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BfN-Skripten 277

2010

Federal Agency for Nature Conservation

2010 International Year of Biodiversity

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Cover picture: Sugar beet (B. Winkel)

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This publication is included in the literature database “***DNL-online***” (www.dnl-online.de)

BfN-Skripten are not available in book trade but can be downloaded in a pdf version from the internet at: http://www.bfn.de/0502_skripten.html

Publisher: Bundesamt für Naturschutz (BfN)
Federal Agency for Nature Conservation
Konstantinstrasse 110
53179 Bonn, Germany
URL: http://www.bfn.de

Printed by the printing office of the Federal Ministry of Environment, Nature Conservation and Nuclear Safety.

Printed on 100% recycled paper.

ISBN .978-3-89624-012-5.

Bonn, Germany 2010

Contents

Introduction

The EU Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms (GMO) requires the assessment of environmental impacts of GMO including direct, indirect, immediate, and delayed effects on the environment. In the case of transgenic crops resistant to specific herbicides, this means that besides evaluating the environmental impacts of the genetically modified plant itself the environmental impacts of the specific herbicide programs and altered agricultural practices associated with this crop have to be assessed.

The introduction of a new herbicide resistant[1] variety can be connected with new agricultural practices. Changes in yields may also occur. Probable changes should be evaluated considering environmental issues such as preservation of biodiversity, soil conservation, pollution and climate change. Effects may be weighted according to their relative importance compared to other impacts and possible measures compromising negative effects.

Changes in herbicide use will unquestionably occur. The combinations of herbicides, amounts and numbers of applications will change. The ecological effects of herbicides on non-target organisms can be direct (toxicity) or indirect (e.g. effects on food chain through exclusion of wild plants and seed set). In addition, herbicide production and application predominantly cause abiotic effects which should be considered by life cycle assessments.

1 Herbicide use in conventional sugar beet

Weed control in sugar beet is done predominantly by herbicides. Mechanical weeding was additionally done on 13 % of the fields in Germany in 2004 (based on an expert survey on agricultural practice, Ladewig et al. 2007). In order to compare herbicide application patterns in conventional and herbicide resistant varieties the range of herbicide active ingredients (a.i.) and the application patterns (frequency and amounts) in conventional varieties is described firstly.

1.1 Combinations of conventional herbicides in currently grown beet varieties

Tab. 1 shows the importance of particular active ingredients and the different active ingredients used. It lists the proportion of herbicide applications containing a given a.i. and the proportion of surveyed farms which applied the given a.i. at least on one field.

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[1] Transgenic herbicide resistance is often characterised as tolerance. Here the term resistance is used as defined by the Weed Science Society of America as an “inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type”.

Tab. 1: Main active ingredients used in German sugar beet fields in 2007

active ingredient	proportion of herbicide applications containing the a.i.	proportion of surveyed farms applying a.i. at least on one field
Metamitron	20.8	100.0
Ethofumesate	20.6	99.2
phenmedipham	20.5	99.4
Desmedipham	12.5	76.1
Chloridazon	6.1	48.1
Quinmerac	5.7	46.2
Triflusulfuron	4.9	49.8
Clopyralid	2.0	29.4
Glyphosate	1.8	30.3
haloxyfop-r (haloxyfop-p)	1.4	21.4
fluazifop-p	1.2	16.2
dimethenamid-p	1.1	9.7
Propaquizafop	0.6	10.1
quizalofop-p	0.4	5.3

(Rossberg, personal communication, 2009)

It can be concluded, that sugar beet fields are often treated with about 6-7 different a.i. per year when the application frequency is relatively high (as it was in 2007, Rossberg et al. 2008). Some herbicides such as metamitron and chloridazon are effective via leaf and soil. According to Madsen & Jensen (1995) a mixture of the first three a.i. in Tab. 1 is widely used in Denmark. It is also a common mixture in many European countries (s. Tab. 2) and can be added by chloridazon and oil (May 2000). When pre emergence applications are done, chloridazon is chosen and can be followed by a mixture of phenmedipham, ethofumesate, desmedipham, and metamitron and a third mixture of phenmedipham, ethofumesate, desmedipham and lenacil (May 2000).
Glyphosate was registered for pre plant or post harvest applications in several countries (see also Tab. 2). There are ample herbicides containing glyphosate. As an example Roundup contains 360 g active ingredient per liter (Bückmann et al. 2006).

Ranking of conventional herbicides based on amounts

A different way of ranking is based on the amounts of a.i. used. The total sprayed amounts (t a.i. year $^{-1}$) of the most prevalent herbicides in European sugar beet fields are listed in Tab. 2. Herbicides are sprayed at a particular dose. Particularly low dose herbicides such as trisulfuron (s. Tab.1) are underrepresented here despite their frequent use. In UK, for example, triflusulfuron was used on about 73% of the sugar beet acreage in 2000 (see Champion et al. 2003).

Tab. 2: T a.i. year -1 of main active ingredients of herbicides used in sugar beet in 2000

a.i.	G	F	UK	NL
metamitron	984	544	158	164
chloridazon	254	169	142	-
ethofumesate	207	114	53	56
phenmedipham	104	113	80	25
glyphosate	103	106	55	25
lenacil	-	26	41	-
quinmerac	42	49	-	-
desmedipham	35	-	6	4
clopyralid	10	0.2	12	7
others	14	19	10	8

values in t a.i. year $^{-1}$ (Märländer 2005)

1.2 Application frequencies, amounts and timing of herbicides used in conventional crops

1.2.1 Application frequency

The *application frequency* (number of sprays) in German sugar beet fields in the years 1994 to 2004 was on average about 3 according to the expert survey of Ladewig et al. (2007). Three applications of herbicide mixtures also gave good control in a field trial in Denmark (Madsen & Jensen 1995). According to a survey conducted in 2000, the national average is 4 in the UK (Champion et al. 2003). Frequencies range between 2 and 4 (see also Märländer 2005).

Frequencies and herbicide combinations vary from year to year and between sites or regions. In addition the agricultural practice has an impact on the application patterns. The average in tilled fields was e.g. 3.7 according to Fuchs in 2004 (2006) and the number rose to 4.4 when tillage was omitted for at least 5 years (see also Chapter 2).

1.2.2 Amounts of herbicides

The amount of herbicide products (a.i. plus surfactant) sprayed in conventional varieties can be up to 6 kg/ha.

The national average of a.i. ha^{-1} sprayed per year in UK was only 2840 g in 2000 (Champion et al. 2003). Considerable variations can be found from year to year and from site to site (Busche 2008, Rossberg et al. 2008, Young et al. 2001). Site to site variations are mostly due to rotations, tillage and management systems with respective short-term and long-term effects (Rossberg et al. 2008).

Tab. 3: Amounts of a.i. used per ha in representative EU countries

	kg a.i. ha^{-1}
Germany	3.7
France	2.7
UK	3.2
NL	2.4
Spain	3.1
Italy	3.1
Belgium	4.1
Denmark	2.5
(average)	(3,1)

(Märländer 2005)

1.2.3 Timing of applications of herbicides

An account of the timing of applications in sugar beet was given by Ladewig et al. (2007) on the basis of an expert survey on agricultural practice from 1994 to 2005:

In Germany the applications of herbicides other than glyphosate and glufosinate are already mostly done post emergence (more than 80% of the beet acreage, 1996-2004, Ladewig et al. 2007). The remaining area was sprayed pre and post emergence. About 25% of the beet acreage was sprayed with non-selective herbicides before seeding or preemergence in 2004 (the rate increased from 17% in 2000).

1.2.4 Indices used to describe the intensity of plant protection

Two indices were developed in order to describe and compare the intensity (amounts, application frequency and proportion of sprayed fields) of pesticide use:

The A*pplication Frequency Index* (AFI) is defined as the number of herbicide applications in a field, independent of the number of products (or a. i.) used per application. This means that if farmers use herbicide mixtures, it still counts as one application. The AFI is computed by the sum of area coefficients which are calculated through the size of the sprayed acreage in relation to the respective field size for the particular herbicide application (Rossberg et al., 2008). If the whole field is sprayed, the quotient for that application is 1. Farmers usually use herbicide mixtures in sugar beet and split the permitted dose of some products using them several times. The *Application Frequency Index* of herbicides in sugar beet was on average 3.92 in Germany in 2007 (Rossberg et al. 2008, 16 regions surveyed in 2007).

Another method to measure the intensity of pesticide applications is the *Treatment Frequency Index, TFI*). The index differs so much from the AFI that no close relation between the two can be found (Rossberg et al. 2008). The TFI for a single herbicide is calculated by the product of the area coefficient (as indicated by the AFI, but for every single herbicide instead of every application of herbicides) and the application rate coefficient which is the proportion of the application rate and the permitted doses (of products or active ingredients).

The partial indices of all herbicide applications are summed up resulting in the TFI for weed control.
Normally one application gives a partial index for one herbicide. But in sugar beet, where the herbicide amounts per season are usually split and sprayed at several times, the approval for the herbicide application rate is also given for the split application. Therefore frequent applications of herbicide mixtures would add up to a seemingly high index even when the total dose of all split applications of the respective product corresponds to the total permitted dose. For that reason a modified calculation was chosen: Repeatedly sprayed herbicide products are only accounted as if sprayed once and the total sprayed amount per season is divided by the total permitted amount per season.
The TFI (of herbicide products) for sugar beet in 2007 was 2.35 using the modified calculation and 5.12 for the standard calculation (Rossberg et al. 2008, 16 regions surveyed). The authors found an increase of 22% (or 12% modified calculation) compared to the TFI in 2005 (TFI 2005: modified calculation: 2.07, standard calculation 4.01). The increase was due to dry conditions which hampered the effects of soil herbicides. In 2000, the modified TFI was 2.5 according to Bruns & Märländer (2006) surveying 13 sites.

2 Potential changes of application patterns in glyphosate resistant sugar beet

2.1 Potential changes of herbicide amounts in fully tilled fields

Amounts

Tab.4: Predicted reductions of herbicide amounts in glyphosate resistant varieties

	Estimated current rates of conventional herbicides	Predicted reduction potential in glyphosate resistant sugar beet
Germany	3.7	-2.0
France	2.7	-0.8
UK	3.2	-1.3
NL	2.4	-0.6
Spain	3.1	-0.9
Italy	3.1	-0.9
Belgium	4.1	-2.6
Denmark	2.5	-1.3
(average)	(3,1)	(-1.3)

kg a.i. ha-1 (Märländer 2005)

Reductions of a.i./ha in glyphosate resistant sugar beet can be 28% to 43% according to calculations of Märländer (2005) based on data of Gianessi et al. (2003) (s. Tab.4). The rate could be reduced by 34% on average in the UK trials (Champion et al. 2003, 3 years 60-75 fields).

2.2 Potential changes of application patterns under different tillage systems

There is a worldwide trend to reduce or omit tillage in order to reduce production costs and erosion. Reduced and zero tillage has also been increasing worldwide due to governmental enforcement. Both systems do not depend on herbicide resistance but some no-till systems largely depend on glyphosate (pre emergence) sprays (van Acker et al. 2003). Tillage systems change with and without herbicide resistant varieties. A direct causality cannot be shown. Surely, the availability of necessary machinery is an important factor for the adoption of conservation tillage systems.

Cover crops / mulch systems which can be combined with conservation tillage reduce wind and water erosion more effectively and can prevent nitrogen leaching which occurs when spring temperatures are relatively high. Cover crops with a high competitive ability (for example legumes, mustard, rye) can suppress weeds. Non winterhardy crops such as *Sinapsis alba, Raphanus sativus* and *Phacelia tanacetifolia* have a lower ability to suppress weeds and prevent leaching. Some cover crops can help to reduce a major sugar beet pest species, the nematode *Heteroda schachtii.*

Reduced tillage was done on 44% of the German beet acreage in 2004, a rate steadily increasing since 1994 (Ladewig et al. 2007). Mulch systems are less commonly used (on about 25% of the German sugar beet acreage data from 2002, Merkes et al. 2003 zit. in Petersen & Röver 2005).

No till production systems are adopted at a lower rate than reduced tillage. In 2004, 20% of 100 surveyed farms planting sugar beet (Fuchs 2006) had adopted no-till systems for more than 5 years. Half of these farms (10% of all farms) planted cover crops. Only a quarter of the farmers who tilled prior to all crops (16%, n=100) planted cover crops (Fuchs et al. 2006). Thus there is a slight tendency to combine no-till systems with cover crops as mulch plants.

Application patterns vary depending on the tillage system. Planting cover crops in *no-till systems* can e.g. increase the number of sprayed active substances from 5.7 to 6.7 (Fuchs et al. 2006).

Changes due to the adoption of glyphosate resistant sugar beet may also depend on the tillage system. When conventional sugar beet fields are tilled, 2-4 post emergence sprays are typical (Tab. 5). Post emergence application will be the standard in tilled fields planted with glyphosate resistant beet too: The tested application pattern suitable for herbicide resistant beet was glyphosate sprays at BBCH 12 and 16 or 14 and 19 (BBCH14=2-leaf stage, BBCH19=4-leaf stage). The first application could be a band application (Petersen & Röver 2005).

The numbers of applications can be reduced in glyphosate resistant varieties seemingly independent from the tillage system at last within the first years of adoption (Tab. 5). However, some findings in Tab. 5 (see [2] and [4]) cannot be generalized because they are based on single investigations.

Tab. 5: Reported application frequencies under different tillage systems

	tilled fields	reduced tillage and mulch	no till	no till and cover crops
conventional herbicides	2-4 [1]	2-4 [2] 4	4.4 [3]	4.1 [3]
glyphosate	2	2	2 [4]	2

1 several authors see chapter 1.2.1; 2 Kainz (1989); 3 (Fuchs et al. 2006); 4 Found by Petersen (2002): applications at BBCH 14 and 19 under conditions of low weed pressure without yield reductions; all other numbers: Petersen & Röver (2005)

A detailed description of changes reveals a complex picture of herbicide use and other weed control measures depending on the tillage practice:

Timing and detailed patterns of weed control in conservation tillage with *conventional* varieties

The conventional application pattern in mulch systems without spring tillage was one pre emergence application of glyphosate followed by 1 to 3 post emergence applications of conventional herbicides (Kainz 1989). According to Petersen & Röver (2005) the standard conventional application pattern in mulch systems and reduced tillage is one pre emergence or predrilling application of glyphosate and 3 further applications of herbicide mixtures at BBCH 12, 14 and 19. Most of the farms (85%) who omit tillage prior to (conventional varieties of) sugar beet use a non selective herbicide at pre emergence (Fuchs et al. 2006).

Timing and detailed patterns of weed control in conservation tillage in *Glyphosate resistant* varieties

Petersen and Röver (2005) investigated different (*reduced and full*) tillage options, herbicide application patterns, and mulch systems planting a glyphosate resistant sugar beet variety. When *non-winterhardy* cover crops were planted or straw mulch was used, the applications in glyphosate resistant sugar beet could be reduced from 4 (in conventional varieties, see above) to two (see Tab. 5) and the first one could be a band spray in the reduced tillage system. In detail, a rotary band cultivator for seedbed preparation substitutes two broadcast passes. When tillage was reduced in *winterhardy* cover crops, the non-selective herbicide (glyphosate) had to be sprayed the latest at emergence and overall (no band spray) in order to prevent yield losses. Pre emergence applications in winterhardy cover crops could not be omitted in reduced tillage systems without yield reduction – thus an overall pre emergence application followed by an application at the BBCH-stage 16 was necessary (Petersen & Röver 2005). Furthermore, in winterhardy cover crops one additional pass with a rototiller is recommended by Petersen & Röver 2005. In addition, glyphosate should be sprayed pre emergence when straw mulch is used and volunteer barley occurs according to Petersen et al. (2002).

In the long run however, conservation tillage systems cause several changes which make it difficult to reduce herbicide amounts (Pallutt & Viehweger 2002). Pallutt & Viehweger (2002) predicted an increasing herbicide use in crop rotations with a high proportion of cereals. This is due to changes in the composition of arable weeds, (old) weeds which survived during winter, and increasing numbers of cereal or oilseed rape volunteers. Also, as described above pre emergence sprays could become more frequent again when winterhardy cover crops are planted.

2.3 Conclusions on potential changes in herbicide use

The predictions or calculations have to be valued or extrapolated with care because the weed flora changes in response to changes in the weed control system (s. also Chapter 6).
And, more important, the toxicity of applied typical doses of different pesticides varies greatly. That is why conclusions on ecological effects cannot directly be drawn from amounts, application frequencies or related indices. There is no connection between the TFI (treatment frequency index) and the degree of effectiveness of herbicides either (Bruns & Märländer 2006, Gutsche et al. 2002). Moreover, the dimension of indirect effects of herbicides on biodiversity (induced by the highly effective destruction of habitats and elimination of food sources) is greater than of direct non-target effects (Körner 1990, DETR 2000) (see Chapter 3.2).

3 Effects of growing herbicide resistant sugar beet on the environment

3.1 Direct effects of changes in herbicide use

3.1.1 General information on glyphosate

Glyphosate ($C_3H_8NO_5P$, N-(phosphonomethyl)glycine, MW 169) is a polar, highly water soluble organic acid (given in acid equivalents a.e.) that inhibits EPSPS (5-enolpyruvylshikimate 3-phosphate synthase). It is a potent chelator that easily binds divalent cations such as calcium (Ca), magnesium (Mg), manganese (Mn), and iron (Fe) forming poorly soluble or very stable complexes (Cakmak et al. 2009). Glyphosate is used as salt, termed the active ingredient (a.i.), mostly as isopropylamine salt (MW 228), less often as trimesium salt (MW 245). The purity of technical grade glyphosate is generally above 90% (WHO 1994). In addition to the active ingredient, formulated pesticides usually contain inert ingredients (also called adjuvants) that aid or modify the action of the active ingredient. For agricultural or other uses, a number of glyphosate products are on the market, such as Roundup, the major formulation, Rodeo, and Vision, all from Monsanto, Touchdown (Syngenta), Credit (Nufarm), Glyfos (Cheminova), Factor (IPCO), Sharpshooter (United Agri Products), and Vantage (Dow Agro Sciences). They may contain different inert ingredients. The products contain various concentrations of acid equivalent (the active moiety): 360, 450, 480, 500, and 540 g a.e./l (Ag-Info Centre 2009).
Unfortunately, literature data are not uniformly given as a.e., instead often a.i. is used in spite of the fact, that, depending on the salt portion, a.i. should have been converted to a.e. In their

review on ecotoxicity of Roundup, Giesy et al. (2000) assumed that 1 mg of Roundup contains 0.31 mg glyphosate a.e. When converting between acid equivalents and active ingredient, 1 mg a.i. was assumed to contain 0.75 mg a.e., meaning 1 mg Roundup is equivalent to 0.41 and 0.45 mg a.i. of isopropylamine salt and trimesium salt, respectively. Recommended application rates do not exceed 5.8 kg a.i./ha and are dependent on the type of use. In Germany, 44 glyphosate products are authorized for use in agriculture (BVL 2009).

3.1.2 Behaviour of glyphosate in soil and water

The environmental fate of glyphosate has been reviewed (Giesy et al. 2000, EC 2002, Solomon et al. 2005). Glyphosate contacts soil via direct application, via removal from plant tissue by rain, and via release from treated plants, either by root exudation (Neumann et al. 2007) or by decomposition of plant material (Locke et al. 2008). Glyphosate decomposition in soils is predominantly performed by microorganisms, in particular by bacteria, such as *Pseudomonas* spp. It takes place under both aerobic and anaerobic conditions (Borggard & Gimsing 2008). Repeated glyphosate applications may be associated with an increase of (gram negative) soil microorganisms capable of metabolizing the herbicide (Lancaster et al. 2009). Oxidative degradation by the manganese oxide birnesite seems to be possible too (Barrett & McBride 2005). Glyphosate is not readily decomposed by light (WHO 2005). Degradation by microorganisms leads either to the formation of aminomethylphosphonic acid (AMPA) and glyoxylate or to sarcosine and glycine (Borggard & Gimsing 2008). Which pathway is most common in soil is not known. AMPA has a much longer half-life than glyphosate (EC 2002) and could accumulate with the extensive use of glyphosate, particularly in loamy sandy soils with low pH (Mamy et al. 2005). The degradation rate of glyphosate increases with temperature, it can be very different from one soil to another. Most reported half-lives range from 3 to 130 days (Viehweger & Danneberg 2005, FAO 2005, EC 2002, Schuette 1998), in some cases reaching up to 240 days (Borggard & Gimsing 2008). Half-lives for AMPA range from 76 to 240 days, but AMPA derived from glyphosate trimesium salt may degrade more slowly with half-lives of up to 875 days (EC 2002).

In general, glyphosate, a small molecule with three polar functional groups (carboxyl, amino and phosphonate), is strongly sorbed on soil minerals, mainly on aluminium and iron oxides that provide more sorption sites than clay silicates. Its sorption, and hence leachability, depends on soil characteristics such as types, contents, and crystallinity of minerals, pH, phosphate content and organic matter (Borggard & Gimsing 2008). Sorption increases with the number of sorption sites of the sorbents (specific surface area), but usually decreases at increasing pH. In phosphate-rich soils, glyphosate sorption can be reduced, since phosphate reacts similarly to glyphosate and may compete for the surface sites. The extent of reduction does not follow a simple pattern: in some soils, phosphate stimulates glyphosate degradation while in others it has the opposite or no effect (Borggard & Gimsing 2008). If *Bacillus thuringiensis* compounds are used together with glyphosate, the persistence of the active ingredient may increase (Accinelli et al. 2004).

Transport of glyphosate and its metabolite AMPA from terrestrial to aquatic environments can occur as solutes or bound to particles. Both dissolved and particle-bound glyphosate can leach through the soil (subsurface) and by surface runoff. The extent of surface runoff versus subsurface transport is unknown, as is the total glyphosate transfer from land to surface waters (Borggard & Gimsing 2008). The compounds end up both in drainage and groundwater and in open waters such as streams and lakes, where they may remain in the aqueous phase or are stored in the sediment.

Glyphosate leaching is strongly affected by soil structure. Limited leaching has been observed in non-structured sandy soils while subsurface leaching was observed in a structured soil with preferential flow in macropores when high rainfall followed glyphosate application (Borggard & Gimsing 2008). Long-term use of glyphosate on coarse-textured soils can lead to glyphosate pollution of groundwater. Oxide-poor sandy soils with low sorption capacity, slow degradation rates, and a shallow groundwater table may also be vulnerable. In addition to soil composition, temperature, and vegetation, heavy rainfall events after glyphosate application affect glyphosate leaching too (Peruzzo et al. 2008). Glyphosate (and AMPA) reach aqueous environments also via spray drift from sprayed areas and via outlets from waste water treatment plants, with AMPA being detected much more frequently than glyphosate (Kolpin et al. 2005). Due to its supposed relative safety, glyphosate is one of only nine herbicides approved for use in aquatic sites in the US (Cerdeira & Duke 2006). Glyphosate may be transported several kilometres downstream from the site of aquatic application (WHO 2005). Its decomposition in water may be slower than in some soils, possibly due to the smaller number of microorganisms in water. Half-lives of 35 - 63 days (Schuette 1998) and from 27 to 146 days (EC 2002) have been reported.

Glyphosate and AMPA are washed out of the root zone of clay-rich grounds in concentrations that exceed the EU level for drinking water of 0,1 µg/l. For glyphosate and AMPA, maximum values of 31 µg/l and 1.6 µg/l, respectively, have been found in soil water of a loamy soil in Denmark (Kjaer et al. 2009). Glyphosate is washed out sooner than AMPA. AMPA was frequently detected as long as two years after application, indicating that it can be retained within the soil and gradually released over a very long period of time. Both substances were also detected in surface water and ground water in Germany and France (Sturm & Kiefer 2007). Reported levels of glyphosate (and AMPA) concentrations in surface waters are in the µg/l to mg/l range (Borggard & Gimsing 2008). In the US, glyphosate and AMPA concentrations in pond water reached up to 1,700 µg/l and 35 µg/l, respectively, and stream water contained up to 1,237 µg/l glyphosate and 10 µg/l AMPA (WHO 2005). In Argentine water bodies, glyphosate levels up to 700 µg/l have been found (Peruzzo et al. 2008). The levels at which glyphosate is tolerated in drinking water differ significantly between countries. In the US, the maximum contaminant level (MCL, the highest level of a contaminant that is allowed in drinking water) of glyphosate is 700 µg/l, higher than for other pesticides (EPA 2009), whereas in the EU the tolerable level for pesticides generally is 0.1 µg/l (EU 2009).

3.1.3 Inert ingredients

Despite their name, inert ingredients may be biologically or chemically active and can influence the behaviour of active ingredients in the environment. They are generally not identified on product labels and are often claimed to be confidential business information. In RoundupReady (RR) sugarbeet, Roundup products such as Roundup OriginalMax and Roundup WeatherMax are used (Weed Resistance Management 2009). They contain polyethoxylated tallow amine (POEA), a surfactant (surface active agent) that promotes the penetration of glyphosate into the plant cuticle (Brausch et al. 2007). POEA, a derivative of tallow, is a complex mixture of long-chain alkylamines synthesized from the fatty tissue of cattle or sheep. Tallow contains a variety of fatty acids including oleic, palmitic, stearic, myristic, and linoleic acids as well as small amounts of cholesterol and other organic acids (Diamond & Durkin 1997). POEA are also characterized by their mass ratio between the oxide portions and the tallowamine portion of the molecule. The ratio ranges from 5:1 to 25:1, with higher ratios becoming more water soluble (Brausch & Smith 2007).

The surfactant is typically 15 % or less of the Roundup formulation. Some formulations, such as Roundup Biactive and Rodeo, do not contain POEA. Here the user adds a surfactant fitting the specific needs of the weed control program (Giesy et al. 2000). Significant higher toxicity of glyphosate formulations and POEA compared to glyphosate alone has been reported (Cox & Surgan 2006), in particular for aquatic organisms (Brausch & Smith 2007, Relyea 2005a, see also below). POEA is more toxic in alkaline than in acid water (Diamond & Durkin 1997). Data from toxicity studies performed with glyphosate alone and over short periods of time may thus conceal undesired effects. In addition, exposure to multiple pesticides in nature can be more lethal than predicted from toxicology studies involving one pesticide at a time (Relyea 2004, Hayes et al. 2006). In general, however, extensive data on natural pesticide concentrations are lacking (Relyea & Hoverman 2006).

3.1.4 Effects of glyphosate-containing herbicides on organisms

Exposure and toxicity values

Based on the broad use of glyphosate and its formulated products, organisms in both terrestrial and aquatic environments are potentially exposed (Giesy et al. 2000). Aquatic organisms are exposed to glyphosate through their diet and via direct uptake of water-borne chemicals. Soil microorganisms, terrestrial invertebrates, and non-target plants are mainly exposed through direct contact with glyphosate during application and through interaction with the soil. The main route of exposure for birds and mammals would be through ingestion of contaminated food. Since small animals have greater rates of metabolism and higher food ingestion rates in relation to their body weight, they are more conservative models for exposure to glyphosate than larger ones. The toxicity of a typical dose of a herbicide or active ingredient differs greatly depending on the tested organisms.

In their review on Roundup ecotoxicity Giesy et al. (2000) based acute exposure levels on Roundup, since in acute exposure, active and inert ingredients co-occur. Under chronic conditions differences in fate of the components could influence exposure. Separate chronic exposure levels for Roundup and glyphosate seem thus reasonable. Toxicity values derived from literature, such as LC50 (concentration resulting in death of 50 % of test organisms) or EC50 (concentration causing a specified effect such as growth decrease in 50 % of the population), have been used to calculate toxicity reference values (TRV), defined as the "maximum exposure concentration that would not cause deleterious impacts on populations of plants, animals, and microorganisms".

Giesy et al. (2000) established acute TRVs using the following process: (1) For each taxonomic group, the most sensitive species was identified based on the least EC50 or LC50 values. (2) If an experimental no observed effect concentration (NOEC) had been identified for that species, then that NOEC was selected as the acute TRV. (3) If an experimental NOEC was not determined, then a no-mortality level (NML, actually a 1 in 10,000 mortality level, equivalent to a probability of mortality of 0.0001) was derived using a 5-fold safety factor (as described in 1986 by Urban & Cook). Chronic TRVs were estimated based on the NOEC from the most sensitive species in chronic tests with glyphosate. If chronic tests were available for Roundup, and the Roundup NOEC was less than the glyphosate chronic NOEC, then the Roundup NOEC (expressed as glyphosate a.e.) was used to estimate the glyphosate chronic TRV (more details in Giesy et al. 2000). Unfortunately, it is not always clear which Roundup formulation has been used in the various toxicity studies.

Microorganisms

Glyphosate influences the soil microflora (Roslycky 1982, Yamada et al. 2009). This may be due to direct effects of glyphosate, differences in the amount and composition of root exudates of treated plants, and indirect effects resulting from altered management practices. In addition, glyphosate is released from the roots of RR crops and dying (weed) plants and can even transfer into non-target plants through the soil (Neumann et al. 2006). EPSPS, the target enzyme of glyphosate, is essential for the biosynthesis of aromatic amino acids not only in plants but also in microorganisms. However, not all microorganisms possess an EPSPS insensitive to glyphosate (Powell et al. 2009). Processes such as nitrification, nitrogen fixation, and urea hydrolysis might be affected.

Pseudomonads known to play an important role in decomposition of glyphosate (Borggard & Gimsing 2008) seem to have an insensitive EPSPS, but some species such as the ubiquitous bacterium *Pseudomonas fluorescens* can be inhibited by glyphosate due to a glyphosate-sensitive EPSPS (Kremer & Means 2009). Studies on effects of glyphosate on total microbial biomass and activity have shown mixed results with most of them finding no significant effects or an increase in microbial activity (Duke & Cerdeira 2005, Cerdeira & Duke 2006, Locke et al. 2008). According to Giesy et al. (2000), toxicity tests with Roundup and Glyphosate applied to soil and performed for 1 – 84 days resulted in no observed adverse effect concentration (NOAEC) levels ranging from 5 to 230 mg a.e./kg dry weight of soil and from 10 to 76.7 mg a.e./kg soil, respectively. A chronic TRV for soil microorganisms was calculated to be 5 mg a.e./kg soil (equivalent to 16 mg Roundup/kg). More detailed studies on specific microorganism species or genera, however, reveal shifts in composition and activity of microorganism populations (Kremer & Means 2009).

A study with Brazilian soils indicated that less sensitive organisms such as *actinomycetes* and fungi are favoured and that soil exposed to glyphosate for several years showed strong response in microbial activity (Araujo et al. 2003). Kremer et al. (2005) observed a generally reduced bacterial growth in the root exudates of RR soy plants treated with glyphosate.

Studying rhizosphere microorganisms in RR and conventional soybeans and maize from 1997 through 2007, Kremer & Means (2009) found significantly less fluorescent pseudomonads in RR crop rhizosphere and even less if the RR crops were treated with glyphosate. Fluorescent pseudomonads are associated with antagonism of fungal pathogens and manganese (Mn) reduction (Mn is taken up by plants in its Mn^{2+} form). In contrast, they observed increases in the proportion of bacteria oxidizing manganese to Mn^{4+} that is not taken up by plants. The micronutrient manganese is essential for plant metabolism and development and is involved in processes such as photosynthesis, nitrogen and carbohydrate metabolism and plays a role in the shikimate pathway and defence reactions (Hänsch & Mendel 2009). Plants undersupplied with manganese can become more vulnerable to pathogens and may show reduced yield (Johal & Huber 2009).

It is known that rhizobia, such as *Bradyrhizobium japonicum*, symbiotic bacteria of the soybean, react sensitively to glyphosate, depending on the herbicide concentration and microbial strain (Abendroth et al. 2002, Schütte 1998, Labes et al. 1999). Glyphosate enters the root nodules and inhibits root development through *B. japonicum* and reduces both their biomass by up to 28% and the leghaemoglobin, important for nitrogen binding in soybean roots, by up to 10% (Reddy & Zablotowicz 2003). In young RR soy plants, glyphosate delayed nitrogen fixation and reduced growth of roots and sprouts, leading to yield decline in less fertile soils and under drought by up to 25% (King et al. 2001). Whereas Powell et al. (2009) did not find negative effects of glyphosate on *B. japonicum* colonization of RR soybean roots, Kremer & Means (2009) observed in their 10 year study that nodulation on RR soybean was always lower with or without glyphosate, compared to conventional varieties with non-glyphosate herbicide or no herbicide. This indicates that glyphosate and perhaps the genetic modification of the RR crop may affect processes associated with nitrogen fixation symbiosis.

Several studies have focused on glyphosate use and its effects on fungi, with contradictory results (Powell & Swanton 2008, Sanyal & Shrestha 2008, Fernandez et al. 2007b). Conflicting study results might be due to pathogen inoculum, selection of study sites, pathogen-weed interactions, herbicide timing, soil properties, tillage, and study design including statistical power. Some fungi express glyphosate-sensitive forms of EPSPS whereas others, including rust fungi and blight fungi, show enhanced growth on glyphosate-amended media. In some cases, inhibitory effects of glyphosate on pathogenic fungi (e.g. *Fusarium solani, Pythium ultimum,* and *Rhizoctonia solani*) have been reported, but not all pathogens and crops are affected similarly, whereas in other cases glyphosate increased severity of fungal diseases (Sanyal & Shrestha 2008). Glyphosate did not affect *Trichoderma* spp. and *Gliocladium* spp., beneficial fungi that favour plant growth and help control plant pathogens, but stimulated *Pythium* spp. and *Fusarium* spp., pathogens that produce toxins harmful for both humans and animals, such as deoxynivalenol DON (Meriles et al. 2006). According to Njiti et al. (2003), *Fusarium* infestation is not related to glyphosate, it rather depends on the particular crop variety. But the statistical power of their study has been questioned (Powell & Swanton 2008).

There is increasing evidence that glyphosate favours fungal diseases caused by *Fusarium* spp.: Kremer et al. (2000, 2005) found that root exudates of treated RR soybeans significantly promote the growth of various *Fusarium* strains and that frequent glyphosate use enhances *F. solani* appearance. Glyphosate interactions with root colonization by *Fusarium* in RR soybean were greatest at the highest soil moisture levels (Means & Kremer 2007). In their 10 year long study, Kremer & Means (2009) found that *Fusarium* root colonization of RR soybean treated with glyphosate was two to five times higher and that of RR maize even three to ten times higher, compared to crops receiving no or a conventional herbicide. There was also a negative relationship between *Fusarium* root colonization and population size of fluorescent pseudomonads demonstrating that glyphosate was involved in altering the microbial composition in the rhizosphere.

In Canadian wheat and barley crops, Fusarium head blight (FHB), caused by *Fusarium* spp., was positively correlated with glyphosate application in the previous 18 months, in contrast to *Cochliobolus sativus*, the most common CRR (common root rot) pathogen (Fernandez et al. 2005, 2007a,b, 2009). In greenhouse studies of RR sugar beet, increased disease severity was observed following glyphosate application and inoculation with certain isolates of *Rhizoctonia solani* and *Fusarium oxysporum* (Larson et al. 2006). Reviews of various studies on the effects of glyphosate on plant diseases indicate that an increase in a number of bacterial and fungal diseases of crop plants, among them diseases once considered efficiently managed, is linked to glyphosate weed control programs (Johal & Huber 2009), but that generalizations about direct effects of glyphosate on plant disease are difficult (Sanyal & Shrestha 2008).

There is no agreement in the literature concerning the mechanisms that might lead to changes in fungal communities and disease development (Powell & Swanton 2008). Exuded glyphosate may serve as a source of nutrition for fungi (P and C as well as energy source). In addition, the increased exudation of soluble carbohydrates and amino acids in treated RR crops could promote fungal growth (Kremer et al. 2005). If antagonistic bacteria, which metabolise these exuded substances and thus restrict fungal growth, are impaired by glyphosate, correcting influences are lacking, with the result of increased growth of pathogenic fungi.

On the other hand, pathogen defence could be compromised since the production of many resistance components involves the shikimic acid pathway that is inhibited by glyphosate: e. g. phenolics such as phytoalexins that accumulate rapidly at the site of infection, structural components (lignin) that fortify cells and ensure isolation of the pathogen at the infection site, and salicylic acid that functions as signal (Powell & Swanton 2008, Johal & Huber 2009). Reduced uptake of Mn will increase predisposition of plants to disease. The observation of Larson et al. (2006) that disease increase in RR sugar beet does not appear to be fungal mediated, could support this latter hypothesis. Combined, these factors may lead to an increased appearance of pathogenic fungi in crops treated with glyphosate. According to Johal & Huber (2009), indirect effects of glyphosate on disease predisposition result from immobilization of specific micronutrients involved in disease resistance, reduced growth and vigour of the plant from accumulation of glyphosate in meristematic root, shoot, and reproductive tissues, altered physiological efficiency, or modification of the soil microflora affecting the availability of nutrients involved in physiological disease resistance.

There have been reports about negative impacts of glyphosate on beneficial fungi. In their review on the impact of GM plants on soil microorganisms, Motavalli et al. (2004) cited studies that found a reduction in growth of several species of mycorrhizal fungi when exposed to glyphosate levels higher than 50 µl/l in culture media. Complete inhibition was found at levels of 5,000 µl/l. Due to the often significant role of mycorrhizal fungi in plant nutrient accquisition, their inhibition may affect soil nutrient transformations. As information about the complex interactions in rhizospheres of glyphosate resistant and treated crops is still limited, Kremer & Means (2009) call for more research into potential impacts of glyphosate on soil microorganisms and, in particular, on mycorrhizal fungi.

Entomopathogenic fungi, useful for combating harmful insects, can also be affected by Roundup when used in field concentrations. Glyphosate alone did not have fungicidal activity on any of the four species tested (*Beauveria bassiana, Metarrhizium anisopliae, Nomuraea rileyi,* and *Neozygites floridana*), but synergistically reinforced the toxic effects of the formulating agents (Morjan et al. 2002). Such non-target effects of herbicide products could impact pest regulation.

Aquatic organisms

For aquatic microorganisms, Giesy et al. (2000) found acute EC50 values ranging from 2.1 to 189 mg Roundup/l (90-fold difference) and calculated an acute TRV of 0.73 mg Roundup/l. Chronic EC50 values for glyphosate ranged from 0.64 to 590 mg a.e./l (900-fold difference) and NOEC levels were between 0.28 and 33.6 mg a.e./l (120 fold difference). The chronic TRV for aquatic microorganisms was estimated to be 0.28 mg a.e./l. Recent microcosm studies representing a worst case scenario revealed that Roundup (6 – 12 mg a.e./l) affected the structure of phytoplankton and periphyton assemblages. Total micro- and nano-phytoplankton decreased in abundance, whereas abundance of pico-cyanobacteria increased by a factor of about 40, at the expense of diatoms (Pérez et al. 2007). Cyanobacteria are known to be particularly resistant to extreme environments and are remarkably tolerant to glyphosate, possibly due to an insensitive form of EPSPS and/or the ability to metabolize glyphosate (Forlani et al. 2008). Should glyphosate add to the phosphorous load in surface waters and lead to a shift in phytoplankton assemblages with an increase of cyanobacteria, harmful cyanobacteria blooms (Paerl et al. 2001, Smith 2003) might result. As Pérez et al. (2007) point out this could adversely affect water quality and human and animal health.

WHO (1994) classified glyphosate without surfactants as being slightly/very slightly toxic to aquatic invertebrates and moderately/very slightly toxic to fish, with carp being the most sensitive species, when exposed to the glyphosate product Sting. There are several reviews on toxicity of glyphosate and its formulations to aquatic organisms such as invertebrates, fish, and amphibians (Giesy et al. 2000, Solomon & Thompson 2003, Solomon et al. 2005). According to Giesy et al. (2000), LC50 of Roundup on aquatic invertebrates ranged from 9.7

to 200 mg/l and NOEC levels from 4.4 to 7.8 mg/l, chronic TRV was 0.5 mg a.e./l for glyphosate and 0.1 mg/l for the surfactant POEA. The most sensitive species was *Daphnia magna*. Toxicity of the glyphosate salts to aquatic invertebrates differs significantly (EC 2002). Acute EC50 and NOEC levels of trimesium salt for invertebrates were 12 and 1.1 mg/l, respectively, and for the isopropylamine salt 930 and 455 mg/l, respectively.

The toxicity order for bacteria, microalgae, protozoa, and crustaceans was POEA > Roundup > technical glyphosate > isopropylamine salt (Tsui & Chu 2003). Microalgae and crustaceans were 4-5 fold more sensitive than bacteria and protozoa. Comparing various formulations, the toxicity order for the crustacean *Ceriodaphnia dubia* and the benthic amphipod *Hyalella azteca* was Roundup (1.5 - 5.7 mg/l) > Roundup Biactive (82 - 120 mg/l) > Rodeo (225 – 415 mg/l), with *H. azteca* being more sensitive than *C. dubia* (Tsui & Chu 2004). Increased organic carbon content in the sediment significantly decreased toxicity of Roundup, unlike Roundup Biactive. Increase of Roundup toxicity to *C. dubia* with pH and suspended sediment concentration (Tsui & Chu 2003) corresponds to the observation that POEA is more toxic in alkaline than in acid water (Diamond & Durkin 1997). Toxicity increase with pH (and with low food availability) has also been found for effects of Vision to the zooplankton species *Simocephalus vetulus* at concentrations lower than 1.4 mg a.e./l (Chen et al. 2004).

POEA surfactant formulations, consisting of a 5:1, 10:1, and 15:1 oxide:tallowamine ratio, exhibited high toxicity to laboratory and field collected fairy shrimp (*Thamnocephalus platyurus*) with 48 hour-LC50 concentrations of 2.01 µg/l, 2.70 µg/l, and 5.17 µg/l for POEA15:1, 10:1, and 5:1, respectively (Brausch & Smith 2007). These LC50 values are considerably lower than the ones reported by Giesy et al. (2000) for Roundup toxicity on invertebrates, ranging from 9.7 to 200 mg/l. Brausch & Smith (2007) believe toxicity most likely to be due to disruption of oxygen transport in respiratory surfaces. Lethal and sublethal toxicity and growth inhibition has also been shown for *Daphnia magna*, with 48 hour-LC50 values of 97.0 µg/l (POEA10:1), 176.4 µg/l (POEA5:1), and 849.4 µg/l (POEA15:1) (Brausch et al. 2007). These results indicate that for *D. magna* POEA10:1 was more toxic than POEA15:1 and that this species was generally less sensitive to POEA than *T. platyurus*. Roundup and glyphosate may also interact with metal ions such as Ag, Cd, Cr, Cu, Ni, Pb, and Zn (Tsui et al. 2005). Combining them with Roundup led to less than additive acute toxicity of the mixture to *C. dubia*, whereas addition of glyphosate alone decreased metal ion uptake and toxicity, but increased Hg uptake significantly.

Mosquito larvae do not seem to be particularly sensitive to Roundup and glyphosate (Giesy et al. 2000). Exposure to sub-lethal concentrations of glyphosate might even increase their tolerance to insecticides, (e.g. imidacloprid and permethrin), as shown for *Aedes aegypti* larvae, possibly through cross-induction of particular genes encoding detoxification enzymes (Riaz et al. 2009). In sea urchin embryos and goldfish erythrocytes, Roundup (Roundup 3plus) and glyphosate have been reported to damage DNA and affect cell division negatively (Marc et al. 2004, Cavas & Könen 2007). For 12 species of fish, acute LC50 values for Roundup ranged from 4.2 to 52 mg/l, for glyphosate from 22 to >1000 mg a.e./l, and for POEA from 0.65 to 7.4 mg/l, with rainbow trout (*Oncorhynchus mykiss*) being the most sensitive species (Giesy et al. 2000). Finding only little chronic toxicity of glyphosate to fish, they calculated a NOEC level/chronic TRV of 0.74 mg a.e./l. In toxicity tests conducted with a mixture of glyphosate and the adjuvant Cosmo-Flux 411F, Solomon et al. (2005) obtained 96 hour-LC50 values for rainbow trout ranging from 1.41 to 2.42 mg a.i./l. Interaction of glyphosate with other stressors has been observed by Kelly et al. (2010) who reported that environmentally relevant glyphosate concentrations of 0.36 mg a.i./l or trematode infection alone did not affect survival of the freshwater fish *Galaxias anomalus*, but simultaneous exposure to glyphosate and parasites reduced their survival significantly.

Amphibians are at higher risk than fish. Shallow temporary ponds, essential to the life cycles of many amphibians, are areas where pollutants can accumulate without substantial dilution (Mann et al. 2003). According to Giesy et al. (2000) Roundup is at best moderately toxic to amphibians and glyphosate non-toxic to slightly toxic: For the tadpoles of the most sensitive species *Litoria moore* the acute LC50 Roundup value was 8.1 mg/l. Chronic TRV of Roundup and glyphosate to amphibians was reported to be 1.6 mg/l and 0.74 mg a.e./l, respectively, suggesting that glyphosate was more toxic than Roundup.

In studies with four Australian frog species, 2d-LC50 values for Roundup ranged from 3.9 to 15.5 mg a.i./l and for technical glyphosate from 108 to 161 mg a.i./l (Mann & Bidwell 1999). For Vision, a POEA containing product, Edginton et al. (2004) found 4d-LC50 values of 1.5 – 4.7 mg a.i./l for three Canadian frog species. Toxicity of POEA containing formulations to amphibians increased with pH (Chen et al. 2004, Edginton et al 2004). Acute toxicity to North American frog species was highest for POEA and decreased in the order POEA > Roundup Original > Roundup Transorb > Glyfos AU (Howe et al. 2004). Toxicity of Roundup Original varied with species and developmental stage, no significant acute toxicity was found with glyphosate or Roundup Biactive, Touchdown, or Glyfos BIO. In general, larval amphibians are more susceptible to glyphosate formulations than other aquatic animals and other amphibian stages.

Treatment of amphibians for a longer period of time (16 d) than usual (up to 4 d) with Roundup Weed and Grass Killer Concentrate led to LC50 values between 0.55 and 2.52 mg a.i./l (Relyea 2005a,b, Relyea et al. 2005), values that are lower than previously reported ones for acute toxicity (1.5 – 15.5 mg a.i./l, Mann & Bidwell 1999, Edgington et al. 2004) and that can be found in actual surface waters (Borggard & Gimsing 2008, Peruzzo et al. 2008, WHO 2005). Roundup concentration of 3.8 mg a.i./l reduced the number of amphibians by up to 70 %, with POEA again playing an important role in Roundup toxicity (Relyea 2005c). Testing Roundup Original Max for 96 h on 13 species of larval amphibians, Relyea & Jones (2009) found that anurans were more sensitive (LC50 values ranging from 0.8 - 2.0 mg a.e./l) than salamanders (LC50 values ranging from 2.7 – 3.2 mg a.e./l). Roundup Weather Max and Roundup Original at 0.57 mg a.e./l also lengthened the larval period of American toads (Williams & Semlitsch 2009). Stressors such as parasites, predators or low food availability synergistically intensify the toxic effects (Kelly et al. 2010, Relyea 2005c, Chen et al. 2004). For this reason, the current distinction between lethal and sublethal pesticide concentrations may be somewhat artificial (Relyea & Hoverman 2006).

Claims that his studies had been completed under non-natural conditions and with dosages that could not be achieved in water in practice (Thompson et al. 2006) have been rejected by Relyea (2006), as the tested dosages corresponded to the manufacturer's data and led to concentrations in water that were in accordance with worst-case scenarios and levels found in many ponds. According to recent unpublished reports from Argentina, glyphosate herbicide diluted by a factor of 1,500 weaker than that used on RR soybean caused malformations in amphibian embryos such as reduced head size, alterations in the nervous system, increased death of cells forming the scull, and deformed cartilage (Valente 2009). Toxicity was attributed to glyphosate, not to the additives. Roundup and glyphosate have been reported to inhibit aromatase, an enzyme that catalyzes the conversion of androgens to estrogens in vertebrates (Gasnier et al. 2009). A different inhibitor of aromatase, the pharmaceutical compound fadrozole, has been shown to lead to sex reversal in the amphibian *Xenopus tropicalis* (Olmstead et al. 2009). Whether such an effect would also be attributed to Roundup and/or glyphosate is unknown.

Aquatic plants may be affected too, EC50 levels for Roundup range from 3.9 to 15.1 mg/l. Based on the EC50 value for the most sensitive species (*Myriophyllum sibiricum*), Giesy et al. (2000) calculated an acute Roundup TRV (0.78 mg/l) and a chronic TRV for glyphosate (0.08 mg a.e./l).

Terrestrial Animals

Soil fauna could be exposed to glyphosate not only by direct application but also via treated plants that excrete glyphosate via their roots (Kremer et al. 2005, Neumann et al. 2007). The high water solubility and low fat solubility of glyphosate reduces the risk of accumulation in animal tissue, and absorbed glyphosate is predominantly excreted (FAO 2005). Whereas indirect effects of glyphosate to soil invertebrates and terrestrial arthropods due to vegetation changes have been shown repeatedly (see Chapter 3.2), direct toxicity is not expected to play a significant role (Cerdeira & Duke 2006, Giesy et al. 2000, Solomon et al. 2005). Roundup is only slightly toxic to earthworms. For glyphosate, Giesy et al. (2000) reported a NOEC level for earthworm behaviour of 118.7 mg a.e./kg soil, with a 14-day NOEC value of 500 mg/kg dry weight. However, glyphosate based herbicides could alter locomotor activity of earthworms in a way that may compromise their survival (Verrell & Buskirk 2004).

Glyphosate and Roundup are slightly toxic to bees when applied either orally or topically with a 2d-LC50 value of >100 µg a.i. per bee (WHO 1994, Giesy et al. 2000, Solomon et al. 2005). No adverse effects of Roundup were found on fertility of green lacewings, and there were no effects of the product Sting on food uptake and mortality of the beetle *Poecilus* (WHO 1994). In a laboratory screening test cited in Giesy et al. (2000), Roundup was found to be harmless to 13 beneficial arthropod species, slightly harmful to 4 species and moderately harmful to 1 species (carabid beetle). Laboratory studies (semifield in one case) provided by industry and reviewed by the EC (2002) have been done with 11 arthropod species of the following taxa: Beetles (5 species, Staphilinidae and Carabidae), flies (2 species, Chrysopidae and Tachinidae), mites (*Typhlodromus pyri* [Phytoseiidea]), bugs

(*Orius insidosius* [Anthocoridae]), spiders (*Pardosa spec*. [Lycosidae]), and aphid parasitoids (*Aphidius rhopalosiphi* [Braconidae]). The mortality of 8 species was measured with glyphosate isopropylamine-containing substrates and of 6 species with glyphosate trimesium containing substrates. A mortality of 53 - 100% was found for half of these species (tested with inert substrate in a field application range of 0.7 to 7.7 kg a.i./ha Larval stages of *Crysoperla carnea* and adults of *Aphidius rhopalosiphi* seemed to be particularly sensitive (EC 2002). Hoverflies (Syrphidae) providing a high level of aphid control have not been covered. The formulated products are also toxic to predatory mites and moderately toxic to some beneficial spiders and (parasitic) wasps (CTB 2000).

Glyphosate is slightly toxic to birds, with an 8d-LC50 of >4,640 mg/kg feed, and 112- to 119-d-NOEC values of >1,000 mg/kg feed (WHO 1994). For chronic toxicity (20 week), the NOAEC value of glyphosate for the mallard and bobwhite quail was 1,000 mg a.e./kg of feed, equivalent to the chronic TRV (Giesy et al. 2000). The toxicity of AMPA, known to be more stable than glyphosate, to birds and arthropods has not been tested

Most mammalian feeding studies reviewed by Giesy et al. (2000) have been performed with rats. Acute single oral LD50 values lie around 5,000 mg/kg/d for Roundup and range from 2,047 to 5,700 mg a.e./kg/d for glyphosate, revealing a more than twofold difference in studies with the same species (rat). In chronic feeding studies (13 weeks to 24 months), for glyphosate NOAEC values from 205 to 1,267 mg a.e./kg/d have been found, whereas in multiple-generation feeding studies NOAEC levels for rat varied from >30 to 666 mg a.e./kg/d. In 1 - 3 month feeding studies with POEA, NOEL levels ranged from 33 - 52 mg/kg/d. Chronic TRV for glyphosate and POEA were estimated to be 410 and 16.5 mg/kg/d, respectively, indicating a 25-fold higher toxicity of POEA.

Reviewing toxicity data for glyphosate and Roundup, Williams et al. (2000) concluded that Roundup does not result in adverse effects on development, reproduction, or endocrine systems in mammals and humans. More recent work, however, indicated that glyphosate-based herbicides are toxic to human cells and act as endocrine disruptors. Enzyme activity may be changed after exposure to glyphosate as reported by Daruich et al. (2001), who found altered specific activity of three cytosolic enzymes in liver, heart, and brain of pregnant rats and their fetuses. In rat hepatoma tissue culture (HTC) cells, treatment with low doses of Roundup resulted in increased lysosome density, morpho-functional modifications of nuclei, and modified mitochondrial membranes (Malatesta et al. 2008).

In human cells, cellular and genetic toxic effects, such as increased chromosome aberrations, have been observed (Monroy et al. 2005, Lioi et al. 1998). Both Roundup Bioforce and glyphosate damage human embryonic cell lines and placental cells, and do so in concentrations at or below the recommended values for agricultural use (Benachour et al. 2007). Comparable results have been reported for dilutions (10 ppm to 2%, 1 – 2% is recommended for agricultural use) of four Roundup formulations (R7.2, R360, R400, and R450) and glyphosate, POEA, and AMPA tested on three human cell types. Within 24h, the treatment caused cell death through inhibition of a mitochondrial enzyme (succinate dehydrogenase) and necrosis (Benachour & Séralini 2009). Apoptosis, DNA fragmentation, nuclear shrinkage and fragmentation were also observed. Roundup400 was the most toxic formulation and POEA the most toxic substance. Like Roundup, POEA and AMPA separately and synergistically damaged cell membranes, but at different concentrations. AMPA was more toxic than glyphosate, e.g. on cell membranes, and amplified glyphosate or POEA toxicity. Benachour & Seralini (2009) concluded that the "inert ingredient" POEA was in fact the "active ingredient" on human cell death and more damaging than glyphosate itself.

In a more recent test series with the human hepatoma cell line HepG2, treatment with the same four Roundup formulations and glyphosate resulted in cytotoxicity, genotoxicity, anti-estrogenic, and anti-androgenic effects (Gasnier et al. 2009). Within 24h, all formulations, in contrast to glyphosate alone, induced in a dose-dependent fashion a rapid decrease in cell viability. R400 was again the most toxic formulation, leading at 5 ppm to 50% DNA strand breaks. Inhibition of aromatase, that plays an important role in the production of steroids and thus the formation of germ cells and reproduction, indicated disruption of androgen to estrogen conversion. Endocrine disruption was reported for R400 at concentrations as low as 0.5 ppm, at 2 ppm the transcription of estrogen receptors was inhibited and at 10 ppm aromatase transcription and activity were disrupted. Glyphosate alone had no anti-estrogenic activity, but was anti-androgenic at sub-agricultural and non-cytotoxic levels. According to Gasnier et al. (2009) the direct glyphosate action is most probably amplified by vesicles formed by adjuvants or detergent-like substances that allow cell penetration, increase its stability, and probably change its bioavailability and thus metabolism.

Effects on non-resistant crop plants

Glyphosate spray drift affecting non-target crop plants is of increasing concern in cropping systems where glyphosate is repeatedly applied. Up to 10% (and in some cases even more) of the sprayed glyphosate may move to non-target plants (Cakmak et al. 2009). There it can impede transport of some essential nutrients. In sunflowers, simulated spray drift led to significantly reduced growth and lower chlorophyll content in young leaves and sprout tips, the transportation of iron (Fe) and manganese (Mn) from root to sprout was almost entirely inhibited within a single day (Eker et al. 2006). Simulated spray drift on non-resistant soybeans (0.6% of the recommended application rate) caused significant reductions of dry weight, chlorophyll contents, and calcium (Ca), magnesium (Mg), and manganese (Mn) concentrations in young leaves (Cakmak et al. 2009). At 1.2% of the recommended application rate, seed concentrations of Ca, Mn and Fe were reduced by 25 - 49%, whereas shoot biomass was nearly 3-fold lower and seed production nearly 9-fold lower. High sensitivities of plant reproductive organs to glyphosate have been observed earlier, e.g. Duke & Cerdeira (2005) reported that glyphosate, sprayed during seed maturation, can dramatically affect seed quality and germination. Seed viability and seedling vigor and establishment may thus be affected.

These studies suggest that, depending on growth stage and the amount of glyphosate absorbed, non-glyphosate resistant crops in neighbouring areas can reveal reduced growth and yield, if they do not die off immediately. This may partly explain why in the US the number of claims for compensation by farmers who are affected by glyphosate drift on their crops has increased rapidly since the introduction of RR crops (Henry et al. 2007). On the other hand, doses of glyphosate that are significantly lower than toxic levels may even stimulate growth of non-resistant plants, as observed by Velini et al. (2008) in short term greenhouse studies (1.8 – 36 g a.e./ha, 21 – 60 days). If this is valid also in the field, spray drift might enhance plant growth under some circumstances, at least in the short term. But it can also make non-resistant plants more susceptible to plant pathogens, presumably by inhibiting production of defence-related compounds derived from the shikimate pathway (Duke & Cerdeira 2005).

Plant residues of glyphosate-treated weeds bear an intoxication risk for subsequent crops. Greenhouse studies on two soils (acidic sandy Arenosol and calcareous loess subsoil) revealed that treating the grass weed *Lolium perenne* with glyphosate shortly before seeding the crop (*Helianthus annuus*) strongly impaired sunflower seedling growth and biomass production with up to 90% reduction in root and shoot biomass (Tesfamariam et al. 2009). Sunflower plants recovered to some extent after three weeks. Detrimental effects were more pronounced after glyphosate weed application, compared to direct soil application. This suggests that the root tissue of glyphosate-treated weeds represents a storage pool for the herbicide. The authors discuss whether the accumulation of glyphosate in young growing tissues of roots and shoots leads to "hot spots" containing high levels of glyphosate that is subsequently released during microbial degradation of the plant residues. Without fast immobilization by adsorption on the soil matrix, glyphosate could intoxicate non-target plants by root contact with these hot spots. In agreement with such an explanation is the observation that sunflower seedlings showed high variation in biomass production, shikimate accumulation, and Mn nutritional status.

Glyphosate toxicity to non-target plants depended also on soil type: in the Arenosol soil with low buffering capacity and lower levels of available manganese, Tesfamariam et al. (2009) observed higher impairment of Mn nutrition. The potential glyphosate pool in treated weeds might thus contribute considerably to its toxity to non-target plants, in particular, if there are only short waiting times between glyphosate treatment and crop seeding. Johal & Huber (2009) discussed links between disease incidence in non-target plants and glyphosate weed control programs: In fruit trees certain bacterial diseases are "emerging" or "reemerging" diseases as glyphosate weed management programs for their respective crops have intensified. Control of citrus variegated chlorosis, for instance, emphasizes elimination of glyphosate and adoption of an alternative grass mulch weed control program.

3.1.5 Ecotoxicity of Roundup compared to ecotoxicity of conventional herbicides used in sugar beet

To compare the environmental impact of different herbicide regimes, various indicators have been developed that convert the data of pesticide used to parameters that can be compared. Some indicators relate only to environmental behaviour, such as leaching to groundwater, while other indicators also take the broader impact of pesticides, e.g. effects on non-target organisms (Kleter et al. 2008) into account. The environmental behaviour of glyphosate has been compared to that of metamitron (and to other herbicides applied in oilseed rape and maize) (Mamy & Barriuso 2005, Mamy et al. 2005). In three tested soil types, glyphosate degraded more rapidly and was more strongly adsorbed than metamitron, the adsorption of which increased with soil organic content. Considering the close relationship between adsorption in soils and herbicide leaching potential the authors estimated metamitron concentrations in soil solution at 0 – 10 cm depth to be one order higher than that of glyphosate, even for the highest rate of glyphosate (3.06 kg/ha). For metamitron, high amounts of non-extractable residues have been found that may accumulate in soil following several applications. In Finnish soils, adsorption of glyphosate was higher than that of phenmedipham, ethofumesate, and metamitron, with metamitron having the highest risk of leaching (Autio et al. 2004). However, according to Mamy et al. (2005), the environmental advantage in using glyphosate due to its rapid degradation is counterbalanced by accumulation of its more persistent metabolite AMPA, specifically in the context of extensive use of glyphosate.

In the Danish pesticide leaching assessment programme, data are collected about leaching risk of 31 pesticides, among them glyphosate, metamitron, ethofumesate, phenmedipham, and desmedipham. Montoring data from 1999 to 2006 show that glyphosate and AMPA leach from the root zone at high average concentrations exceeding 0.1 µg/l on loamy soils, with maximum values of 31 µg/l and 1.6 µg/l, respectively, at one site (Kjaer et al. 2009), but they have been rarely detected in monitoring screens below the depth of the drainage system. Metamitron, its metabolite metamitron-desamino, and ethofumesate also leached through the root zone at high average concentrations in loamy soils with maximum values of 26.3 µg/l, 5.5 µg/l, and 12 µg/l, respectively, and, in some cases, have been found in both drainage system and groundwater monitoring screens. Phenmedipham and desmedipham did not leach significantly and were found only in concentrations <0.1 µg/l. According to these data, glyphosate and AMPA may not pose a lower risk of leaching from the root zone than the main herbicides used in conventional sugar beet cultivation.

In a study of PAN Germany (2002), the toxicology of pesticide residues was evaluated using acknowledged classification systems such as that of the EU. Risk phrases used in the EU classification range from R50 to R59:

- R50: very toxic to aquatic organisms (LC50 of 1 mg/l)
- R51: toxic to aquatic organisms (LC50 values 1 - 10 mg/l)
- R52: harmful to aquatic organisms (LC50 values 10 – 100 mg/l)
- R53: may cause long-term adverse effects in the aquatic environment
- R54: toxic to flora
- R55: toxic to fauna
- R56: toxic to soil organisms
- R57: toxic to bees
- R58: may cause long-term adverse effects in the environment
- R59: dangerous for the ozone layer

For aquatic environments, they are based on LC50 concentrations for fish and *Daphnia* (96h), and algae (72h).

Glyphosate, metamitron, pyrazon/chloridazon, ethofumesate, and quinmerac are herbicides used in sugar beet and included in the PAN list. The risk phrases assigned to glyphosate and ethofumesate read R51/R53. Metamitron carries R50/55 and pyrazon/chloridazon R50/53. Quinmerac does not carry a risk phrase. According to this classification, glyphosate and ethofumesate are equally toxic to aquatic environments, whereas metamitron and chloridazon are more toxic to aquatic environments, metamitron is also toxic to fauna.

Dietsch (2002) compared the ecotoxicity of herbicides used in conventional sugar beet with ecological effects expected when sugar beet resistant to glyphosate and glufosinate would be grown in Germany. He based his calculations on agronomic data (e.g. soil type, weed coverage, and precipitation) and herbicide use (metamitron, ethofumesat, phenmedipham, desmedipham, chloridazon, clopyralid, quinmerac, haloxyfop-R, trisulfuron-methyl, and rape oil) from nine different sugar beet production sites and assumed Roundup Ultra applications to be 2 x 2 l/ha or 3 x 2 l/ha. Risk-indicator models, such as the Dutch EYP model (Environmental Yardstick for Pesticides) and the German SYNOPS 2 model (Synoptisches Bewertungsmodell für Pflanzenschutzmittel Version 2), have been applied. To assess impacts on groundwater, the simulation model PELMO 3.20 (Pesticide Leaching Model Version 3.20) was used. According to the model EYP, ecotoxicity impacts of glyphosate were significantly lower for soil and surface water and groundwater than those of conventional herbicide application. The model SYNOPS 2, developed to calculate both short- and long-term predicted environmental concentrations (PEC) of pesticides, their sorption to soil matrix and water sediment, and acute and chronic biological risk for terrestrial and aquatic reference organisms, gave similar results. Chloridazon and metamitron application resulted in particularly high PEC values. Using the model PELMO 3.20 for calculation of cumulated herbicide concentrations in subsurface soil and leaching water, Dietsch (2002) found very low values for glyphosate. Higher values for conventional herbicides have been found only in the case of quinmerac. Groundwater did not seem to be at risk to be contaminated by any of the herbicides, with exception of quinmerac.

Main reasons for favourable results found for glyphosate in the model EYP, compared to the application of the conventional herbicides, were according to Dietsch (2002): the rapid degradation of glyphosate, the lack of accumulation in soil even after a number of applications over several years, low sorption on clay and humic complexes, and very low leaching potential despite its high water solubility. Recent research, however, indicates that glyphosate and AMPA may be more stable in soils than previously assumed, that they can be washed out of the root zone and reach groundwater and surface waters, where concentrations ranging from sub- µg/l to mg/l have been found (Borggard & Gimsing 2008, Kjaer et al. 2004, 2009). Reference organisms in the SYNOPS 2 model are earthworms, *Daphnia*, algae, and fish for which, based on LC50 values, acute and chronic predicted environmental concentrations are calculated. Dietsch (2002) did not discuss whether these reference organisms were representative for aquatic and terrestrial organisms exposed to glyphosate and conventional herbicides. In particular, amphibians, shown to be very sensitive to Roundup, have not been included in the analysis.

Bennett et al. (2004, 2006) performed a life-cycle assessment (LCA) to compare the potential environmental and human health impact of growing glyphosate-resistant sugar beet in the UK and Germany with that of conventional sugar beet varieties. According to their analysis, the ecotoxicity of glyphosate (a.i) is lower than that of the mixtures of conventional herbicides used, but this assessment was based on a literature review up to the year 2000. Newer data about glyphosate toxicity have not been considered.

3.2 Indirect effects of changes in herbicide use

In general, the toxicity of herbicides for wildlife is less crucial than the indirect effects of removing the arable flora (Körner 1990). Less species richness and abundance of arable plants and less seeds in the soil seedbank resulted in large biodiversity losses of many species. Plants are existential for the whole food web and provide habitats for many arthropods. The use of economic threshold models can limit the degree of weed control to an economically reasonable extent (see also Chapter 4). UK studies on the significance of weed abundance for biodiversity distinguish between weeds and non-target weeds, evaluating the relative importance of these species for biodiversity (DEFRA 2001).

3.2.1 Effects of weed control in glyphosate resistant sugar beet on the wild agricultural flora

Glyphosate is even more effective and less selective than currently used conventional herbicides with the exception of atrazine. Over 95% weed control is achieved by non selective herbicides like glyphosate and glufosinate (Westwood 1997). However, a 95% control is not necessary for the exclusion of competitive effects of weeds and non-target or beneficial wild plants to crops (Korr et al. 1996, Pallutt et al. 1997, Busche 2008).

The effects of the HR cropping-technique on abundance and species-diversity were investigated in a large-scale trial (60-75 fields, 3 years, size of plots: half fields) on fields selected to represent the variation of geography and “intensity” of management across Britain (Firbank et al. 2003, Squire et al. 2003). These “Farm Scale Evaluation” (FSE) trials are unique according to the range of indicators, sampling methods, sampling intervals, and its whole methodology.

The density, biomass, and seed rain of the agricultural flora in herbicide resistant beet were reduced by a factor of three to six relative to conventional practice and the soil seed bank (for 19 out of 24 species) decreased by 20% (Heard et al. 2003a,b). The emergence of 8 species was lower in HR beet. The losses regarding plant abundance and the soil seed bank, which have been found in herbicide resistant varieties, would result in large decreases in population densities of the field flora compounded over time according to Heard et al. (2003 b). The soil seed bank will be depleted if there is only little replenishment by ripe weed seeds due to clean weeding.

The field boundary was also adversely affected by the new agricultural practice in herbicide resistant varieties. The wild plant cover at field margins was about 30% lower on average and seeding was about 40% lower in herbicide resistant beet. The scorching of vegetation at margins was more than doubled (Roy et al. 2003). Spray drift can also damage hedgerows and trees growing close to arable fields, these habitats being very important for arthropods and birds for food, shelter, and nesting (Roy et al. 2003).

Impacts of conservation tillage

The composition of weed species will change in conservation tillage systems. Effects of conservation tillage on the arable flora in glyphosate resistant crops have not yet been studied. In general, the weed population shifts to perennial and grass weed species in systems with reduced or zero tillage. Broad-leaved annual plants providing nectar and pollen for agriculturally relevant predators, e.g. aphid predators, may further decrease in glyphosate resistant varieties.

3.2.2 Effects of weed control in glyphosate resistant sugar beet on the wild agricultural fauna

Arthropods

Less field flora resulted in decreasing forage or habitat and consequently less arthropods compared to fields sprayed with conventional herbicides.

The numbers of within-field above-ground (epigeal and aerial) arthropods were smaller in HR-beet due to forage reductions (Buckelew et al, 2000, Haughton *et al*. 2003, Brooks *et al*. 2003). Herbivores, pollinators (e.g. bees, butterflies) and beneficial natural enemies of pests were reduced (Hawes *et al*. 2003). Their numbers changed in the same direction as their resources (Hawes *et al*. 2003). According to Haughton *et al*. (2003) population densities of these arthropods will decline when forage is reduced over large HR-crop areas.

Impacts of conservation tillage

A positive effect of reduced tillage on invertebrates was mostly proved in conventional crops with cover crops. Effects are quite small without plant cover (Krück et al. 1997, Makeschin 1997, Stippich & Krooß 1997, Wardle et al. 1999) and mixed in the case of ground beetles (Stinner and House 1990, Kromp, 1999). Populations of beneficial organisms (except spiders to some extent) will not significantly increase in fields with conservation tillage unless plant coverage mitigates cold temperature in winter (Bürki and Hausammann 1993, Stippich and Krooß 1997).

The amount and diversity of living and dead mulch is more important for many soil-associated arthropods than reduced soil disturbance (Krück et al. 1997, Wardle et al. 1999). In this way herbicides indirectly affect them more negatively than disturbance by tillage can do (Wardle et al. 1999).

Vertebrates

In glyphosate resistant sugar beet fields the abundance of weed, weed seeds, and invertebrates as forage is reduced (compared to conventionally sprayed beet fields) and may lead to a further decrease of the field fauna, e.g. birds. Important food sources for 16 of 17 bird species would markedly be reduced if beet, spring, and winter rape would be largely displaced by herbicide resistant varieties managed as in the FSE trials according to Gibbons et al. (2006).

Studies from Switzerland show that numbers of birds increased when food plants were planted (Jenny et al. 2003). Smart et al. (2000) quantified changes in abundance of food plants for farmland birds from 1978 to 1990 and found significantly more decreases than increases in food plants for 9 of 12 decreasing bird species. In conclusion, food seemed to be an important factor. The greater coincidence of decreasing food plants and bird numbers occurred for the most strictly herbivorous species (however these species require invertebrate food for juveniles too).

Analyses of large data pools show that there also is a correlation between the abundance of important arable weeds and invertebrates (Marshall et al. 2001) and between the abundance of invertebrates and birds (Benton et al. 2002). Hart et al. (2006) found a relationship between breeding performance (success) of birds and arthropod abundance in their case study on yellowhammer. Thus, decreasing key host plants for invertebrates as chick feed may be an important and underestimated driving factor for bird decline besides the seed of other plants, nesting habitats and weather.

Butler *et al*. (2007) raised the question, whether the nationwide conservation status of typical farmland birds would be affected after a nationwide introduction of HR crops in the UK. Their result from a highly aggregated model was that only one bird species of 39 will change to a less favorable conservation status. However this does not mean that regional declines of bird populations would not occur. Stronger evidence is likely to come from more detailed spatial coincident data. Furthermore, the decrease of particular key host plants for invertebrates, that serve as chick feed, could have been under-represented by the model of Butler et al. (2007, see main components of the model). In addition Butler et al. (2007) did not take the decrease in arthropod abundance (see above) in herbicide resistant varieties into account.

Impacts of conservation tillage

Impacts of mechanical weeding on ground nesting birds and hares are likely, depending on the timing of operations. Nesting birds and small mammals are frequently killed or injured by tillage operations. However, as Cowan (1982) showed for spring planted crops, a clear positive effect of no-till systems on birds could only be seen, when farmers carefully avoided crushing nests and covering the eggs during seeding operations.

3.3 Abiotic effects

Emission of pollutants and greenhouse gases

Bennett et al. (2006) performed a life-cycle assessment comparing emissions when glyphosate or conventional herbicides are used in sugar beet. The emission of greenhouse gases, the ozone depleting potential, airborne nitrification and pollution of air, soil and water were assessed. Glyphosate production, transport and applications resulted in slightly lower impacts. The emissions were mainly related to herbicide manufacture. However, the reduction of abiotic impacts through growing glyphosate resistant beet is supposed to be very small. For example if conventional varieties were totally replaced in the UK, CO_2 and NO_x emissions would fall by an estimated 0.0006%. This outcome is also wholly dependent upon the spraying frequency and amounts of herbicides used which may increase in the long run (see Chapter 2).

Effects of conservation tillage on climate gas emissions

In general, cultivated soils contain about 50% to 75% of the original soil organic carbon of natural soils, due to mineralization/oxidation, leaching and erosion. According to two studies cited in Lal (2008) there is a potential to sequester 0.4-1.2 Pg C per year (one Pg [petagram] = one billion metric ton = 1000 x one billion kg) when tillage is omitted (provided that about 1600 M ha of cropland is under no-till practice). This is equivalent to 5-15% of the current global fossil fuel emissions. However, these calculations are not verified and there are at least two studies questioning them: Baker et al. (2007) analyzed the case studies on which the calculations were based. The soil samples were taken from a depth of 20-30cm in nearly all cases, where conservation tillage was found to sequester C. When soil sampling extended 30cm, there was no consistent accrual of soil organic matter by conservation tillage. Furthermore, Conant et al. (2007) discussed long-term impacts and the reversibility of C-sequestration in agricultural soils: Climate change, warmer temperatures or single tillage passes after long periods of no-till can partly reverse gains.
It can be concluded from the current state of knowledge, that C sequestration by conservation tillage is still questionable, whereas afforestation, diverse cropping, continuous cropping, manure application of organic manure, formation of charcoal (used as fertilizer, Fowles 2007 cited in Lal 2008) are unquestioned means of terrestrial biotic C sequestration (Lal 2008).

3.3 Conclusions on environmental impacts

Direct toxicological impacts

Despite decades of glyphosate use, there is still insufficient knowledge about glyphosate behaviour in soil and water and its effects on aquatic and terrestrial organisms. The degradation rate depends very much on soil conditions and mineral composition. Leaching of glyphosate and its metabolite AMPA has been shown. Reported levels in surface waters can exceed the EU tolerable level of 0.1 µg/l (Borggard & Gimsing 2008). Study results so far indicate that glyphosate can affect soil and aquatic microorganisms. In face of the widespread use of glyphosate, more research analyzing potential links between glyphosate and fungal crop diseases (Powell & Swanton 2008, Johal & Huber 2009) and between glyphosate and phytoplankton assemblages (Pérez et al. 2007) should be initiated. Several studies have shown that the surfactants added to increase the herbicidal activity can be toxic on their own. In particular, POEA, the main surfactant in Roundup, exhibits significantly higher toxicity than glyphosate alone to aquatic invertebrates and to amphibians. Larval amphibians are generally more susceptible to glyphosate formulations than other aquatic animals examined and other amphibian stages. Negative effects of glyphosate on certain terrestrial invertebrates seem possible. Roundup and POEA have also been shown to be toxic to human cells and to induce endocrine disruption (Gasnier et al. 2009). The reassessment of glyphosate-based herbicides with regard to their effects on development, reproduction, and endocrine systems of mammals (and humans) seems most necessary.

Comparisons of environmental impacts caused by glyphosate with those of herbicides used in conventional sugar beet production depend very much on the type of indicators and the quality of data used for the assessment. Some of the studies found that impacts of glyphosate were significantly lower than those of conventional application. Results of other comparisons were not as straightforward. Therefore, more extensive assessments and carefully selected relevant indicators that take recent data into account seem necessary. Glyphosate, AMPA and Roundup should be assessed referring to their behaviour in soil and water, their toxicity to terrestrial and aquatic organisms, and in particular to amphibians.

Indirect biotic impacts

Biodiversity losses reported after 1980 involve more subtle and indirect effects than the poisoning of wildlife by pesticide residues (Krebs et al. 1999). There is much evidence that the soil seedbank, wild flora and whole food webs in agricultural fields will further be reduced, if herbicide resistant beet are planted and sprayed with broad-spectrum herbicides. Reduced abundance of wild plants and arthropods in field and at field margins will affect other organisms feeding on them.

Abiotic impacts

Life cycle assessments of the different herbicides used in glyphosate versus conventional beet concerning emissions of pollutants and climate gases resulted in slightly lower impacts of glyphosate resistant varieties. However this result is dependent upon the application pattern, which is subject of change.

Tillage impacts

Biodiversity effects of herbicide resistant sugar beet grown in reduced and no till systems:
The effects of spraying regimes in conservation tillage using herbicide resistant sugar beet on flora and fauna are not well studied. The soil seedbank of the arable flora may increase when tillage is reduced, but this effect can be reversed in the long run if the input of new weed seeds is reduced by clean weeding with non-selective herbicides. Herbicides can cause larger impacts on soil fauna than tillage can do.

4 Potential effects of alternative application patterns in sugar beet

Alternative spraying regimes for glyphosate in resistant varieties

Delayed spraying had only transient positive effects in herbicide resistant beet, and only on sites with a rich soil seed bank (Dewar et al. 2000). The weed seed bank (important for bird food and for conserving the arable flora) is still reduced in the long term as discussed by Freckleton et al. (2004). Band applications (20cm band over the rows at 10-20% ground cover within the rows) followed by a late overall treatment (at 550-950° Cd = accumulated day degrees above 3°C) as tested by May et al. (2005) resulted in yields equivalent to conventional herbicide regimes but seed return of the field flora was reduced. This will lead to reductions in abundance of the arable flora in the coming years.

Management for environmental benefit

Glyphosate resistant beet

A single early (in May at 200-250° Cd) overall treatment gave similar or better (1 of 4 fields) yields and a significantly higher seed return than conventional regimes. However delaying the single treatment (up to 450° Cd) increased yields and decreased seed return sharply (May et al. 2005). When 2 overall sprays were applied, yields increased by 11% but seed return was lowest. Hence, the single early spray option is not realistic without incentives.

Conventional beet

Sugar beet yield in integrated production systems was not influenced by 15% ground coverage of the associated weed flora. The weed flora was managed by one or two herbicide sprays (row spraying) and by additional cutting or hoeing of large weeds between the rows. The ground cover can even lead to a 7 % higher yield because of an effective aphid control by natural antagonists. The associated flora attracts the aphid predators (Häni et al. 1990, Schäufele 1991).
Furthermore, there are reduction potentials in conventional sugar beet varieties when economic threshold models for weed control (see Chapter 3.2) are applied:
Busche (2008) found a reduction potential of herbicide amounts in sugar beet of about 20% (ranging from 9% to 40%) without economic losses. 10 arable weeds per m^2 at the BBCH stage 31 had no negative influence on yields. In the UK Green and Ogiloy (2001) investigated alternative spraying regimes in sugar beet. They reduced the numbers of full label applications of single herbicides by 35% and omitted insecticides and nematicides. The yield reductions of 18% were due to insect pests and nematodes according to the authors. In any case, threshold evaluations of weeds are time consuming and not realistic without incentives.

Organic agriculture

The demand for organic sugar is rising. In 2007, 336 ha (0.09%) of the total 391,496 ha sugar beet acreage were planted with organic beet in Germany (IFZ 2008, Triebe 2008). The Südzucker AG produces organic sugar and is improving the weeding technique.
The current practice of mechanical weeding in sugar beet is not as effective as herbicide use and causes yield reductions. These reductions are difficult to estimate (see Chapter 5), because organic sugar beet is harvested during the first half of September - weeks before the conventional beet. Thereby a mixture with conventional sugar during production is avoided.
Field tests with high-tech systems are being conducted on 50ha near Leipzig. The prevailing aim is to reduce the costs of hand weeding, which makes up most of the management costs.

Hand weeding hours per ha range between 70-200 h with an average of 150 h (Müller 2008). The aim is to reduce them to less than 100 h (Müller 2008).
Perennial weeds are normally controlled within the crop rotation. A typical rotation is: 2 years alfalfa, winter wheat and then sugar beet. The weed control system tested near Leipzig reduces hand weeding hours to 70-80 per ha while 4-6 varying hoeing passes take place. After sowing a curry comb is used (full area, 1cm depth). When soils are dry, a rotary hoe is used afterwards (at 1-8 leaf phase of sugar beet plants). Under wet conditions a between-rows hoe and a hoe for within the rows (“finger-hoe”) is taken instead. Two alternative hoes (*Winkelscharen* at 4 leaf phase, *Hacksterne* at 6 leaf phase) can be chosen depending on the specific soil conditions. The intensity of weeding within the row can be varied using different fingers and changing the hoeing angle (Müller 2008).

5 Yields

Conventional herbicides can cause root yield reductions due to phytotoxic effects up to 6% or 8% according to Wilson (1999) and sugar yield reductions of about 5% according to Märländer & Tiedemann (2006) compared to glyphosate resistant beet sprayed with glyphosate. These effects are avoided in organic beet production. Differences in yields can also result from the varieties used, tillage, harvest time, test site conditions, weed infestation and effectiveness of control.
Reduced tillage (maximum of 10cm depth) leads to yield losses, which can partly be compensated by N-fertilization. The increased fertilization however worsens the N-balance but not necessarily the risk of N-leaching (König et al. 2005). On loessial sites, yields in conservation tillage are also smaller than in tillage systems. Mean yield losses of about 4% in mulch systems and 12% in direct drilling (no-till) were found in field tests (Koch et al. (2009).
Sugar beet yields per ha can exceed 90 t (IFZ 2008). The average yield (2002 to 2006) in Germany was 59.2 t/ha. In 2007 conventional sugar beet yields were on average 64.2 t/ha (17.6% sugar, 9.965% pure sugar) (IFZ 2008).
Organic beet yields were on average 45 t/ha (17.3% sugar) in 2007 (Müller 2008). However, organic beets are harvested much earlier (first half of September, König et al. 2005) for production reasons. Thus the yield potential is higher and data are not fully comparable. When high tech hoeing is done (see above) early harvested organic beet yields can reach 55 t/ha (18% sugar) according to Triebe (2008). When delaying the harvest by one month (end of October instead of end of September) pure sugar yields would rise by about 24% as tested by Märländer (1991).

Findings from yield comparisons between herbicide resistant and conventional beet on tilled fields (partly in combination with different application regimes such as timing and band spray) as well as in conservation tillage - and mulch systems are listed in Tab. 6.
In general, yields are slightly increased in tilled herbicide resistant beet fields but merely equivalent in mulch and conservation tillage systems. But according to Märländer (pers. communication) these data are insufficient for statistical analysis. In general, more repetitions under comparable conditions and at least 3 years of testing are required.

Table 6: Yield comparisons between conventional herbicide systems and glyphosate used in herbicide resistant (HR) varieties

Reference	country	aim of study	yield differences
Wevers 1998	NL	HR and conv. Technique compared	HR: small increase
Wilson et al. 2002	USA	HR and conv. Technique compared	HR: 15% increase in pure sugar yield,
May 2003	UK	HR and conv. Technique compared	HR: small increase
Bückmann et al. 2000	Germany	weed control in HR sugar beet	conventional herbicides: 100%; glyphosate: 103,1% (late treatment) to 105,2% (early treatment) [not significant]
Dewar et al. 2000	UK	effects on weeds and pests in HR sugar beet: different herbicide systems	conventional herbicides: 100%; glyphosate: 68% (late treatment) to 105% (early treatment)
Dewar et al. 2003	UK	weed control in HR sugar beet, different timing of treatments compared	2 overall glyphosate applications resulted in an average of 9.7% higher yield, result with band spray similar to May et al. 2005
May et al. 2005	UK	weed control in HR sugar beet, different timing of treatments compared	2 overall glyphosate applications resulted in at least 11% higher yield at 3 of 4 sites, band sprays: see[1]
Petersen et al. 2002	Germany	herbicide systems with cover crops and mulch in HR sugar beet	<u>mulch:</u> conventional herbicides: 100% / glyphosate: 96,3% <u>cover crop:</u> conventional herbicides: 100% / glyphosate: 96,8% to 110% (depending on cover crop and herbicide timing)
Petersen & Röver 2005[2]	Germany	herbicide systems with cover crops and straw mulch in HR	no differences

[1] When the first treatment was a band spray (at 10-20% ground cover between rows), yields were similar to sites where conventional herbicides were used.

[2]Yields in conservation tillage or mulch systems: There was no difference in yields when glyphosate resistant sugar beet were planted and weed control was done with conventional herbicides (including one pre emergence application of glyphosate and 3 further applications at BBCH 12, 14 and 19) compared to glyphosate sprays (one pre emergence and one post emergence = application at BBCH 16) (in reduced tillage and winterhardy cover crops). In conventional tillage and mulch, no difference occurred (glyphosate sprayed post emergence instead of conventional herbicides).

6 Resistance management

6.1 Weed resistance to glyphosate

Due to their remarkable genetic variability within species, weeds have been and will be able to avoid the strategies designed to control them (Johnson et al. 2009). Therefore, to base weed control solely on herbicides is the key to selecting herbicide-resistant wild plants. Within the last decades, herbicide resistance in weeds has increased dramatically. The Weed Science Society of America (WSSA) and the industry-sponsored Herbicide Resistance Action Committee (HRAC) regularly update data on resistance development. In February 2010, 195 weed species (115 dicot and 80 monocot species, found in 346 biotypes) are listed being resistant to at least one herbicide, infesting over 330,000 fields worldwide (HRAC 2010). With 131 resistant species (67 %) recorded, the US is number one among the countries listed. The extent of resistance correlates with the use of the respective herbicides: the more widely spread and the longer the period of a herbicide´s application, the more frequent the development of resistant weeds. The actual number of resistant weed populations and the acreage infested with them are probably higher, since the WSSA/HRAC system is a passive one that depends on academic weed scientists to provide their data on resistant populations (Benbrook 2009). Strict standards have to be met for verifying resistance, which may delay or prevent likely cases from being reported.

It was long assumed that glyphosate was an exception to the rule. The first reports on glyphosate-resistant weed species did not appear until the mid-nineties (rigid ryegrass (*Lolium rigidum*) found in 1996 in Australia in non-glyphosate-resistant crops), even though the product had been released on the market in 1974. Reasons for late appearance of resistant weeds were thought to be: fast decomposition of glyphosate, its limited adsorption through the soil, and its particular mode of action (Johnson et al. 2009). Furthermore, before glyphosate-resistant crops were introduced, glyphosate was mostly used in alternation or in combination with other herbicides reducing selection pressure to some extent (VanGessel 2001).

But to date, at least 17 cases of glyphosate-resistant weed species (more than 90 populations) have been confirmed, observed at different locations and in various countries and increasingly associated with RR crop cultivation (HRAC 2010). The total area infested amounts to millions of hectares, many of them in the US. Multiple resistances have been observed too: 15 glyphosate-resistant populations, members of 11 species, express also resistance to other herbicide classes, such as ALS inhibitors, ACCase inhibitors, or paraquat. Resistance can spread through out-crossing, as proven by the successful hybridisation of the glyphosate-resistant horseweed (*Conyza canadensis*) with the related species *Conyza ramosissima* (Zelaya et al. 2007), and by transport of resistant seeds through e. g. farm equipment, animals, wind, and floods (Norsworthy et al. 2008).

Given the widespread cultivation of RR crops on millions of hectares, the list of glyphosate-resistant species and biotypes is expected to grow (Powles 2008). Among the currently known 17 glyphosate-resistant weed species, horseweed (*C. canadensis*), palmer amaranth

(*Amaranthus palmeri*) and giant ragweed (*Ambrosia trifida*) are the weeds most frequently observed in RR crops. Since 2000, when the first glyphosate-resistant horseweed population was described in Delaware after only three years of RR soybean cultivation, this resistant species has infested hundreds of thousands of hectares in 16 US states (HRAC 2010). It has highly effective spreading mechanisms, is very well adapted to ploughless soil tillage and has developed tolerances to numerous herbicides (Zelaya et al. 2007). At least two of the glyphosat-resistant horseweed populations are tolerant to another herbicide (ALS inhibitors and paraquat). Glyphosate-resistant palmer amaranth and giant ragweed increasingly create control problems in glyphosate-resistant soybean, cotton, and corn crops as they have spread on hundreds of sites in at least 12 US states (Benbrook 2009).

Increasing numbers of glyphosate-resistant weeds, such as horseweed, hairy fleabane (*Conyza bonariensis*), and *Euphorbia heterophylla*, have also been reported from Argentina and Brazil, where RR soy is grown on millions of hectares (Vila-Aiub et al. 2008b). Glyphosate-resistant Johnson grass (*Sorghum halepense*), covering at least 10,000 ha, has become a major problem in Argentina (Vila-Aiub et al. 2008a, Binimelis et al. 2009). In Europe, no glyphosate-resistant crop is authorized for cultivation at present. However, due to glyphosate application in other areas, such as orchards and vineyards, several glyphosate-resistant biotypes have evolved in Europe, among them horseweed in Spain (2006 in orchards) and the Czech Republic (2007 on railways) (HRAC 2010, Chodová et al. 2009). Spain is the most afflicted country, where, besides horseweed, three other glyphosate-resistant species have been found on hundreds of hectares: Italian ryegrass (*Lolium multiflorum*), rigid ryegrass (*Lolium rigidum*), and hairy fleabane (*C. bonariensis*). Resistant rigid ryegrass has also been observed in France and Italy.

Some resistant weeds can tolerate up to a nineteen-fold quantity of the glyphosate dose tolerated by herbicide-sensitive plants (VanGessel 2001, Jasieniuk et al. 2008, Legleiter & Bradley 2008). Palmer amaranth was shown to have an LD50 (lethal dose to kill 50 % of plants) up to 115-fold greater than that of sensitive biotypes (Norsworthy et al. 2008). The glyphosate-resistance in weeds is based on differing molecular and genetic mechanisms. Resistance mechanisms confirmed so far are higher EPSPS mRNA levels, lower sensitivity of the target enzyme EPSPS, and modified translocation of glyphosate in the plant. In resistant populations of hairy fleabane from Spain (the first reported case of glyphosate-resistant biotypes in Europe, 2004), EPSPS mRNA levels have been found about double that of sensitive plants (Dinelli et al. 2008). Recently, glyphosate-resistant palmer amaranth (*Amaranthus palmeri*) biotypes from Georgia have been found to contain 5-fold to more than 160-fold more copies of the EPSPS gene (Gaines et al. 2010). Gene amplification correlated with increased EPSPS transcript and protein levels.

A mutation inside the critical amino acid sequence (target site) of the EPSPS enzyme has been reported for several biotypes. The exchange of one amino acid with another may modify the electric charge and/or folding of the target site such that the enzyme is no longer or less inhibited by glyphosate. In resistant Malaysian goosegrass (*Eleusine indica*) and certain populations of rigid ryegrass (*L. rigidum*) and Italian ryegrass (*L. multiflorum*), the replacement of proline at position 106 of EPSPS with serine, threonine, or alanine seems to decrease sensitivity of EPSPS to glyphosate (Powles & Preston 2006, Kaundun et al. 2008, Wakelin & Preston 2006a, Simarmata & Penner 2008, Jasieniuk et al. 2008).

In other cases, observed in resistant horseweed, hairy fleabane, rigid ryegrass, and Italian ryegrass populations, translocation of glyphosate from the leaves to other parts of the plant, including the roots, is slowed down (Wakelin et al. 2004, Dinelli et al. 2008, Feng et al. 2004, Chodová et al. 2009). The mechanisms leading to reduced glyphosate translocation are not fully understood, putative transporter molecules and (phosphate) pumps have been suspected to play a role (Preston & Wakelin 2008, Shaner 2009). This type of resistance can confer high resistance levels and will be favored under intense glyphosate selection, although it may come with a fitness penalty. Reduced absorption of glyphosate by the leaves may also help plants to tolerate glyphosate (Nandula et al. 2008).

Resistance is mainly inherited as a nuclear single-gene mutation in semi-dominant or dominant inheritance (Powles & Preston 2006, Wakelin & Preston 2006b), but may also be related to multiple genes (Simarmata et al. 2005). In resistant palmer amaranth from Georgia, EPSPS gene amplification was heritable. Fluorescence in situ hybridization (FISH) revealed that EPSPS genes were present on every chromosome. Therefore, gene amplification was likely not caused by unequal crossing over, but perhaps by a transposon- or RNA-mediated mechanism (Gaines et al. 2010).

Resistance mechanisms can co-occur, not only in the same species, but also in the same biotype: the resistance of an Italian ryegrass biotype from Chile results from lower spray retention, lower foliar uptake from the abaxial leaf surface, and altered translocation (Michitte et al. 2007). In hairy fleabane biotypes from Spain, higher EPSPS mRNA levels are combined with impaired glyphosate translocation (Dinelli et al. 2008). Resistance mechanisms not based on target site mutations are considered particularly problematic, as they could favor evolution of resistance to other herbicidal modes of action (Yuan et al. 2007). In Californian glyphosate-resistant horseweed populations, air pollution, e.g. ozone, might have accelerated fixation of glyphosate resistance alleles (Grantz et al. 2008).

Species shift among the weed flora, caused by the selection pressure of the herbicides, is another very important aspect (Owen 2008, Duke & Powles 2008, Johnson et al. 2009), but its extent is unknown, since the HRAC/WSSA do not report cases of species shifts on their webpage (Benbrook 2009). Glyphosate does not affect all weed species to the same extent and not all plants are coated in the same manner. Within a plant species, e.g. common lambsquarter (*Chenopodium album*), biotypes may exhibit differential susceptibility, partly depending on parental exposure (Kniss et al. 2007). Common lambsquarter biotypes tolerant to glyphosate showed a higher growth rate early in the season and no fitness penalty in seed production (Westhoven et al. 2008).

Less sensitive species and populations can survive sprayings and subsequently accumulate, whereas more sensitive species disappear. Some weeds adapt their growing cycles so that they only germinate after the usual spraying date and others germinate over a longer period of time or are persistent species that shoot continuously (Scursoni et al. 2007). If early germinators have grown quite tall, they may not be fully eliminated and be able to re-germinate and set seed. Soil nitrogen status could influence survival rates too: under low nitrogen, glyphosate effectiveness on velvetleaf (*Abutilon theophrasti*) and common lambsquarter (*C. album*) was reduced (Mithila et al. 2008). Annual broadleaf weed species such as ragweed (*Ambrosia* spp.), waterhemp (*Amaranthus* spp.), lambsquarter (*Chenopodium* spp.), horseweed (C. canadensis) and morningglory (*Ipomoea* spp.) appear to have increased in difficulty for many producers of glyphosate-resistant corn, soybean, and cotton (Johnson et al. 2009). Weed shift has also been reported from Argentina: After just a few years of RR soybean cultivation, 37 weed species have gained in significance, while only 18 species have decreased (Vitta et al. 2004).

6.2 Management of weed resistance to glyphosate

Despite these troubling developments, many US farmers growing RR crops do not seem to be particularly worried about glyphosate-resistant weeds (Johnson & Gibson 2006, Johnson et al. 2009). This is even true for Delaware, where the first glyphosate-resistant horseweed population in RR soybean fields was found in 2000, and where resistant plants are widespread (Scott & VanGessel 2007). According to Benbrook (2009), the broad adoption of glyphosate-resistant crops in the US has increased herbicide use since 1996 by a total of 174 million kg, part of this increase has been attributed to the emergence of resistant weeds. Control of glyphosate-resistant weeds causes additional costs ranging from $5 to over $40 per hectare (Mueller et al. 2005, Foresman & Glasgow 2008), with a tendency to rise even higher (Benbrook 2009). In Argentina, glyphosate-resistant Johnson grass could double herbicide costs and increase the price of soy production by 160 to 950 million dollars per year (Romig 2007).

For years, weed experts have recommended that the evolution of herbicide-resistant weeds can only be prevented, or at least slowed, by diversity on the field and the combination of different approaches to weed control. For that reason, glyphosate-resistant monocultures and the repeated application of glyphosate should be avoided (Powles 2008, Duke & Powles 2008, Beckie 2006, Buhler 2002, Ghersa et al. 2000) and integrated weed management practices should be adapted (Sanyal et al. 2008, Bastiaans et al. 2008).

Necessary measures recommended by scientists include:

- Crop rotation that changes the weed population
- Reduction of herbicide use
- Rotation of the herbicidal mode of action in order to reduce selection pressure
- Rotation of control measures in order to reduce the dependence on herbicides, including mechanical control and cultivation
- Change of sowing times, in order to provide crop plants with a head start on weeds
- Enhanced crop competitiveness

- Increased scouting of weeds in order to improve knowledge of weed communities
- Integrated weed management – taking into account seed bank dynamics, weed thresholds, critical period of weed emergence etc.
- Cleaning of harvesting machines in order to avoid the spreading of weed seeds
- Ploughing in low light in order to suppress light-induced germination
- Other measures: for example cover crops, mixed cropping, fallow land

In spite of the recommended strategies to diversify in crops, rotations, herbicides, and weed management, continuous glyphosate-resistant crops are realized in some areas in the Americas. Farmers there resort to increased herbicide doses and other, often "old", herbicides. They rather focus on short-term weed control, not on prevention of herbicide resistance in weeds by integrated pest management practices (Wilson et al. 2008, Sanyal et al. 2008). A considerable portion of farmers surveyed in different US states do not scout their fields for problematic weeds (Johnson et al. 2009), and nearly two-thirds of growers do not express a high level of concern for glyphosate-resistant weeds despite frequent occurrence of glyphosate-resistant horseweed (Johnson & Gibson 2006).
Surveys show that higher doses and additional applications of glyphosate, as well as tank mixtures with other herbicides, are being implemented, procedures that serve to increase the selection pressure. In soybean, among others, the herbicides paraquat and the synthetic auxins 2,4 dichlorophenoxy acetic acid (2,4-D) and dicamba are recommended for use in tank mixtures or in rotation with glyphosate (Beckie 2006, Eubank et al. 2008). Incidentally, 24 of the 346 listed herbicide-resistant weed populations are already resistant to the herbicide group of paraquat, among them two that are already resistant to glyphosate (rigid ryegrass and hairy fleabane). 28 populations are resistant to synthetic auxins (HRAC 2010). Although herbicide rotations are generally recommended to ease selection pressure, they may also exacerbate resistance problems by selecting for general (metabolic) resistance in weeds (Neve 2007). Neve also points out that low herbicide doses have the potential to rapidly select for high levels of resistance, as observed in rigid ryegrass (*Lolium rigidum*) in Australia.

Many farmers may rely on the development of a new herbicide within the next few years (Scott & VanGessel 2006, Foresman & Glasgow 2008), but most experts disagree as it is becoming increasingly difficult to find suitable herbicidal substances that are compatible with the intensified requirements for new chemicals (Rüegg et al. 2007, Service 2007, Johnson & Gibson 2006, Kudsk & Streibig 2003). In addition, development costs have increased dramatically. Industry rather tends to modify well-known active ingredients and to stay, for instance, in the class of the ALS or ACCase inhibitors.

Biotech companies have acknowledged that glyphosate-resistant weeds create increasing control problems. Monsanto (2009a), Syngenta (2009) and the Glyphosate Stewardship Working Group (2009) have set up web sites, where farmers can acquire information about resistance management strategies and herbicide solutions. To lower the potential for new glyphosate resistance to occur, Monsanto recommends that growers start with clean fields, scout fields before and after herbicide application, add other herbicides and cultural practices, prevent weeds from setting seeds, clean equipment before moving from field to field, and use new commercial seed free from weed seeds (Gustafson 2008). As an additional method for adding other herbicides into a continuous RR system, rotating to other RR crops was suggested. Monsanto scientists argue also for a high-dose-strategy, so that the weeds with low resistance levels are destroyed (Sammons et al. 2007). To withstand higher doses without damage, soybeans with a higher resistance to glyphosate are being developed (Service 2007).

Use of multiple herbicide-resistant crops has been advocated as a new solution to herbicide resistance in weeds, e.g. the introduction of resistance to dicamba into soybean (Behrens et al. 2007). Biotech companies expect that the era of the single herbicide resistance trait will soon be over, replaced by stacked traits conferring resistance not only to glyphosate, but also to other active ingredients, such as glufosinate, ALS inhibitors, ACCase inhibitors, synthetic auxins, and others (Green 2009, Green et al. 2008). Monsanto (2009b) has announced that “SmartStax” corn, combining resistance genes to glyphosate and glufosinate, together with six Bt insecticidal toxin genes, will be commercialized in 2010. But this process might result in a “transgenic treadmill”, as Binimelis et al. (2009) called it, an extension of the pesticide treadmill.

6.3 Conclusions on resistance management

Weeds resistant to glyphosate have evolved rapidly since the late nineties. Controlling them by herbicides becomes increasingly difficult and leads to higher costs. Farmers in the Americas apparently are reluctant to adhere to resistance management strategies proposed by weed experts. But it becomes apparent that only diversity on the field, less reliance on chemical weed control and a combination of different approaches to control weeds will lead to sustainable crop production.

7 Overall conclusions

Research shows that glyphosate and glyphosate-based herbicides directly affect soil and aquatic organisms and plant health. They enhance, for instance, certain fungal crop diseases and endanger amphibians. As shown by the only large scale and long-term experiment on biodiversity effects which included an eligible set of methods, field sites and indicators (FSE tests in the UK), even the lower application rates and amounts of a.i. in the glyphosate spraying regimes had more negative impacts on biodiversity than the conventional spraying regime with several herbicide products. These impacts were predominantly indirect ones due to the reduction of the agricultural flora including their soil seed banks, which has consequences for the whole food web. Therefore it can be concluded that net (direct and indirect) biodiversity effects of growing glyphosate resistant varieties are negative.

Weed control systems should be developed which do not further decrease wild plant abundance and particularly not of open flowering non-target weeds.

Field tests as done in the UK are extremely costly. And as there is no connection between the treatment frequency index (TFI) and the degree of effectiveness (Bruns & Märländer 2006, Gutsche et al. 2002), which leads to net biodiversity losses, we need to find a better and less expensive way of measuring biodiversity impacts (Nistrup Jorgensen pers. communication 2009). Any evaluation predominantly based on artificially measured direct toxic effects and omitting indirect effects of the exclusion of plants lacks of sound science. Furthermore, current toxicological assessments did not take into account more recent findings on toxicological effects of glyphosate and POEA (surfactant) on aquatic organisms, leaching of AMPA, effects on soil microorganisms, incidence of disease and on endocrine systems of mammals. There is also no consensus on the choice of indicators for these tests and the degree of their relevance.

The emission of greenhouse gases, the ozone depleting potential, airborne nitrification and pollution of air, soil, and water were slightly lower for the production system of glyphosate resistant beet. This result was found for the current application pattern in glyphosate resistant beet. A review of resistance management measures in glyphosate resistant varieties and findings on changes of the weed flora under conservation tillage suggest that applications may increase after some years.

Conservation tillage in conventional and in glyphosate resistant beet can help to reduce emissions and erosion, but also likely leads to critical changes in the arable flora resulting in biodiversity losses.

It becomes apparent that only diversity on the field, less reliance on chemical weed control and a combination of different approaches to control weeds will lead to sustainable crop production.

Open questions

Yields seem to be similar independent from the use of glyphosate or conventional herbicides but data are not sufficient for a serious comparison.

The effects of spraying regimes in tilled herbicide resistant sugar beet on erosion have not yet been studied in field. Also, C sequestration by no-till is yet to be proved by deep soil samples. Another question is how to implement seeding operations which avoid crushing nests.

Also, the effects of using broad-spectrum herbicides in minimum-tillage systems on wild plants (and their soil seedbanks) are not well understood. According to DEFRA (2001), the challenge is to develop weed management systems which allow biodiversity to be maintained in the crop. One important step is to further assess the biodiversity importance of common weeds (DEFRA 2001). Selective herbicides or selective and spatial spraying techniques may be a solution. Pidgeon et al. (2007) suggested that 4% of the fields should be left unsprayed in glyphosate resistant varieties to mitigate biodiversity losses. However, this practice is not realistic without incentives for farmers.

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