interval)	11.7 (7.5-16.3)	2.8 (1.7-3.9)
HC5 crop / HC5 non-crop	20.8	2.8

This conclusion is in accordance with findings by Boutin et al. (2004) who compared HC5 values for non-crop species (15 species) with the HC5 values for crop species for a number of herbicides. A 15 to 21 (depending on the concentration of the active ingredient) and 1.4 times higher sensitivity of the non-crops species were found for glyphosate (9-11 tested crop species) and dicamba (10 tested crop species), respectively.

However, ER_{50} values of crop and non-crop data of both analyses (the present report, Boutin et al 2004) were obtained from different experiments. Data on crop plant sensitivity from standard Tier II tests mainly originated from the US EPA (Environmental Protection Agency) database (for the analysis by Boutin et al. 2012), the ECOTOX database created by US EPA (for the analysis by Strandberg, 2012) or were provided by the UBA and included endpoints from draft assessment reports (for the present analysis). In contrast, data for wild non-crop plant species of all three analyses came mainly from a rate-response study performed by Boutin et al. (2004) and – in the case of the present analysis

– from additional rate-response studies performed by Vielhauer (2010), Geisthardt (2012) and Strandberg (2012). Although data of all these studies have been obtained following protocols for standard plant tests (OECD guideline), several test conditions such as the number of replicates, number of plants per pot, pot size, soil type, climatic conditions, watering, time of exposure, spray equipment, and the harvest are not always well documented and may vary (Strandberg et al. 2012). For instance, it was demonstrated that the sensitivity of *Abutilon theophrasti* to glyphosate depends on temperature and water availability (Zhou et al. 2007). Therefore, it cannot be ruled out that these potential differences in test conditions may be the main reason for the differences in sensitivity calculated in previous studies (Boutin et al. 2004; Strandberg et al. 2012) and also in our re-analysis.

To overcome the weakness of the comparisons of sensitivity of crop species and non-crop species that are based on analyses of data from different studies Strandberg and coworkers (2012) conducted a rate-response experiment with ten crops and two non-crops species. To be able to compare the results to a larger dataset of wild non-crop plants, both wild plants chosen by Strandberg et al. (2012) were already tested by Boutin et al. (2004; only wild plants were tested) and study conditions were in accordance with that study as well. Sensitivity for the two wild plants (*Centaurea cyanus* and *Papaver rhoeas*) was similar in both studies. The crop plants in the study by Strandberg et al (2012) however showed very low sensitivities, in the range of the wild plant species. This differs markedly from the dataset of glyphosate that we used, where oats show a value of 874 g/ha (see Appendix 2) compared to approximately 75 g/ha (Figure 4.2., p. 53 of Strandberg et al. 2012). This difference also highlights the importance to improve the current OECD test protocol with additional standardized test conditions.

Apart from the varying test conditions, the cultivars of the used crop plants may also have had an influence on the different results. White & Boutin (2007) demonstrated that the range in herbicide sensitivity among cultivars of the same crop can be quite extensive and that, depending on the cultivar included in a risk assessment, conclusions regarding the phytotoxicity of any given herbicide may differ. Likewise, a study by Boutin et al. (2010) revealed that variation in sensitivity exist also among different ecotypes of different plant species. Given these differences in herbicide sensitivity between cultivars or ecotypes of the same species, the phytotoxicity of any given herbicide may differ depending on the cultivar or ecotype chosen for inclusion in the toxicity test. Therefore, the endpoint obtained in a standard Tier II non-target plant study can vary depending on whether the chosen cultivar or ecotype for risk assessment is quite tolerant or quite sensitive to the tested herbicide (White & Boutin. 2007). This range in species-specific sensitivity, coupled with the supposed arbitrary selections of cultivars for testing could alter the outcome of the risk assessment and ultimately result in a threat for non-target plant species (White & Boutin 2007).

Another major difference between the data points not mentioned in previous critical comments lies in the herbicide formulation assessed in the tests. The available data only mention the active ingredient glyphosate and dicamba, although especially for glyphosate a multitude of formulation products are available. These formulations might vary in their effects and therefore additionally influence the ER_{50} values and muddle a combined database from multiple tests.

To estimate if results based on testing performed in the greenhouse are representative for field situations, we compared datasets obtained by greenhouse and field tests. However, only for metzachlor suitable data (at least comparable data of the same endpoint for 4 different plant species) were available. While the two Poaceae species (*Lolium multiflorum* and *Avena sativa*), were found to be more sensitive in the greenhouse tests, *Allium cepa* and *Lactuca sativa* were found to be more sensitive in the field tests (**Table 3**). No conclusion can be drawn for *Brassica napus* and *Pisum sativum* since the endpoints of the greenhouse tests are higher than the given inaccurate (">") ER_{50} values for the field test (**Table 3**). Given the small dataset available, a detailed calibration study (field and greenhouse tests for a number of plant species and herbicides under comparable conditions) would be required to estimate whether results based on testing in the greenhouse are representative for field situations. However, taken into account that some species (two of four comparable datasets) were more sensitive in the field tests a safety factor should be applied for tests performed under greenhouse condition (safety factor of at least 5, based on the comparison for *Lolium multiforum*, **Table 3**). However the results should be interpreted with caution since test duration was different.

Table 3 Comparison of ER_{50} values (shoot biomass) of six different plant species for vegetative vigor tests after post emergence exposure to metzachlor. The shown greenhouse ER_{50} values are means of 2-3 different tests.

plant species	field tests (45 days)	greenhouse tests (21 days)
Avena sativa	> 750 g a.i /ha	224 g a.i /ha
Lolium multiflorum	415 g a.i /ha	82 g a.i /ha
Allium cepa	634 g a.i /ha	1042 g a.i /ha
Lactuca sativa	130 g a.i /ha	469 g a.i /ha
Brassica napus	> 750 g a.i /ha	1042 g a.i /ha
Pisum sativum	> 750 g a.i /ha	938 g a.i /ha

3.2. Selection of appropriate test species for herbicide risk assessment

In the non-target plant risk assessment it is assumed that the selected test species are representative for all plant species that are found within non-target habitats.

The examination of non-target plant tests performed for the risk assessment of herbicides (obtained from draft assessment reports and datasets provided by the Federal Environmental Agency) revealed that in total only 20 plant species, representing 9 families were used in 54 examined datasets. In contrast, 249 species of 41 different flowering plant families occurring in field margins, habitats that are susceptible for the effects of herbicide drift, were found in a large-scale survey in Germany (Roß-Nickoll et al. 2004). A species list is provided in Appendix 3. Table 4 shows the different plant families, the number of recorded species per family and the dominance of each family (given as the percentage of the number of species of the complete dataset in the respective family; e.g. 39 species (or 15.9 %) out of a total of 249 species belong to the Asteraceae) in comparison to the total number of different species used in the examined plant test for the risk assessment.

Table 4 Comparison of flowering plant families, the respective species number and their percentage of the total species number recorded in field margins (non-target plants) and used in plant tests performed for the risk assessment of herbicides. Families are dominant in field margins (with more than 10 species) but were not used as test species in bold.

	non-target plants		**tested species**	
Family	species number	percental share	species number	percental share
Asteraceae	39	15.9	2	9.1
Poaceae	27	10.8	6	27.3
Fabaceae	23	9.2	2	9.1
Caryophyllaceae	**16**	**6.4**	not tested	
Brassicaceae	16	6.4	5	22.7
Lamiaceae	**15**	**6.0**	not tested	
Apiaceae	13	5.2	1	4.5
Rosaceae	**13**	**5.2**	not tested	
Scrophulariaceae	**10**	**4.0**	not tested	
Boraginaceae	6	2.4	not tested	
Liliaceae	6	2.4	1	4.5
Polygonaceae	6	2.4	1	4.5
Ranunculaceae	6	2.4	not tested	
Geraniaceae	4	1.6	not tested	
Rubiaceae	3	1.2	not tested	
Violaceae	3	1.2	not tested	
Campanulaceae	3	1.2	not tested	
Cyperaceae	3	1.2	not tested	
Hypericaceae	3	1.2	not tested	
Plantaginaceae	3	1.2	not tested	
Primulaceae	3	1.2	not tested	
Valerianaceae	3	1.2	not tested	
Convolvulaceae	2	0.8	not tested	
Gentianaceae	2	0.8	not tested	
Onagraceae	2	0.8	not tested	
Urticaceae	2	0.8	not tested	
Caprifoliaceae	1	0.4	not tested	
Chenopodiaceae	1	0.4	not tested	
Cornaceae	1	0.4	1	4.5
Crassulaceae	1	0.4	not tested	
Dipsacaceae	1	0.4	not tested	
Equisetaceae	1	0.4	not tested	
Euphorbiaceae	1	0.4	not tested	
Fumariaceae	1	0.4	not tested	
Juglandaceae	1	0.4	not tested	
Juncaceae	1	0.4	not tested	

Table 4 continued.

Family	non-target plants		tested species	
	species number	percental share	species number	percental share
Linaceae	1	0.4	not tested	
Malvaceae	1	0.4	not tested	
Papaveraceae	1	0.4	not tested	
Plumbaginaceae	1	0.4	not tested	
Resedaceae	1	0.4	not tested	
Salicaceae	1	0.4	not tested	
Saxifragaceae	1	0.4	not tested	
Amaranthaceae	not recorded		1	4.5
Solanaceae	not recorded		1	4.5
Cucurbitaceae	not recorded		1	4.5
All families	**249**	**100**	**22**	**100**

The results in Table 4 demonstrate that the selected test species for risk assessment procedures are not representative for all plant families that are found within the agricultural fields and in surrounding habitats such as field margins since most plant families are not covered in the tests. Among them are several dominant plant families where a high number of species were recorded in field margins such as Caryophyllaceae, Lamiaceae, Rosaceae, and Scrophulariaceae. In contrast, no members of three of the nine plant families that were assessed in Tier II non-target plant tests were recorded in the non-target habitat (Amaranthaceae, Solanaeceae, and Cucurbitaceace). However, native species of all three families exist and may occur in other non-target habitats not assessed by Ross-Nickoll et al (2004). Moreover, apart from flowering plants, ferns and lichens may also occur in the field margins, dry walls and hedges bordering fields that potentially are affected by herbicide drift from neighboring crop fields but are never used in phytotoxicity testing. However, a test conducted by Boutin et al. (2012) revealed a high sensitivity of ferns to herbicides. Also, the sensitivity of lichens was clearly demonstrated (Newmaster & Bell 2002; Juuti et al. 1996).

White & Boutin (2007) recommended that when selecting plant species for testing for regulatory risk assessment, those that are known to be insensitive should not be included (among them those that the herbicide is intended for use). According to the study of Boutin et al. (2004), species sensitivity varies greatly for the herbicides tested, but Asteraceae species generally exhibit less sensitivity while species of Lamiaceae were clearly among the most sensitive. Considering that Lamiaceace are also among the dominant families of non-target plants in field margins in Germany it should be recommended to use them in testing.

In order to find the more sensitive species used so far in phytotoxicity tests a dataset of non-target plant tests was analyzed to examine the sensitivity of different crop species to a range of herbicides. However, it should be noted, that no information about cultivars was considered since they were not documented in most cases, even though they may have had a significant influence on the results of the tests as discussed above (White & Boutin 2007). To obtain comparable datasets we only considered absolute ER_{50} values of vegetative vigor tests with the endpoint biomass of standard tests performed by following protocols of the OECD guideline. Since there are no absolute ER_{50} values (i.a. only "> values") in most performed tests, only 14 datasets out of 54 fulfilled these requirements (Appendix 4)

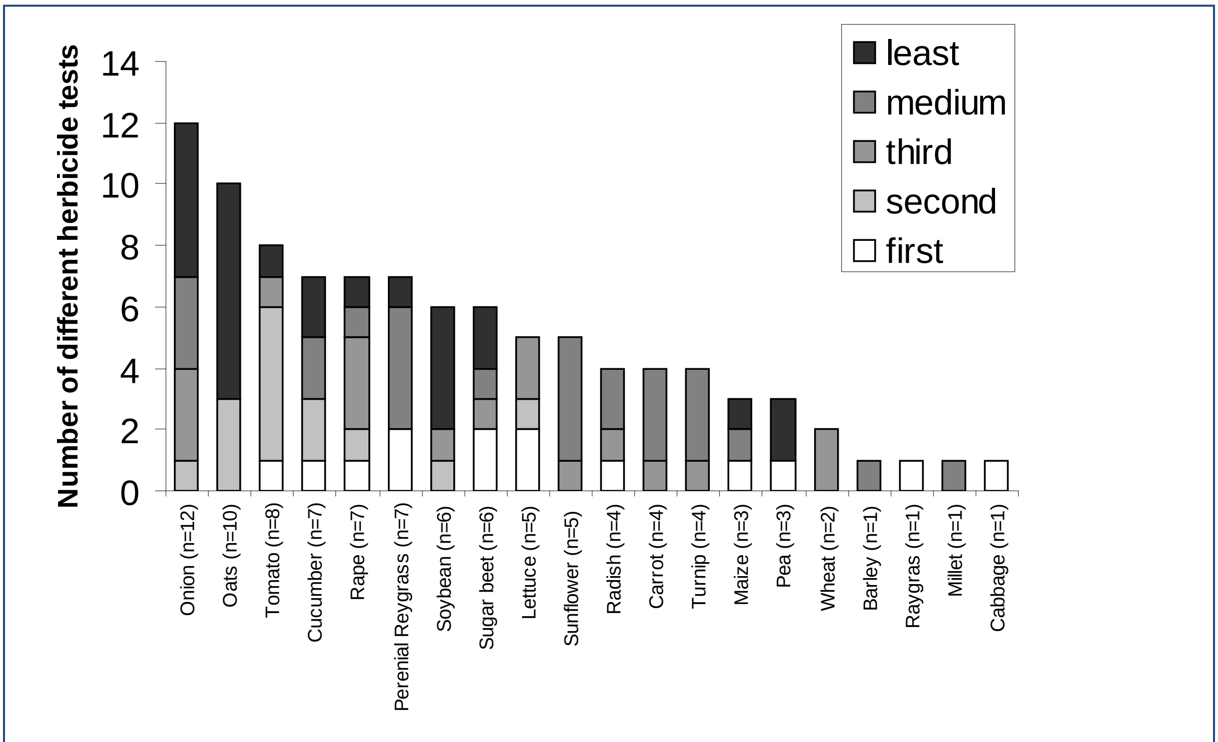

Figure 3 Sensitivity ranking of tested plant species for 14 different herbicides (data derived from Draft assessment reports and UBA reports). The figure indicates the position in the sensitivity distribution for each species: first (=most sensitive), second, third, medium (rank 4-11 according to the number of used plant species per test) and least sensitive.

Figure 3 shows the ranking of test species for their sensitivity towards 14 herbicides. For instance, oats was the least sensitive crop plant in seven out of ten herbicides studied. Onion was also the least sensitive test species in five of 12 studies and soybean also tends to be among the more insensitive crop species. Lettuce, sugar beet and ryegrass showed highest herbicide sensitivities in two studies. The herbicide dependent effect on species-specific sensitivity can be seen in maize which was the least sensitive species for one herbicide but the most sensitive for another.

The tested monocotyledons were in general among the less sensitive species (onion, oats, and perennial reygrass). A number of studies demonstrated that, compared to dicotyledons, monocotyledons are in general more tolerant to herbicides (Fletcher et al. 1985; Boutin & Rogers 2000; McKelvey et al. 2002; White & Boutin 2007). Grass species tend to respond to chemicals in a more similar way than species of other families and, therefore, test effort should focus on the area of greater uncertainty, which in this case means non-grasses (Boutin & Rogers 2000). Thus, when selecting representative species for testing, fewer grass species should be included and more broad-leaved plant species, with the provision that for herbicides known to be exclusively grass killers, a more extensive dataset of grass species would be needed (Holst & Ellwanger 1982; Boutin & Rogers 2000).

Species sensitivity varies greatly with the herbicide tested since no species was the most or least sensitive plant in all analyzed tests. Quite a number of species were the least sensitive species for one herbicide, but the most sensitive for another herbicide, such as tomato, rape, perennial ryegrass, sugar beet, maize, and pea (Fig. 3). That conclusion is in accordance with other studies, where plant sensitivity was shown to be both herbicide and species dependent with no existing obvious pattern (Marrs et al. 1989; Flechter et al. 1990; Pestemer & Zwerger 1999; Boutin et al. 2004; Strandberg et al. 2012). However, the analyses demonstrated that several crop species appear to be less sensitive to herbicides than others, among them onion, oats, perennial reygrass, soybean, sugar beet, sunflower, raddish, carrot and turnip since all of them being at least in one third of all examined tests the least or a medium sensitive species (by only considering species which were used in at least 4 tests). On the other hand, only three species, tomato, cucumber, and lettuce were among the most and second sensitive species in one third of all examined test (by only considering species which were used in at least 4 tests). As a conclusion, it should be recommended not to select the less sensitive crop species for testing, all above not oats and soybean, which were even in half of all test the least sensitive species, and onion which was in more than one third of all test the least sensitive species (Figure 3).

Herbicide sensitivity appears to be unrelated to family level. In the case of Asteraceae, for instance, lettuce appears to be quite sensitive, while the sunflower seems to be less sensitive (Figure 3). In accordance, Franzaring et al. (2012) found that response to rates of the herbicide imazamox were unrelated to the taxonomy of the tested species. Strandberg and co-workers (2012) as well as Vielhauer (2010) demonstrated that other characteristics such as morphological characteristics or plant traits such as leaf shape, leaf thickness, hairs or wax layer may be of higher importance for the differences in sensitivities. However, little work has been done so far to relate ecological traits of plants to their herbicide sensitivity. Fletcher et al. (1990) found that plants belonging to the same genus or taxonomically similar species had a higher degree of similarity for species' sensitivity to herbicides than taxonomically more distant species. This finding was supported by Boutin and Rogers (2000). Hence, it should be recommended to test a set of taxonomical different species with different ecological traits.

The results of tests with herbicides of the same mode of action were compared to examine if a relation between species sensitivity and the mode of action of the herbicides exists. For four different modes of action suitable data of at least two different herbicide active ingredients tested were available (Table 5). A direct comparison of species sensitivities was impossible since different plant species were used in the tests. However, in several cases the first or the second most sensitive species was tested in two different tests of herbicides belonging to the same mode of action. In three such cases similar results were found for the sensitive species (Table 5, green background). However, in three other cases the first or second most sensitive species of one test were found to be the least sensitive species in other tests with herbicides of the same mode of action (Table 5, red background). The latter cases indicate that the species sensitivity distribution differs even between different herbicides of the same mode of action.

Therefore, no recommendations for the selection of sensitive species or families for tests for herbicides of a given mode of action can be given a priori. A wide range of different species of different families have to be tested under the same study conditions in parallel with different herbicides representing different MOAs to be able to generalize the risk for sensitive species.

Table 5 Sensitivity ranking of tested plant species for herbicides of four different modes of action. Green colored background indicates that results for two herbicides of the same mode of action were similar for the two most sensitive species. Red colored background indicates that results for two herbicides of the same mode of action were different for the two most sensitive species. The numbers 1, 2, 3 indicate that the corresponding species was the first (= most sensitive), second, third sensitive species of a test, while M, L indicates that the species was the medium (rank 4-6 according to the number of used plant species per test) and least sensitive species (in several cases more than one species were equally least sensitive). The species are grouped into families (a = Asteraceae, b = Fabacea, c = Apiaceae, d = Liliaceae, e = Cucurbitaceae, f = Solanaceae).

		Poaceae				Brassicaea					a		b		c	d	e	f
Mode of Action	**Herbicide (a.i.)**	*Avena sativa*	*Lolium multiflorum*	*Lolium perenne*	*Zea mays*	*Brassica napus*	*Brassica rapa*	*Brassica oleracea*	*Beta vulgaris*	*Raphanus sativus*	*Helianthus annuus*	*Lactuca sativa*	*Glycine max*	*Pisum sativum*	*Daucus carota*	*Allium cepa*	*Cucumis sativus*	*Lycopersicon esculentum*
PS II Inhibitors	Chlortoluron	L		M	L	3			M			1	L		M	L	2	
	Metamitron			M			3				M		L			2	1	
	Diuron					3			L					1		M		2
Affecting Cell Division	Dimethenamid-P	M		1	M		M					2	L			3		L
	Metazachlor	2	1			L	M		L			3		L	M	M		
	Pethoxamid	L				2			1							3		
Growth Regulators	Fluroxypyr	L		M		M			M		3		2			L	L	1
	Dicamba	L							1	3						M		2
Pigment Synthesis Inhibitors	Sulcotrione	L		L					3	M	M	1					M	2
	Tembotrione	L		M		3		1		M	M					L	M	2

In summary, more research is needed to reduce the uncertainties in species-specific differences in the sensitivities to herbicides. Yet it is quite clear that much uncertainty remains by continuing to test the crop species that are currently used. Moreover, it seems almost unfeasible to select representative plants considering the variability in response and the large number of species in existences.

Therefore, a more promising approach by ensuring ecological relevance could be the testing of sensitive native plant species that are representative for the ecosystem to be protected (e.g. field margins). In order to identify species that are sensitive to herbicides, Strandberg et al. (2012) proposed to look for species that have disappeared from agricultural fields and their margins during the second half of the last century. For instance, a study that compared the number of weed species in Danish agricultural fields between 1967/1970 and 1987/1989 demonstrated a significant decrease of a number of plant species such as *Anagalis arvensis*, *Arenaria serpyllifolia*, *Atriplex patula*, *Cerastium caespitosum*, *Galium aparine*, *Plantago major*, *Silene noctiflora* and several species of the genus *Veronica* (Andreasen et al. 1996; Strandberg et al. 2012). So far, toxicological endpoints are only available for *Anagalis arvensis* (Boutin et al. 2004) and *Plantago major* (Geisthardt, 2012), both of them being among the more sensitive plants (Appendix 2). Thus, even though other factors might have caused the observed decline, testing these decreasing and therefore threatened species may be a suitable way to select sensitive species. Also Olszyk et al. (2008) suggested that, on the basis of importance in different habitats, woody species should be included in phytotoxicity testing protocols because of their regional ecological and economic importance (especially if present in hedges and seed or nut bearing) and potential exposure to herbicide drift. Wild plant species are often thought to be difficult to grow. However, apart from the difficulty to grow and test woody species, native wild plant species show good germination rates if treated appropriately and can be grown and maintained easily in a greenhouse for toxicity testing for risk assessment purposes (e.g. Boutin et al. 2004; White & Boutin 2007; Olszyk et al. 2008).

3.3. Optimal number of test plant species

The number of species actually tested should adequately represent the diversity of responses that will be observed among all non-target plants. However, given the uncertainty discussed in chapters 3.1 and 3.2 the question arises how it is possible to estimate the risk of herbicide when only around six to ten plant species are tested. Apart from the basic requirement that the number of plant species tested must provide an adequate level of statistical confidence, the number of species and replicates must also be manageable. Boutin & Rogers (2000) estimated that at least 40 plant species should be tested.

Newman et al. (2000) used a bootstrap procedure to estimate the approximate number of species sensitivity needed to approach the point of minimal variation about the HC5 values for several published ecotoxicological datasets. However, the approach was criticized since the applied resample size (100) was larger than the actual sample size (between 21 and 91), which was demonstrated leading to statistical inconsistencies (Verdonck et al. 2001).

We modified Newman's approach by not exceeding the sample size with the resample size. Bootstrap estimates of the HC5 and its 95% confidence intervals were obtained with the program Resampling Stats (Resampling Stats. 1995). Only for two herbicides, glyphosate and dicamba, sufficient datasets with enough plant species sensitivities (ER_{50} endpoints) were available. To estimate the HC5 values for each data set, the available data were sampled randomly with replacement to create a resample set of sensitivities of the size of the total sample set (42 for glyphosate and 19 for dicamba). These sensitivities were ranked from smallest to largest and, following the approach of Newman et al. (2000), the value ranked at the fifths was selected as the HC5. This resampling and ranking approach was repeated to produce 10,000 estimates of the HC5 and, subsequently, these 10,000 estimates were ranked and the value corresponding to 50% was taken as the best estimate of HC5 (Newman et al. 2000). The estimates corresponding to 2.5 and 97.5% were used as the 95% bootstrap confidence intervals. Subsequently, to estimate the number of species-sensitivity values needed to approach the point of minimal variation of the HC5 estimate, the above approach was repeated by resampling data sets of size six to the approximate maximum sample size (42 instead of 41 for glyphosate and 18 instead of 19 for dicamba) in increments of six. The resulting 2.5, 50, and 97.5% values were plotted against sample size (Figure 4 & 5).

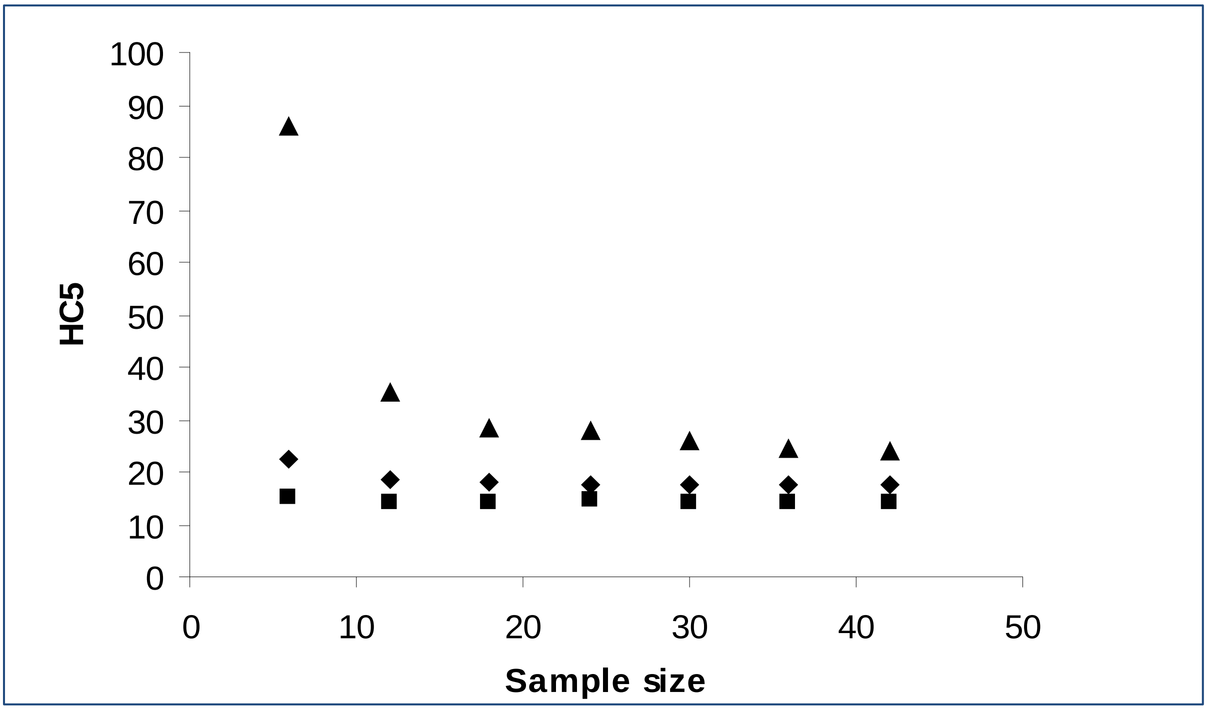

Figure 4 Curves for estimating the sample size (number of species sensitivity values) needed to approach the point of minimal variation of the HC5 values for glyphosate. The symbols indicate the HC5 values ranked at 50% (diamonds), 2.5% (squares), and 97.5% (triangles) of the 10,000 values generated by bootstrapping.

The confidence interval of the HC5 estimate decreased as sample size increased until the first point of minimal improvement was reached. According to Newman et al. (2000) this is the point at which an approximate sufficient sample size was reached (approximately 20 in the case of glyphosate and 15 in the case of dicamba) (Figure 4 & 5).

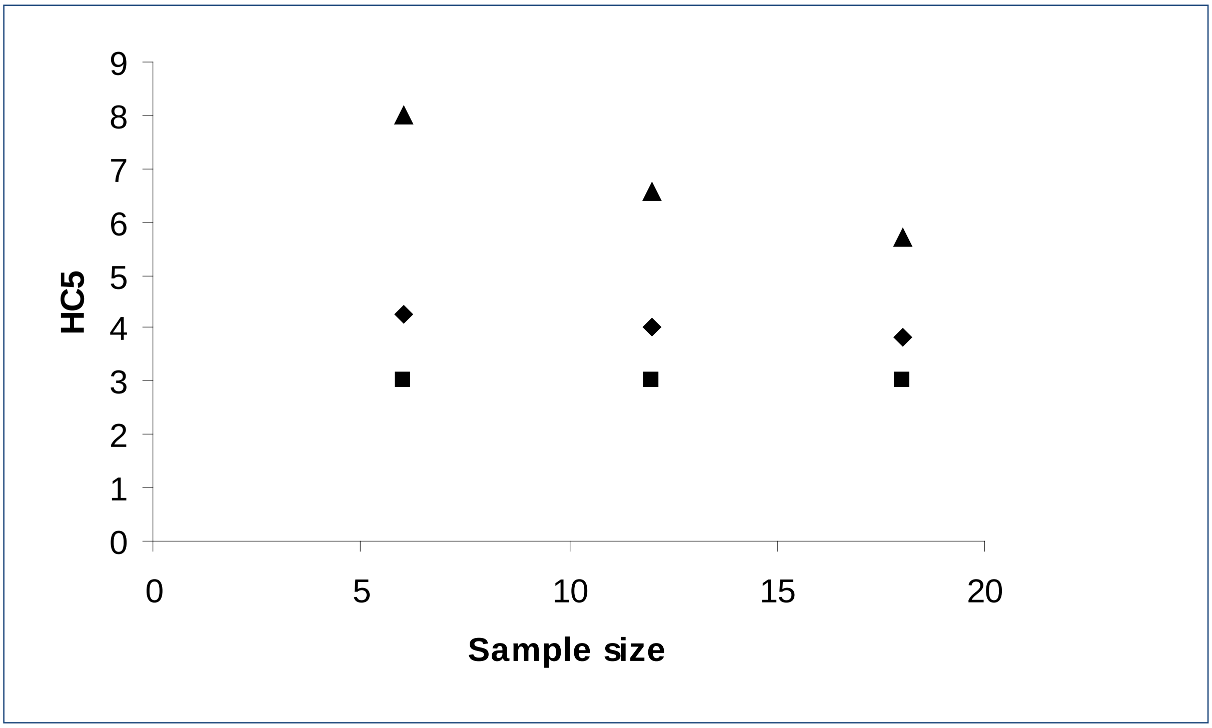

Figure 5 Curves for estimating the sample size (number of species sensitivity values) needed to approach the point of minimal variation of the HC5 values for dicamba. The symbols indicate the HC5 values ranked at 50% (diamonds), 2.5% (squares), and 97.5% (triangles) of the 10,000 values generated by bootstrapping.

However, the improvements of the confidence intervals are based on the upper confidence interval (97.5% value) since the lower confidence interval (2.5%) appears to be almost unchangeable. The same effect can be seen in the figures depicted by Newman et al. (2000). This effect is not due to the certainty of the estimate of the lower confidence intervals but is an artifact of the applied method. Due to relative small sample sizes, in combination with the high number of estimates (10,000) and the way to obtain the HC5 confidence intervals by ranking all estimates this approach will automatically result in an estimate of the lower confidence interval being the same as the lowest possible HC5 estimate. Given that in the case of risk assessment the lower confidence intervals (i.a. lower concentration) is more important than the upper confidence intervals (i.a. higher concentration) we consider Newman's approach impractical, even when using a resample size similar to the real sample size (the published critic of this approach by Verdonck et al. 2001).

Instead, we propose a new approach based on worst case assumptions. First, the HC5 value and its confidence intervals (2.5%, and 97.5%) were calculated with the program ETX. Subsequently, the same was repeated for the 6, 7, 8,... most insensitive species (worst case scenario of selecting 6, 7, 8,... species out of the given dataset). The resulting HC5 values and the respective confidence intervals were plotted against sample size and the HC5 value of the complete dataset was added as a straight line (Figure 6-9). The point where the lower confidence interval reached HC5 of the complete data set (straight line) was considered the necessary sample size. This means that with this sample size, even in the worst case scenario (only insensitive species were tested) its lowest estimate of the HC5 (i.a. the lower confidence interval) contains the hazard concentration of the complete data set. In the case of glyphosate a sample size of 29 plant species would be necessary (Figure 6), while in the case of dicamba already six species would be enough (Figure 7).

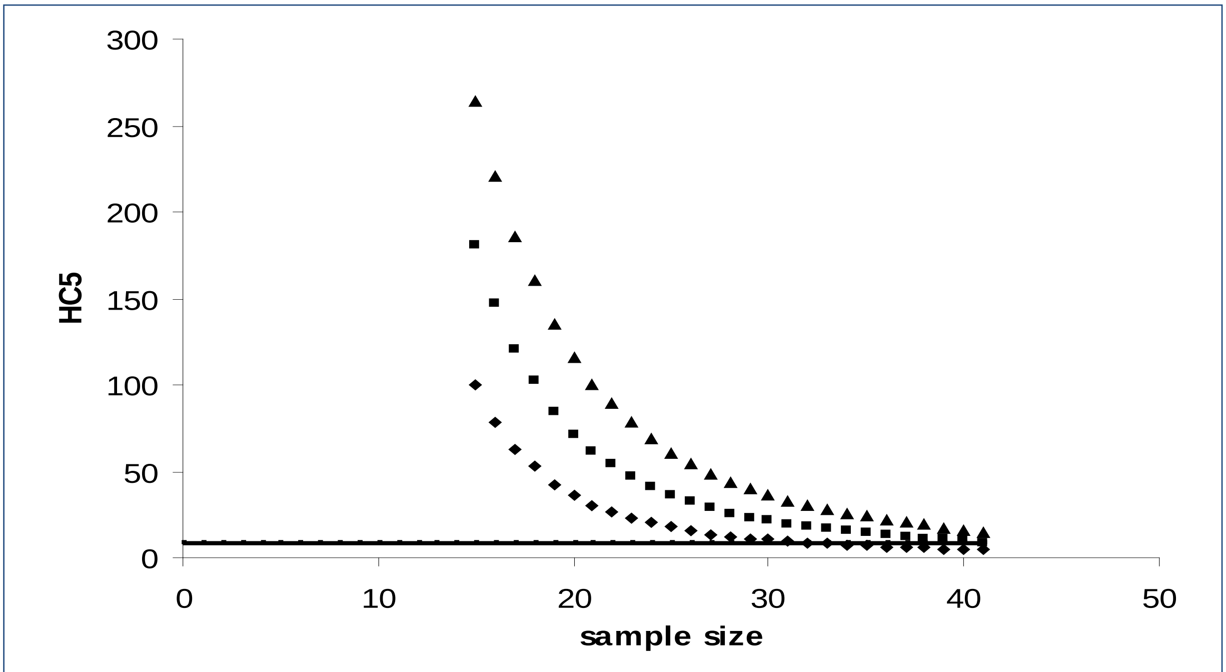

Figure 6 Curves for estimating the sample size (number of species sensitivity values) needed where the lower confidence interval reached the HC5 for glyphosate of the complete data set (straight line).The symbols indicate the HC5 values ranked at 50% (diamonds), 2.5% (squares), and 97.5% (triangles). Only data for more than 15 plant species are shown due to the large confidence intervals for estimations for fewer species.

This is related to the fact that only suitable values for four crop species were available for dicamba (all of them being insensitive compared to the non-crops; see data in Appendix 2) while the remaining species-sensitivities of the non-crop plants were narrowly distributed. Therefore, the HC5 for the six most insensitive species (four crop species and two more sensitive non-crop plant species) result in such large confidence intervals that the lower confidence interval is already below the HC5 for the complete dataset.

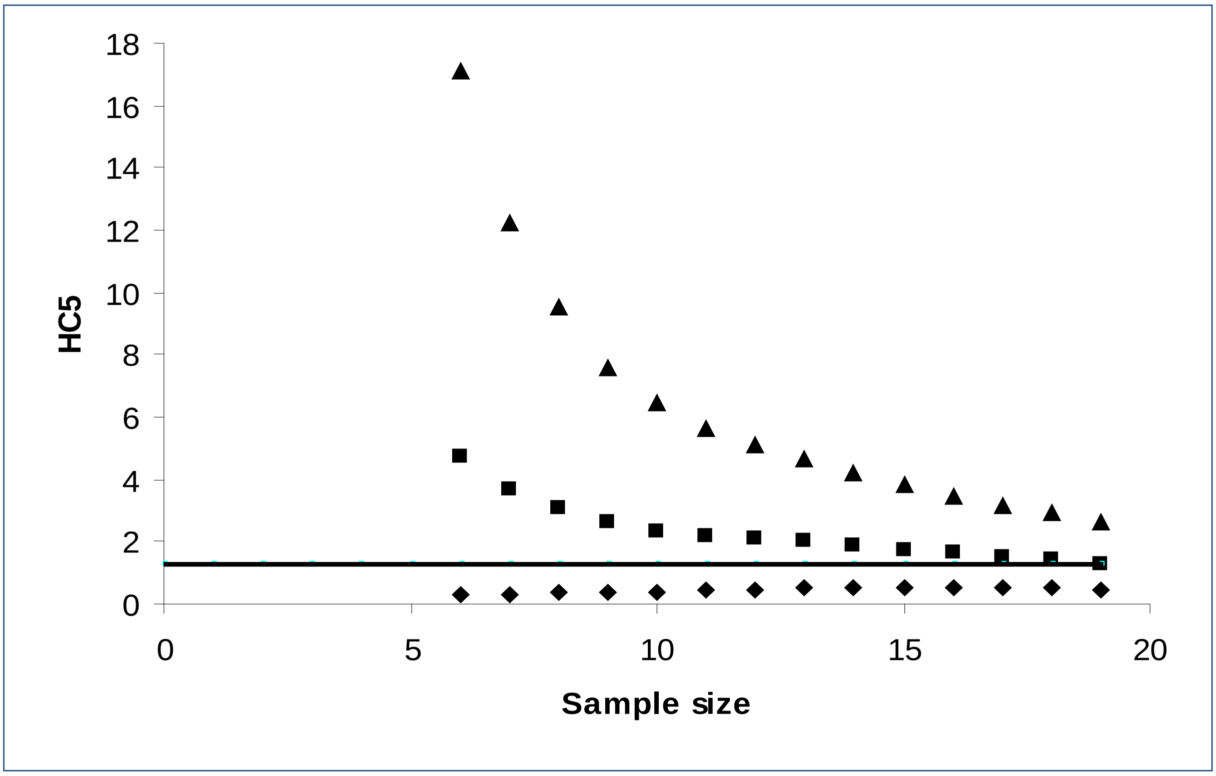

Figure 7 Curves for estimating the sample size (number of species sensitivity values) needed where the lower confidence interval reached the HC5 for dicamba of the complete data set (straight line).The symbols indicate the HC5 values ranked at 50 % (diamonds), 2.5 % (squares), and 97.5 % (triangles).

The rather large required sample size in case of glyphosate depends on the wide range of measured sensitivities. Given the fact that all crops were more insensitive than the non-crop species we repeated the approach by only considering non-crops (Figure 8-9).

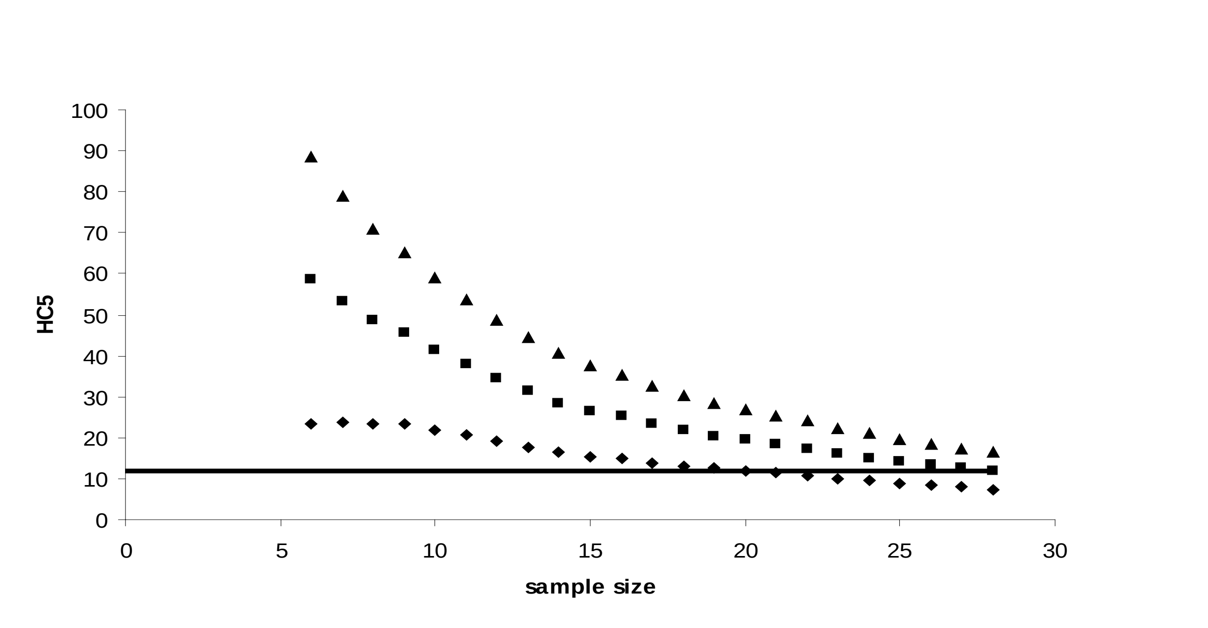

Figure 8 Curves for estimating the sample size (number of species sensitivity values) needed where the lower confidence interval reached the HC5 for glyphosate (straight line) by only using data for non-crop plants. The symbols indicate the HC5 values ranked at 50% (diamonds), 2.5% (squares), and 97.5% (triangles).

In the case of glyphosate a sample size of 18 non-crop plant species would be necessary (Fig. 8), while in the case of dicamba, again, already 6 species of non-crop plants would be enough to estimate an appropriate HC5 value (Figure 9). The differences between both herbicides are caused by the much narrower species-sensitivity difference to dicamba compared to glyphosate (see EC_{50} values in Appendix 2).

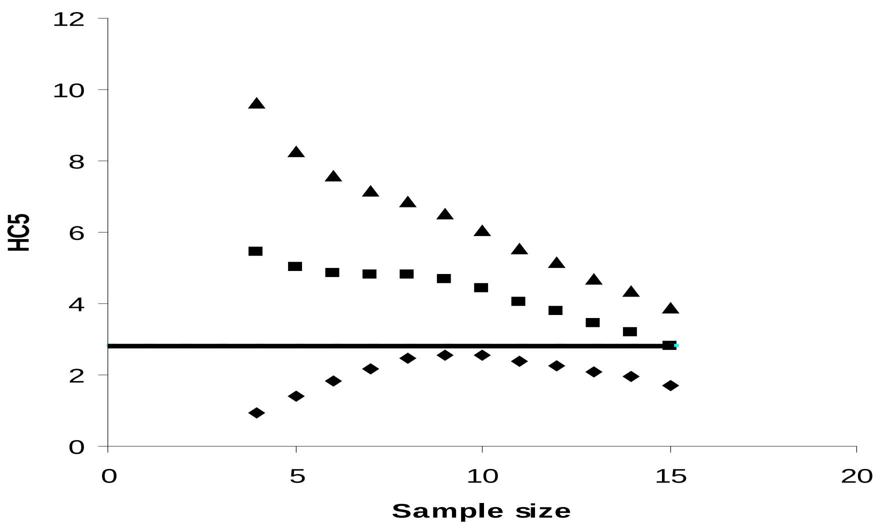

Figure 9 Curves for estimating the sample size (number of species sensitivity values) needed where the lower confidence interval reached the HC5 for dicamba (straight line) by only using data for non-crop plants. The symbols indicate the HC5 values ranked at 50% (diamonds), 2.5% (squares), and 97.5% (triangles).

Apart from the approach of testing a large enough number of species for the estimation of an appropriate HC5, safety factors could be applied to take into account the uncertainties obtained by tests using a small number of species which, generally, are only crops (including *Lolium perenne*).

In the following we calculate the range of possible HC5 values for different numbers of tested crop species for glyphosate, the only substance where ER_{50} values for a number of crops and non-crops were available (see chapter 3.1). HC5 values were calculated for different numbers of crop species. The ER_{50} values of the 4, 5,..., 12 most sensitive and, respectively, least sensitive crop species were calculated to demonstrate the full range of possible HC5 values out of a dataset of 13 crop species (including *Lolium perenne*). The differences in sensitivities between crops and non-crops were calculated by comparing the obtained HC5 values with the calculated HC5 of 11.7 g glyphosate / ha for non-crops (see 3.1). The differences in sensitivities range from 17.9 to 53.3 for the 4 most sensitive and, respectively, the 4 least sensitive crop species (**Table 6**). The upper estimation of the sensitivity differences varies more in dependence of the number of crops tested than the lower estimation (**Table 6**) due to the high differences in the ER_{50} values of the least sensitive species (see Appendix 2).

When 6 species are tested (as it is common in the current risk assessment) our results indicate a sensitivity difference between 18.2 and 47.9 which is about 4 to 10 times higher than the currently used safety factor of 5. When considering the available endpoints of all 13 different crops species, a safety factor of about 21 would still be necessary to bridge the differences between crops and non-crops. This is still more than 4-times higher than the currently used safety factor of 5.

Table 6 HC5 values for vegetative vigor tests after post emergence exposure to glyphosate (greenhouse tests) based on ER_{50} values (data are given in Appendix 2). The HC5 values of the 4, 5,…, 12 most sensitive and least sensitive crop species, respectively, were calculated to demonstrate the full range of possible HC5 values selected out of a dataset of 13 crop species (including *Lolium perenne*). To indicate the differences in sensitivities between crops and non-crops the obtained HC5 values for the crops were compared with the HC5 of 11.7 (g glyphosate / ha) for 28 non-crops (see 3.1).

Number of crop species	**HC5** (g glyphosate / ha) Most sensitive – least sensitive	**HC5 difference crops / non-crops** Most sensitive –least sensitive
4	210 - 624	17.9 – 53.3
5	215 - 596	18.4 – 50.9
6	213 - 561	18.2 – 47.9
7	219 - 532	18.7 – 45.5
8	227 - 505	19.4 – 43.2
9	235 - 448	20.1 – 38.3
10	244 - 398	20.9 – 34.0
11	253 - 335	21.6 – 28.6
12	251 - 285	21.5 – 24.4
13	243	20.8

Without having more than 6 sutiable ER_{50} values (to allow subsampling) and suitable ER_{50} values for several non-crops, it is impossible to estimate if similar safety factors would be necessary for given numbers of crop species tested with other herbicides. These data are not available. Without having any data on non-crops, the analysis of crop data does not allow any conclusion on the question how many crop-species should be tested to receive a reliable estimate of the HC5 value. The reason for that is the fact that the ratios between the HC5 values for the crops and non-crops differ between different herbicides as demonstrated for dicamba and glyphosate (see table 2). ER_{50} data for several herbicides need to be generated in studies containing many crop and non-crop species in order to examine if ratios between the HC5 values for the crops and non-crops are similar for herbicides of a given mode of action. If that is the case, it may could become possible to recommend the number of crops that should be tested for a herbicide of a certain type of mode of action.

3.4. Summary and discussion

As specified in the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC the risk of non-target plants is considered acceptable if the TER for the most sensitive species is greater than 5. Given that in the examined plant test performed for the risk assessment of herbicides only crop plant species were used in combination with the fact that crop species are much less sensitive to herbicides (differences up to 178 between crops and non-crops have been shown), a safety factor of 5 is certainly not adequate to bridge the differences in sensitivity seen in plants in Tier II test. A safety factor of 48 (based on the results of glyphosate) should be applied when only 6 crop species were tested. However, it should be considered that this estimate is based on the comparison of HC5 values of different dataset for a single herbicide. To get a more precise estimate of this uncertainty ER_{50} data for several herbicides need to be generated in studies containing many wild and crop species in one study set up with exactly matching conditions. So far these data are not available.

The testing of sensitive species could help to avoid uncertainties. In the present study an appropriate estimate of the HC5 was achieved by using sensitivity data of at least 18 non crop species (again this estimate is based on the worst case assumption derived from the analysis of only two datasets) (see chapter 3.2). Species of different families and with as many different ecological traits should be selected, among them Fabaceae, Caryophyllaceae, Scrophulariaceae, Asteraceae, Poaceae, Brassicaceae Lamiaceae, Apiaceae, and Rosaceae, the most dominant plant families in field margins (Table 4). Several crops such as oats, onion and soybean, which have been shown to be insensitive to most herbicides, should be avoided for non-target plant testing for risk assessment purposes.

4. State of knowledge of higher tier studies

A higher tier non-target plant study (Tier III) is required, when a potential risk at the lower Tier II level is identified (European Commission 2002). According to the Guidance Document for Terrestrial Ecotoxicology a potential risk occurs when the Toxicity to Exposure Ratio (TER)-value of the Tier II study is below 5, when the deterministic approach with at least six plant species is used. If the probabilistic approach is used at the Tier II level, a species sensitivity distribution (SSD) of the species is calculated and the HC5 parameter (hazard concentration for 5% of the species) is obtained. This parameter is an Effect Rate, where 5% of the population of the species is affected. If the HC5 parameter is below the highest predicted exposure value, the risk for terrestrial plants is assumed to be not acceptable (see chapter 1.2 Risk assessment of herbicides).

To refine the risk assessment a Tier III study, a semi-field or field assay, can be performed. However, to date no standard protocol for field or semi-field studies is available. Therefore, notifiers of pesticides might wish to discuss the study protocol and details on the test design with the responsible authority of a Rapporteur Member State (European Commission 2002). Generally, in a Tier III study effects on non-target plants should be observed during realistic applications and spray drift conditions or at exposure levels simulating different spray drift rates in the field. However, since semi-field and field studies are time-consuming and expensive it is noted in the Guidance Document to check whether there are options for the refinement of exposure and/or effects. Furthermore, a Tier III study is not required, if the risk based on the Tier II level could be managed by risk mitigation strategies (European Commission 2002). These strategies can also be discussed on Member State level (European Commission 2002). Therefore, mainly Tier II studies are performed for non-target plant risk assessment and registration of herbicides. The result of this policy is that to date Tier III studies were only in some occasions conducted (Olszyk et al. 2004, UBA 2012).

This chapter aims to summarize the current knowledge of higher tier testing, including mono-species and multispecies field tests, microcosms and field studies. Literature searches were performed as described in chapter 2 and in total 15 studies were obtained, which assessed the effects of herbicides using field or microcosm studies with selected individual or several cultured plant species. Additionally, we found four studies addressing the effects of herbicides on natural plant communities (Kleijn & Snoeijing 1997, De Snoo et al. 2005, Strandberg et al. 2012, Schmitz et al. 2013). These studies are presented in the following chapter and are listed separately, since they consider the complexity of natural community responses.

In the following the above mentioned 15 higher tier studies are described and discussed. These studies are divided in three categories (Table 7). In addition, it is listed, whether the plants were exposed to a realistic drift in the field (plants placed in different distances from the treated field) or to a simulated spray drift (direct overspray), since it is often mentioned that drift in the field differs from direct spray due to different droplet sizes and concentration (Koch et al. 2004, Strandberg et al. 2012, De Snoo et al. 2005).

Categories of higher tier studies:

1. Realistic drift studies with individual plant species in pots placed at different distances from the treated field during field application
2. Microcosm studies with several plant species planted together and exposed in greenhouse or field
    a) with realistic drift
    b) with simulated spray drift
3. Simulated spray drift studies with individual or several plant species on an experimental study site in the field

Table 7 Summary of Tier III studies with selected individual or several cultured plant species. The studies are divided in three categories: realistic drift studies with individual plant species, microcosm studies with several plant species planted together and exposed in greenhouse or field with realistic or with simulated spray drift, and field studies (simulated drift studies) with individual or several plant species. * Drift rates were calculated according to Rautmann et al. (2001) for field crops; DAT = days after treatment, WAT = weeks after treatment, n.d. = no data.

	Authors	Test design	Herbicide	Plant species		Measurements (test duration)	Main results
				Crop	Non-crop		
realistic drift studies with individual plant species	Marrs et al. 1989	individual plant species in pots placed at different distances from the treated field (0-20 m)	5 herbicides		23	sublethal and lethal effects (several weeks-months after treatment)	lethal effects up to 6 m, effects on flowering up to 10 m (6 m ≙ 0.48% drift of the field rate, 10 m ≙ 0.29% drift of the field rate*) → buffers zone of 5-10 m are needed
	Marrs et al. 1991a	individual plant species in pots placed at different distances from the treated field (0-4 m)	3 herbicides		5	plant biomass, lethal effects (20 WAT)	effects occur up to 4 m, young plants were more affected than old ones (4 m ≙ 0.71% drift of the field rate*) → buffers zone of 5-10 m are needed
	Marrs et al. 1993	a) 1 species in trays (140-250 seedling/tray) were placed at different distances from the treated field (0-40 m)	1 herbicide		1	sublethal and lethal effects (28 DAT)	10% mortality occurred at 10 m distance to the treated field (10m ≙ 0.29% drift of the field rate*)
		b) individual plant species (seedlings) in pots placed at different distances from the treated field (0-20m)	1 herbicide		15	sublethal and lethal effects (28 DAT), LDist50-value = distance to the treated field where 50% mortality occurred	wide range of response, one species had a LDist50 value of 15-20 m (20m ≙ 0.15% drift of the field rate* → buffer zones of 20 m needed to protect seedling regeneration
	De Jong & Haes 2001	individual plant species in pots were placed at different distances from the treated field (0-20 m)	4 herbicides	1	1	plant biomass (21 DAT)	significant effects were found regularly up to a distance of 8 meters, and in one experiment even at 16 m (8m ≙ 0.36% drift of the field rate*) → an optimized test setup was proposed

		Authors	Test design	Herbicide	Plant species: Crop	Plant species: Non-crop	Measurements (test duration)	Main results
microcosm studies	realistic drift	Marrs et al. 1991b	2-year field study: several plant species grown in microcosms were placed at different distanced from the treated area (0-8 m)	1 herbicide		9	sublethal (flowering) and lethal effects (28 DAT), plant biomass (3 month after treatment)	damage effects up to 4 m, flower suppression up to 2 m (10 m ≙ 0.29% drift of the field rate*) → buffer zones between 6-10 m are adequate to protect established plants
microcosm studies	realistic drift	Marrs & Frost 1997	4-year field study: several plant species grown in microcosms were placed at different distanced from the treated area in each of 4 years (0-8m)	3 herbicides		9	sublethal and lethal effects, plant biomass (3 month after treatment), flower number, seed production (1 and 2 years after the first treatment)	most effects (lethal, no. flowers, seeds) were confined to 8 m (8m ≙ 0.36% drift of the field rate*) → buffer zones of 8 m are adequate to protect surrounding vegetation
microcosm studies	simulated drift	Reuter & Siemoneit-Gast 2007	greenhouse study: several plants grown in microcosms and individually in pots were treated with different herbicide rates; dose-response experiments	2 herbicides		6	plant biomass, foliar injury (14, 28, 42 DAT)	species respond differently in pot and microcosms (further information see Research Box 3) → some species showed a higher sensitivity in microcosms than in single-species test
microcosm studies	simulated drift	Riemens et al. 2008	greenhouse study: several plants grown in microcosms and individually in pots were treated with different herbicide rates; dose-response experiments	1 herbicide		8	plant biomass, visual effects (28 DAT)	dicotyledons showed a higher sensitivity than monocotyledons in microcosms, shielding effects of monocots was observed → species respond differently in pot and microcosms
microcosm studies	simulated drift	Dalton & Boutin 2010	comparison of herbicide effects on plants grown individually in pots and in microcosms in greenhouse and outdoors; dose-response experiments	2 herbicides		16	plant biomass (28 DAT)	species in greenhouse microcosms were more sensitive than species in single species tests → sensitivity is dependent on interactions between species and the conditions

	Authors	Test design	Herbicide	Plant species Crop	Plant species Non-crop	Measurements (test duration)	Main results
field studies with simulated drift	Kjaer et al. 2006a	study site: hedgerows randomized spray experiment with two spray timings (at flower bud stage and flowering)	1 herbicide		1	leaves, buds, flowers, green berries, mature berries (n.d.)	hedgerows were most sensitive when sprayed at bud stage → 100% berry reduction at 5% drift of the field rate
	Kjaer et al. 2006b	study site: hedgerows effects on hedgerow the year after spray drift (Kjaer et al. 2006a)	1 herbicide		1	leaves, buds, flowers, green berries, mature berries (1 year after treatment)	effects on growth and reproduction was measured 1 year after exposure → risk assessment is likely to overlook effects on perennial plants
	Pfleeger et al. 2008	study site: potato fields 2 x 2 factorial design (application time and herbicide rate) in randomized blocks	6 herbicides	1		plant growth (14 DAT), tuber yield and quality (125 DAT)	tuber yield and quality were more affected by low herbicide rates than plant height → vegetative response did not predict yield and quality responses of tubers
	Pfleeger at al. 2012	a) study site: different fields on two farms, plants were transplanted in plots, randomized design	2 herbicides		4	plant growth (measured every 2 weeks during the growing season (May-July)	the most sensitive species in the field was *Cynosurus echinatus*
		b) single pot experiments, VV-Test (Tier II) with the four species used at the field site, greenhouse vs. field				plant growth (12 DAT)	the most sensitive species in the greenhouse was *Prunella vulgaris* → mixed relationships between field and greenhouse responses
	Gove et al. 2007	study side: woodland margins, individual plants in pots were treated with the herbicide and transferred to field plots; second treatment: fertilizer	1 herbicide		6	number of flowers and seeds, plant biomass (1 year after treatment)	drift rates increased mortality, reduced biomass and fecundity in all species, fertilizer treatment did not significantly alter flowering in any species → buffer zones of 5 m are needed
	Perry et al. 1996	study site: simulated field margin, community with six species were treated with diff. fertilizer and herbicide rates, randomized block design	1 herbicide		6	plant cover abundance (before and after treatment, measured every month during March-August)	fertilizer and herbicide treatment reduced the cover of species significantly → fertilizer and herbicide affect the plant community

4.1. Realistic drift studies with individual plant species

The studies of category 1 consist of realistic drift studies performed during an herbicide application in a crop field with in-situ bioassays with individual plant species placed at different distances from the treated field. Thus, the plants received different drift exposure rates. In total four studies were obtained using this study design (Table 7).

Marrs and co-workers conducted a series of tests to investigate the effects of herbicide drift on native plant species of conservation interest and to determine in-field buffer distances to protect the vegetation of field margins (Marrs et al. 1989, 1991 and 1993). A preliminary study was performed in 1989, in which the effects of five herbicides (asulam, chlorosulfuron & metsulfuron methyl, glyphosate, MCPA and mecoprop) on 23 non-crop plant species[1] were studied (Marrs et al. 1989). Single seedlings (information on leaf stage not given) in pots were arranged at different distances from the treated area (0, 1, 2.5, 5, 10, 20, 50 m) downwind of a tractor-mounted Team sprayer with a 6 m boom, which was fitted with 12 fan nozzles (80 cm above the ground) during application. The aim was to assess the impacts (lethal and sublethal effects such as flower suppression (flowering/not flowering) or damage effects (e.g. reduction in size, leaf chlorosis, discoloration) of the resulting spray drift. Five replicates per species and distance were used and plant performance was assessed several months after spraying (dates of assessment are not given).

The assessments showed that lethal effects were present up to 6 m away from the treated field. This distance is similar to an estimated drift of 0.48% of the field rate for field crops according to Rautmann et al. (2001), which is always added in the following text and marked with an asterisk (*). Effects on flowering and seed production were found up to 10 m (≙ 0.29% drift of the field rate*) and the greatest distance at which damage effects (e.g. reduction in size, leaf chlorosis, discoloration) were detected was 20 m (≙ 0.15% of the field rate*) for *Prunella vulgaris*. In general, Marrs et al. (1989) found no particularly sensitive indicator species for the different herbicides tested, but some species appeared to be consistently more sensitive than others, e.g. *Cardamine pratensis*,

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[1] *Allotria petiolata, Cardamine pratensis, Centaurea nigra, Circaea lutetiana, Digitalis purpurea, Dipsaeus fullonum, Filipendula ulmaria, Galium mollugo, Geum urbanum, Hypericum hirsutum, Lamiastrum galeobdolon, Leontodon hispidus,Lychnis.flos-cuculi, Medicago lupulina, Plantago media, Primula elatior, Primula veris, Primula vulgaris, Prunella vulgaris, Ranunculus acris, Silene dioica, Staehys officinalis, Teucrium seorodonia*

Centaurea nigra, *Digitalis purpurea*, *Lychnis flos-cuculi*, *Medicago lupulina* and *Prunella vulgaris*. As a main result, they suggested in-field buffer zone distances of 5-10 m for ground applications in arable crops to minimize the risk of herbicide impacts on the vegetation of field margins.

In a subsequent study, Marrs et al. (1991) considered plant age an influencing factor for exposure and effect. Therefore, pots with individual plant species in different development stages (seedlings or established plants, exact information on the leaf stage was not given) were placed downwind of a tractor-mounted Team sprayer (0, 1, 2, 4 m). In total five different species (*Digitalis purpurea, Leontodon hispidus, Lotus corniculatus, Lychnis flos-cuculi, Primula veris*) were studied with five replicates at each distance. Visual observation of the plants four weeks after treatment showed that all species had some symptoms of damage (reduced size, chlorosis, leaf discoloration, necrosis, epinasty). Lethal effects occurred up to 2 m downwind from the sprayer (≙ 1.4% of the field rate*). At the end of the growing season (20 weeks after treatment) the plants were harvested. Two of five species (*Lychnis flos-cuculi* and *Lotus corniculatus*), which had been placed 4 m downwind of the sprayer (≙ 0.71% drift of the field rate*), showed significant biomass reductions (dry weight). Generally, young plants were more affected than older ones.

Marrs et al. (1993) investigated the effects of herbicide drift on the seedlings stage of one species (*Lychnis flos-cuculi*). Therefore, several seedlings of this species (140-250) were grown in trays (220 mm x 175 mm x 55 mm) and exposed downwind of a sprayer (0-40 m). At 10 m distance to the treated field (≙ 0.29% of the field rate*) a mortality of 10% was observed 28 days after treatment. In another experiment of Marrs et al. (1993), seedlings of 15 different plant species[2] were also arranged at different distances from the treated area (0-40 m). This experiment indicated a wide sensitivity to spray drift of herbicides with one species (*Hypericum perforatum*) affected (50% mortality) between 15 and 20 m downwind of the sprayer (≙ 0.19% and 0.15% of the field rate*). Therefore, Marrs et al. (1993) concluded that buffer zones for established plants could be set at 6-10 m, but where seedling regeneration is important a buffer zone of 20 m is needed.

[2] *Betonica officinalis, Digitalis purpurea, Galium verum, Geum urbanum, Hypericum perforatum, Lotus corniculatus, Lycopus europaeus, Pimpinella saxifrage, Plantago media, Primula elatior, Primula vulgaris, Ranunculus acris, Silene alba, Teucrium scorodonia, Verbascum thapsus*

The study of De Jong & Haes (2001) aimed at developing a standardized field trial for assessing the short-term impact of low rates of herbicides on vascular plants. At first various designs for bioassays had been tested in a greenhouse setting and thereafter a test method was chosen: Experiments were conducted with three herbicides (glyphosate, bentazone and diquat) and as test species *Brassica napus* and *Poa annua* were used, since they can easily be handled in bioassay set-ups. For *B. napus* 150 rape seeds were placed in separate compartments in a multi-compartment tray (30 x 50 cm, 10 x 15 compartments) and for *P. annua* a 10 L pot was used, which was divided into three parts. In each part of the pot 0.075 g *P. annua* seeds were sown. The plants were grown in the laboratory for approximately two weeks and then the plants were transferred to the field for spraying. Therefore, the trays and pots were placed in a 5 x 10 m test plot to obtain the full application rate, as well as at distances of 2, 4, 8 and 16 m downwind. The test plot was sprayed with a knapsack sprayer, which was connected to a 1 m spray boom. A further tray (control) was situated at a large distance (> 500 m) from the treated area. Two hours after spraying, the trays and pots were returned to the cultivation rooms, where 20 randomly chosen plants of *B. napus* were harvested after 7, 14 and 21 days and the aboveground biomass (wet and dry weights) were determined. For *P. annua* one measurement was performed on 30 plants to get an accurate measurable amount since individual plants of *P. annua* were too small. Additionally, the deposition rate of the applied spray volume was determined using water-sensitive papers.

Considerable differences in biomass were detected between species as well as between herbicides. For glyphosate the distance at which 50% biomass reduction occurred was between 5 and 6 m (≙ 0.57% and 0.48% of the field rate*) from the test plot for both species. Diquat led to a 50% biomass reduction at this distance only in *B. napus*. However, in some of the experiments, a 50% effect was even found at the furthest sampling point (≙ 16 m from the sprayed test plot), even though no deposition with water-sensitive papers was measured there with the used method (≙ 0.18% of the field rate*).

De Jong & Haes (2001) concluded that these bioassay tests were suitable to assess impacts of herbicide drift on plants. Additionally, they discussed four methodical aspects (number of individual plants, variations between trays, variation in time and time intervals of effect measuring) to be considered in further tests and proposed an optimized test design:

- Tests should be performed using climate chamber reared plants

- Plants should be approximately 2 weeks old, at least two leaves should be present
- One bioassay should be placed inside the treated plot and at 2, 4, 8 and 16 m downwind
- Two unexposed controls should be placed at least 500 m away
- Tests should be conducted in dry weather conditions and high temperatures should be avoided
- Compounds should be in a formulation used in practice
- Equipment according to standard practice should be used
- Droplet deposition should be measured to check that plants are indeed exposed to a compound
- At least three assessments should be conducted: one third of the plants should be harvested at the moment of clear visible effect on the 100% treated unit, one week later the next third should be harvested, followed by the last harvest again one week later

Summary and discussion of the realistic drift studies

The experiments performed by Marrs et al. (1989, 1991a and 1993), as well as the study of De Jong & Haes (2001) used similar test designs. They placed individual plant species in pots at different distances from a treated field and investigated the effects of the resulting spray drift. After spraying, the test plants were transferred to a holding area in the field or to the greenhouse and were monitored for the development of damage and biomass reduction. In general, the cultivation and replication of such bioassays are comparatively simple and inexpensive and therefore, these experiments are easy to perform and can be repeated to a certain degree. The variation in spraying conditions (actual temperature and wind speed and direction) during the application will always result in slightly different results. According to De Jong & Haes (2001) these bioassay are appropriate and sensitive methods, which can probably be used to assess effects of herbicides on non-target plants (De Jong & Haes 2001).

However, these tests do not differ strongly from Tier II studies and give little additional information. The difference is that the proposed bioassays allow natural exposure of plants under realistic drift conditions, but they give, as well as the Tier II studies, no information on plant community processes since single-species tests cannot capture the large variations found in natural systems (Dalton & Boutin 2010). Furthermore, with the proposed test setup of De Jong & Haes (2001) only short-term effects up to 21 days on individual plants in young development stages can be assessed. Effects of reproduction and recovery could not be detected with this method, even though former studies of Marrs and co-workers (e.g. Marrs et al. 1989) showed that also flowering and seed production of plant species can be suppressed by drift up to 10 m from the treated field. Since numerous plants have to produce viable seeds to persist in field margins, it is necessary to consider specifically this endpoint (flowering, seed production) in herbicide drift studies.

Therefore, if bioassays according to the design suggested by De Jong and Haes (2001) should be used in registration processes it seems crucial to include also established plants at older phenological stages beside seedlings and young plants. Additionally, it seems important to extent the assessment period after treatment to the time of seed maturity. This time span is species dependent and should be decided on a case by case basis. However, it must be recognized that results of these studies can never be assumed to be representative of the response of plant species in plant communities. They can only provide useful information of the sensitivity of a species to a particular herbicide or to generate hypothesis for further semi-field or field studies (Dalton & Boutin 2010).

4.2. Microcosm Studies

The second category of studies consists of so called microcosm studies. In microcosm studies the realism in contrast to single-species tests should be increased and therefore microcosm experiments are performed with more than one plant species per plot or planting tray. With this method interaction effects among species should be investigated. Furthermore, the response of a plant species in microcosms can be affected by neighboring plants, for example through a shielding effect. In particular species with a relative small stature can probably be shielded to herbicide exposure by leaves of taller growing plants (Riemens et al. 2008). To date, there has been very little research on the effects of herbicides on non-target plant species grown in microcosms. In total only five microcosm studies with non-target plants could be located (Table 7).

From 1987 to 1988, Marrs and co-workers (Marrs et al. 1991b) conducted a two year pilot study with microcosms to investigate the effects of herbicide drift on plant communities. Therefore, eight native dicotyledonous species (*Digitalis purpurea, Filipendula ulmaria, Galium mollugo, Hypericum hirsutum, Lychnis flos-cuculi, Primula veris, Ranunculus acris, Stachys sylvatica*) and one perennial grass (*Lolium perenne*) were used. The dicotyledonous plants were raised separately. When the plants had developed four expanded leaves, one individual plant per species was transferred in a defined arrangement in the microcosms (tallest species were positioned on the downwind side). As microcosms, trays with 27 cm diameter and 12 cm depth were used. Half of the microcosms were sown with the grass *Lolium perenne* (20 kg seed/ha) to create an additional treatment comparison (grass vs. no grass). A few weeks later the microcosms were exposed to drift of the herbicide mecoprop. In accordance with previous studies of Marrs et al. (1989) and (1991a), the effects of herbicide drift were investigated under realistic drift conditions. Therefore, the microcosms were placed at different distances from the treated area (0, 1, 2, 4 and 8 m, 5 replicates at each point). Two applications were conducted in two years. After spraying the microcosms were placed in a greenhouse for 24 h to prevent that the herbicide washed off and then the microcosms were transferred to a holding area (open-air plunge bed).

The results show, that the effects on the microcosm plant community were stronger in the second year in comparison to the first year : Lethal effects near the sprayer, as well as damage effects up to 4 m (≙ 0.71% drift of the field rate*) were found already in the first year, but effects on reproduction (presence/absence of flowering) for three species (*Lychnis flos-cuculi, Primula* *veris* and *Ranunculus acris*) were detected up to 2 m (≙ 1.4% of the field rate*) only in the second year. Additionally, the growth of two species (*Stachys sylvatica* and *L. perenne*) was enhanced near the sprayer and six species showed a reduction in performance or biomass after the second exposure. A further and general conclusion was that different species showed different responses and that there are differences in the response of dicotyledons in microcosms sown with grass and those left unsown. According to Marrs et al. (1989) these differences are maybe caused by variations in interception of herbicide drift by the inter-sown grass and the different plant densities in the microcosms. Thus some dicotyledonous plant species have a benefit or a disadvantage of the presence of a grass. A final conclusion of this study is that in-crop buffer zones of 6-10 m are needed to protect established plants from spray drift (Marrs et al. 1989).

In a subsequent microcosm study, Marrs & Frost (1997) investigated the cumulative impacts of herbicide drift on plant communities over 3-4 years. Therefore, microcosm experiments with the same test design (eight dicotyledonous species and one perennial grass per microcosm) as used before (Marrs et al. 1991b) were carried out. The microcosms were placed downwind of the sprayer (0-8 m) in each year and were exposed to one of three herbicides (glyphosate, mecoprop, MCPA). The results were in line with the results of Marrs et al. (1991b). The effects (reduced biomass) became stronger with the course of time. This means, that herbicide drift affects the balance of species from the second year of exposure and that most effects (reduced biomass, suppression of flowering) were confined within an 8 m zone.

Reuter & Siemoneit-Gast (2007) also performed a microcosm study with the aim to develop a methodological approach to test effects of herbicides on wild plant species. In contrast to the experiments of Marrs and co-workers (Marrs et al. 1991, Marrs & Frost 1997) this microcosm study was conducted under greenhouse conditions. Additionally, single-species tests were performed to compare the effects of herbicides on plants grown separately and in mixture. Therefore, monocultures and artificial plant communities (microcosms) were treated in a dose-response design with simulated drift rates (see Research Box 3).

Riemens et al. (2008) also performed a dose-response experiment with microcosms under standardized greenhouse conditions. As microcosms 5 L pots were used. Each microcosm consisted of four monocotyledons (*Poa annua, Echinochloa crus-galli, Elymus repens, Panicum miliaceum*) and four dicotyledons

(*Solanum nigrum, Stellaria media, Chenopodium album, Centaurea cyanus*). Seeds of the species were seeded in such a manner that emergence of the species would coincide. Mono- and dicotyledons were placed alternately in the pots and thinned to eight plants per species per pot after emergence. Four weeks later the microcosms were sprayed with different rates of the herbicide glufosinate ammonium in a spray chamber. Five herbicide rates and one control with eight replicates each were used. First visual symptoms of herbicides were recorded two days after treatment and four weeks later the fresh weight of the plants was determined.

The ER_{50} values of all monocotyledons were higher than the ER_{50} values of the dicotyledons. Generally, the monocotyledons in the microcosms were less affected by glufosinate ammonium compared to the dicotyledons in the same microcosms. Consequently in natural vegetation a shift in the species composition may occur. Furthermore, some species (e.g. *S. media*) can benefit from the sheltering effect of other species and thus this species has a reduced exposure. Additionally, Riemens et al. (2008) performed single species tests with four species used in the microcosm experiments *(C. album, S. media, P. annua, E. crus-galli)* under greenhouse and field conditions. Therefore, species in 0.5 L pots (4 individuals per pot) were treated two or four weeks after emergence with the same herbicide as used in the microcosm experiments. The results show that the greenhouse-grown plants had lower ER_{50}-values than the field grown plants, which is maybe caused by different environmental conditions. Another outcome of the study was that the sensitivity of species grown individually and in mixtures differs from each other due to inter- and intraspecific interferences and shielding effects in mixtures, which cannot be separated from each other.

Dalton & Boutin (2010) conducted a microcosm study with nine terrestrial plant species (biennial species: *Alliaria petiolata, Rudbeckia hirta;* perennial species: *Euthamia graminifolia, Fragaria virginiana, Geum canadense, Leucanthemum vulgare, Solidago rugosa, Symphyotrichum lateriflorum, Symphyotrichum novae-angliae)* and seven wetland plants (perennial species: *Asclepias incarnata, Chelone glabra, Eupatorium maculatum, Eupatorium perfoliatum, Lycopus americanus, Phalaris arundinacea, Verbena hastata).* The objective was to compare the response of the plants to the herbicides glyphosate and atrazine when grown separately in pots versus under different microcosm set ups. In total three different microcosm conditions were used:

- Greenhouse microcosm experiments (test duration 28 days after treatment)

- Outdoor microcosm experiments (test duration 28 days after treatment)
- Long-term greenhouse microcosm experiments (test duration 60-70 days after treatment)

All microcosm experiments were conducted separately for each herbicide and each habitat type (terrestrial and wetland). 5 L round plastic pots were used as microcosms and one seedling of each species were transplanted in the microcosms with a standardized planting arrangement where one species (*A. petiolata* for terrestrial microcosm and *P. arundinacea* for wetland microcosm) was planted in the middle of the pot and the others were randomly assigned in a circular arrangement around the perimeter of the pot. The plants were sprayed with a track sprayer in a spraying chamber when they reached a size comparable to the 4-6 leaf stage typically used with crop plants in herbicide testing (4-15 leaves depending on the size and growth form of the species). Five herbicide rates and one control with six replicates each were used.

The greenhouse microcosms were the most sensitive test with the largest biomass reduction. In the single species tests a similar overall biomass reduction as in the long-term or outdoor microcosm was observed. Sensitivity was found to be dependent on interactions between species and test conditions. For example greenhouse plants were taller, greener and had more leaves than outdoor plants. Additionally, the temperature in the greenhouse was higher. These differences maybe increased the translocation of the herbicide in the greenhouse plants and increased their sensitivity. The outdoor plants had smaller leaves and maybe thicker cuticles, which may have contributed to a decreased herbicide absorption and a resulting lower herbicide toxicity (Dalton & Boutin 2010).

RESEARCH BOX 3: Extended method for assessing the risk to terrestrial non-target plants exposed to herbicides

BACKGROUND: The data requirements for testing the toxicity of plant protection products for non-target organisms are defined in Annex II and III of Council Directive 91/414/EEC, following, in principle, a tiered approach. Phytotoxicity tests for non-target plant risk assessment are performed mainly with crop plants, which are cultivated under standardized conditions and individually for each test species (mono species test). In order to gain further information for the risk assessment for non-target plants and plant communities, a research project was initiated by the German Federal Environment Agency, (Umweltbundesamt = UBA) on the inclusion of relevant non-crop plants in the assessment scheme under more realistic, but still standardized conditions. Artificial plant communities were studied to assess competition and recovery effects.

METHODS: The study was performed in three stages: 1) selection of appropriate test plants, 2) formation of stable artificial communities and development of an evaluation scheme, and 3) trial of the developed test system with herbicides of different mode of actions. In total 231 commercially available non-crop species were cultivated under uniform conditions to select species which fulfil the requirements for seedling emergence under standardized conditions. Out of this germination test 74 plant species had a seedling emergence rate of > 50% after 14 days. Six plant species were selected to establish the community for the trial: *Leontodon hispidus, Silene nutans, Trifolium pratense, Galium mollugo, Bromus erectus, Cynosurus cristatus.* For the microcosm experiment plant trays were used (17cm x 17cm, filling height 5cm) and plants were sown in 2.5 cm distance to each other. Each plant species was sown 8 times in a uniform arrangement on the trays. Since in this arrangement, one space was left, it was decided to sow alternately one species of the 6 test plants in each microcosm replicate. Thus, there were 49 individual plants per microcosm. For the evaluation only the 24 plants (4 x 6 test species) in the middle of the trays were considered. In addition to the microcosm experiments, the selected plant species were separately cultivated in pots (single-species design: Ø 7cm, filling height 5 cm, 4 plant individuals per pot) to investigate the effects of the herbicides in monocultures.

The monocultures and the artificial plant communities were treated in a dose-response design (5 treatments of application and simulated drift rates, 1 control) with two different herbicides. The non-selective herbicide Roundup Ultra (a.i. glyphosate 360g/l) and the selective herbicide Monitor (a.i. sulfosulfuron 800g/kg) was chosen. The test plants were treated at the 2-4 leaf growth stage in the greenhouse. The treatments of the monocultures were replicated 4 times and the treatments of the plant communities were replicated 3 times. Effects were assessed on three days at 14-day intervals with total test duration of 42 days. The influence of competition within the plant stand was determined by comparison with the same plants in monoculture.

RESULTS: The total fresh weight of the plant community was significantly affected by the herbicide applications. The non-selective herbicide Roundup had a greater impact on the fresh weight of the plant community than the non-selective herbicide Monitor (Fig. 1).

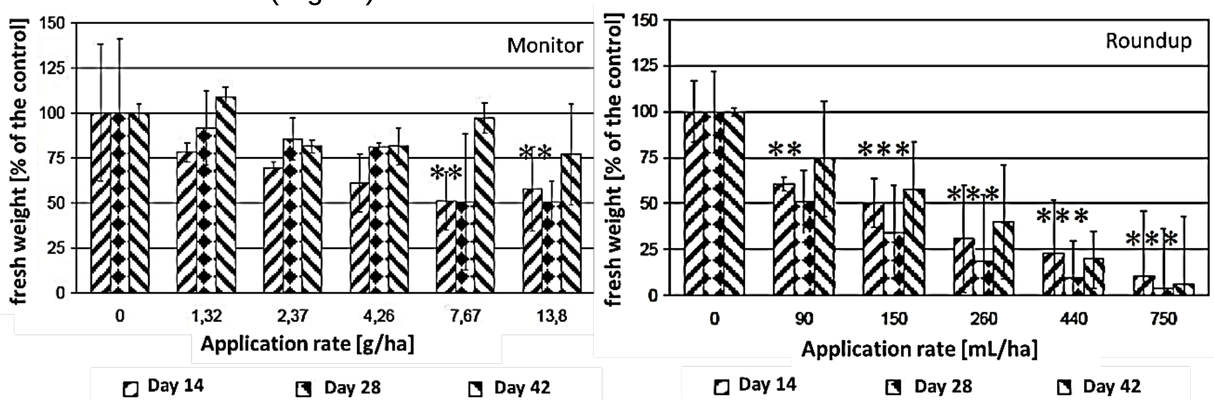

Figure 1: Fresh weight (± SD) of the plant community per treatment (Reuter & Siemoneit-Gast 2007)

Roundup affected the plant growth significantly in all treatments in comparison to the control, while the non-selective herbicide Monitor affected only a few species. This is shown in the results of the monoculture experiments (M) and the estimated ER_{50} values based on the fresh weight of plant species (Table 1). The plant species *G. mollugo, C. cristatus, L. hipidus* and *S. nutans* showed a progressive damage for both herbicides. The species *B. erectus* and *T. pratense* were less sensitive in comparison to the other test plants, especially to the non-selective herbicide Monitor. Consequently these two species had a competitive advantage in the plant community experiments (rows C in table 1) treated with the non-selective herbicide, where they revealed an increased biomass.

The comparison of the ER_{50} values of the plant species grown in monoculture (M) and in community (C) show 42 days after treatment, that 3 out of 5 species treated with Roundup and 2 out of 5 species treated with Monitor revealed a higher sensitivity in the plant community than in the monoculture (Roundup = *G. mollugo, L. hispidus, S. nutans*; Monitor = *G. mollugo, L. hispidus*). Especially *S. nutans* and *L. hispidus* showed a 3 times higher sensitivity in the plant community than in the monoculture. The process of recovery was also recorded with the study design. A recovery of the test plants occurred, when the estimated ER_{50}-values at 42 days after treatment (DAT) were much higher than the prior determined values (DAT 14 and 28). As it can be seen in Table 1 a few species showed a recovery 42 days after treatment and others did not respond in the extended growth period. However, two species showed a higher sensitivity 42 days after treatment than 28 days after treatment. The sensitivity towards Roundup and Monitor increased over time for *G. mollugo* (in M treated with Roundup and Monitor) and *S. nutans* (in C treated with Monitor).

Generally, phytotoxicity tests based on OECD guidelines have a test duration of 21-28 days and therefore in both species effects would have not been detected in the standard test duration.

Table 1: Estimated ER_{50}-values (fresh weight) of Round up (a.i. Glyphosate 360g/L) and Monitor (a.i. Sulfosulfuron 800g/kg) for the plants species grown in monoculture (M) and in plant communities (C) (grey columns) at 14, 28 and 42 days after treatment (DAT). The last column shows the ER_{50}-values for the total plant community; R = Recovery: - no recovery; o not clear; + recovery. n.d. = not determined. Plants are arranged by increasing ER_{50}s (Data were taken from Reuter & Simoneit-Gast 2007).

				non-selective				selective			
				Round up Ultra [mL/ha]; ER50				Monitor [g/ha]; ER50			
species	**common name**	**family**	**Test**	**14 DAT**	**28 DAT**	**42 DAT**	**R**	**14 DAT**	**28 DAT**	**42 DAT**	**R**
G. mollugo	white bedstraw	Rubiaceae	M	120	106	93	-	n.d.	1.4	1.4	-
			C	n.d.	n.d	79	o	1,5	1.6	0.9	-
C. cristatus	crested dog's-tail	Poaceae	M	88	84	101	o	2,1	1.0	5.9	+
			C	n.d.	n.d	n.d.	o	4,9	4.0	7.9	+
L. hispidus	hawkbits	Asteraceae	M	159	108	127	-	n.d	1.7	6.6	+
			C	95	63	115	+	1,1	1.7	1.9	-
S. nutans	nottingham catchfly	Caryophyllaceae	M	81	137	237	+	6,6	1.4	1.4	-
			C	77	78	92	o	2,2	3.5	1.7	-
B. erectus	upright brome	Poaceae	M	313	319	340	o	> 13.8	> 13.8	> 13.8	+
			C	288	217	420	+	> 13.8	> 13.8	> 13.8	+
T. pratense	red clover	Fabaceae	M	308	214	380	+	> 13.8	> 13.8	> 13.8	+
			C	376	256	470	+	> 13.8	> 13.8	> 13.8	+
plant community			**C**	140	93	185	+	> 13.8	11.8	> 13.8	+

CONCLUSION: The study aimed to provide a methodological approach to supplement tests to assess the risk to terrestrial plants exposed to herbicides. For some species higher sensitivities were revealed when planted in communities, although water and nutrient stress was limited in this study. Effects in the field can be more pronounced due to more complex community composition (more species, interactions) and severe competition for light, water and nutrients. It can be concluded that generally effects measured with only a few species in microcosms cannot be easily transferred to the field situation.

SOURCES:

Reuter & Siemoneit-Gast 2007: Entwicklung einer weiterführenden Methode zur Bewertung des Risikos für terrestrische Pflanzen durch Exposition mit Pflanzenschutzmitteln und ihren Wirkstoffen. Umweltforschungsplan des Bundesministeriums für Umwelt, Naturschutz und Reaktorsicherheit. Im Auftrag des Umweltbundesamtes.

Siemoneit-Gast S, Reuter S, Kubiak R, Höllrigl-Rosta A. 2007. Development of an extended method for assessing the risk to terrestrial plants exposed to plant protection products and their active ingredients. Conference Title: *13th Symposium Pesticide Chemistry*, Conference paper*: Environmental fate and ecological effects of pesticides 2007*. Piacenza, Italy, pp 649-656

Summary and discussion of the microcosm studies

Three of the five evaluated microcosm studies were performed in greenhouses (Dalton & Boutin 2010, Reuter & Siemoneit-Gast 2007, Riemens et al. 2008). These greenhouse microcosm experiments were conducted under standardized conditions with a precise control of environmental conditions. As test species young wild plant species were used, which were treated a few weeks after emergence, approximately at the 4-6 leaf stage (Reuter & Siemoneit-Gast 2007, Riemens 2008) or when they reached a size comparable to the 4-6 leaf stage (Dalton & Boutin 2010). The number of species used in these microcosm experiments was in a similar range (6-9 species). However, the individuals per species and microcosms differed strongly between these three experiments. Dalton & Boutin (2010) used in total seven wetland or nine terrestrial plant species, but only one individual plant per species and microcosm. In contrast, Riemens et al. (2008) used eight different plant species and eight individual plants per species and microcosm. Thus, a microcosm of Riemens et al. (2008) consisted of 64 individual plants. Since both microcosm experiments used a 5 L pot as test system, the plant density differed considerably between these two microcosm experiments. Generally, a higher plant density increases interaction effects between plants (e.g. competition) and, additionally, shielding effects can occur. For example Riemens et al. (2008) could detect a shielding effect for e.g. the small species *Stellaria media*. Probably this species was in advantage because it received less of the applied herbicide rate due to shelter provided by other species grown in mixture, which could not be predicted from single-species tests (Riemens et al. 2008). Reuter & Siemoneit-Gast (2007) used in total six different plant species and also eight individual plants per species and microcosm. Additionally, they used alternately one species of the six test plants in each replicate and thus, they had 49 plants per microcosm. As test systems they used trays of 17 cm x 17 cm with a filling height of 5 cm. Therefore, this test system differed slightly from the test system of Dalton & Boutin (2010) and Riemens et al. (2008), which used 5 L pots. However, the size of 17 cm x 17 cm roughly corresponds to the size of 5 L pots. Generally, such sizes of microcosms are small enough in scale to be used in dose-response experiments with an appropriate number of replications. The evaluated microcosm studies used 4-8 replicates. However, since community analyses are complex and it cannot be excluded that variations between microcosms can occur, the number of replicates should be increased whenever possible (Fraser & Keddy 1997).

Besides the number of species and replicates, it is also important to consider the taxonomic group of species. Dalton & Boutin (2010) used only dicotyledons in the terrestrial microcosms. Riemens et al. (2008), as well as Reuter & Siemoneit-Gast (2007) used a mix of dicotyledons and monocotyledons. This mix of broad-leaf species and grasses seems to be important and necessary since most herbicides have a specific mode of action, targeting specifically on mono- or dicotyledons (Riemens et al. 2008). Furthermore, the presence or absence of monocotyledons in the vegetation can influence the response of the dicotyledons, maybe due to different interceptions of herbicides (Marrs et al. 1997). Moreover, the vegetation of field margins consist of broad-leaf species and grasses and therefore, microcosms should also consist of both groups.

Another area of concern lies in the test duration of the microcosm experiments and the assessed endpoints. Test durations of 28 days as used in current non-target plant testing could underestimate the risk of herbicides on plants. For example Reuter & Siemoneit-Gast (2007) showed that the sensitivity of some species increased over time (higher sensitivity in an extended test period, e.g. two of six species showed a higher sensitivity 42 days after treatment than 28 days after treatment). In contrast, Dalton & Boutin (2010) detected no increase in the sensitivity by using long-term (70-90 days after treatment) microcosm experiments. However, in all studies only young plant species were used and as endpoint biomass reduction was determined. Effects on reproduction were not investigated.

In the field, herbicides are often applied when plants are in other phenological stages (e.g. just before flowering) and then, effects on reproduction were observed (for example Schmitz et al. 2013, Marrs et al. 1991b). Also a few other studies have indicated that reproductive structures such as flowers, pollen, fruits, seeds are particularly sensitive to herbicide exposures (Fletcher et al. 1996, Marrs et al. 1993, Kjaer et al. 2006, Strandberg et al. 2012). Moreover, Strandberg et al. (2012) found that seed production is a more sensible endpoint for risk assessment of herbicides than biomass independently of time of exposure. Therefore, besides tests with species in young development stages, tests with established species or species in older phenological stages are necessary to assess also effects on reproduction. A risk assessment based only on biomass and visual effects presumably underestimate the sensitivity of non-target plants (Strandberg et al. 2012). Additionally, older phenological stages that are more field relevant to the point of application of herbicide in spring or summer will better mirror field relevant shielding effects that seem to be important for the community composition and the presence of specific plant species as shown by Riemens et al. (2008) for *Stellaria media*.

The two microcosm studies of Marrs and co-workers (Marrs et al. 1991, Marrs & Frost 1997) investigated the effects of herbicides on wild plant species in the field. They used microcosms (pot: 27 cm diameter x 12 cm deep) with nine different plant species (one individual plant per species and microcosm) and placed these microcosms in different distances from the treated field. Thus, the plants were exposed to herbicide drift under realistic drift conditions. In contrast, the microcosms in the greenhouse experiments received an overspray in spraying chambers with simulated spray drift rates, which differs from real spray drift: The substantial difference may be caused by different droplet size (Strandberg et al. 2012).

Drift consist of smaller droplets with possibly higher concentrations of the pesticide. The droplets deposited in the field are larger and have maybe a higher penetration power of the vegetation than spray drift (Koch et al. 2004). However, to date it is not possible to conclude whether these differences in exposure produce different effects on the plants (Strandberg et al. 2012). Furthermore, drift in the field is extremely influenced by meteorological conditions (e.g. wind speed, temperature, relative humidity) and technical factors (e.g. boom height, driving speed, nozzles). These factors can vary from application to application and may produce different effects. A comparison between plants exposed to realistic drift and spray drift was not done in the discussed studies. However, the main advantage of direct spraying is that the application can be performed carefully under controlled and repeatable conditions. Furthermore, with this type of application the amount of spray volume can be kept constant. Thus, simulated spray drift conditions probably represents worst-case conditions.

Another difference between the studies of Marrs and co-workers and the three other greenhouse experiments is the test duration. Marrs et al. (1991) and Marrs & Frost (1997) performed perennial field studies (2-4 years) and investigated the effects of repeated herbicide exposures. Damage and lethal effects were noted in the first year, but effects on species compositions, as well as effects on reproduction (flowering, seed production) were firstly noted after the second year of exposure. These studies could demonstrate the importance of perennial field studies and Marrs & Frost (1997) noted that such microcosm approaches are probably the most efficient way of investigating the cumulative effects on plant communities to successive exposures to spray drift. This approach is very time-consuming and expensive and, in addition, in field microcosms an invasion by new species growing in adjacent areas can occur (but this could be handled, when necessary). Generally, plants in field margins are also exposed to repeated pesticide applications every year leading to cumulative effects which can only be assessed with perennial experiments.

A comparison between the sensitivity of plants grown in field and greenhouse microcosms was performed only by Dalton & Boutin (2010). They showed that the short-term greenhouse microcosm experiments were more sensitive than short-term microcosm experiments in the field. This is maybe a result of differences in environmental conditions (higher temperature in the greenhouse), which increased the translocation of the herbicide in the greenhouse plants. Moreover, the outdoor plants had smaller leaves and maybe thicker cuticles which may have contributed to a decreased herbicide adsorption in the field. Riemens et al. (2008) found similar results with single species tests performed in greenhouse and in the field. They detected that the greenhouse grown plants were more sensitive than the field grown plants, maybe also as a result of differences in environmental conditions, such as temperature, relative humidity and light intensity. A low relative humidity and low light intensity reduce the performance of the herbicide glufosinate ammonium, which they had used in their study. Riemens et al. (2008) described that a high relative humidity increases the efficacy of the herbicide due to the hydration of the cuticle and thus, water soluble compounds can penetrate the cuticle more easily. A low relative humidity in the field, results in a reduced uptake of the herbicide (Riemens et al. 2008).

In addition, Riemens et al. (2008) compared the results of the single species tests with the results of the microcosm test, in which the same species were used. They found that results from single species tests could not be translated to effects on these species grown in mixture since species showed a species-specific response to the habitat (due to interactions with other species, shielding effects etc.). Dalton & Boutin (2010) also detected a higher sensitivity of species grown in microcosms than in single-species test and concluded that it is unlikely that single-species tests could predict the risk of herbicides on non-target plant communities. These results are also in agreement with the results of Reuter & Siemoneit-Gast (2007). A comparison of the sensitivity of the plant species grown individually in pots with the sensitivity of the species grown in microcosms showed that three out of five species treated with glyphosate and two out of five species treated with sulfosulfuron revealed a higher sensitivity in the microcosms than in monoculture. Even two species (*Silene nutans* and *Leontodon hispidus*) showed a three times higher sensitivity in the plant community than in the monoculture (Reuter & Siemoneit-Gast 2007).

Hence, it seems that microcosms represent an appropriate testing system for higher tier studies since they have several advantages over single-species tests (Table 8).

Table 8 Advantages and disadvantages of microcosm studies under greenhouse conditions.

Advantages of microcosm studies	Disadvantages of microcosm studies
• more realistic than single species testing, due to interaction effects • precise control over environmental variables • possibility to manipulate the parameters and treatments under investigation • background variability and vegetation heterogeneity can be minimized • ease of replication • acceptable costs • insect pests can be managed	• over-simplification compared to natural conditions • only a few "representative" species can be studied • cannot capture the large variation of natural systems

Microcosms are important and useful investigative tools for examining relationships between plant species (Dalton & Boutin 2010). Additionally, they increase our understanding of natural processes by simplifying the complexity of our natural environment (Fraser & Keddy 1997). However, before microcosms can be used as standardized studies for pesticide registration risk assessment a proper establishment and validation of a testing method should be undertaken. Additionally, clear testing protocols/guidelines are needed, as well as a decision whether the microcosms should be conducted in the field or in greenhouse, since test conditions (light, temperature, relative humidity) seem to be important factors, which can influence the sensitivity of plant species.

Moreover, microcosms represent an oversimplification of natural communities since only a few representative species can be used (see previous chapter on sensitivity of plant species). Microcosm experiments also provide optimal conditions for recovery from negative herbicide effects as do the standard test designs. The plants are grown in the greenhouse or under controlled conditions, and are tested at uniform growth stages and sufficient nutrients are provided. In natural communities the plants are generally exposed to more stressors (e.g. herbivores, competition for limited water and nutrient resources), which is expected to reduce their ability to recover. Therefore, it is conceivable that the effects could be stronger in natural communities, in particular for sensitive plant species. Nevertheless, microcosms can be useful for measuring the effects of a particular herbicide on several plant species grown in a mixture.

Based on the described experiments, general factors which should be considered in microcosm experiments, as well as recommendations for the design and performance of a microcosm study with non-target plants are provided in Box 1.

Box 1: General factors and recommendations for the design and performance of a microcosm experiment with non-target plants

- **Species:** The vegetation of non-target areas (e.g. field margins) consists of dicotyledons and monocotyledons, annual and perennial species. Therefore, a mix of these types of species seems to be appropriate. Only wild plant species should be used, no crop species. It is also important to consider the plant traits of the species.
- **Number of Species:** The number of plants used in the evaluated microcosm studies ranged from 6-9 species. We recommend using a minimum of 6 species. However, plant communities have many species, and the more used in a study, the greater the realism (Fraser & Keddy 1997).
- **Individuals per species and microcosm:** The number of individual plants is dependent on the size of the test system and the number of species used in the microcosm experiments. In the evaluated microcosms up to 8 individuals per species were used. An appropriate plant density is important for investigating interaction effects. In this topic more information is needed.
- **Development stage of the test species:** Beside young developed plant species (2-6 leaf stage), it seems extremely important to use also plant species in older phenological stages, e.g. just before flowering. Numerous studies showed that herbicides can affect the reproduction of wild plant species. Therefore, effects on reproduction should also be assessed.
- **Test duration:** In some cases a test duration of 28 days can underestimate the effects of herbicides on plant communities. Especially when effects on reproduction and plant compositions should be assessed. Hence, it would be important to extent the assessment period after treatment to e.g. the time of seed maturity. This is species depend and needs to be decided on a case by case basis.
- **Size of the test system:** The size of the test systems is related to the size of the test plants and their phenological stages. The evaluated microcosm studies used test systems of 5 L pots or trays of 17 cm x 17 cm. However, Fraser & Keddy (1997) recommend using areas not smaller than 25 cm x 50 cm for microcosm experiments.
- **Number of replicates:** The number of replicates in the evaluated microcosm experiments ranged from 4-8. Since community analyses are complex it is important to increase replication whenever possible (Fraser & Keddy 1997).
- **Pest infestations:** Plants in microcosms can be infested by pests (e.g. aphids, spider mites, fungus gnats). Pest populations that occur during the experiment can be managed with biological pest control. When a biological control is used, all treatments should be treated equally.
- **Fertilization:** The amount of fertilization depends on the used soil/substrate and the time duration of the test system. However, an over-fertilization can also influence the species sensitivity and therefore it is extremely important to set up general regulations for the fertilization. More information is needed on this aspect

4.3. Field studies

The third category of experiments with non-target plants consists of studies with one or several plant species treated with herbicides under natural conditions in an experimental field study. In our literature search attention was paid on studies that investigated the effects of herbicides at different rates (covering the range of rates measured in spray drift situations) on plants. Several studies were found, which assessed these effects on plants in the field (Obrigawitch et al. 1998, Olszyk et al. 2004, Gilreath & Chase 2001, Fagliari et al. 2005, Al-Khatib et al. 2003, Felix et al. 2009, Romanowski et al. 1980). However, many of these studies dealt exclusively with the negative effects of herbicide drift on neighboring crops and the related yield loss (e.g. Gilreath & Chase 2001, Fagliari et al. 2005, Al-Khatib et al. 2003, Felix et al. 2009). Hence, the main focus of these studies was not the protection of wild plant species in field margins, but rather the economic losses of other crops due to herbicide drift. The reviews by Obrigawitch et al. (1998) and Olszyk et al. (2004) provided an overview of field studies investigating the effects of herbicide drift on yield or reproductive responses of plants. In the reviewed studies also mainly crop species were treated with different herbicide rates and over two-thirds of these studies indicated reproductive or development effects (yield reduction) at less than field application rates (Clark et al. 2004). However, in these studies only a limited number of plant species were tested (Clark et al. 2004, Obrigawitch et al. 1998). Our literature search focused mainly on further studies which are not listed in the above mentioned reviews. Moreover, we were particularly interested in studies, which probably provide useful information for higher tier testing with non-target plants. Therefore, a detailed literature search as described in chapter 2 was performed. However, to date there has been very little research on the effects of herbicides on non-target plant communities in the field (exposure experiments) that is comparable to a higher tier testing approach. In total six field studies were found, which met these criteria (Kjaer et al. 2006a, 2006b, Pfleeger et al. 2008, 2012 and Gove et al. 2007, Perry et al. 1996). In these studies the risk assessment of herbicides on non-target plants was addressed and field studies to investigate the negative effects of herbicide drift on plant species were performed (Table 7).

Kjaer and co-workers (2006a) investigated whether the simulated drift of herbicides has negative effects on hawthorn (*Crataegus monogyna*), a woody shrub, in hedgerows near agricultural fields. They performed a fully randomized spray experiment in seven different hawthorn hedgerows. Effects of four different rates of the herbicide metsulfuron, equal to 5 to 40% of the field rate and a zero control, on individual shrubs were included in the experiment. Spraying was conducted at the bud stage or at early flowering and four endpoints (leaves, flowers, green berries and mature berries) were recorded by measuring their number and weight.

The results showed that hawthorn was most sensitive when it received a spray application at the bud stage. Spraying at this stage of development caused a highly significant reduction in number and dry weight of berries, whereas it had no effects on leaf and flower production. A 100% berry reduction was even found at spray rates of 5% of the recommended field rate. The spraying at early flower stage also reduced the number of berries significantly, although the effect was less pronounced than after the bud stage spraying. However, since hedgerow berries are important for wild berry eating birds, the decrease in number of berries could lead to food reductions with further ecological consequences. Therefore, Kjaer et al. (2006a) concluded that there is a need of hedgerow protection, which could take place voluntarily or through regulation of herbicide spraying practices and the implementation of a buffer zone.

The year after the application described in Kjaer et al. (2006a) the hedgerow trees were revisited and the effects of the herbicide drift on the same endpoints as the year before were measured (Kjaer et al. 2006b). Significantly effects on growth (number and weight of leaves) and reproductive endpoints (flowers, berries) were observed even though other influencing factors (e.g. herbivory, different pollination) were not eliminated in the field experiments. Based on these results the authors concluded that present day risk assessment of effects on non-target plants is likely to overlook effects since the risk assessment focus on results from short-term laboratory studies and effects on reproduction are not assessed.

Another study also focused on parameters of plant reproduction (Pfleeger et al. 2008). However, in this study herbicide effects on a crop species were assessed and only vegetative propagation (asexual reproduction) was considered. As test species potatoes were used since they have, beside economic importance, also a short life cycle. Additionally, potatoes are simple to grow and number of tubers is an easy-measured endpoint (Pfleeger et al. 2008). To determine the effects of herbicide drift on potatoes, a field trial with a 2 x 2 factorial design (application time and herbicide rate) in randomized blocks was performed. Each plot had a size of 7.6 x 2.6 m and contained three rows 86 cm apart from each other and were sprayed once at flowering, i.e. either 14 or 28 days after plant emergence (DAE), using a plot sprayer. In total six different herbicides (sulfometuron methyl, imazapyr, glyphosate, cloransulam-methyl, bromoxynil or MCPA) were used and the applied herbicide rates were equivalent to 0.0056%, 0.032%, 0.18% and 10% of the field application rate. Each treatment was replicated four times. Assessments of foliar injury and biomass were measured 14 days after treatment (DAT), and tuber yield, number and quality (tuber size) was measured 120 DAT.

The assessments showed that yield and quality of potatoes were generally more affected by herbicides applied 14 DAE than 28 DAE. Moreover, tuber yield and quality parameters were more affected by lower herbicide rates than plant height and injury. Therefore, Pfleeger et al. (2008) concluded that plant reproduction can be a more sensitive indicator of herbicide effects than biomass. Hence, they suggested considering reproductive responses in phytotoxicity test protocols for pesticide registration.

The objective of another study of Pfleeger and co-workers (2012) was to develop a regional and simple Tier III field test that is economic and can investigate important ecological interactions. To validate the usefulness of this experiment the authors conducted a field trial with two herbicides, at two sites, and in multiple years. The experiments were conducted at two farms 15 km apart from each other in a flood plain in Oregon. In these farms different fields (cover crop *Trifolium incarnatum*) were used, in which individual test plots of 60 cm x 60 cm were prepared by removing vegetation within 1 m^2 using a propane burner followed by hand weeding. As test species three native plant species (*Festuca roemeri, Clarkia amoena, Prunella vulgaris*), and one introduced species (*Cynosurus echinatus*) were used and grown in the greenhouse for approximately 21 days. Then, the plants were moved to the field and one of each plant species were planted in a square in the middle of each plot 10 cm apart from each other. The transplanting took place in mid-April. A few weeks later (at the beginning of May) the plots were treated once with either the herbicide glyphosate or aminopyralid. In total three different treatments (application rates) were used: glyphosate was applied at 1%, 10% and 20% of the field application rate and aminopyralid at 4%, 13% and 50% respectively. Each treatment was replicated 10 to 14 times. Assessments of the plant height and width were performed every 2 weeks during the growing season (May to July). Additionally to the field experiment, Pfleeger et al. (2012) conducted a vegetative vigor Tier II test in greenhouse under controlled conditions with the four species used in the field experiments following the OECD protocol (Series 850.4150) (US EPA 1996). The aim was to compare the results of the field experiment with the standard greenhouse tests.

The results of the field experiment showed that plant height decreased with increasing glyphosate rates for all species, and for nearly all fields. With aminopyralid, one species died at nearly all concentrations, sites and years, while the effects on the other three species were less pronounced and variable (Pfleeger et al. 2012). However, the comparison of the field and greenhouse tests showed that the glyphosate sensitivity among species in the field differed from the ranking from greenhouse studies. In the field *Cynosurus echinatus* was the most sensitive species, but *Prunella vulgaris* was the most sensitive species in the greenhouse tests, which indicated that the response of the selected plant species is affected by the different growth conditions. Therefore, the authors conclude that greenhouse tests cannot predict the exact response of plants in communities in the field. However, simple field tests can be used as an appropriate design to investigate the ecological effects of herbicides on plant communities.

Gove and co-workers (2007) performed a field study to investigate the effects of glyphosate on non-target plants. However, beside the herbicide effects, they were also interested in the effects of fertilizer on non-target plants, since the vegetation of field margins can be affected by both agrochemicals. Therefore, they exposed six weeks old herbaceous woodland plant species (*Mercurialis perennis, Primula vulgaris, Galium odoratum, Viola riviniana, Carex remota, Geranium robertianum*) potted separately and cultivated in a greenhouse to different rates of glyphosate (1, 5, 10, and 25% of the median field application rate (= 6 L/ha; 360 g a.i/L)). Then, half of the test plants remained in the greenhouse and the other half was transplanted into 1 m^2 plots in woodland margins. Before, the plants were introduced the plots were cleared and fenced and weeding was carried out over the course of the experiment to remove any competing plants. The experimental design consisted of two rows of 10 1m^2 plots, given 20 plots in total, each separated by 1 m of bare ground. Every plot contained one replicate of each herbicide treatment for all six species. The species were randomly allocated to a grid position (5 herbicide treatments x 6 species = 30 plants per plot). Half of the plots were then additionally treated with a pelleted NPK (14-13-13) fertilizer with one rate equivalent to 50% of the application rate for wheat (140 kg N/ha). 1 year later the number of flowers per plant were recorded and the plants were harvested and weighted. The plants, which remained in the greenhouse, were also treated with the same fertilizer as used in the field or with distilled water (control). Each treatment was replicated 10 times and the plants were harvested 10 weeks later.

Gove et al. (2007) found a considerable agreement between the greenhouse and the field experiment. The results showed an increased mortality, reduced biomass and reduced fecundity in all six species treated with herbicides relative to control. Already, glyphosate drift rates of 5% showed reduction in the proportion of flowering plants. In contrast, the fertilizer treatment did not significantly alter flowering or affect the biomass of any plant species.

Another study, which investigated the effects of fertilizer and herbicides on non-target plants, was conducted by Perry and co-workers (1996). They started their experiments 1994 in Shropshire, England, to determine the effects of herbicide spray drift (glyphosate) and the misplacements of fertilizer (ammonium nitrate fertilizer with 34.5% N) on a simulated field margin community containing three grasses and three herbaceous plants. The experiment was laid out in four replicated blocks, each containing twelve 2 x 3 m plots separated by 70 cm. The plots were sown by hand in May with a mixture of *Elymus repens* (1.3 g/m2), *Arrhenatherum elatius* (1 g/m2), *Bromus sterilis* (4 g/m2), *Ranunculus repens* (2 g/m2), *Silene latifolia* (0.6 g/m2) and *Galium aparine* (2.8 g/m2). Plots were hand weeded during the first year of establishment to prevent invasion by other plant species. A randomized block design with 12 treatments and 4 replicates was chosen. The treatments consisted of three different fertilizer rates (0, 50, 200 kg N/ha), four different herbicide rates (0, 45, 90, 180 g a.i./ha) and all combinations of these treatments. In March 1995 (11 month after the establishment of the study site), the plots were treated with fertilizer and in June 1995 the plots were treated with the herbicide. The monitoring of the plots started in March 1995 to assess plant cover (abundance) and plant architecture. The assessments were carried out with a 1 m high point quadrat frame, which contained ten pins. In each plot the frame was randomly positioned three times and the numbers of touches of each species on each pin were recorded at height intervals of 5 cm. The assessments were repeated monthly from March to August. At the beginning of the assessment (March), there were no visible differences between the plots. However, *R. repens* and *G. aparine* failed to establish and therefore these two species were not taken into further consideration.

The results showed that fertilizer and herbicide applications had a significant effect on the four established plant species. All fertilizer treatments caused a significant reduction in cover of *S. latifolia* and *A. elatius* and all rates of glyphosate significantly reduced the cover of the sown grasses. These effects became stronger in the course of time. Interaction effects between the fertilizer and herbicide treatments were not found. However, the authors supposed that this could change with time.

Summary and discussion of the field studies

In general, the objective of a Tier III non-target plant field study should be to determine if a pesticide will have negative effects on the non-target plant community. Therefore, beside the effects on individual plant species, it is especially important to investigate the ecological effects, including intra- and inter specific interactions between plants, but also the effects on organisms, depending on these plant species (e.g. pollinators and herbivores). Hence, it seems to be essential to take account of sublethal effects of herbicide applications on non-target plants, such as a suppression of flowering and seed production or even germination rates of harvested seeds. Vegetative endpoints (e.g. biomass or plant height) are often not appropriate to predict effects on reproduction. For example, Kjaer and co-workers (2006a and 2006b) showed that an herbicide application simulating a drift of 5% of the application rate caused a 100% berry reduction, whereas leaf production was not affected. Pfleeger and co-workers (2008) concluded that plant reproduction development responses can be more sensitive than vegetative endpoints. However, they performed a field test with only one plant species and interspecific competition effects were not included. The test design of the field studies of Pfleeger et al. (2012) and Gove et al. (2007) were composed of four and six different plant species respectively, but interspecific competition effects in these studies were also limited since plant density in the field plots was not very high. In the study of Pfleeger et al. (2012) four individual plant species were planted 10 cm apart from each other in a 60 cm x 60 cm plot and Gove et al. (2007) used a 1m^2 plot with 30 plants. In natural communities plant density is much higher and thus interaction effects caused by intra and interspecific competition and shielding are stronger. The study of Perry et al. (1996) used bigger plots (2 m x 3 m), in which seeds of six species were sown with a specific density, but it was not described how many plant individuals germinated after sowing and therefore no information about the plant density was available. Moreover, two species (*Ranunculus repens* and *Galium aparine*) failed to establish (Perry et al. 1996), probably because no pretreatment of the seeds was undertaken. A lot of wild plant species require a stratification period (e.g. cold temperature, darkness) before they can germinate. However, with appropriate information for the plant species high germination rates can be achieved (Pallet et al. 2007, White et al.

2009, Olszyk et al. 2008). Another simple way to stimulate the seed germination is to use the plant hormone Gibberellic acid (Gibberellin A3), which can trigger germination in seeds that would otherwise remain dormant.

In all discussed field studies only four to six different species were used. This seems to be a very small number of plant species and even in the previously presented microcosm experiments more species (6 to 9 species) were used (Reuter & Siemoneit-Gast 2007, Dalton & Boutin 2010, Riemens et al. 2008, Marrs et al. 1991b, Marrs & Frost 1997). Generally, a Tier III non-target plant field study should be more complex in the design than a microcosm study to increase realism. Therefore, it would be necessary to use an appropriate number of plant species, which are representative for natural plant communities. To date, it is not clear how many plant species are needed for such investigations. In this area more studies are required to increase our knowledge. In addition, an appropriate number of individuals per plant species, as well as a mix of grasses and herbaceous plants have to be considered. These topics were discussed in the previous chapter (see e.g. Box 1). Nevertheless, the six presented field studies suggest that higher tier tests with non-target plants can be designed and performed in the field in a simple and successful way. Moreover, the used test designs were not very expensive (Pfleeger et al. 2012) and could easily be handled since only small plots (60 x 60 cm, 1m^2, 2 m x 3m) were used. Furthermore, when the plant species were firstly cultured in greenhouses and then transferred to the field, standardized plots can be established. With this method it would be comparatively easy to create artificial plant communities in the field, which can be used for Tier III field tests.

In general, it can be proposed that a field study should be performed in geographic locations where the herbicide is expected to be applied (Pfleeger et al. 2012) and the time of applications should be in agreement with label recommendations. Moreover, the phenological development stages of plant species during the time of the herbicide application have to be identified and should be implemented in field studies. Moreover, when an artificial plant community should be established, it can be necessary to prepare the field plots sufficiently early, as done by Perry et al. (1996). However, another possibility would be to perform a field study directly on a natural field (e.g. a meadow with a plant community of 40 to 50 different species) (Schmitz et al. 2013, see Research Box 5 for further information). Such a meadow could be used as an experimental study site and can be regarded as an original habitat that is not contaminated with agrochemicals and therefore as presenting the plant community of a surrogate field margin without this influence (Schmitz et al.

(2013). Another point, which must be considered in field studies, is the repeated exposure of plants to pesticide applications during a growing season. If the pesticide label suggests repeated applications, e.g. over the life cycle of the plants, then the field study should account for this (Pfleeger et al. 2012). Therefore, a field study can be considerably longer than a Tier II study, also due to the fact that ecological effects should be considered in Tier III field tests (Oslzyk et al. 2004). Furthermore, the vegetation of field margins can be affected by fertilizer inputs, which can also affect plant composition and might interact with herbicide effects, especially in the long run (Perry et al. 1996, Schmitz et al. 2013, Strandberg et al. 2012). To date, there are no regulations for fertilizer applications next to field margins and therefore it seems necessary to consider the nutrient inputs on plant communities and their interactions with herbicides as well. According to Olszyk et al. (2004) a complete standardization of field tests is unrealistic because the type of field study depends on the question being asked, but some basic guidelines are required and should be developed. Based on the described field experiments, general suggestions for the design and performance of a Tier III field study with non-target plants are provided in Box 2, which have to be further validated.

In short:

The literature search revealed three different types of studies, which fit to a higher tier testing approach:

- Realistic drift studies with individual plant species in pots placed at different distances from the treated field: These studies are basically an outdoor vegetative vigour test exposed to realistic drift conditions. Such tests can easily be performed, but provide little additional information compared to a Tier II test conducted under greenhouse conditions.
- Microcosm studies with several plant species: The evaluated experiments suggest that microcosms can be a useful testing system for measuring the effects of a particular herbicide on several plant species grown in mixture since interaction effects between species (interspecific competition) are present. General factors, which should be considered in microcosm experiments, as well as recommendations for the design and performance of a microcosm study with non-target plants, were proposed. However, further validation of appropriate testing methods should be undertaken.
- Field studies with one or several plant species on an experimental study site: The evaluated field studies showed that generally higher tier tests with non-target plants can be designed and performed in the field in a simple and successful way. However, to date no appropriate testing methods exist. Further research is needed to develop a field testing approach to address the effects of herbicides on non-target plant communities. However, general suggestions for the design and performance were proposed, which have to be further validated.

Box 2: Suggestions for the design and performance of Tier III field study

- **Study site:** The experimental study site should not be contaminated with agrochemicals (pesticides, fertilizer). An appropriate study site would be a meadow with a relative homogenous distribution of approx. 30 to 50 different plant species. Such a meadow can be regarded as an original habitat that is not contaminated with agrochemicals and therefore as presenting the plant community of a surrogate field margin.
- **Test design:** The design of a field experiment and its statistical analysis are intimately connected. Therefore, the experimental test design has to be well elaborated. In addition, a test design has to take account of potential underlying environmental gradients. An appropriate test design would be for example a randomized block design.
- **Size of test plots:** The size of the test plots is dependent on the number of species of the study site and the homogenous distribution of these species. However, the size of the test plots should be not too small.
- **Number of replications:** The number of replicates in the evaluated field studies ranged from 4-14. Since community analyses are complex it is important to increase replication whenever possible (Fraser & Keddy 1997).
- **Application:** The time and number of applications should be in agreement with label recommendations. The herbicide product should be applied and not only the active ingredient.
- **Assessment of vegetation:** The vegetation of the study site should be assessed before and after treatments in different time intervals. Plant community assessments have to be performed with a method that is appropriate to document changes in the plots over time. In addition, it is important to use a method with which uniform plant community assessments can be obtained, independent of the technicians. In the end of the growing season, biomass samples of each plot (e.g. above ground biomass of 1 m x 1m) should be taken and measured.
- **Assessment of reproduction:** Numerous studies showed that herbicides can affect the reproduction of wild plant species. Therefore, effects on reproduction (flowering, seed set) should also be assessed.
- **Test duration:** The test duration have to be considerably longer than in a Tier II study. Since effects on reproduction and plant composition should be investigated, which are often firstly apparent in the second experimental season or still in the year after application, it seems necessary to extent the assessment period after treatment to the next growing season (one year after treatment).
- **Fertilization:** There are no regulations for fertilizer applications next to field margins. Therefore, it seems necessary to consider the nutrient inputs on plant communities and their interactions with herbicides as well.

RESEARCH BOX 4: Agrochemicals in field margins – an experimental field study - Effects of herbicides and fertilizer on the common buttercup *Ranunculus acris*

BACKGROUND: Field margins (not cultivated strips adjacent to fields) comprise the majority of the semi-natural habitats in the intensively farmed agricultural landscape and thus they can benefit the conservation of biodiversity in agroecosystems. Field margins can enhance the plant diversity within farmland and act as corridors for the movement of fauna and possibly flora. However, field margins can be affected by pesticides and fertilizer through direct overspray and spray drift from the adjacent field applications. A perennial field study (2010-2012) simulated the inputs of agrochemicals in the first width meter of a winter wheat field margin to investigate the direct and indirect effects as well as the cumulative effects (due to the annual application sequence) of the misplacement of pesticides and fertilizer on the flora and fauna of field margins. In the following the effects of the repeated herbicide applications in the years 2010 and 2011 on the common buttercup *Ranunculus acris* in field margins are demonstrated.

METHODS: A randomized block design with seven treatments (I: insecticide (Karate Zeon, a.i. lambda-Cyhalothrin 7.5 ml a.i./ha), H: herbicide (Atlantis WG, a.i.: 30 g/kg Mesosulfuron-methyl; 6 g/kg Iodosulfuron-methyl-natrium), F: NPK fertilizer, H+I, F+H, F+I, F+H+I) and one control have been established on a low productive meadow (Figure 1). Each treatment was replicated eight times in plots of 8 m x 8 m with 2 m distance to each plot. The used fertilizer concentrations (25% of the field rate) and pesticide concentrations (30% of the field rate) are consistent with their inputs (drift + overspray) in the first meter of a field margin directly adjacent to the field under Good Agricultural Practices.

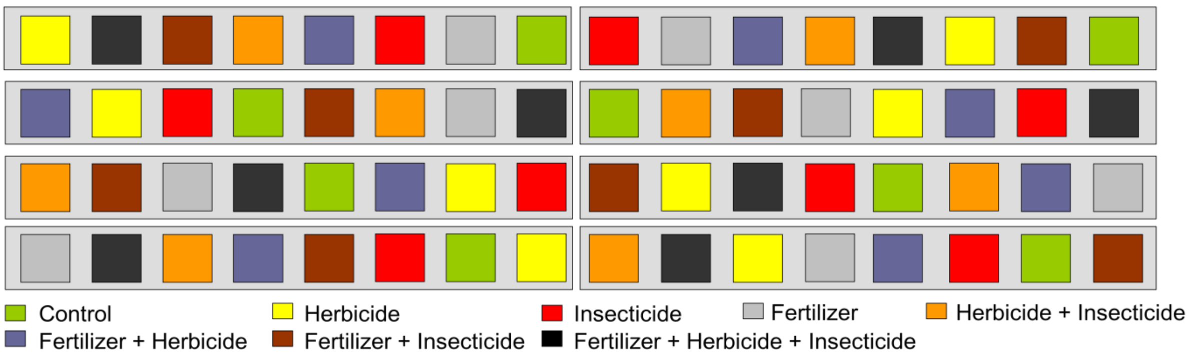

Figure 1: Randomized block design.

To detect the effects of the agrochemical applications on *R. acris* vegetation assessments and a photo-documentation of the flowering intensity of *R. acris* was performed in May 2010 and 2011, two weeks after the herbicide applications.

Additionally in May 2011 the field experiment was accompanied by field monitoring of *R. acris* (presence/absence data) in field margins around the study area.

RESULTS: Following the applications in 2010 and 2011 the plant density of *R. acris* was significantly affected by the fertilizer treatments (Fig. 2A). During these years the herbicide had no effect on the plant density of this species. However, the herbicide caused a sublethal effect by reducing flower intensity by 85% (Fig. 2B). Reduced flowering intensity results in a reduced seed production and consequently *R. acris* will probably disappear in plots (and field margins) which are treated with herbicides over the years.

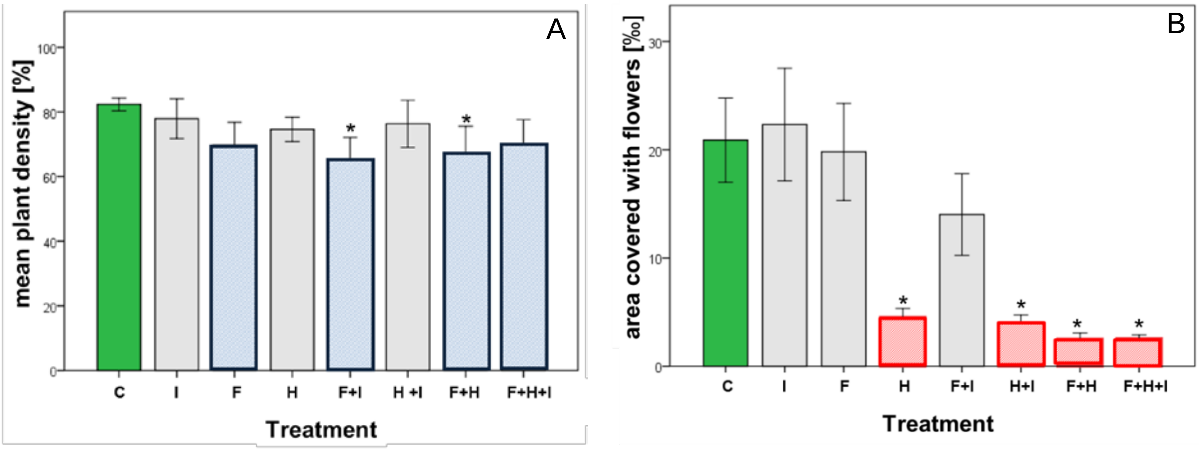

*Figure 2: A) Mean plant density (± SE) of R. acris in May 2011 per Plot and Treatment; n per Treatment = 48. Plots treated with fertilizer are highlighted with a blue frame and the green bar represents the control. B) Mean (± SE) area covered with flowers of R. acris in May 2011 per Plot and Treatment; n per Treatment = 48. Plots treated with herbicides are highlighted with a red frame and the green bar represents the control. *: significant difference to the control (p<0.05, nested PerAnova (C = Control, I = Insecticide, F = Fertilizer, H = Herbicide).*

Monitoring data to investigate the presence and absence of *R. acris* in field margins support the assumption of the disappearance of this plant species. In total, 1130 monitoring points were recorded in field margins; 844 data points (75%) were located in field margins next to cereal fields whereas the other data points were recorded adjacent to vineyards, hedges, orchards or extensively managed meadows (Table 1). In total, *R. acris* was recorded 76 times, however in negligibly small proportions in field margins adjoining cereal crops and vineyards. Presence of *R. acris* in field margins adjacent to cereal crops differed significantly from all other field margins.

Table 1: Monitoring points (m.p.) and the occurrence of R. acris in field margins adjacent to different cropped areas or hedges. Different letters indicate significant differences (PerAnova, $p < 0.05$) between the occurrences of R. acris in different field margins.

neighboring crop / structure	**m.p.**	**m. p. with *R. acris***		**Significance**
		N	**[%]**	
cereal	844	16	2	A
vine	172	12	7	B
orchard	46	14	30	C
hedge	42	12	29	C
meadow	26	22	85	D
Overall	**1130**	**76**		

CONCLUSION: The misplacement of herbicides in field margins can cause sublethal effects, which will cause disappearance of specific plants and related community shifts in agricultural field margins in the long run. Besides these implications for the plants the sublethal effects can also cause secondary effects for e.g. flowering visiting insects. Because many flies, bees and butterflies use *R. arcis* as the source of nectar. If *R. arcis* has failed in the development of flowers it will consequently reduce the presence of pollinators and thereby negatively affect the biodiversity of the agricultural landscape.

Source:

Schmitz et al. (2013): Agrochemicals in field margins – Assessing the impacts of herbicides, insecticides and fertilizer on the common buttercup *Ranunculus acris. Environmental Toxicology and Chemistry, Vol 32, No.5, pp. 1124-1131.*

5. Evaluation of the current risk assessment approach for terrestrial non-target plants in the field

The risk assessment of herbicides aims to protect non-target plant species in off-crop habitats (e.g. field margins) from adverse effects of pesticides. Therefore, guidelines for the conduct of relevant studies for pesticide registration were implemented (European Commission 2002). These guidelines are based on laboratory and greenhouse tests with individual plant species grown in small pots. For this purpose, generally young annual crops species (2-4 leaf stage) are used, although non-crop species (wild annual and perennial species) are to be protected in field margins. The extrapolation from these data (acute ER_{50} values) based on mono-species tests to natural plant community involves many uncertainties (e.g. the use of crop plants as surrogates for non-crop or native plant species, the use of only a few test species to represent the highly diverse terrestrial plant community, etc.; see chapter 3 and 6 for further uncertainties). Furthermore, competition effects between plants cannot be assessed with single-species tests. In addition, greenhouse tests provide optimal conditions for growing and recovery. However, in the field the plants are exposed, and are maybe affected by further stressors such as herbivores, competition, increased nutrient supply through fertilization, water stress due to high temperature etc. Therefore, in risk assessment a Safety Factor (SF) has to be applied, which should take account of the above mentioned uncertainties. In the herbicide risk assessment a factor of 5 is applied to extrapolate from acute ER50-values to no effect levels (Füll et al. 2000). However, there are indications that a safety factor of 5 is probably not adequate (see chapter 3 for more information), and thus the question arises whether the current risk assessment represents a sufficient safeguard for the protection of non-target plant communities in field margins.

To assess the credibility of the current risk assessment approach, an examination of the protectivity is necessary. Hence, there is a need to identify and to use refined reference testing systems ("reference tiers" such as "terrestrial model ecosystems = TMEs"), which investigate the effects of herbicides on the composition of plant communities in the field (EFSA 2010, UBA 2012). Generally, these higher Tier studies would not be practical for routine use in a Tier I risk assessment procedure, but can be used to calibrate lower tier studies using simplified approaches (EFSA 2010, UBA 2012). To date, little is known about the performance of such reference tiers in the risk assessment of herbicides for terrestrial non-target plants. Therefore, the objective of this chapter was to summarize the current knowledge on the effects of herbicides on non-target plant communities in the field. A detailed literature search as described in chapter 2 was performed. In the following table (Table 9) and text section the identified field studies are presented and the effects of herbicides in natural plant communities that are also affected by fertilizer and interaction are evaluated.

Table 9 Summary of field studies evaluating the effects of herbicides on natural plant communities. ***WAT = weeks after treatment, DAT = days after treatment, n.d. = no data.***

	Authors	Test design	Agrochemicals	Plant species		Measurements (test duration)	Main results
				Crop	Non-crop		
field studies with natural plant community	Kleijn & Snoeijing 1997	a) 3-year experiment on a meadow randomized block design	Starane 200 NPK fertilizer 1 treatment/year		natural community (approx. 44 species)	assessments of vegetation composition (once a year in May/June), biomass (August)	fertilizer decreased the species richness significantly → fertilizer effects became stronger in the course of time
		b) 3-year experiment on a fallow arable field randomized block design	Starane 200 NPK fertilizer 1 treatment/year		30	assessments of vegetation composition (twice a year May and September), biomass (August)	fertilizer and herbicide affected the species richness significantly → herbicide and fertilizer effects were additive (reduction of species no. by 37%)
		c) pot experiments with individual plant species from the field in greenhouse, VV-Test	Starane 200		18	plant biomass (6 WAT)	results differ from the field studies → extrapolation of the results of pot experiments to natural communities and field conditions is inappropriate
	De Snoo et al. 2005	3-year experiment on road verges and ditch banks, randomized block design	Liberty 2 treatment/year		natural community (species no. n.d)	phytotoxic effects (10 DAT), assessments of vegetation composition (May and August), plant biomass (August),	effects on biomass and species composition were observed → low herbicide rates resulted in phytotoxic effects
	Strandberg et al. 2012; Damgaard et al. 2011	long-term experiment on a fallow field (start 2001) randomized block design	Roundup Bio nitrogen fertilizer 1 treatment/year		31	plant cover, vertical density (3 times a year: before treatment, 2 WAT and at the end of the growing season)	herbicide and fertilizer treatments affected the species number negatively → interaction effect of fertilizer and herbicide were demonstrated
	Schmitz et al. 2013	3-year experiment on a meadow randomized block design	Atlantis WG NPK fertilizer 1 treatment/year		natural community (approx. 50)	plant density, flower intensity (every year in May and June), seed production of four species at seed maturity	fertilizer and herbicide decreased the plant density, herbicide reduced flower intensity by 85% → both agrochemicals lead to community shifts

Kleijn and co-workers (Kleijn et al. 1997) conducted two three-year experiments in the Netherlands to assess the effects of herbicide drift and fertilizer misplacements on the botanical diversity of an arable field boundary. For this purpose, plots in a low productive meadow (exp. 1) and plots in a high productive fallow arable field were treated annually (exp. 2). Both experiments started in March/April 1993. In Experiment 1, 48 quadrats of 2 m x 2 m and 0.5 m apart from each other were established on a low production meadow, dominated by *Festuca rubra* ssp. *commutate* and *Holcus lanatus* (in total approx. 44 species). During the experiment cutting and removing the vegetation once a year in autumn was maintained. Each year a fertilizer (NPK 15-12-24, field rate 110 kg N/ha) and an herbicide (fluroxypyr; a.i. pyridyloxyacetic acid; field rate 200g fluroxypyr/ha) were applied in spring (May/June), when vegetation height was approximately 20 cm. The fertilizer was applied evenly by hand and the herbicide with a pressurized houseplant sprayer. In total three different fertilizer rates (0, 25 and 50% of the field rate) and four different herbicide rates (0, 5, 10, and 50% of the field rate) were used. Each treatment, a combination of each fertilizer and herbicide rate, was replicated four times in a randomized block design (12 x 4 = 48 plots). Vegetation assessments were made in the central square meter of the plots every year in May from 1993 to 1996. The plant biomass was measured at the end of August every year (1993-1995), by cutting two 0.3 x 0.3 m quadrats of the plots. The samples were separated into grasses and herbaceous plants and their dry weight was measured.

In the second experiment, an arable field, which had been cultivated for the last decades (before 1993), was ploughed and a seedbed preparation was conducted. Afterwards plots were established and a mixture of 30 grassland herbaceous plants (representing vegetation types on a fallow arable land) was sown (rate of seed mixture 1 g/m) and treated each year with a fertilizer and an herbicide. The plot size, treatment levels, date of applications and test duration was the same as in experiment 1. Vegetation assessments were made twice a year (May and September) and plant biomass was determined in August.

In addition to these two field experiments, Kleijn et al. (1997) performed a greenhouse test with 18 species from the field experiments. The aim was to investigate the sensitivity of these plants species grown individually in pots to the herbicide fluroxypyr. The same herbicide rates (5, 10 and 50% of the field rate) as used in the field experiment plus the control were used. The plants were treated when four real leaves were developed and each treatment was replicated four times and arranged in a randomized block design in greenhouse. Plant biomass was determined six weeks after treatment.

In the first experiment, all fertilizer treatments (25% and 50% of the field rate) resulted in a statistically significant decline in species richness by a loss of species of low stature (e.g. *Trifolium repens, Hieracium pilosella, Leontodon autumnalis*) while a single herbicide treatment had no effects on the vegetation composition. However, the treatment combination of herbicide and fertilizer showed a reduction: in experiment 1 the control plots were most species rich witch 15 species/m^2 and the plots treated with a combination of herbicide and fertilizer (10% herbicide + 50% fertilizer, and 50% herbicide + 50 fertilizer) had the least species richness with 9 species/m^2.

The results of experiment 2 showed similar fertilizer effects and these effects became even stronger after the second experimental season (in both experiments, exp. 1 and 2). The fertilizer treatments increased grass biomass production in all years and decreased the occurrence of some plant species. However, in contrast to the experiment 1, significant herbicide effects on plant species richness were found in experiment 2 in the third experimental season: in control plots 30.8 species/m^2 were found and in the herbicide treated plots (5% of the field rate) approx. 24 species (data taken from a figure) were determined. However, the treatment combination of fertilizer and herbicide (50% herbicide + 50% fertilizer) resulted in a species reduction of 37% (19.5 species/m^2). Species with a significantly lower mean presence in the herbicide treated plots were *Galium mollugo, Hypericum perforatum* and *Leonurus cardiac*. Based on these results, the authors concluded, that the fertilizer effects on species richness, biomass production and the abundance of individual species were far more severe and constant in comparison to the herbicide effects. However, they also noted, that the herbicide and fertilizer effects appeared to be additive, since the decline in species numbers increased with levels of both herbicide and fertilizer.

In the third experiment, all three herbicide treatments (5, 10 and 50% of the field rate) produced statistically significant effects for three plant species (*Galium mollugo*, *Galium verum* ssp. *verum*, *Hypericum perforatum*) in mean dry weight. The 50% treatment resulted in almost 100% mortality in these species. All other plant species showed at least some reaction (e.g. curling of leaves), but recovery was detected at the end of the assessment period (6 weeks after treatment). Therefore, the authors concluded, that the pot experiments did not correspond well with the results of the field experiment since in the field also other plant species (*Leonurus cardiaca*) were statistically significantly affected by the herbicide treatment. In addition, the abundance of *Daucus carota* was positively influenced in the second experiment in the herbicide treated plots (higher abundance in the 50% treated herbicide plots in comparison to the control plots, exact data not available), while this species was not affected in experiment 3. In contrast, G*alium verum* was only affected in experiment 3, but not in the field, maybe due to different leaf morphologies between seedlings and adult plants. Seedlings of *G. verum* have lanceolate leaves and adult plants needle-like leaves and thus, interception and efficacy of the herbicide is probably much higher for seedlings of this species (Kleijn et al. 1997). Therefore, the authors pointed out, that short-term, monoculture pot experiments have a limited predictive value and that the extrapolation of the results of pot experiments to field conditions is inappropriate (Kleijn et al. 1997).

In the Netherlands, another field experiment studied the effects of herbicide drift on off-crop vegetation (e.g. field margins) (De Snoo et al. 2005). However, in contrast to Kleijn et al. (1997) they used not a meadow as a study site, but species rich road verges and ditch banks adjacent to pastures, which were not adapted to a history of herbicide use. They started their field experiment in 2000 at four different locations in the Netherlands. Two sites were road verges and the other two sites were ditch banks (exact numbers of vegetation composition or plant species at the study sites are not given in the available literature). The study sites were divided into plots of 25 m^2 (1 m x 25 m) and the plots were arranged in blocks to minimize within site variation. One block contained five plots, treated twice with one herbicide rate (glufosinate ammonium; 2, 4, 16, 32, 64% of the maximum field rate of 800 g a.i./ha) in 2000 and 2001. The repeated treatment during one growing season was chosen, because a worst-case scenario should be simulated. Spraying was performed with a handheld knapsack sprayer, with an attached spray boom in May and June. The interval between the first and the second spraying was 15-20 days and control plots were treated only with water. The number of replications per treatment over all four sites was 20. Phytotoxic effects were quantified eight to ten days after treatment, biomass samples (three samples of 30 x 30 cm per plot) were taken in August/September and vegetation assessments (species cover and number of species with the method of Braun-Blanquet) were carried out in May (before spraying) and in August (after spraying) every year (2000-2002).

The results showed significant phytotoxic effects at all herbicide rates, even at the lowest herbicide rate of 2% of the filed application rate. The lowest two concentrations (2% and 4% of the field application rate) had most impact when applied in May. At high rates (64% of the field rate) a statistically significant decrease of biomass of 22% in 2000 and 32% in 2001 in comparison to the control was observed. The lower herbicide rates had no significant effect on the biomass. Effects on the composition of the plant community were found only at application rates of 32% of the field rate or higher. However, the authors noted, that the results should be handled with care since in some instances there were effects and clear negative trends, although not statistically significant.

A further large-scale field experiment was performed by Strandberg and co-workers (Strandberg et al. 2012, Damgaard et al. 2011) in Denmark. This experiment was designed to investigate the ecological processes, including establishment, survival and competitive interactions, in a semi-natural ecosystem, treated with an herbicide (glyphosate) and fertilizer (nitrogen) (Damgaard et al. 2011). The field experiment was already established in 2001 on a former agricultural field, which laid fallow a couple of years prior to the start of the experiment in 2001. The field was quadrangular and surrounded by small parts of forest and separated from adjacent fields by 5 meter wide hedgerows. In 2001, the field was deep ploughed and prepared by harrowing and rolling. Afterwards (in spring), 31 grassland species covering different life form strategies were sown (no information to the seed rate available). As a test design a randomized block design with twelve treatments and ten replicates was chosen: four glyphosate treatments (0, 1, 5, 25% of the field rate of glyphosate (1440 g a.i./a)), three fertilizer treatments (0, 25 and 100 kg N/ha) and all combinations of fertilizer and herbicide rates. Each plot had a size of 7 m x 7 m and was separated by 1.5 m to the next. The herbicide treatment was conducted with an experimental field sprayer and a spray boom of 3 m length. The granular fertilizer was spread by hand. The first herbicide application was performed in August 2001, when the vegetation was established. Afterwards, the plots were treated with herbicide and fertilizer once a year in spring. Vegetation assessments were carried out during two periods: 2005-2007 and 2007-2009. In the first assessment period, sampling was made with six randomly selected 0.75 m x 0.75 m quadrates to study the effects of the herbicide treatments on the vegetation composition. For the second assessment period one permanent 0.5 m x 0.5 m quadrat was placed within each plot in June 2007. This quadrat was used to study the dynamic between the two perennial grass species *Agrostis capillaris* and *Festuca ovina* since these two species are known to differ in their responses of both treatments. In addition, plant cover and vertical density of all plants were measured non-destructively with the pin-point method. Therefore, they used a 0.5 m x 0.5 m frame with a 5 x 5 grid. This resulted in 25 pin-positions (intersections) regularly placed at a distance of 10 cm. At each intersection a sharply pointed pin was passed vertically through the vegetation. The percent cover of vascular plants was obtained by recording the first interception of the pin with the canopy of the different species. For selected species (e.g. *Agrostis capillaris, Festuca ovina, Elytrigia repens*) every contact between pin and vegetation was recorded. The total number of intercepts gives an estimate of the projected plant area (PPA), which correlates highly with plant biomass. The vegetation assessments were carried out three times a year: before herbicide and fertilizer treatment, approximately two weeks after herbicide treatment and at the end of the growing season (August).

The vegetation of the experimental site was dominated by grasses regardless of the treatments and covered at least 50-60% of the ground. Here, the three grasses *Agrostis capillaris, Festuca ovina* and *Elytrigia repens* made up the main part of the vegetation. However, these grasses (cover) were affected differently by the fertilizer and herbicide treatments: the abundance of *Elytrigia repens* was affected negatively at low and intermediate nitrogen levels (0 and 25 kg/ha), but in plots receiving 100 kg N/ha it became dominant; except for the treatment combination of nitrogen and high glyphosate rates (25% of the field rate). In contrast, the cover of *Festuca ovina* was reduced at the highest nitrogen treatment (100 kg N/ha), as well as in the treatment combination of nitrogen (100 kg/ha) and glyphosate (1 and 5% of the field rate). An interaction effect of nitrogen and glyphosate was found for *Agrostis capillaris*, which had the highest density at intermediate levels of both nitrogen and glyphosate. Furthermore, the results of the vegetation assessments showed that the vegetation (species richness and species composition) gradually changed over the years. Both applications of herbicide and fertilizer affected species number negatively with increasing rates. The lowest glyphosate treatment (1 and 5% of the field rate) resulted in sublethal effects (e.g. curly, yellow-colored or dead leaf lips) and the highest rate (25% of the field rate) caused mortality resulting in dead plant material and uncovered soil. However, at the highest nitrogen level (100 kg/ha) the application of low doses of glyphosate to some extent counteracts the negative nitrogen effect. Therefore, the authors concluded, that fertilizer interacts with effects of herbicide spray drift in natural and semi-natural habitats by affecting the species competition (Strandberg et al. 2012).

Schmitz and co-workers (Schmitz et al. 2013, see Research Box 4) investigated also the effects of fertilizer and herbicide misplacements in field margins and their ecological effects on plant community composition. They performed a perennial field study, which started in 2010 and ended 2012. The field experiment was carried out on a low productive meadow, which had been extensively managed for feed for horses by mowing twice a year without any fertilizer additions. The vegetation of the meadow was homogenous and consisted of tall grasses (e.g. *Holcus lanatus*, *Arrhenatherum elatius*) and herbaceous plants like *Galium mollugo, Ranunculus acris* and *Lathyrus pratensis* (in total approximately 50 different plant species). The objective of the study was to detect short and medium-term effects of fertilizer and pesticide inputs in narrow winter wheat field margins. As test design a randomized block design with seven treatments (I: insecticide (Karate Zeon, a.i. lambda-Cyhalothrin 7.5 ml a.i./ha), H: herbicide (Atlantis WG, a.i.: 30 g/kg Mesosulfuron-methyl; 6 g/kg Iodosulfuron-methyl), F: NPK fertilizer, H+I, F+H, F+I, F+H+I) and one control was used. Each treatment was replicated eight times in plots of 8 m x 8 m with 2 m distance to each plot. The used fertilizer concentrations (25% of the field rate) and pesticide concentrations (30% of the field rate) were consistent with their inputs (drift + overspray) in the first meter of a field margin directly adjacent to the field under Good Agricultural Practices. Beside the effects of the agrochemical applications on the plant composition, they also investigated the effects the herbicide applications on the reproductive capacity of selected plant species (e.g. flowering intensity of *Ranunculus acris* and seed production of *Lathyrus pratensis, Vicia sepium,* and *Rumex acetosa*).

The results of the plant community assessments showed that the plant density of the four species was significantly affected by the fertilizer and herbicide applications (see Fig.1. in Research Box 5). The plant density of *R. acris* and *L. pratensis* was affected stronger in the fertilizer treatments (single as well as in combination with the herbicide and the insecticide) than the herbicide treatment alone. However, the herbicide treatment also decreased the plant density significantly and in addition the herbicide caused a sublethal effect by reducing flower intensity of *R. acris* by 85% (see Research Box 4). Consequently seed production also decreased (see Research Box 5). The plant density of *R. acetosa* and *V. sepium* showed a similar average decrease in the fertilizer and herbicide treatments (Fig. 1, Research Box 5). However, it appears that the treatment combinations resulted in a stronger plant density reduction (see Research Box 5 for further information).

RESEARCH BOX 5: Agrochemicals in field margins – an experimental field study - Effects of herbicides and fertilizer on plant density and reproduction

BACKGROUND: Current phytotoxicity tests are short-term tests performed with crop plants to predict the sensitivity of wild plant species in non-target areas (e.g. field margin) to herbicides. Due to the short test duration of 21-28 days effects on reproduction cannot be detected with these test methods. Furthermore, the phytotoxicity tests are performed under standardized greenhouse conditions that differ markedly from the field conditions (e.g. intra- and interspecific competition for resources). Additionally, possible cumulative effects of agrochemicals and repeated exposures are ignored in phytotoxicity tests for risk assessment.

The present study was undertaken to investigate the effects of the misplacement of pesticides and fertilizer on the flora and fauna of field margins. The study is a perennial field study, which started in 2010. More information to the field study can be found in Research Box 4. In the following, the effects of herbicides and fertilizer on the plant density of selected plant species after three years of treatment (2010-2012) are presented. In addition, the reproductive capacities of these species were assessed.

METHODS: The field study was carried out on a low productive meadow. As test design a randomized block design with seven treatments (I: insecticide (Karate Zeon, a.i. lambda-Cyhalothrin 7.5 ml a.i./ha), H: herbicide (Atlantis WG, a.i.: 30 g/kg Mesosulfuron-methyl; 6 g/kg Iodosulfuron-methyl-natrium), F: NPK fertilizer, H+I, F+H, F+I, F+H+I) and one control was used. Each treatment was replicated 8 times (plots) (See Research Box 4 for further information).

Four plant species were selected to study the effects of the herbicide and fertilizer treatments: *Ranunculus acris*, *Lathyrus pratensis, Vicia sepium,* and *Rumex acetosa.* Plant density assessments were carried out in June 2012. Additionally, the seed production of the species was assessed in 2012. For this, at maturity the seeds of 6 fruits from 6 different plants (1 fruit per plant) per plot were harvested. Thus, seeds of 48 individuals (fruits) per species and treatment were collected (6 fruits x 8 plots). The seeds were stored in a dry place over several weeks and then the seeds were counted and weighted.

RESULTS: The plant density of the four species was significantly affected by the fertilizer and herbicide applications (Figure 1). The plant density of *R. acris* and *L. pratensis* was affected stronger in the fertilizer treatments (single as well as in combination with the herbicide and the insecticide) than the herbicide treatment alone. However, the herbicide treatment also decreased the plant density significantly (Fig.1) and in addition the herbicide caused sublethal effects (Table 1).

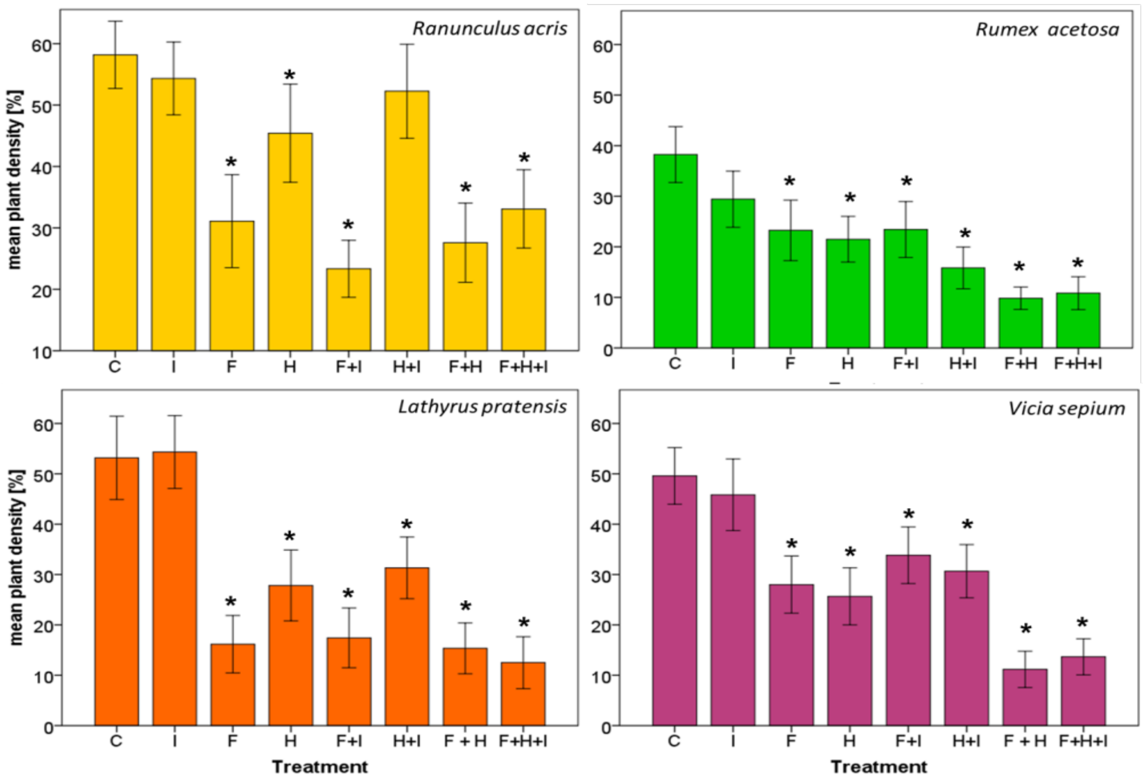

*Figure 1: Mean plant density (± SE) of R. acris, R. acetosa, L. pratensis and V. sepium in June 2012 per plot and treatment; n per treatment = 48. *: significant difference to the control (p<0.05, nested PerAnova). C: Control, I: Insecticide, F: Fertilizer, H: Herbicide.*

It appears that the fertilizer and herbicide treatment caused a similar average decrease in the plant density of *R. acetosa* and *V. sepium* (Fig. 1). However, the treatment combination of F+H, as well as the F+H+I treatment resulted in a stronger reduction of the plant density of these two species and an additive effect seems likely.

The results of the assessment of the seed production are shown in Table 1. It was not always possible to find 6 fruits in the herbicide treated plots (H, H+I, F+H, F+H+I) because the herbicide already suppressed the formation of flowers of *R. acris, L. pratensis* and *V. sepium*. Thus, the total seed production of these three species in the herbicide treated plots was reduced and therefore their reproductive capacity. In the control, insecticide and fertilizer treatments enough fruits could be found. Additionally, to the reduction of seeds per plot due to lower flowering, the weight of seeds per fruit and treatment differed for *R. acris*. The herbicide influenced seed production by reducing seed weight which may influence germination negatively.

Table 1: Number of determined fruits, seeds per fruit, and 1-seed weight of the species R. acris, R. acetosa, L. pratensis, and V. sepium in the control and treatment plots. n.d. = not determined.

		C	I	F	H	F+I	H+I	F+H	F+H+I
R. acris	No. of fruits	48	48	48	8	48	2	0	1
	Mean no. of seeds/fruit	28	29	31	24	33	32	n.d.	30
	Mean 1-seed weight [mg]	1.6	1.5	1.3	0.6	1.4	0.7	n.d.	0.5
R. acetosa	No. of fruits	48	48	48	48	48	48	48	48
	Mean no. of seeds/fruit	30	26	32	30	25	29	35	29
	Mean 1-seed weight [mg]	0.8	0.8	0.8	0.8	0.9	0.76	0.8	0.8
L. pratensis	No. of fruit	48	48	48	0	48	5	3	0
	Mean no. of seeds/fruit	5	6	5	n.d.	5	6	3	n.d.
	Mean 1-seed weight [mg]	9.5	11.6	11.5	n.d.	9.1	8.8	14.6	n.d.
V. sepium	No. of fruits	48	48	48	25	48	33	12	14
	Mean no. of seeds/fruit	4	4	4	4	4	4	4	4
	Mean 1-seed weight [mg]	19.4	17.5	18.3	16.2	17.7	18.2	19.2	18.2

CONCLUSION: Herbicide and fertilizer misplacement in field margins causes negative effects on the plant density and reproductive capacity of wild plant species. In addition, plants in field margins are exposed to repeated agrochemical inputs during a growing season over several years, and these application sequences are additive in the long run.

SOURCE:

Schmitz et al. (in prep.): Agrochemicals in field margins – Assessing reproduction effects

Schäfer, K (in prep.): Auswirkungen von feldsaumrelevanten Herbizideinträgen auf das Vorkommen, die Blütenbildung und Reproduktion von verschiedenen Nichtzielpflanzen. Diplomarbeit, Institut für Umweltwissenschaften, Universität Koblenz-Landau. Campus Landau.

Summary and discussion of the field experiments

We found only four studies, which investigated the effects of herbicides on natural plant communities in the field. This finding itself is surprising since field studies with pesticides are in other areas, e.g. for arthropod communities, standard higher tier studies and also a lot of research findings are published in scientific journals on this aspect. Plant community ecology seems not seeing the sense of this study design since researchers are maybe not aware of the effects of agrochemicals on natural plant communities bordering crop fields.

Three out of the four located studies performed a field experiment on a meadow or a fallowed field (Kleijn et al. 1997, Strandberg et al. 2012, Schmitz et al. 2013) and one study was conducted on two different road verges and two different ditch banks (De Snoo et al. 2005). Hence, De Snoo and co-workers have combined and compared the data of four different locations. However, the combining of data from different sites can be problematic, since generally the vegetation composition is different at all sites. In addition, the effects of a test substance on different vegetation can vary from each other. Therefore, it appears to be more appropriate to perform a field experiment on one study site (e.g. on a low productive meadow). The study sites in the field experiments were not treated with herbicides or fertilizer before the experiments started. Therefore, botanical changes within these habitats over the time of the field experiment could be assigned to the treatments and the changes demonstrated in the vegetation are likely to occur in field margins as well (Kleijn & Snoeijing 1997). All discussed field experiments had chosen a randomized block test design, which is very useful to conduct dose-response experiments. However, the plot sizes were different in the studies and ranged from 2 m x 2 m to 8 m x 8 m. Generally, the size of a test plot is dependent on the size of the study site, the number of species, and in particular the distribution of the species over the study site. However, plant communities can be complex and dynamic and hence it seems necessary to increase the size of the test plots and their replications whenever possible, especially when sub-samples (several plant community assessments per plot) should be carried out. Therefore, a plot size of 7 x 7 or 8 m x 8 m as used by Strandberg et al. (2012) and Schmitz et al. (2012) seems to be appropriate.

The three field experiments conducted by Kleijn & Snoeijing (1997), Strandberg et al. (2012) and Schmitz et al. (2013) studied not only the herbicide effects on the plant community, but also the effects of fertilizer. This is an important aspect since these studies showed that relevant herbicide drift rates, but also low fertilizer rates as caused by realistic misplacement affected the plant communities negatively (Strandberg et al. 2012, Kleijn & Snoeijing 1997). Here, especially the fertilizer resulted in a relative immediate measurable decrease of plant species diversity since fertilizer increase the availability of nutrients and promotes plants with a high nutrient uptake (Kleijn & Snoeijing 1997, Schmitz et al. 2013).

The herbicide treatment in contrast, caused a mortality of particular plant species and resulted in sublethal effects (phytotoxic effects, flower suppression) and these sublethal effects reduced the reproductive capacity of certain plant species

(Schmitz et al. 2013). In general, such sublethal effects will need more time to be measurable in the density of a particular plant species since firstly the seed production is reduced. However, over a longer time span these sublethal effects will also cause the disappearance of the affected species (Schmitz et al. 2013). Therefore, long-term field studies are particularly important to assess the whole herbicide effects on non-target plant communities and, also, because repeated agrochemical applications over several years intensify the effects. Thus, the field experiments demonstrated that effects in the field are complex; interaction effects between species, as well as, interaction effects between agrochemicals (e.g. herbicides and fertilizer) can occur and are certainly important for the sensitivity of species to agrochemicals.

Evaluation of the field study designs

The study designs of the field experiments conducted by Kleijn & Snoeijing (1997), Strandberg et al. (2012) and Schmitz et al. (2013) can be evaluated as appropriate to study plant community responses, including intra- and inter specific interactions between plants. With such studies also ecological effects on organisms depending on affected plant species (e.g. pollinators and herbivores) can be assessed. More studies in this area are needed and if possible study duration should be longer than the general funding period for a Ph.D. project of three years to be able to detect long term changes in the plant community.

Protectivity of the risk assessment approach

In the following, we aim to compare the herbicide effects on natural plant communities found in the field studies (lowest field rates at which effects could be detected) (Kleijn & Snoeijing 1997, De Snoo et al. 2005, Strandberg et al. 2012, Schmitz et al. 2013) with the “regulatory acceptable concentration (RAC)” of the used herbicides. The RAC is an assessment endpoint, that is used in risk assessment and is expressed as an environmental concentration (or rather a field rate for non-target plants) of an active substance expected to have no unacceptable adverse effects on the environment (Brock et al. 2009, for aquatic risk assessment). This value (field rate) can be calculated with the ER50 values of the most sensitive test species used in risk assessment procedures and the application of a Safety Factor (RAC = ER50/SF)[3] (Brock et al. 2009). The ER50 values of the tested plant species in risk assessment procedures can be obtained from herbicide authorization documents (e.g. Draft Assessment Reports).

In the four evaluated field studies the herbicides Starane 200 (a.i. fluoroxypur), Liberty (a.i. glufosinate-ammonium), Roundup Bio (a.i. glyphosate) and Atlantis WG (a.i. mesosulfuron-methyl + iodosulfuron-methyl) were used. For these herbicides the ER50 values of the most sensitive test species used in regulatory processes were selected (data were obtained from UBA and Draft Assessment Reports, except for Atlantis since no data were available for ER50 values) and the regulatory acceptable rate were calculated (Table 10).

In the next step the calculated acceptable rate was compared with the lowest field rate at which significant effects on the plant community in the evaluated field studies were found (Table 10). However, a comparison is difficult since in the field studies not the same endpoints as in single species tests (fresh or dry weight of one species) were determined. Generally, in field studies the entire plant community is considered and it needs a long time span to determine changes in community structures when low herbicide rates were used. For example Kleijn & Snoeijing (1997) found a significant herbicide effect on the plant species richness firstly in the third experimental season at a field rate of 10 g fluroxypyr/ha (=5% of the recommended field rate). This rate is higher

[3] The equation can also be derived from the TER approach (TER = toxicity exposure ratio). The TER is calculated by using the estimated ER50 value and determined PEC (predicted environmental concentration). If the TER-value is >5 (trigger value) effects on plants are considered acceptable (TER = ER50/PEC > 5) (European Commission 2002). Accordingly, the equation for the regulatory acceptable rate for off-field habitats is ER50/trigger value.

than the calculated regulatory acceptable rate for this herbicide based on a single species test. However, it has to be considered that Kleijn & Snoeijing (1997) have not tested a lower herbicide rate and therefore, it cannot be excluded that lower field rates cause also effects on the plant community. Additionally, in the field often older phenological development stages than the 2-4 leaf stage, which are used in single species tests, are present during the time of application. The older phenological stages are often affected by low herbicides rates, which resulted in sublethal effects (e.g. phytotoxic effects, flower suppression) (De Snoo et al. 2005, Strandberg et al. 2012, Schmitz et al. 2013) and not in mortality. However, over time these sublethal effects (e.g. flower suppression) are also expected to cause the disappearance of the affected species and lead to shifts in plant communities (Schmitz et al. 2013).

Table 10 *ER_{50}-values (dry weight determined 21 days after treatment, vegetative vigour test) and the calculated regulatory acceptable rate. The ER_{50} value of the most sensitive test species used in regulatory processes are listed (data taken from UBA and from Draft Assessment Reports). The last column lists the lowest field rate at which significant effects on the plant community in the evaluated field studies were found. SF = Safety Factor (=5)*, WAT = weeks after treatment.

Herbicides used in field studies (a.i.)	ER50-value [g ai/ha] (test species)	Regulatory acceptable rate (ER50/SF)	Significant effects found in field studies (references)
Starane 200 (fluroxypyr 200g/L)	19.4 (*Glyxine max*)[A]	3.9 g a.i./ha	reduced species number in the third experimental season: 10 g a.i./ha (= 5% field rate) (1)
Liberty (gulfosinate-ammonium 800g/ha)	101.0 (*Veronica persica*)[B]	20.2 g a.i./ha	decreased biomass (8 WAT): 512 g a.i./ha (2) sublethal effects (phytotoxic effects 8 WAT): **16 g a.i/ha (= 2% field rate)** (2)
Roundup Bio (glyphosate 360 g/L)	145.7 *(Lycopersicon esculentum*)[C]	29.1 g a.i./ha	sublethal effects (phytotoxic effects): **14.4 g a.i./ha (=1% field rate)** mortality: 360 g a.i./ha (3)
Atlantis WG (mesosulfuron-methyl 30 g/kg + iodosulfuron-methyl 6 g/kg)	no data available		sublethal effects (flower suppression): 120 g Atlantis WG/ha (= 30% field rate) mortality effects (e.g. *Rhinanthus minor*): 120 g/ha Atlantis WG (4)

(1) Kleijn & Snoeijing 1997; (2) De Snoo et al. 2005; (3) Strandberg et al. 2012; (4) Schmitz et al. 2013 [A] ER50 value taken from UBA (UBA ICS 76501), [B] ER50 value taken from DAR, [C] ER50 value taken from UBA (UBA ICS 44219)

A comparison between the lowest field rates at which sublethal effects in the field studies were found and the regulatory acceptable rates (Table 10) shows that field rates lower than the acceptable rates can cause sublethal effects on the vegetation (e.g. curly, yellow-coloured or dead leaf lips). Generally, these phytotoxicity effects at low herbicide rates were mainly short-term effects and recovery was good (De Snoo et al. 2005, Strandberg et al. 2012). However, these sublethal effects occurred at field rates (1% and 2% of the recommended field rate, see Table 7), which were much lower (1.2 – 2 times lower) than the calculated regulatory acceptable field rate for the herbicides. Hence, it might be possible that herbicide rates equivalent to the regulatory acceptable field rate cause stronger effects, which is not investigated until now. It was also noticed in the evaluated field studies that at species level large differences in phytotoxic effects occurred (e. g. phytotoxic effects on *Rumex acetosa* seem to be stronger than for *Ranunculus repens*) (De Snoo et al. 2005) and that competitive interactions between species having different sensitivity to herbicides are important for the species response in natural habitats, which cannot easily be extrapolated from single species tests (Strandberg et al. 2012). Hence, it seems possible that the current risk assessment provides not sufficient protection of non-target plant species and their habitats. The literature search located only four field studies, and therefore, the data set is limited. Further research in this topic is needed to make more accurate statements. In order to detect the protectivity of the current risk assessment procedure, field studies with experimental application rates equivalent and below the regulatory acceptable rate of an herbicide would maybe useful. However, it must be taken into account that plants in field margins are exposed to herbicide mixtures and repeated sublethal herbicide rates, which can be additive in the long run and are not considered in risk assessment procedures. Furthermore, interaction effects with herbicides and fertilizer can occur (Strandberg et al. 2012, Schmitz et al. 2013) and therefore, it would be important to consider these influence factors in field studies, too.

In short:

- The literature search located only four field studies, which investigated the effects of herbicides on non-target plant communities.
- Three of these studies also considered the effects of fertilizer effects on the plant community.
- Effects in the field are complex due to interaction effects between species and agrochemicals (herbicides and fertilizer).

- Long-term field studies are particularly important to assess herbicide effects on non-target plant communities. Only a few studies are conducted so far and further studies using RACs as experimental rates seem necessary.
- Single-species tests (Tier II studies) cannot predict the sensitivity of plant species grown in natural plant communities and therefore, it seems that the current risk assessment probably provides insufficient protection of non-target plant species and their habitats.

6. General discussion

6.1. Sensitivity of crop and wild non-crop plants

In chapter 1 of this report we assessed the differences between mortality and biomass endpoints for wild (non-crop) and crop plant species that were used at the seedling stage in Tier II standard tests according to the OECD guidelines 208 & 227 (OECD 2006). The ER_{50}-values for the datasets that were obtained from publicly available literature and EFSA databases as well as unpublished reports for the registration of a herbicide to be processed by UBA. However, only for two herbicides, glyphosate and dicamba, suitable data for several crop and non-crop plant species (at least four of each group) were available. Of all datasets only two relatively large ones remained – one for glyphosate with many data on wild plant species and one for dicamba with some non-crop plants tested. In these data sets it became obvious that crop plants were always at the non-sensitive end of the species sensitivity distribution (SSD). The difference between the ER_{50} values for biomass reduction of crop plants and the more sensitive wild non-cop plant species was up to a factor of 178 (most sensitive wild plant to least sensitive crop plant). This was true for both datasets available and we are not aware of any other.

The datasets for crops and non-crop plants for both herbicides came from different studies although all approximately followed the OECD guideline and therefore test conditions are expected to be similar. However, Strandberg et al. (2012) stated that several test conditions may vary and are not documented and that these potential differences in test conditions may be the main reason for the differences in sensitivity calculated in previous studies based on data of different datasets (e.g. Boutin et al. 2004 and the re-analysis by Strandberg et al. 2012). A recent study by Strandberg et al (2012) suggested that crop plants are less sensitive than non-crop species to a number of common herbicides (among them as well glyphosate and dicamba, probably based on a similar database). This was in accordance with the finding by Boutin et al. (2004). Strandberg and co-workers (2012), therefore, performed a calibration study to compare results with Boutin et al (2004) and then concluded that sensitivity between crop and non-crop species does not differ, although there remains an argument about the non-crop species that were used being weed species (*Centaurea cyanus* and *Papaver rhoeas*). However, it was also demonstrated that study results differ depending on the crop cultivar used (White & Boutin 2007) and it cannot be ruled out that these crop-specific differences are, at least partly, responsible for the different crop species sensitivities found by Strandberg et al. (2012) compared to the US

EPA data used by Boutin et al. (2004). Additionally, different herbicide formulations influence the combined data set (see chapter 3).

Recommendation: ER_{50} data for several herbicides need to be generated in studies containing many crop and non-crop species in order to examine if ratios between the HC5 values for the crops and non-crops are similar to that of glyphosate or dicamba. Moreover, further research is needed to study the influence of different test condition possible under OECD guideline and the used cultivar. If these studies confirm Strandberg's conclusion the currently used OECD test protocol guideline should be improved.

6.2. Wild plants in field margins and in standard tests

It seems that a relative small number of wild non-crop plant species were ever tested for their sensitivity towards herbicides and that this important research field is covered by only a few scientists and research groups globally. In the following we analyze the taxonomic representation of wild plant species in OECD tests for the regulation of herbicide and their occurrence in field margins. Fletcher et al. (1990) found that the ER_{50} values of taxonomically closely related species had a higher degree of similarity than taxonomically more distant species (although Strandberg et al. 2012 argued that plant morphology is a better predictor of sensitivity than relatedness. See also Research Box 1). Composition of natural field margin plant communities was assessed at different sites in Germany by Ross-Nickoll et al. (2004). In this large-scale survey 274 species of 41 different flowering plant families occurring in field margins were found among them families, which seems to be sensitive towards herbicides such as the Lamiaceae (Boutin et al. 2004). However, it has to be kept in mind that the field margins monitored by Ross-Nickoll et al. (2004) were already influenced by agrochemicals and herbicides since the 1960s potentially depleting the diversity of wild plants (e.g. Andreasen et al. 1996; Andreasen & Streibig, 2011). In contrast, the examination of non-target plant tests performed for the risk assessment of herbicides (obtained from draft assessment reports and datasets provided by the Federal Environmental Agency, see above) revealed that in total only 20 plant species, representing 9 families, were used in 54 examined datasets. The narrow taxonomic range of the test species indicates that our knowledge on species sensitivity is limited.

Recommendation: Further wild plant species (annuals and perennials), selected from the known communities of field margins, need to be studied with products of different mode of action to improve the protectivity of the risk assessment based on tier II studies.

6.3. Realistic drift in Tier III non-target plant studies

As a refinement of Tier II standard non-target plant studies, plants are exposed in the field to realistic drift with smaller droplet sizes and higher concentration of herbicide in the droplets than in the test applications. The analyzed studies with exposure of plants in different distance to a field applied at full application rates with a commercial sprayer set up or similar device showed that significant effects on flowering occurred up to 10 m from the field border. This is similar to the drift of 0.29 % of the field application rate (according to Rautmann et al. 2001). Studies using realistic drift are difficult to compare since the variation in spraying conditions (e.g. temperature, wind speed and direction) during the application will always result in slightly different results as shown by Koch et al. (2004) and Strandberg et al. (2012). These two studies conducted exposure experiment in spraying chambers and agricultural fields and Koch et al. (2004) found that different levels of drift deposition occur at different distances depending on the drift potential of the application technique, wind, and other canopy related factors (density, height). Moreover, Koch et al. (2004) concluded that the formation of drift deposit on a single plant is unpredictable under field conditions. Generally, it can be expected that a worst case scenario with relatively high drift will occur at cloudy skies and cool temperatures (low evaporation and volatilization) and high wind speed, which can be up to 5 m/s under good agricultural practice. Low herbicide drift on the other hand will occur in sunny weather with higher temperatures and low wind speeds. Therefore, we expect large variations in spray drift depositions depending on weather conditions and additionally differences in the technologies used will also come into play. For registration purposes Tier III realistic drift non-target plant studies should always be performed under a realistic worst-case scenario. Generally this approach is questionable since differences in droplet size and in herbicide concentration within droplets between spray drift and direct spray is strongly dependent of environmental and technical factors as already mentioned. Exposure in spraying chambers did not result in any differences in the effects observed on plants at a given herbicide dosage (see Strandberg et al 2012). Therefore, a simulation of spray drift (direct overspray) seems to be a useful method, which is additionally easy to replicate.

<u>Recommendation</u>: The direct overspray is a conservative approach. If refinement is needed, studies on realistic drift and their effects need to be performed.

6.4. Tier III microcosm studies with 6-9 plant species allowing interaction effects

Microcosm studies with more than one plant species planted in a container potentially allow interaction such as intra- and interspecific competition for resources for nutrients, water and light and may affect exposure due to shielding, where higher and denser plant species are more exposed than lower growing plants. For example Riemens et al. (2008) could detect a shielding effect for the small wild plant species *Stellaria media*. Probably this species was in advantage because it received less of the applied herbicide rate due to shelter provided by other species grown in mixture (Riemens et al. 2008).

Interaction effects cannot be predicted from single-species tests. The main conclusion of the analyzed studies using a realistic drift scenario was that in-crop buffers of 8-10 m would be required to avoid growth and survival effects on plants (Marrs et al. 1991b, Marrs & Frost 1997). The simulated drift studies comparing single potted plants and plants grown in a mixture came to the conclusion that it is not possible to predict effect size or direction from standard Tier II non-target plant studies to microcosms (e.g. Riemens et al. 2008, Dalton & Boutin 2010). An additional factor for the composition of the plant community is fertilizer misplacement in field margins that is not addressed in microcosm studies. Fertilizer is increasing the growth of some plant species whereas others cannot tolerate high nutrient levels. Strandberg et al. (2012) performed an experiment with glyphosate and fertilizer, and Schmitz et al. (2013) (see Research Box 4) with the sulfonyl urea herbicide Atlantis WG and fertilizer. In both studies the fertilizer interacts with the effects of herbicides. The nutrient level in microcosms is a crucial factor for effects and recovery of plants and therefore, needs to be carefully selected and documented. It is also a factor that is so far not considered in the risk assessment for non-target plants even though it may render some species more sensitive to common agricultural practice than expected based on data from standard plant tests (Strandberg et al. 2012).

The evaluated microcosm studies used plant densities far lower than recorded in natural communities. We could expect intra- and inter specific effects to be less pronounced and the resulting uncertainty might be another factor affecting the extrapolation from microcosm data to natural plant communities.

<u>Recommendation</u>: The density effect in Tier III studies might underestimate the negative competition effects in natural communities. Therefore, further research is required to improve the risk assessment based on tier III studies.

6.5. Tier III non- target plant field studies

The analyzed field studies used plants, which were either germinated in the greenhouse and transplanted at young development stages into small plots in the field (Gove et al. 2007, Pfleeger et al. 2012) or which were sown and cultured directly in experimental plots (Pfleeger et al. 2008, Perry et al. 1996). Afterwards the plants were exposed to simulated spray drift rates in the field with an appropriate field sprayer (except for the plants of Gove et al. (2007), who treated the plants already in the lab before transplanting). The plants in the field plots were growing under realistic conditions allowing pesticide break down under natural influences (photolytic decomposition, washing-off by rain,…). However, plant density was again low leading to the same uncertainty as mentioned above. Especially intra-specific competition was not present since mostly only one or a small number of individuals of each plant species were planted in the mixture. Plant density also affects shielding and herbicide exposure. To improve the realism of field studies some suggestions for improvement are presented (see Box 2).

In non-target plant studies woody plant species are rarely included although hedges surrounding agricultural fields can be exposed to herbicide spray drift. The study by Kjaer and co-workers (2006a) investigated whether the drift of herbicides has negative effects on hawthorn (*Crataegus monogyna*), a common shrub species in hedges in the agricultural landscape. In this study a sublethal endpoint was evaluated: When herbicide exposure occurred at the budding stage a 100% berry reduction was found at drift rates of 5% of the application rate. This is a clear example where the reproductive endpoint berry production is very sensitive, whereas the drift exposure did not lead to mortality or leave reductions of the shrubs. The authors also concluded that this effect is not only reducing the fitness of the plants but may influence also other organisms like berry eating birds (Kjaer et al. 2006a).

<u>Recommendation</u>: To establish a factor for effects under realistic herbicide exposure (including break down, shielding, washing off …) more field studies are required. Especially woody plant species under realistic exposure seem to be understudied.

6.6. Reproduction as an endpoint in non-target plant testing

The analyzed studies (Kjaer et al. 2006a, Marrs et al. 1989, Marrs et al. 1991b, Marrs & Frost 1997, Pfleeger et al. 2008, Gove et al 2007), but also the research projects by Schmitz et al. (2013) Figure 10, Research Box 4 & 5) and Strandberg et al. (2012) showed that reproductive endpoints as flowering and seed production are highly sensitive.

Figure 10 Comparison of the flower intensity of *Ranunculus acris* in a control plot (left photo) and a plot treated with 30% of the herbicide Atlantis WG (right photo). The flower formation was suppressed in the herbicide treated plots (Schmitz & Brühl 2010, Schmitz et al. 2013).

There is general agreement in the analyzed studies that seed production is a more sensible endpoint for risk assessment of herbicides than growth and survival for both annual and perennial species (see also Strandberg et al 2012). Endpoints of biomass and damage assessments as typically performed in Tier II but also many Tier III non-target plant studies are not able to predict the effects on reproductive endpoints.

These findings are not really surprising since it is a common basic ecological knowledge that according to the concept of resource exploitation resource depletion or a stressor first limits reproduction, further reduction affects individual growth and further limitation finally leads to death (Figure 11, Smith & Smith 2009).

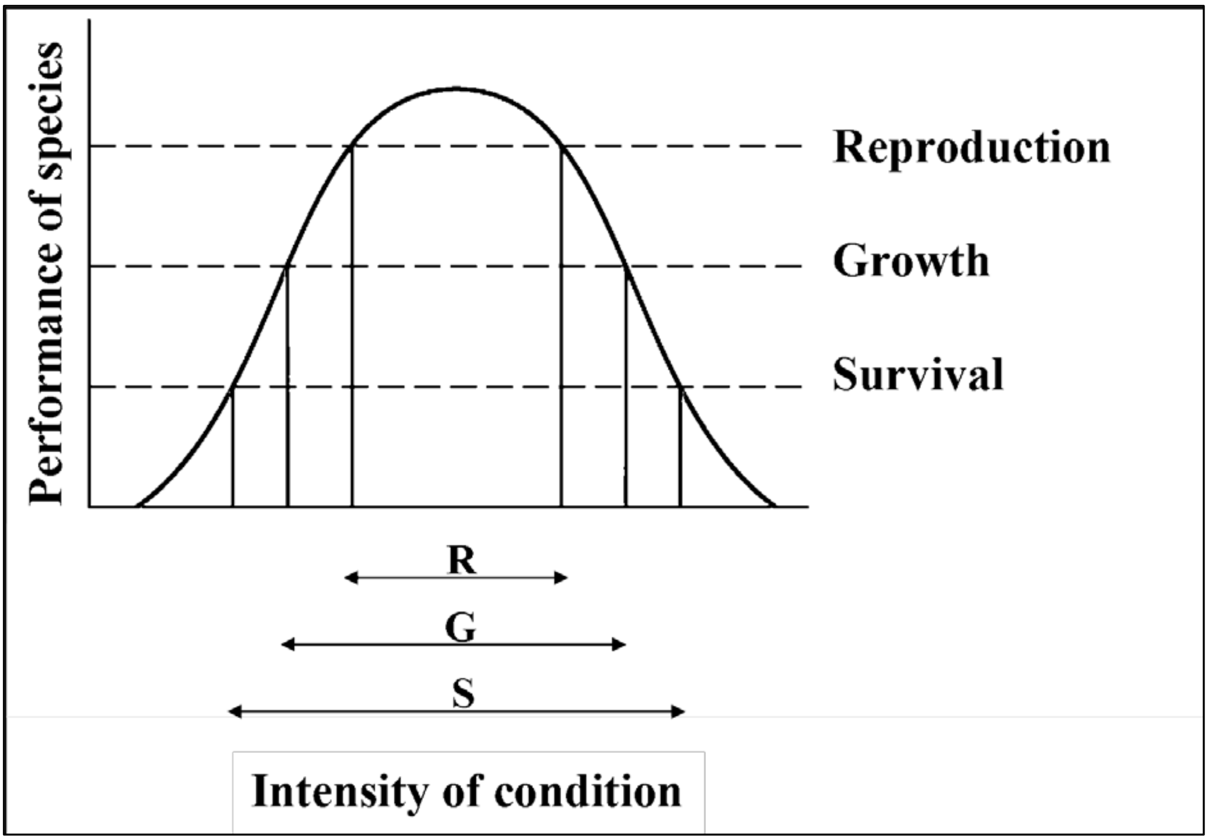

Figure 11 Intensity of a condition and influence on the performance of a species (individual). S = survival, G= growth, R= reproduction (adapted from Smith & Smith 2009). Conditions have to be in an optimum and a maximum of resources need to be available to allow reproduction. With increasing intensity a stressor like an herbicide should first affect reproduction, then growth and then lead to mortality.

Non-target plant studies for regulatory risk assessment so far only account for mortality and growth (biomass, length) but never include reproductive endpoints like flowering or seed production, although the latter (and the germination rate of these seeds) is vital for the persistence of the population. Since it is unknown by what factor these endpoints differ with only a few studies available it is impossible to extrapolate from available biomass endpoints to reproductive risk.

Recommendation: To identify a factor for the risk assessment using standard tier II studies, further research is required. Further wild plant species need to be tested in a reproductive stage with herbicides of different mode of action. These life-cycle studies are lasting for a long period of time to allow assessment of seeds and the germination probability to assess viability of populations.

6.7. Effects on other organisms

Plants as primary producers form the basis of any food web in an ecosystem. In agricultural landscapes herbivorous animals such as hares or voles and a multitude of insects (e.g. grasshoppers and butterfly caterpillars) consume various parts of plants. But not only the green leaves are eaten, also nectar and pollen is a resource used by many insects. For the common buttercup *(Ranunculus acris)*, where reductions in flowering were observed in a study by Schmitz et al. (2013) (see Research Box 4 & 5) 117 flower visiting insects were recorded in Germany for this plant species alone (Weiner et al. 2001) and more than 70 insect species were recorded to feed on *Stellaria media* (Marshall et al. 2003). Host specifity can be especially high in some insect groups. For example 60% of sap sucking cicadas and 70% of leaf mining moths in the UK are monophagous, that means they are specialized on one plant species only. It is therefore, immediately conceivable that a loss or density reduction of certain wild plant species has a negative effect on the population size of especially herbivorous insects. Herbicides are not directly killing herbivorous insects like an insecticide, but would affect them indirectly by reducing their food source (Figure 12)

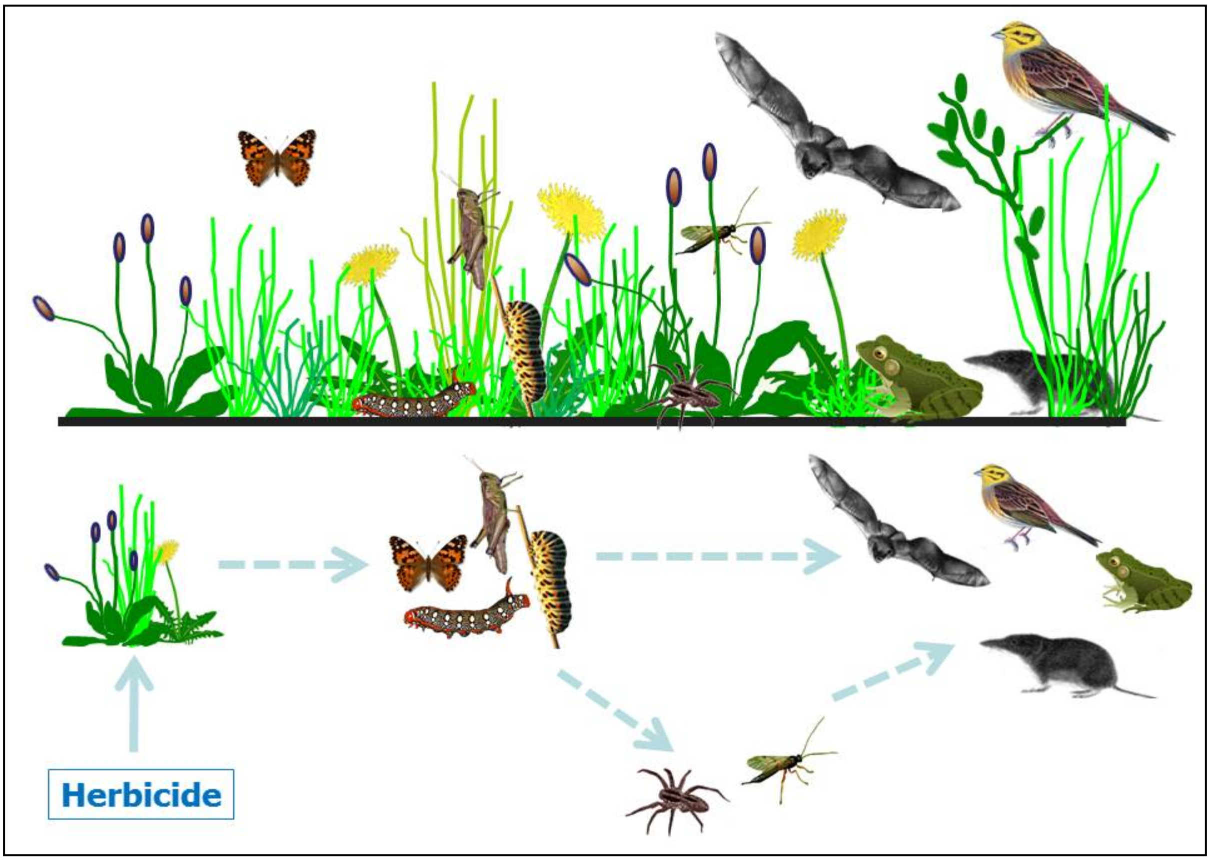

Figure 12 Herbicides and their effects on agri-ecosystems. Solid line: direct effect, dashed line: indirect effect.

Herbivorous insects represent the food of other predatory arthropods such as spiders, parasitoid flies and wasps. Together all arthropods are essential food for insectivorous birds and mammals, though their reduction might lead to food resource reduction at these higher trophic levels.

But not only insectivorous animals feed on insects. Also granivorous birds such as the Yellowhammer *(Emberiza citronella)* or the grey partridge *(Perdix perdix)* need insects for their nestlings (Morris et al. 2005, Rands 1985). For the latter species, that shows one of the most dramatic reductions in population size throughout Europe in the last decades, the impact on herbicides on the food availability of insects for the chicks was demonstrated in an experimental design (Rands 1985). Herbicides do not only affect the plant communities neighboring agricultural fields, but also the higher trophic levels in the food web and finally the ecosystem of agricultural landscapes. But not only the mere number of species and individuals – biodiversity – reduced. With herbicide related reductions of host plants of herbivorous insects, these species (e.g. butterflies and moths) also reveal lower numbers and changes in their communities. Since the adults are pollinating plant species, there is a feedback loop on plant reproduction and a general reduction of the ecosystem service of pollination (of human's crops) possible.

Indirect effects of herbicides can also be more subtle. For herbivorous insects the plant quality is essential especially for the growth of larval stages. We tested the hypothesis that herbicides act as stressors on plants, which results in an increase in the production of plant defense chemicals. For the common buttercup *(R. acris)* treated with a sublethal 3% field application rate of Atlantis WG (a drift occurring at approx. 1 m distance from the field border according to Rautmann et al. 2001), we observed smaller caterpillar weight and longer time spans to pupation of the cabbage moth *Mamestra brassicae* (see Research Box 6). In a field situation a longer development time results in a higher predation risk for the caterpillar. Other tested wild plant species did not reveal this effect and therefore, it seems to be highly species specific. However, with a multitude of registered herbicides and all the plant- insect interactions present, chemical interruption of feeding processes seems a likely process. For this kind of herbicide effect we would not necessarily detect any changes in the composition of the plant community. Plants would be present, showing recovery after sublethal effects of an herbicide application, however their internal chemistry is altered and their quality as a food plant reduced.

Recommendation: Plants form the basis of the food chain and sublethal effects such as chemical alteration in plant defense products might have large effects on the food web. To understand the disruptive effects of herbicides on the entire food web research programs need to be developed. Chemical changes in plants leading to lower food quality for herbivores need to be included in the non-target plant risk assessment.

RESEARCH BOX 6: Herbicide-effects on host plant quality

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

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

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

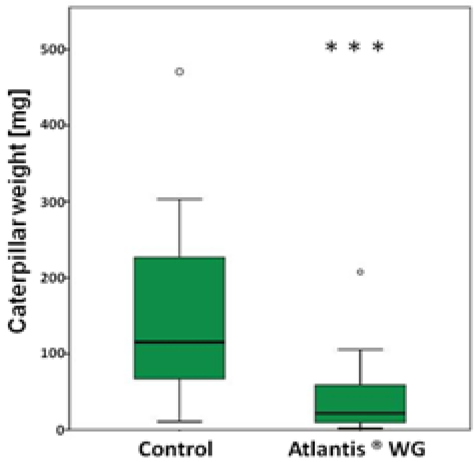

Figure 1: Comparison of the weights of *Mamestra brassicae* caterpillars (17 days old) reared on untreated control plants and plants treated with the herbicide Atlantis WG (10% in-field application rate). ***: $p<0.001$ (Wilcoxon-Test).

SOURCE:

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

6.8. Risk assessment of non-target plants – prediction of field effects from standard tests

The risk assessment for non-target plants uses endpoints derived from seedling emergence or vegetative vigor Tier II tests of a selected range of species tested as seedling or at an early life stage with only a few leaves present. In most cases crop plants are tested and endpoints include survival and growth endpoints such as biomass and some measurement of plant lengths or number of leaves and comparisons are made between a range of test rates and an untreated control. These standard non-target plant tests form the effect data and together with exposure assessments and predictions they are included in the risk assessment and are assumed to protect natural plant communities.

Our analysis of Tier II studies and especially the data sets compiled for glyphosate and dicamba revealed difference by a factor of up to 178 between the sensitivity of the different species, with the non-crop plant species always being more sensitive. This is in accordance with many other studies (e.g. Boutin et al. 2004), however Strandberg et al. (2012) conclude that there is not such a difference between the two different plant types, and differences are related to between studies variation instead. Although study dependent variation is a point we argue that all endpoints analyzed by us were conducted to a standard protocol (OECD 208 & 227) and therefore variations should be acceptable, otherwise the test protocol would need revision. Since no specific study is carried out for a few herbicides to answer this question, using a range of wild plant species, ideally from many different families that are or were present in agricultural plant communities, we consider the evidence provided in chapter 3 to be valid and conclude that wild non-crop plants are more sensitive than crop plants. When calculating a SSD for glyphosate the HC5 for wild plants differs by a factor of 21 from the crop plants, for dicamba this is a factor of 3. We do not want to discuss the validity of the SSD approach in risk assessment, nevertheless it should be noted that the HC5 value means that we accept loosing 5% of the most sensitive species of our plant community. We are not sure if this approach is in accordance with the protection of biodiversity and its reestablishment that is an aim globally agreed on.

The analyzed studies and recent research by Strandberg et al. (2012) and Schmitz et al. (2013) (see Research Box 4 & 5) showed that reproduction is a highly sensitive endpoint, which is in accordance with ecological theory. Effects on reproductive endpoints such as seed or berry production as well as flowering were also observed in other studies up to a distance of 10 m from the field edge

(≙ spray drift of 0.29% of the field application rate according to Rautmann et al. 2001) (Marrs et al. 1989, Marrs et al. 1991b). It is not possible to recover from reproductive effects in a growing season which is the case for damage effects as e.g. reduction or chlorosis of leaves. The reproductive effects will, over time with re-occurring exposure events, result in the depletion of the seed bank and consequently a reduction in population size. Unfortunately, we cannot provide a factor to account for this uncertainty and therefore, it is not possible to extrapolate from standard survival and growth endpoints to reproductive effects.

In plant communities sublethal effects due to phytotoxicity were noticed at experimental rates below the regulatory acceptable rates. Hence, it seems that the current risk assessment most likely does not provide sufficient protection of non-target plant species and their habitats.

In natural communities, plant density is much higher than in laboratory or microcosm studies. We expect intra- and interspecific competition as well as shielding to play a structuring role in natural plant communities, which is not included in standard tests. Additionally, wild plants in natural communities are facing stress due to extreme conditions (cold spell, drought) and are competing for limited resources such as light, water and nutrients. In Tier II and III studies plants usually are kept under optimal conditions for growth and reproduction. An additional stressor such as an herbicide is expected to have a more substantial effect on a stressed plant than a plant grown under optimal conditions. Due to our limited knowledge of this relationship it is impossible to provide any factor that could be used to include natural stress for extrapolation. In natural systems we also encounter a high proportion of herbivory as a common stressor, which is entirely excluded from standard laboratory test designs. The standard tests performed according to established protocols (OECD 2006, US EPA 1996) usually do not include fertilizer treatments, which could pose another stressor in natural communities. Additionally, since risk assessments are carried out for one specific compound the influence of tank mixes or multiple herbicide applications with different compounds and resulting exposure of wild plant communities are not accounted for in the current risk assessment approach and pose a further uncertainty.

The current risk assessment for non-target plants aims to protect the flora. However, only so called higher or vascular plants are tested. We are only aware of very few assessments of herbicide effects on lower plants such as soil growing algae, mosses and ferns although many of their species occur in agricultural landscapes in field margins and on dry stone walls. The sensitivity of ferns towards herbicides was recently assessed by Boutin and co-workers (Boutin et al. 2012). They recorded a high sensitivity to metsulfuron methyl and to a lesser extent to glyphosate. Also mosses seem to react sensitive to herbicide exposure (Newmaster & Bell, 2002).

Additionally, another group of organisms could also be included in non-target plant risk assessments: lichen. Lichen are composite organisms consisting of a fungus and a photosynthetic plant partner. Their sensitivity is established for air quality measurement but they are not studied in risk assessment approaches although a first study indicates also high sensitivity for lichens in Europe (Juuti et al, 1996). Lichen play an important role in nitrogen fixation and many insect species depend on lichen as a food source.

Recommendation: Since only a few field studies on herbicide effects on natural plant communities are available further research is necessary to establish a solid factor for extrapolation to be used in a risk assessment approach. This is especially true since entire plant groups such as ferns and mosses as well as lichens are so far not included in the risk assessment.

In the following table (Table 11) the uncertainties presented in the previous paragraphs and their impact on the safety factor are summarized.

Table 11 Uncertainties in the current risk assessment, relevant paragraphs for the scientific reasoning, impact on safety factor (+ safety factor increases,- safety factor decreases) and recommendations.

Uncertainties	Scientific reasoning	Influence on SF	Recommendations
The use of crop plants as surrogates for non-crop or native plant species	**chapter 6.1**, page 101, , chapter 3.1, 3.2, page 17-36	+	To get a more precise estimate of these uncertainties ER_{50} data for several herbicides need to be generated in studies containing many wild and crop species in one study set up with exactly matching conditions. → More research is needed to evaluate the current safety factor
The use of a few test species to present the highly diverse terrestrial plant community	**chapter 6.1,** page 101, chapter 6.2, page 102, chapter 3.3, page 37	+	
The difference in plant sensitivity between studies, test conditions	**chapter 6.1,** page 101, chapter 3.4, page 45,	+	
Different crop cultivars are used in different studies	**chapter 6.1**, page 101, chapter 3.1, page 27, 28,	+	
The use of annual plants as surrogates for perennial plants	**chapter 6.2**, page 102	+	The narrow taxonomic range of the test species indicates that the knowledge on species sensitivity is limited. → More research is needed to be able to extrapolate between species and especially information on perennials is needed
Difference in plant exposure to herbicides in the field and greenhouse	**chapter 6.3**, page 103, chapter 4.2, page 63-65,	-	Plants in greenhouse experiments receive an overspray in spraying chambers with simulated spray drift rates, which differs from real spray drift due to different droplet sizes. However, a simulation of spray drift (direct overspray) seems to be a useful method, which is easy to replicate. → Real drift might lead to a reduction of SF
Interaction effects between species in the field	**chapter 6.4,** page 104, chapter 4.2, page 56, page 62-68, chapter 4.3, page 75	+	To date only a few field studies were performed. Inter- and intraspecific competition can influence the composition of natural plant communities and are not covered by the standard tests. → More research is needed.

Uncertainties	Scientific reasoning	Influence on SF	Recommendations
Greenhouse = optimal conditions	**Chapter 6.5.** page 105	-	Plants in the field grow under realistic conditions, which allow pesticide break down under natural influences (photolytic decomposition, washing-off by rain,..). These factors may reduce effects in the field. However, to due to our limited knowledge it is not possible to provide any factor that could be used.
	Chapter 6.5, page 105, chapter 6.8, page 111-113	+	Wild plants in natural communities are facing stress due to extreme conditions (cold spell, drought) and are competing for limited resources such as light, water and nutrients. Due to our limited knowledge of the effects of these stressors it is impossible to provide any factor that could be used to include natural stress for extrapolation → More research is needed.
Only species in young development stages (approx. 2-4 leaf stage) are tested	**chapter 6.6**, page 106 chapter 5, page 83 and 98,	+	In the field often older phenological development stages than the 2-4 leaf stage are present during time of application. The older phenological stages are often affected by low herbicides rates, which result in sublethal effects (e.g. phytotoxic effects, flower suppression) → Older phenological stages could be included in RA or be addressed in a specific research project
Reproductive endpoints are not addressed in RA	**chapter 6.6,** page 106 chapter 5, page 95 -98 chapter 6.8, page 112	+	Endpoints of biomass and damage assessments as typically performed in Tier II non-target plant studies are not able to predict the effects on reproductive endpoints. → Reproductive endpoints should be included in RA

Uncertainties	Scientific reasoning	Influence on SF	Recommendations
Effects on other organisms	**chapter 6.7**, page 108	+	Indirect effects of herbicides on biodiversity, trophic levels (primary consumers = e.g. herbivorous insects, secondary consumers = e.g. birds) and ecosystem functions are not considered.
Protectivity of the current RA approach	**chapter 6.8**, page 112, chapter 5, page 83, page 97ff, chapter 7, page 115	+	So far only four field studies with natural plant communities were conducted. Phytotoxic effects were observed in natural plant communities at experimental rates below the regulatory accepted rates. One solution to evaluate the protectivity of the current risk assessment scheme is the conduct of targeted field studies that focus on plant community composition shifts using regulatory accepted rates of various herbicides. It is not enough to carry out literature reviews. → More research is needed to establish values for a general extrapolation approach
Extrapolation of laboratory results to field conditions	**chapter 6.8,** page 112, chapter 3.1, page 28, 29	+	With our current knowledge of natural plant communities in agricultural ecosystems and the impacts of pesticides we are not able to extrapolate from Tier II standard tests to the field situation. To date only four field studies with natural plant communities were conducted. → More research is needed.
Interaction effects between agrochemicals (e.g. repeated herbicides and fertilizer applications per year) in the field	**chapter 6.8**, page 112 chapter 4.3, page 70, chapter 5, page 83	+	The field studies demonstrated that effects in the field are complex. Interaction effects can occur in the field and are important for the sensitivity of species to agrochemicals. → More research is needed to address multiple applications and other agrochemicals

7. Conclusion

The current risk assessment for the regulation of pesticides in the EU aims to protect the flora in non-target areas outside the cropped fields and requires that effects of herbicides on non-target plants are addressed. Therefore, Tier II non-target plant studies according to established protocols are conducted (OECD 2008, US EPA 1996).

By analyzing available datasets for non-target plants from published literature and from EFSA draft assessment reports only for the herbicides glyphosate and dicamba more than four crop and non-crop plant species were tested and data on wild plants were available. In both datasets it was obvious that wild plants were 100 times more sensitive than crop plants. We also revealed that a test using six crop plant species does not cover the full range of the SSD but only addresses the upper end of the curve (least sensitive). It is therefore questionable if the current testing scheme is addressing the sensitivity of the plants in natural communities correctly. If the risk assessment scheme is chosen to be developed along the current lines, data on wild plant species for other herbicides are urgently needed to address the issue of species specific sensitivity properly. Ideally this research should be conducted in one laboratory specifically to avoid the influence of other factors of growing conditions. So far the available database is highly restricted with only a few working groups addressing this issue actively.

However, the approach of developing a better prediction using the same test system by including more plant species or a proportion of wild plants is not likely to improve the protection of the flora in total since it was concluded by a few authors and is also in accordance with ecological theory that reproductive endpoints are always more sensitive than endpoints obtained from mortality or growth assessments. Reproductive endpoints are relevant to protect the existence of a plant population. We therefore conclude that the current risk assessment based on Tier II non-target plant studies with mostly crop plant species and survival and growth endpoints used most likely does not provide a sufficient protection of natural plant communities. The same conclusion was reached by Strandberg and co-authors in a recent report published by the Danish Ministry of Environment (Strandberg et al. 2012).

Additionally, with our current knowledge of natural plant communities in agricultural ecosystems and the impacts of pesticides we are not able to extrapolate from Tier II standard tests to the field situation. As we could demonstrate in the previous chapter only a few relevant field studies were conducted. Phytotoxicity effects were observed in the natural plant communities of two studies at experimental rates below the regulatory accepted rates. One solution to further evaluate the protectivity of the current risk assessment scheme is the conduct of targeted field studies that focus on plant community composition shifts using regulatory accepted rates of various herbicides. So far these studies were never performed and the question of the protectivity of the current risk assessment scheme can therefore not be answered fully.

To extrapolate from endpoints obtained by conducting standard Tier II non-target plant studies to natural plant communities we would need to include additional safety factors in the risk assessment. Using a safety factor of 48 derived from the HC5 approach for the glyphosate dataset based on the assumption that 6 crops species are tested as currently practiced (see chapter 3), we only address the variation of plant sensitivity between crop and wild non-crop plant species based on the endpoint vegetative vigour at the seedling state.

To additionally address interactions among plants and with other stressors and the higher sensitivity of reproductive endpoints we possibly reach even higher values for safety factors. Factors like exposure reduction through shielding, intra- and interspecific competition for often limited resources come additionally into play and are not covered by the standard tests but are also not addressed by most Tier III microcosm and field studies since plant densities are generally lower then recorded in natural communities. Furthermore, the interaction with other factors such as nutrient input by fertilizers and herbivory are not accounted for, although they play an enormous role in structuring natural plant communities. Additionally plant communities might be exposed by different herbicide applications per year which is never addressed in the regulation currently in place. All these factors are meant as additional factors increasing the uncertainty in risk assessment and are not addressed in a safety factor.

To address the protectivity of the current risk assessment scheme correctly a specific research program needs to be designed and conducted. It is not enough to carry out literature reviews and evaluate the available knowledge that can be deduced from the scientific literature since the research questions addressed are very likely not related to the current regulatory requirements. To be able to evaluate the protectivity of the present EU risk assessment scheme for the regulation of herbicides the research program needs to include laboratory tests with a range of wild plant species and herbicides of different modes of action as well as long-term field studies to be able to evaluate effects on reproduction and other sublethal endpoints and their translation into community composition shifts. So far funding for applied research questions targeted to answer regulatory issues is limited or not available and therefore the interest of scientist in these questions is lacking. This dilemma is not unique for non-target plant risk assessment but also obvious in other areas, but especially in issues relevant for the terrestrial guidance document (e.g. pollinators other than honey bees, amphibians, non-target arthropod, indirect effects to mention a few).

Some of the analyzed higher tier studies suggested an 8-10 m wide non-spray buffer to the non-target area to avoid effects on wild plants, although even at 10 m effects on reproductive endpoints were recorded (Marrs et al. 1989, Marrs et al. 1991b, Marrs & Frost 1997). An in-field buffer of this dimension could be expected to conserve a major part of the plant community, especially in narrow field margins that so far do not receive any protection at all. In Germany, field margins below 3 m wide are not considered as a non-target terrestrial habitat and therefore, no drift reducing technology has to be used or distance during the application process is needed (Kühne et al. 2000) However, these narrow field margins (up to 3 m) are the typical margins remaining in the agricultural landscape and made up 85% of the total field margin length in a winegrowing area in Rhineland-Palatinate, which was determined in a recent project that used a quantitative approach using aerial photographs and a GIS evaluation (Hahn et al. 2010). Additionally, even the few wider margins do not have to be treated as non-target area if there is a certain proportion of so called small structures (“Kleinstrukturen”) such as hedges or patches of natural vegetation present on community-level, which is the case for most agricultural areas in Germany. Since the application in an arable scenario is conducted right up to the border of the field, the neighboring margin not only receives drift but also a partial overspray (Figure 13).

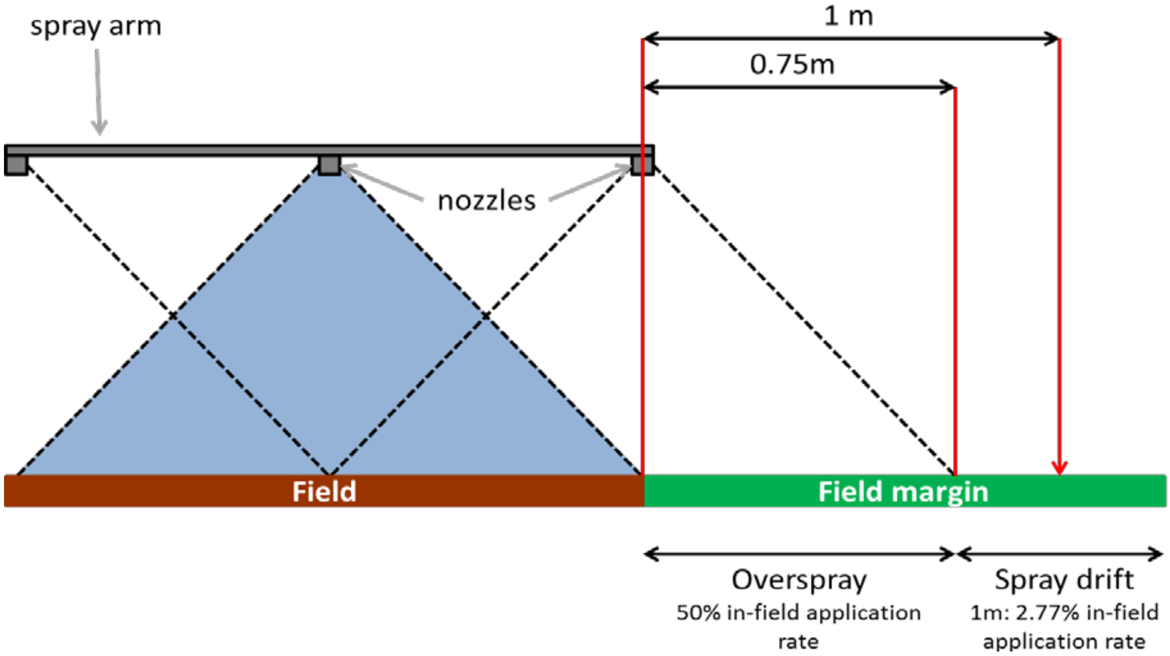

Figure 13 Schematic of the pesticide inputs via overspray and spray drift in cereal field margins. The blue colored area illustrates the spray cone of one nozzle (Schmitz et al. 2013).

A mandatory in-field buffer for all herbicide applications could be a potent measurement to conserve the flora in agricultural landscapes. It would be worth while to develop risk management strategies in parallel to further adaptations of the risk assessment scheme since a majority of margins would then also be protected from negative influences from all herbicide products. However, the design of field margin management strategies and their implementation is still in its infancy and resources for applied research projects are needed to improve current schemes and to develop easy to use applications for farmers and landscape planners alike. This kind of risk mitigation and management scheme would not only help to conserve the natural plant community but would additionally ensure the food resources for the entire food web from herbivorous insects to farmland birds and bats. It would therefore help to restore biodiversity in agricultural ecosystems and reestablish dwindling ecosystem services such as pollination and biological pest control.

8. Literature

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9. Appendices

9.1. Appendix 1

Summary of greenhouse studies (pot experiments with individual species) evaluating the effects of herbicides on non-target plants (see following pages). Since the test duration in the evaluated greenhouse studies range from short-term studies (assessments were made 14 days after treatment (DAT)) to studies with a test duration of 21-28 DAT (according to OECD guidelines), studies including assessments of reproduction effects (e.g. seed set), or studies, which assessed the effects of herbicide vapour, the evaluated literature is arranged accordingly.

Appendix 1 Summary of greenhouse studies (pot experiments with individual species) evaluating the effects of herbicides on non-target plants. Since the test duration in the evaluated greenhouse studies range from short-term studies (assessments were made 14 days after treatment (DAT)) to studies with a test duration of 21-28 DAT (according to OECD guidelines), studies including assessments of reproduction effects (e.g. seed set), or studies, which assessed the effects of herbicide vapour, the evaluated literature is arranged accordingly (yellow rows = short term studies (14 DAT), blue rows = assessments made 21-28 DAT, green rows = reproduction effects were assessed, purple rows = herbicide vapour studies), (VV = Vegetative Vigour, SE = Seedling Emergence). Studies are marked with an asterisk (*) when an ER25 or ER_{50} value was generated.

	Reference	Test design	Herbicide	species		Measurements	Results
				Crop	Non-crop		
1	Flechter et al. 1985	Phytotox Database evaluation, VV-Test	wide range of herbicides	23		toxicological comparison for 6 species; (duration: n. d.)	oat and wheat were most sensitive
2	Fletcher et al. 1990*	Phytotox Database evaluation: greenhouse vs. field, VV-Test	17 herbicides	6	7	mortality; 20 comparisons of sensitivity of greenhouse and field treated plants (test duration: no data.)	in 11 cases plants in field were more sensitive; sensitivity is correlated to taxonomic classification
3	Humphry et al. 2001*	pot experiment, VV-Test, (2 or 64 plants per pot)	1 herbicide		1	plant biomass (14 DAT)	the plant density affected the sensitivity, a high density increased the sensitivity
4	Maeghe et al. 2004*	pot experiments, SE-Test	2 herbicides	6	4	plant biomass (14 DAT)	sugar beet, red clover and lettuce showed a high sensitivity to herbicides
5	Jüttersonke 2004	pot experiments, VV-Test	2 herbicides		4	plant biomass (18 DAT)	species respond differently, reduced biomass with increased application rates
6	Blakeley-Smith 2006*	pot experiments, VV-Test	3 herbicides		17	plant biomass (14 DAT)	non-crop species have a broad range of susceptibility to herbicides
7	Olszyk et al. 2008*	pot experiments, VV-Test	1 herbicide	5	5	plant growth and biomass (14 DAT)	non-crop species vary more in sensitivity than crop species
8	Franzaring et al. 2012*	pot experiments, VV-Test	1 herbicide		22	plant growth and biomass (14 DAT)	a tenth of the field rate affected 50% of the non-crop species

Appendix 1 continued.

9	Birnie 1984	pot experiments; screening, VV-Test	7 herbicides	17		plant growth (28 DAT)	most susceptible species were *Sisymbrium officinale* and *Poa trivialis*
10	Breeze et al. 1992*	pot-experiments, VV-Test	4 herbicides		14	plant biomass (28 DAT)	no species was the most sensitive to all herbicides
11	Boutin & Rogers 2000	Phytotox Database evaluation: Canadian and U.S. EPA	10 herbicides	134 species (no data to no. of crop and non-crop species)		sensitivity of crop vs. non-crop species (test duration: followed U.S. EPA guidelines)	crop species were not consistently more, or less, sensitive to herbicides than non-crop species
12	McKelvey at al. 2002	pot experiments, VV- and SE-Test	10 herbicides		65	comparison of crop and non-crop species (test duration 14-28 DAT)	crop species are more sensitive than non-crop species
13	Boutin et al. 2004*	pot experiments with non-crop species, comparison with crop plants from databases	6 herbicides	22	15	visual effects (14 DAT, plant biomass (21 DAT)	non-crop species were more sensitive than crop species
14	Reuter & Siemoneit-Gast 2007*	pot and microcosm experiments, VV-Test	2 herbicides		6	plant biomass (14, 28 and 42 DAT)	species respond differently in pot and microcosms (further information see Research Box 3)
15	White & Boutin 2007*	pot experiments, comparison of crop species and their wild relatives, VV-Test	5 herbicides	10	10	visual effects, plant biomass (28 DAT)	no sig. difference in sensitivity between crop and related wild species

Appendix 1 continued.

16	Damgaard et al. 2008*	a) pot experiments, VV-Test	1 herbicide		2	plant biomass (21 DAT)	both species were affected, but one more than the other
		b) competition experiment, series of plant densities, VV-Test	1 herbicide		2	plant biomass (21 DAT)	single species test and competition experiment show discrepancies
17	Riemens et al. 2008*	pot experiments, VV-Test in greenhouse and field	1 herbicide		4	plant biomass (28 DAT)	higher sensitivity for species grown in greenhouse than in the field
18	Tesfamariam et al. 2009	pot experiments, VV-and SE-Test	1 herbicide	1	1	plant biomass, root biomass (25 DAT)	higher sensitivity of plants in the VV-Test than in the SE-Test
19	Boutin et al. 2010*	pot experiments, variability in phytotox testing, several ecotypes, VV-Test	2 herbicides		8	plant biomass (28 DAT), seed weight and germination tests with the ecotypes	sig. differences in sensitivity among ecotypes, different ecotype seeds differed in weigh, germination etc.
20	Vielhauer 2010*	pot experiment, VV-Test	1 herbicides	3	3	plant biomass (21 DAT)	higher sensitivity of non-crop species (further information see Research Box 1)
21	Geísthard 2012*	pot experiments, VV-Test	2 herbicides		5	plant biomass (28 DAT)	high sensitivity of non-crop species (further information see Research Box 2)
22	Strandberg et al. 2012*	pot experiments, VV-Test	3 herbicides	10	2	plant biomass (21 DAT)	crop species are less sensitive to metsulfuron than non-crop species
23	Boutin et al. 2012*	4 experiments were presented	4 herbicides	7	26	plant growth, plant biomass (28 DAT)	herbicide toxicity responses were similar when comparing a suite of crop versus wild species

Appendix 1 continued.

24	Fletcher et al. 1996	pot experiments; VV-Test	4 herbicides	3	1	plant growth, reproduction, biomass; treatment at 3 different development stages; test duration: 1-3 month after treatment	species respond differently; reduced plant growth and reproductive effects
25	Boutin et al. 2000	pot experiment, effects on growth and reproduction (diff. growth stages)	1 herbicide		5	biomass of vegetative and reproductive parts (time of harvest: upon seed set of the control plants)	species exhibited effects on growth, reproduction when sprayed with 10% field rate
26	Zwerger & Pestemer 2000	pot experiments, VV-Test	3 herbicides	2	2	plant biomass (3 and 6 WAT), seed production (10 WAT), seed variability, seed weight, germination (4-20 WAT),	reduced plant growth, no effects on recovery
27	Olszyk et al. 2009*	pot experiments, effects on growth and reproduction	7 herbicides	1		plant height, leaf injury (14 DAT), plant biomass, seed production (21 DAT)	pea is maybe a model species for reproductive effects, seed production is a more sensible endpoint than biomass
28	Carpenter & Boutin 2010*	pot experiments, short-term (juvenile) and long-term (repro-duction stage) tests	1 herbicide	10	10	plant biomass (21 DAT), plants were grown until fruit/seed production	no difference between the sensitivity of crop/non-crop species, but reproductive endpoint were more sensitive than biomass
29	Olszyk et al. 2010a*	pot experiments, VV-Test	7 herbicides	1		plant height (14 DAT), plant biomass, tuber production (28 DAT)	tuber production is more sensible endpoint than biomass for potatoes
30	Olszyk et al. 2010b*	pot experiments, VV-Test	5 herbicides	1		plant height (14 DAT), plant biomass, seed production (21 DAT)	seed production is a more sensible endpoint than biomass, *B. rapa* could be used to indicate reduced seed production in VV-Tests

Appendix 1 continued.

31	Blake 2011	pot experiments, VV-Test	1 herbicide		11	emergence, phytotoxicity and above-ground biomass (28 DAT)	caused reductions in seedling emergence and increased phytotoxicity
32	Pfleeger et al. 2011*	pot experiments, VV-Test; greenhouse vs. field grown plants	4 herbicides	3		plant growth (14 DAT), plant biomass, reproduction (tuber, pod) (different dates, depending on species)	greenhouse and field results were similar, reproductive endpoints were more sensitive than vegetative ones
33	Strandberg et al. 2012*	pot experiments, effects on growth and reproduction	3 herbicides		6	plant biomass (21-28 DAT), seed production (at maturity)	seed production is a more sensible endpoint than biomass
34	Franzaring et al. 2001	fumigation experiment	2 herbicides		14	chlorophyll fluorescence (2 DAT), foliar injury (7 DAT), plant biomass, plant growth (14 DAT)	vapours have adverse effects on non-target plants
35	Follak & Hurle 2003	exposition of plants to airborne herbicides in a wind tunnel	2 herbicides	1		quantum yield (PSII) (1 h after treatment), newly developed leaves (48 h after treatment), plant biomass (16 DAT)	plants are affected by sublethal concentrations, plants are at risk to airborne herbicides
36	Follak et al. 2005	a) exposition of plants to airborne herbicides in a wind tunnel	2 herbicides	1		plant growth, quantum yield (PSII) (2, 8, 16 and 24 DAT)	plants are affected by sublethal concentrations, plants are at risk to airborne herbicides
		b) exposition of plants to airborne herbicides in different distances to the treated field	1 herbicide	1		air sampling (10, 14 and 24 h after treatment) plant growth, quantum yield (PSII) (1 DAT)	concentrations outside the treated field was too low to cause effects
37	Egan & Mortenson 2012	exposition of plants to vapour drift	1 herbicide	1		plant injury; phytotoxic effects	vapour drift was detected at 21 m away from the treated plot

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9.2. Appendix 2

ER_{50} values for biomass of vegetative vigor test performed by following protocols for standard plant tests according to OECD guidelines for Glyphosate and Dicamba used in the performed SSD and HC5 analyses.

(a) Glyphosate

species	crop / non crop	ER_{50}	unit	data source
Achillea millefolium	non-crop	63,0	g ai /ha	Vielhauer 2010
Achillea millefolium	non-crop	36,4	g ai /ha	Strandberg et al. 2012
Allium cepa	crop	1793,4	g ai /ha	UBA ICS 35146
Anagallis arvensis	non-crop	17,5	g ai /ha	Boutin et al. 2004
Avena sativa	crop	874,3	g ai /ha	UBA ICS 35146
Bellis perennis	non-crop	14,3	g ai /ha	Boutin et al. 2004
Brassica oleracea	crop	739,8	g ai /ha	UBA ICS 35146
Centaurea cyanus	non-crop	29,2	g ai /ha	Boutin et al. 2004
Cucumis sativus	crop	896,7	g ai /ha	UBA ICS 35146
Daucus carota	non-crop	151,0	g ai /ha	Vielhauer 2010
Daucus carota "sativa"	crop	651,0	g ai /ha	UBA ICS 52213
Daucus carota "sativa"	crop	247,0	g ai /ha	Vielhauer 2010
Digitalis purpurea	non-crop	64,7	g ai /ha	Boutin et al. 2004
Gernanium molle	non-crop	28,6	g ai /ha	Strandberg et al. 2012
Gernanium robertianum	non-crop	130,9	g ai /ha	Strandberg et al. 2012
Glycine max	crop	975,1	g ai /ha	UBA ICS 35146
Glycine max	crop	659,0	g ai /ha	UBA ICS 52213
Helianthus annuus	crop	296,0	g ai /ha	UBA ICS 52213
Hypochoeris radicata	non-crop	113,0	g ai /ha	Vielhauer 2010
Inula helenium	non-crop	43,5	g ai /ha	Boutin et al. 2004
Lactuca sativa	crop	762,2	g ai /ha	UBA ICS 35146
Lactuca sativa	crop	250,0	g ai /ha	Vielhauer 2010
Lactuca serriola	non-crop	312,0	g ai /ha	Vielhauer 2010
Leonorus cardiaca	non-crop	35,8	g ai /ha	Boutin et al. 2004
Lolium perenne	crop ?	1345,0	g ai /ha	UBA ICS 35146
Lycopersicon esculentum	crop	145,7	g ai /ha	UBA ICS 35146
Lycopersicon esculentum	crop	534,0	g ai /ha	UBA ICS 52213
Mentha spicata	non-crop	17,9	g ai /ha	Boutin et al. 2004
Nepeta cataria	non-crop	39,7	g ai /ha	Boutin et al. 2004
Papaver rhoeas	non-crop	18,5	g ai /ha	Boutin et al. 2004
Plantago lanceolata	non-crop	36,0	g ai /ha	Geisthardt 2012
Plantago major	non-crop	36,0	g ai /ha	Geisthardt 2012

Appendix 2 continued.

Polygonum convolvulus	non-crop	15,8	g ai /ha	Boutin et al. 2004
Prunella vulgaris	non-crop	28,0	g ai /ha	Boutin et al. 2004
Ranunclus acris	non-crop	108,0	g ai /ha	Geisthardt 2012
Raphanus sativus	crop	246,6	g ai /ha	UBA ICS 35146
Raphanus sativus	crop	262,0	g ai /ha	UBA ICS 52213
Rudbeckia hirta	non-crop	24,7	g ai /ha	Boutin et al. 2004
Rumex acetosa	non-crop	54,0	g ai /ha	Geisthardt 2012
Rumex crispus	non-crop	27,5	g ai /ha	Boutin et al. 2004
Silene noctiflora	non-crop	74,7	g ai /ha	Strandberg et al. 2012
Silene vulgaris	non-crop	67,4	g ai /ha	Strandberg et al. 2012
Sinapis arvensis	non-crop	19,3	g ai /ha	Boutin et al. 2004
Solidago canadensis	non-crop	24,1	g ai /ha	Boutin et al. 2004
Tripleurospermum inodorum	non-crop	76,8	g ai /ha	Strandberg et al. 2012
Triticum aestivum	crop	648,0	g ai /ha	UBA ICS 52213
Zea mays	crop	751,0	g ai /ha	UBA ICS 35146
Zea mays	crop	640,0	g ai /ha	UBA ICS 52213

(b) Dicamba

species	crop / non-crop	ER50	unit	data source
Anagallis arvensis	non-crop	8,3	g ai /ha	Boutin et al. 2004
Bellis perennis	non-crop	11,5	g ai /ha	Boutin et al. 2004
Beta vulgaris	crop	24,0	g as/ha	Annex IIIA 10.8 / 002
Centaurea cyanus	non-crop	3,9	g ai /ha	Boutin et al. 2004
Digitalis purpurea	non-crop	4,9	g ai /ha	Boutin et al. 2004
Glycine max	crop	590,0	g as/ha	Annex IIIA 10.8 / 002
Inula helenium	non-crop	3,3	g ai /ha	Boutin et al. 2004
Leonorus cardiaca	non-crop	8,2	g ai /ha	Boutin et al. 2004
Lycopersicon esculentum	crop	49,0	g as/ha	Annex IIIA 10.8 / 002
Mentha spicata	non-crop	5,5	g ai /ha	Boutin et al. 2004
Nepeta cataria	non-crop	10,0	g ai /ha	Boutin et al. 2004
Papaver rhoeas	non-crop	5,8	g ai /ha	Boutin et al. 2004
Polygonum convolvulus	non-crop	8,3	g ai /ha	Boutin et al. 2004
Prunella vulgaris	non-crop	7,7	g ai /ha	Boutin et al. 2004
Raphanus sativus	crop	213,0	g as/ha	Annex IIIA 10.8 / 002
Rudbeckia hirta	non-crop	6,5	g ai /ha	Boutin et al. 2004
Rumex crispus	non-crop	10,7	g ai /ha	Boutin et al. 2004
Sinapis arvensis	non-crop	3,5	g ai /ha	Boutin et al. 2004
Solidago canadensis	non-crop	30,8	g ai /ha	Boutin et al. 2004

9.3. Appendix 3

Species list of the 249 flowering plant species recorded in field margins in different areas of Germany (Roß-Nickoll 2004; Roß-Nickoll pers. communication).

Species	Family	Group
Aegopodium podagraria	Apiaceae	Apiales
Anthriscus sylvestris	Apiaceae	Apiales
Chaerophyllum hirsutum	Apiaceae	Apiales
Chaerophyllum temulum	Apiaceae	Apiales
Conium maculatum	Apiaceae	Apiales
Daucus carota	Apiaceae	Apiales
Falcaria vulgaris	Apiaceae	Apiales
Heracleum sphondylium	Apiaceae	Apiales
Pastinaca sativa	Apiaceae	Apiales
Pimpinella saxifraga	Apiaceae	Apiales
Selinum carvifolia	Apiaceae	Apiales
Torilis japonica	Apiaceae	Apiales
Achillea millefolium	Asteraceae	Asterales
Arctium lappa	Asteraceae	Asterales
Artemisia vulgaris	Asteraceae	Asterales
Carduus acanthoides	Asteraceae	Asterales
Carduus crispus	Asteraceae	Asterales
Centaurea cyanus	Asteraceae	Asterales
Centaurea jacea agg.	Asteraceae	Asterales
Centaurea scabiosa	Asteraceae	Asterales
Cichorium intybus	Asteraceae	Asterales
Cirsium arvense	Asteraceae	Asterales
Cirsium vulgare	Asteraceae	Asterales
Conyza canadensis	Asteraceae	Asterales
Crepis biennis	Asteraceae	Asterales
Crepis capillaris	Asteraceae	Asterales
Echinops sphaerocephalus	Asteraceae	Asterales
Erigeron acris	Asteraceae	Asterales
Hieracium lachemalii agg.	Asteraceae	Asterales
Hieracium umbellatum	Asteraceae	Asterales
Hypochoeris maculata	Asteraceae	Asterales
Inula conyza	Asteraceae	Asterales
Lactuca serriola	Asteraceae	Asterales
Lapsana communis	Asteraceae	Asterales
Leontodon autumnale	Asteraceae	Asterales
Leontodon hispidus	Asteraceae	Asterales
Leucanthemum vulgare agg.	Asteraceae	Asterales
Mycelis muralis	Asteraceae	Asterales
Senecio erucifolius	Asteraceae	Asterales

Appendix 3 continued.

Senecio inaequidens	Asteraceae	Asterales
Senecio jacobea	Asteraceae	Asterales
Serratula tinctoria	Asteraceae	Asterales
Solidago canadensis	Asteraceae	Asterales
Solidago gigantea	Asteraceae	Asterales
Sonchus asper	Asteraceae	Asterales
Sonchus oleraceus	Asteraceae	Asterales
Tanacetum vulgare	Asteraceae	Asterales
Taraxacum officinale agg.	Asteraceae	Asterales
Tragopogon pratensis agg.	Asteraceae	Asterales
Tripleurospermum inodora	Asteraceae	Asterales
Tussilago farfara	Asteraceae	Asterales
Anchusa arvensis	Boraginaceae	Euasteriden 1
Cynoglossum officinale	Boraginaceae	Euasteriden 1
Echium vulgare	Boraginaceae	Euasteriden 1
Lithospermum arvense	Boraginaceae	Euasteriden 1
Myosotis arvensis	Boraginaceae	Euasteriden 1
Myosotis discolor	Boraginaceae	Euasteriden 1
Alliaria petiolata	Brassicaceae	Brassicales
Arabidopsis thaliana	Brassicaceae	Brassicales
Armoracia rusticana	Brassicaceae	Brassicales
Barbarea intermedia	Brassicaceae	Brassicales
Bunias orientalis	Brassicaceae	Brassicales
Capsella bursa-pastoris	Brassicaceae	Brassicales
Cardamine hirsuta	Brassicaceae	Brassicales
Cardamine pratensis	Brassicaceae	Brassicales
Cardaria draba	Brassicaceae	Brassicales
Descurainia sophia	Brassicaceae	Brassicales
Erophila verna	Brassicaceae	Brassicales
Erysimum cheiranthoides	Brassicaceae	Brassicales
Hirschfeldia incana	Brassicaceae	Brassicales
Sinapis arvensis	Brassicaceae	Brassicales
Thlaspi arvense	Brassicaceae	Brassicales
Thlaspi perfoliatum	Brassicaceae	Brassicales
Campanula patula	Campanulaceae	Asterales
Campanula rapunculus	Campanulaceae	Asterales
Campanula rotundifolia	Campanulaceae	Asterales
Symphoricarpus orbicularis (ang.)	Caprifoliaceae	Dipsacales
Arenaria serpyllifolia	Caryophyllaceae	Caryophyllales
Cerastium arvense	Caryophyllaceae	Caryophyllales
Cerastium brachypetalum	Caryophyllaceae	Caryophyllales
Cerastium glomerata	Caryophyllaceae	Caryophyllales
Cerastium holosteoides	Caryophyllaceae	Caryophyllales
Dianthus carthusianorum	Caryophyllaceae	Caryophyllales
Dianthus deltoides	Caryophyllaceae	Caryophyllales

Appendix 3 continued.

Saponaria officinalis	Caryophyllaceae	Caryophyllales
Silene dioica (rot)	Caryophyllaceae	Caryophyllales
Silene latifolia	Caryophyllaceae	Caryophyllales
Silene vulgaris	Caryophyllaceae	Caryophyllales
Silene x hampeana	Caryophyllaceae	Caryophyllales
Stellaria graminea	Caryophyllaceae	Caryophyllales
Stellaria holostea	Caryophyllaceae	Caryophyllales
Stellaria media	Caryophyllaceae	Caryophyllales
Pisifera sativa	Caryophyllaceae	Caryophyllales
Chenopodium album	Chenopodiaceae	Caryophyllales
Calystegia sepium	Convolvulaceae	Solanales
Convolvulus arvensis	Convolvulaceae	Solanales
Cornus sanguinea juv.	Cornaceae	Cornales
Sedum maximum	Crassulaceae	Saxifragalis
Carex flacca	Cyperaceae	Poales
Carex hirta	Cyperaceae	Poales
Carex spicata	Cyperaceae	Poales
Knautia arvensis	Dipsacaceae	Dipsacales
Equisetum arvense	Equisetaceae	Equisetales
Euphorbia cyparissia	Euphorbiaceae	Malpighiales
Mercurialis annua	Euphorbiaceae	Malpighiales
Astragallus glyciphyllos	Fabaceae	Fabales
Coronilla varia	Fabaceae	Fabales
Lathyrus pratensis	Fabaceae	Fabales
Lotus corniculatus	Fabaceae	Fabales
Medicago falcata	Fabaceae	Fabales
Medicago lupulina	Fabaceae	Fabales
Medicago sativa	Fabaceae	Fabales
Melilotus alba	Fabaceae	Fabales
Melilotus officinalis	Fabaceae	Fabales
Onobrychis viciifolia	Fabaceae	Fabales
Ononis spinosa agg.	Fabaceae	Fabales
Ornithopus perpusillus	Fabaceae	Fabales
Trifolium campestre	Fabaceae	Fabales
Trifolium dubium	Fabaceae	Fabales
Trifolium pratense	Fabaceae	Fabales
Trifolium repens	Fabaceae	Fabales
Vicia angustifolia	Fabaceae	Fabales
Vicia cracca	Fabaceae	Fabales
Vicia grandiflora	Fabaceae	Fabales
Vicia hirsuta	Fabaceae	Fabales
Vicia sepium	Fabaceae	Fabales
Vicia tenuifolia	Fabaceae	Fabales
Vicia tetrasperma	Fabaceae	Fabales

Appendix 3 continued.

Fumaria officinalis	Fumariaceae	Ranunculales
Centaurium erythrea	Gentianaceae	Gentianales
Gentiana cruciata	Gentianaceae	Gentianales
Geranium columbinum	Geraniaceae	Geraniales
Geranium dissectum	Geraniaceae	Geraniales
Geranium pratense	Geraniaceae	Geraniales
Geranium pusillum	Geraniaceae	Geraniales
Hypericum dubium	Hypericaceae	Malpighiales
Hypericum hirsutum	Hypericaceae	Malpighiales
Hypericum perforatum	Hypericaceae	Malpighiales
Juglans regia juv.	Juglandaceae	Fagales
Luzula campestris	Juncaceae	Poales
Ajuga genevensis	Lamiaceae	Lamiales
Ballota nigra	Lamiaceae	Lamiales
Galeopsis tetrahit	Lamiaceae	Lamiales
Glechoma hederacea	Lamiaceae	Lamiales
Lamium album	Lamiaceae	Lamiales
Lamium amplexicaule	Lamiaceae	Lamiales
Lamium maculatum	Lamiaceae	Lamiales
Lamium purpureum	Lamiaceae	Lamiales
Origanum vulgare	Lamiaceae	Lamiales
Prunella vulgaris	Lamiaceae	Lamiales
Salvia pratensis	Lamiaceae	Lamiales
Stachys palustris	Lamiaceae	Lamiales
Stachys recta	Lamiaceae	Lamiales
Stachys sylvatica	Lamiaceae	Lamiales
Thymus pulegioides	Lamiaceae	Lamiales
Allium schoenoprasum	Liliaceae	Liliales
Allium sphaerocephalon	Liliaceae	Liliales
Allium vineale	Liliaceae	Liliales
Asparagus officinalis	Liliaceae	Liliales
Gagea pratensis	Liliaceae	Liliales
Ornithogalum umbellatum	Liliaceae	Liliales
Linum catharticum	Linaceae	Malpighiales
Malva moschata	Malvaceae	Malvales
Epilobium adnatum	Onagraceae	Myrtales
Epilobium angustifolium	Onagraceae	Myrtales
Papaver rhoeas	Papaveraceae	Ranunculales
Plantago lanceolata	Plantaginaceae	Lamiales
Plantago major	Plantaginaceae	Lamiales
Plantago media	Plantaginaceae	Lamiales
Armeria elongata	Plumbaginaceae	Caryophyllales
Agrostis tenuis	Poaceae	Poales
Agrostis stolonifera	Poaceae	Poales

Appendix 3 continued.

Alopecurus myosuroides	Poaceae	Poales
Alopecurus pratensis	Poaceae	Poales
Anthoxanthum odoratum	Poaceae	Poales
Apera spica-venti	Poaceae	Poales
Arrhenatherum elatius	Poaceae	Poales
Avenella flexuosa	Poaceae	Poales
Brachypodium pinnatum	Poaceae	Poales
Calamagrostis epigeiios	Poaceae	Poales
Dactylis glomerata	Poaceae	Poales
Elymus repens	Poaceae	Poales
Festuca arundinacea	Poaceae	Poales
Festuca pratensis	Poaceae	Poales
Festuca rubra	Poaceae	Poales
Helictotrichon pubescens	Poaceae	Poales
Holcus lanatus	Poaceae	Poales
Holcus mollis	Poaceae	Poales
Lolium multiflorum	Poaceae	Poales
Lolium perenne	Poaceae	Poales
Phalaris arundinacea	Poaceae	Poales
Phleum pratense	Poaceae	Poales
Picris hieracioides	Poaceae	Poales
Poa pratensis agg.	Poaceae	Poales
Poa trivialis	Poaceae	Poales
Secale cereale	Poaceae	Poales
Trisetum flavescens	Poaceae	Poales
Polygonum amphibium	Polygonaceae	Caryophyllales
Rumex acetosa	Polygonaceae	Caryophyllales
Rumex acetosella agg.	Polygonaceae	Caryophyllales
Rumex crispus	Polygonaceae	Caryophyllales
Rumex obtusifolius	Polygonaceae	Caryophyllales
Rumex thyrsiflorus	Polygonaceae	Caryophyllales
Anagallis arvensis	Primulaceae	Ericales
Lysimachia nummularia	Primulaceae	Ericales
Primula veris	Primulaceae	Ericales
Myosurus minimus	Ranunculaceae	Ranunculales
Ranunculus acris	Ranunculaceae	Ranunculales
Ranunculus auricomus agg.	Ranunculaceae	Ranunculales
Ranunculus bulbosus	Ranunculaceae	Ranunculales
Ranunculus ficaria	Ranunculaceae	Ranunculales
Ranunculus repens	Ranunculaceae	Ranunculales
Reseda lutea	Resedaceae	Brassicales
Agrimonia eupatoria	Rosaceae	Rosales
Bromus hordeaceus	Rosaceae	Rosales
Bromus inermis	Rosaceae	Rosales
Bromus sterilis	Rosaceae	Rosales

Appendix 3 continued.

Crataegus monogyna juv.	Rosaceae	Rosales
Fragaria vesca	Rosaceae	Rosales
Geum urbanum	Rosaceae	Rosales
Potentilla anserina	Rosaceae	Rosales
Potentilla argentea agg.	Rosaceae	Rosales
Potentilla reptans	Rosaceae	Rosales
Potentilla tabernaemontani	Rosaceae	Rosales
Rubus fruticosus agg.	Rosaceae	Rosales
Rubus idaeus agg.	Rosaceae	Rosales
Galium aparine	Rubiaceae	Gentianales
Galium mollugo agg.	Rubiaceae	Gentianales
Galium verum	Rubiaceae	Gentianales
Salix caprea juv.	Salicaceae	Malpighiales
Saxifraga granulata	Saxifragaceae	Saxifragalis
Linaria vulgaris	Scrophulariaceae	Lamiales
Melampyrum pratense	Scrophulariaceae	Lamiales
Scrophularia nodosa	Scrophulariaceae	Lamiales
Verbascum densiflorum	Scrophulariaceae	Lamiales
Veronica arvensis	Scrophulariaceae	Lamiales
Veronica chamaedrys	Scrophulariaceae	Lamiales
Veronica hederifolia	Scrophulariaceae	Lamiales
Veronica persica	Scrophulariaceae	Lamiales
Veronica sublobata	Scrophulariaceae	Lamiales
Veronica triphyllos	Scrophulariaceae	Lamiales
Parietaria judaica	Urticaceae	Rosales
Urtica dioica	Urticaceae	Rosales
Valeriana repens	Valerianaceae	Dipsacales
Valeriana wallrothii	Valerianaceae	Dipsacales
Valerianella locusta	Valerianaceae	Dipsacales
Viola arvensis	Violaceae	Malpighiales
Viola hirta	Violaceae	Malpighiales
Viola odorata	Violaceae	Malpighiales

9.4. Appendix 4

Sensitivity ranking of the tested plant species for 14 different herbicides (data derived from Draft assessment reports and UBA reports). The position in the sensitivity distribution for each species is indicated by 1 (= most sensitive), 2 (= second), 3 (= third), M (= medium) and L (= least) sensitive species (in case where several species had "> values" they were all indicated as least sensitive).

Herbicide	*Raphanus sativus*	*Lycopersicon esculentum*	*Glycine max*	*Hordeum vulgare*	*Daucus carota*	*Avena sativa*	*Lactuca sativa*	*Cucumis sativus*	*Brassica napus*	*Beta vulgaris*	*Lolium perenne*	*Allium cepa*	*Zea mays*	*Brassica rapa*	*Lolium multiflorum*	*Pisum sativum*	*Helianthus annuus*	*Triticum aestivum*	*Sorghum bicolor*	*Brassica oleracea*
Diquat	1	2	3	M	M	L														
Chlortoluron			L		M	L	1	2	3	M	M	L	L							
Dimethenamid-P		L	L			M	2				1	3	M	M						
Metazachlor					M	2	3		L	L		M		M	1	L				
Pethoxamid						L			2	1		3								
Quizalofop-P					3	2		L					1				M			
Fluroxypyr		1	2			L		L	M	M	M	L					3			
Nicosulfuron		3	L			M		2	1			L				L		M	M	
Metamitron			L					1			M	2		3			M			
Diuron		2							3	L		M				1		M		
Sulcotrione	M	2				L	1	M		3	L						M			
Dicamba	3	2				L				1		M								
Metazachlor						2	3				1	L		M						
Tembotrione	M	2				L		M	3		M	L					M			1