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Scale Implications for Environmental Risk Assessment and Monitoring of the Cultivation of Genetically Modified Herbicide-Resistant Sugar Beet: A Review

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Abstract

Genetically modified herbicide-resistant (GMHR) sugar beet (*Beta vulgaris* L.) has been cultivated in the US for several years and an application has been submitted for cultivation in Europe. Concerns have been raised about how GMHR sugar beet cultivation might impair the agro-environment.

European legislation for GM plants requires, prior to their commercial import and/or cultivation, a stepwise reduction of the containment and a gradual increase in the scale of release. Experimental results gained during this procedure enter an environmental risk assessment; after the GM plant approval, a systematic monitoring of potential adverse environmental effects is required.

We collected information on sugar beet biology and cultivation and the HR technology. We categorised the literature findings, evaluated the evidence of agro-environmental effects and indicated adverse effects. The impacts are directly and indirectly linked to sugar beet biology and/or to the HR technology. Most likely are a) adverse herbicide effects on field organisms, aquatic communities and soil microbial communities, b) persistence of the GM plant triggered by a potential selective advantage and/or genetic drift after hybridisation of GMHR cultivated, feral and weed beet with neighbouring beets and wild relatives, c) the increase of HR in weeds and subsequent increase and/or change in the herbicide application regime after several years of glyphosate application, and d) decline in agrobiodiversity (weed communities, herbivores, pollinators and beneficial species).

Our study reveals a lack of experimental data on potential agro-environmental effects. This suggests that the principle of a stepwise scale increase of release is inadequately applied to the GMHR sugar beet approval process. The adverse effects identified should prompt further research experiments to gain information for the ERA and/or specific monitoring activities at the respective identified spatial scale levels.

Keywords: Sugar beet, Genetically modified herbicide resistance, Risk assessment, Monitoring, Spatial scales

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1 Introduction

Genetically modified herbicide-resistant (GMHR) sugar beet (*Beta vulgaris* L.) was deregulated in 2006 and introduced to the US and Canadian market in 2007. It was rapidly adopted by farmers and now accounts for nearly 95 percent of the acreage of all sugar beets produced in the US. The GMHR sugar beet is resistant to the herbicide active ingredient glyphosate. An application for cultivation of the variety H7-1 was made in Europe in 2008 and is currently in the approval process under Directive 2001/18/EC (European Commission, 2001). This regulatory framework specifies a systematic environmental risk assessment (ERA) and mandatory post-market environmental monitoring (PMEM) after approval. This is designed to handle uncertainties about potential adverse environmental effects still remaining after the ERA, which is primarily based on short-term and field-scale releases of the GM crops.

Various public and scientific concerns have been raised both in the US and in the EU about potential adverse agro-environmental effects of widespread GMHR sugar beet cultivation, leading to its provisional ban in some US states (Burkett, 2010). The concerns include cross-pollination with both conventional non-GM and organic *Beta vulgaris* varieties cultivated for sugar or for seed production (Lange *et al.*, 1999; OECD, 2006), thereby reducing or even destroying their market value. Hybridisation with and gene flow to related wild species within the *Beta* section may occur (Bartsch *et al.*, 2003; Sukopp *et al.*, 2005), forming normally vigorous HR hybrids that may spread in the environment. The modified herbicide application regime with glyphosate includes multiple changes in cultivation practice (Graef, 2009) that may adversely impact the agro-environment and cause negative weed flora changes (Heard *et al.*, 2003b,a; Roy *et al.*, 2003), direct toxic environmental effects (Benachour and Séralini, 2009; Brausch and Smith, 2007; Relyea, 2005a,b,c), or the development of weed resistance through selection pressure (Heap, 2009; Zelaya *et al.*, 2007). The concerns cover a wide range of environmental, agronomic, economic, and social aspects which are often partly interlinked, but our review focuses primarily on potential environmental and agronomic aspects of GMHR sugar beet cultivation.

Currently, the only GM crops approved for cultivation purposes in the EU are the Bt-Maize MON 810, which has been cultivated primarily in Spain, and the Amflora potato, which was approved in early March 2010. Despite their regulation under Directive 2001/18/EC (European Commission, 2001) and under the Supplementing Guidance Notes (European Commission, 2002), both the general approaches to ERA and the PMEM of GM crops are under continuing scientific debate. The quality and quantity of data provided on the ERA process presently does not satisfy scientific and technical standards (Dolezel *et al.*, 2009). Moreover, the monitoring plans submitted and the monitoring reports presented by the applicants (BVL, 2007) still lack a profound science-based design. More general guidance on the ERA was developed by EFSA (2008). PMEM guidance was presented by ACRE (2004), more sharply delimiting the different monitoring intensity categories of general surveillance and case-specific monitoring.

Various additional scientific aspects have been developed and outlined in the last few years. These include a more targeted ERA involving well-defined hypotheses, precisely defined and prioritized hazards and quantifying elements of exposure (Andow and Hilbeck, 2004; Johnson *et al.*, 2007; Wilkinson *et al.*, 2003), the selection of test species for the risk assessment for non-target organisms (Hilbeck *et al.*, 2008a), the selection of indicator organisms for the PMEM (Hilbeck *et al.*, 2008b), systematic approaches for landscape-scale or ecoregion-based PMEM (Graef *et al.*, 2005a,b), a science-based systematic step-by-step approach in PMEM (Züghart *et al.*, 2008), the enhanced quality of statistics required for the ERA (Lövei and Arpaia, 2005; Perry *et al.*, 2009), and a proposal for the definition of environmental damage (Bartz *et al.*, 2009). Nonetheless, open questions and shortcomings in the present ERA and monitoring practice remain. Key issues to be further tackled are

- improvements in guidance and standardization of risk assessment methodology, e.g., guidance on selecting representative locations for the assessment of agronomic and environmental behaviour of a particular GM crop, on the details of field trial designs, and on the risk assessment of long-term and cumulative effects (Dolezel *et al.*, 2009),
- normative indications and thresholds for ecological hazards and damages associated with GM crops (Bartz *et al.*, 2009; Breckling *et al.*, 2009; Regal, 1994),
- the necessary field test and PMEM design required to yield scientifically sound data, (De Jong, 2010; Graef *et al.*, 2005b; Lövei and Arpaia, 2005; Perry *et al.*, 2009; Züghart *et al.*, 2008)
- the ERA and monitoring data management and technical implementation using structured databases (Reuter *et al.*, 2010a),
- the methodology of the ERA and monitoring of GM crops with multiple stacked transgenic events within one crop (De Schrijver *et al.*, 2007),
- the methodology of data upscaling and interpretation as more field testing and monitoring data become available with more widely spread GM crop cultivation (ACRE, 2004; Breckling *et al.*, 2009; Squire *et al.*, 2009),
- the monitoring data coordination and harmonisation at national and/or EU levels (Finck *et al.*, 2006; Graef *et al.*, 2008),
- the applicability and use of existing national and/or EU environmental monitoring programmes and data infrastructure schemes for genetically modified organism (GMO) monitoring (Graef *et al.*, 2005b; EU Monitoring Working Group, 2010).

The uncertainty connected to these key issues is also reflected in the often contradictory comments of EU member state experts during GM crop approval processes. Research is underway to tackle some of these shortcomings, for instance in national research programmes or as part of the Framework Programme on research by the European Commission.

So far, environmental risk-related data on GM plants is mainly concentrated on the lower levels of spatial extension such as molecular detection, laboratory trials, and short-term greenhouse or field studies to assess effects on the population level. Experiments on larger landscape scales are sparse, the most prominent being the Farm Scale Evaluations in the UK (Firbank *et al.*, 2003). The usual ERA practice is to analyse and assess the greenhouse and field-scale results and extrapolate them to the European scale of (future) crop cultivation, potentially entailing large inference errors. Extrapolating ecological effects of GM crops from field scale to larger landscape scales, however, requires an up-scaling approach based on reliable data on various scales of GM exposition. This has been demonstrated in a special issue of the journal “Ecological Indicators” with GM oilseed rape (*Brassica napus* L.) (Breckling *et al.*, 2009; Middelhoff *et al.*, 2010; Reuter *et al.*, 2010b). Many of those results are generally valid for other GM crops such as for GMHR sugar beet: it has certain biological features in common with oilseed rape, for instance wind-pollination, hybridisation with wild relatives, and persistent seeds in the soil.

It is important mentioning that not only GM crop but also the nonGM crop cultivation may entail various environmental effects that can occur on different spatial and temporal scales. Furthermore, the ecological importance of environmental effects is difficult to determine and may vary depending on the type of effect. Little focus has been placed on ERA and PMEM of sugar beet. This literature review is designed to identify likely adverse effects of GMHR sugar beet cultivation at the various spatio-temporal scales relevant for the ERA and the PMEM. The key question is whether experimental greenhouse- or field-scale-based data on specific potential adverse effects of

GMHR sugar beet cultivation are scientifically-based and sufficient to be upscaled to larger areas such as landscapes. We thus identify the results and shortcomings of small- (field-) scale findings and indicate whether they enable inferring the outcomes at larger scales and on required PMEM.

In the context of this paper the term 'field organism' is defined as all organisms living in or visiting the field and its margins, such as plants, epigeic and endogeic invertebrates, birds, mammals and amphibians. The term 'herbicide resistance' is applied according to the WSSA (Weed Science Society of America) definition as the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type. In plants, resistance may be naturally occurring or induced by techniques such as genetic engineering or selection of variants produced by tissue culture or mutagenesis. The term 'agro-environment' is used here as the cultivated area along with neighbouring fields and biotopes. The different terms for scale levels used in this review are a) 'laboratory scale', b) 'greenhouse scale', c) 'field scale', which applies to pre-commercial experimental and large-scale field trials (European Commission, 2002) that are limited in number, their extension, and the duration of observations, and d) 'landscape scale'. The latter is the commercial GM plant cultivation scale and may range from smaller ecoregions, for instance at the 1:50,000 scale (Graef *et al.*, 2005b), to larger climatic regions or ecoregions (Bailey, 2002; EFSA, 2008) or even (bio)geographical regions at the national or European scale (Eiden *et al.*, 2000; Metzger *et al.*, 2005).

2 Legal framework

In accordance with the precautionary principle, the Directive 2001/18/EC (European Commission, 2001) regulates the releases, imports and cultivation of GM crops, applying a stepwise and systematic case-by-case assessment of the risks to human health and to the environment. Annex III B of the Directive 2001/18/EC specifies that the ERA shall encompass an evaluation of a) biological features of the parental plants such as reproduction, dissemination, survivability and geographical distribution, b) details of their genetic modification, c) harmful effects on human or animal health arising from the GM food/feed, d) interactions of the GM plant with the biotic and abiotic environment, and e) impacts of the specific cultivation, management and harvesting techniques. Important elements of this ERA are ecotoxicological tests at the research and development stage, investigating adverse effects of a GM plant in ecosystems that could be affected (Preamble 25 of Directive 2001/18/EC). It has to be demonstrated at each level that the risk for the environment is zero or negligible. In terms of duration and scales of release (Figure 1) the Directive distinguishes between a) experiments in the contained use system, b) deliberate release for experimental purposes, and c) release onto the market including cultivation.

Preamble 24 of the Directive 2001/18/EC recommends that "the introduction of GMOs into the environment should be carried out according to the 'step by step' principle." This means that the containment of GMOs is reduced and the scale of release increased gradually, step by step, but only if evaluation of the earlier steps in terms of protection of human health and the environment indicates that the next step can be taken. Risk-related research should thus be carried out initially in laboratories and greenhouses and then be followed by release-related research and monitoring. In premarket field trials, field plots are limited in time, number and space.

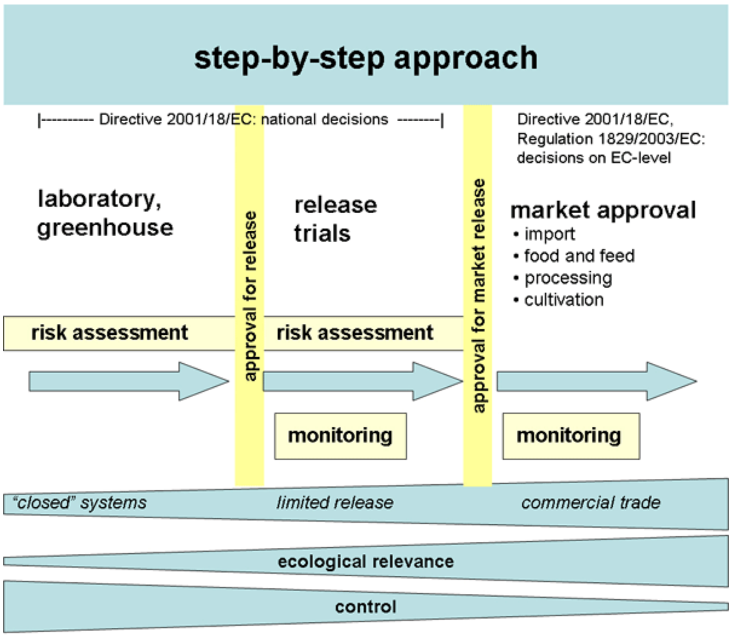

Figure 1: 'Step by step' principle for the release of GMOs (Züghart *et al.*, 2008, modified).

In regulatory practice the step-by-step principle is often not followed. Especially the field testing of GMO in those ecosystems that could be affected in the case of commercial release is incomplete and, if done, the parameters assessed are mostly of agricultural and rarely of environmental value (Dolezel *et al.*, 2009).

For market-approved GMOs the Directive distinguishes (a) a case-specific monitoring (CSM) which focuses on direct and indirect, immediate and delayed potential effects on human health and the environment, identified in the preceding ERA process, and which is limited to a specified time period in which to obtain results and (b) a general surveillance (GS) that aims to identify and record indirect, delayed and/or cumulative adverse effects that have NOT been anticipated in a preceding ERA. In science, regulatory and monitoring practice, full non-anticipation for GS is not feasible. This is because, for any GS activity, there automatically is an effect hypothesis. In contrast to CSM, general surveillance should aim at identifying unforeseen and long-term effects and therefore be conducted over a longer time period and possibly wider area. The general definitions of CSM and GS leave some room for interpretation because cumulative effects, for example, may be either anticipated (then inducing CSM) or unforeseen (leading to GS). There are gradual differences in predictability among the effects. For instance, local effects on cropland can be more easily assessed than effects beyond cropland and on larger scales.

The monitoring results of marketed GMOs contribute to decisions regarding approval or additional precautions, and can enhance the certitude of prognosis for a future ERA. GM crop monitoring is intended to serve as an early warning system to react in case of reported adverse effects and then help in the decision-making process about countermeasures.

Once environmental changes are identified, it is essential to determine whether they are harmful or not. Adverse changes cannot always be attributed to a GMP because there are numerous influencing environmental and agricultural practice covariables (Graef, 2009; Hails, 2002; Stein and Ettema, 2003). If harmful, more in-depth studies are envisaged in order to detect causal relationships. In case of a relationship between a GMP and an adverse effect, measures to avoid or minimise effects must be taken. At the same time a new ERA is required. The subsequent results are the basis for decisions on extending GMP approvals, withdrawal of approval, modified risk management, and adaptations of the monitoring plan.

In the case of GMHR crops, there is an overlapping of competencies between the pesticide Directive 91/414/EEC (European Commission, 1991) and the Directive 2001/18/EC on the deliberate release of GMOs. Direct herbicide effects such as for glyphosate are regulated by Directive 91/414/EEC, but adverse effects due to a GMHR plant are not covered by the pesticide Directive. The European Commission therefore recommended that ERA and monitoring of herbicide effects of GMHR crop cultivation, as compared to conventional varieties, be done in the framework of Directive 2001/18/EC (DOC NR ENV/03/23). Some of the adverse effects discussed in the following will thus also fall into the remit of the pesticide Directive.

As outlined by Bartsch *et al.* (2009), the Directive 2004/35/EC on environmental liability specifically includes the handling and cultivation of GMO that potentially may cause environmental damage such as to protected species and natural habitats under Directive 92/43/EC (Natura 2000 habitats). Any damage that has significant adverse effects on reaching or maintaining the favourable conservation status of these legally defined protection goals must therefore be avoided. Some EU states and/or regions are therefore imposing cultivation bans for Bt-Maize and GM crop release restrictions for field tests within up to 1 km distance from Natura 2000 sites. BfN (2010) is hosting a mapping service in Germany to conform to these distance regulations.

3 The sugar beet biology, geographical distribution and gene flow

The genus *Beta* is classified into four genetically differing sections, namely Beta, Corollinae, *Nanae* and *Procumbentes* (Lange *et al.*, 1999; Frese, 1998). The species *B. vulgaris* ssp. *vulgaris* comprises the cultivated forms sugar beet (var. *altissima*), fodder beet (var. *crassa*), Swiss chard (var. *vulgaris*) and red beet (var. *conditiva*). The sugar beet has cultivar, wild and weed forms. Wild beet species are quite common among the Beta section, comprising the subspecies *Vulgaris, Maritima, Adanensis* and the closely related species *B. patula* and *B. macrocarpa.* They occur along the coasts of northern and western Europe and in the Mediterranean area including north-west Africa and the Canary Islands, the Balkans, the Caucasus and from Asia Minor to Bangladesh (Table 1) (Frese, 1998).

Sugar beets are most often self-incompatible and usually wind-pollinated, although insect pollination is also possible (Bartsch *et al.*, 1999). Sugar beet hybridises easily with cultivated and wild forms of *Beta vulgaris*. It has normally vigorous and fertile progeny without incompatibilities with members within the Beta section (OECD, 2006). Natural interspecific hybridisation between the species of section Beta is also possible, but is very unlikely with species of the other three Beta sections. If hybridisation between *B. vulgaris* and other sections occurs either naturally or through artificial techniques, the resulting progeny is not viable and does not reach the generative phase (Van Geyt *et al.*, 1990). Thus, a gene transfer from cultivated beets to wild beets is likely only for the Beta section.

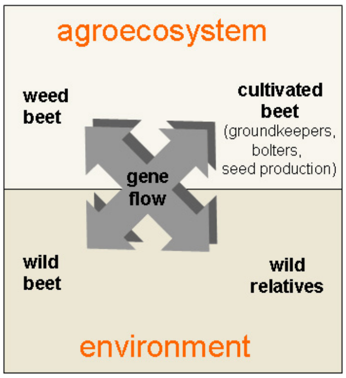

Figure 2: Intra- and interspecific gene flow of cultivated, weedy, wild sugar beet and related wild species within and between agricultural fields and the environment (Bartsch *et al.*, 2003, modified).

Sugar beets are biennial plants forming a beet in their first season. If the beets are not harvested, flowering shoots appear in the second year after vernalisation. However, the formation of flowering shoots and completion of the whole life cycle in the first year is possible (bolters) (probability $< 0.05\%$) due to the genetic constitution of the plants and/or certain weather conditions such as drought or frost (Keller *et al.*, 1999; Geisler, 1980). In practice, bolters are usually removed or destroyed before flowering. Seeds from bolters that have not been removed before seed maturity fall off and pass into the soil seed bank. From these seeds, weed beets can emerge within and between rows in the crop stand in the following years (May, 2009). The seeds can have a life span of over 10 years, but are depleted with time or may germinate under favourable conditions. Small beets or beet sections left in the field after harvesting can regenerate (groundkeepers) (Elliott and Weston, 1993) depending on the part of the plant, size, depth of placement, survival ratio, and management of the following crop (Buddemeyer and Petersen, 2002). Bolters and volunteers in some years with special conditions can become overwhelming, hindering their management or eradication by farmers. Hence, gene flow via seed, pollen or clonal plant parts within and between agroecosystem and the environment is possible in various ways and directions (Figure 2).

Table 1: Biological properties relevant to the ERA and PMEM across different spatial scale levels.

Biological properties of *Beta vulgaris* L.	**Chain of potential agro-environmental effects**	**References**[1]			**Evidence**[2]
		laboratory or greenhouse experiments	field trials or observations	landscape-scale experiments or observations	
widespread geographical distribution of *Beta vulgaris* L., wild beets and bastards in some EU regions	→ potential of HR sugar beet to hybridise with neighbouring cultivated, feral and weed beets	1	1, 8	1, 2, 3	high
widespread geographical distribution of related wild species of the genus *Beta* L.	→ potential of HR sugar beet to hybridise with wild relatives			3, 4, 13	high
formation of bolters	→ in case of flowering: hybridisation of HR sugar beet with neighbouring cultivated, feral and weed beets, and with related species → seed production	2, 5, 6,10, 11	2, 5, 6, 7, 8, 10, 11, 14, 15, 16, 31, 32	2, 7, 8, 9, 10, 11, 12, 20, 27	high
regeneration of vegetative plant residues for the next season (groundkeepers)	→ in case of flowering: hybridisation of HR sugar beet with neighbouring cultivated, weed and wild beets, and with related species → seed production	2, 5, 6, 18	2, 5, 6, 7, 8, 14, 17, 18, 25, 26, 31, 32	7, 8, 9, 12, 20, 27,	high
stable weed beet populations	→ increase of HR seed bank, → hybridisation with neighbouring cultivated and/or wild relatives → in case of a selective advantage and/or genetic drift: HR sugar beet and related species may persist and become invasive		12, 14, 16, 19, 25, 26; 31, 32	19, 20, 30	high
	→ unpredictable combinatory effects with cultivated and/or wild relatives		23		low
	→ adverse effects on neighbouring habitats such as ecosystem food chains; impacts on biodiversity		24	28	low
horizontal gene transfer	→ transgenes may be transferred to other species such as bacteria	21, 22, 29			medium

[1]**References legend (E: Expert opinions; M: Models; R: Review; O: Original data):** 1 (Drießen *et al.*, 2001 / O, R); 2 (OECD, 2006 / R); 3 (Frese, 1998 / R); 4 (Frese *et al.*, 2001 / O, R); 5 (Van Geyt *et al.*, 1990 / R); 6 (Bartsch and Schmidt, 1997 / O); 7 (Bartsch *et al.*, 1999 / O); 8 (Bartsch *et al.*, 2003 / O, R); 9 (Sukopp *et al.*, 2005 / O, R); 10 (Keller *et al.*, 1999 / R); 11 (Geisler, 1980 / R); 12 (Beckie, 2006 / E); 13 (De Bock, 1986 / R); 14 (May, 2009 / E); 15 (Lehnert, 2007 / E); 16 (Viard *et al.*, 2002 / O); 17 (Elliott and Weston, 1993 / E); 18 (Buddemeyer and Petersen, 2002 / O); 19 (Soukup *et al.*, 2002); 20 (Desplanque *et al.*, 2002 / O, E); 21 (Heinemann and Traavik, 2004 / O); 22 (Nielsen and Townsend, 2004 / O); 23 (Pessel *et al.*, 2001); 24 (Watkinson *et al.*, 2000 / M, E); 25 (Märländer *et al.*, 2010 / R); 26 (Viard *et al.*, 2002); 27 (Boudry *et al.*, 1993); 28 (Züghart and Breckling, 2003 / R); 29 (Rensing *et al.*, 2002 / O); 30 (Cerdeira and Duke, 2006 / R); 31 (Arnaud *et al.*, 2003 / O); 32 (Arnaud *et al.*, 2009 / O)

[2]Evidence among the references based on the data quality aspects a) how closely the measured or observed features, effects and indicators resemble the actual features, effects and indicators about which information is desired; b) quality, mode and accuracy of the methodological design and the degree to which empirical or expert observations have been used to produce the data; c) statistical design, number of replications, spatio-temporal representativeness (Graef, 2009)

4 HR technology with GM sugar beet and agricultural practice changes

Experience with GMHR sugar beet cultivation is limited to the US and Canada, where this GM plant has been grown since 2007. Nonetheless, some conclusions can also be drawn from the long-term cultivation of GMHR oilseed rape (Graef, 2009). The introduction of GMHR sugar beet cultivation and its HR technology in Europe will alter existing cropping systems and lead to various practice changes (Benbrook, 2009; Champion *et al.*, 2003) that may entail agro-environmental effects (Table 2). Locally, GMHR sugar beet cultivation may be expanded to areas that, due to weed pressure, were less suitable for cultivation before.

Herbicide application pattern: In HR sugar beet cultivation, only the broad-spectrum herbicide glyphosate is applied, usually first at the post-emergence stage and second until 60 – 70% canopy closure. This makes timing more flexible and simplifies weed control (Champion *et al.*, 2003). In conventional agriculture, usually three to four (up to six) herbicide applications are done, with glyphosate often applied at the pre-seeding or pre-emergent stage to clear fields and postharvest for volunteer control (Märländer, 2005; Schütte and Mertens, 2010), while other herbicides are applied during crop development. With HR sugar beet cultivation, the aim is also to reduce the active ingredient (ai) amount and the number of herbicides. For the case of GMHR oilseed rape in the US and Canada, this holds true only for the first years of cultivation (Champion *et al.*, 2003; Benbrook, 2009).

Similarities between sugar beet and oilseed rape rotations (break crops in cereal-dominated rotations typically grown one year in every three, four or five years) and biology (hybridisability, volunteer occurrence, wild relatives) allow changes in agricultural practice to be postulated after years of continued HR technology: a) weeds may become herbicide-tolerant through selection pressure and adaptation, especially if different HR crops resistant to glyphosate are cultivated in the same rotation (Beckie *et al.*, 2006; Owen and Zelaya, 2005), b) the composition of weed communities and their seed bank will change (Heard *et al.*, 2003a; Owen and Zelaya, 2005), and consequently, c) ai rates, application frequencies, and numbers of ai may increase again, particularly in low-disturbance seeding systems (Senior and Dale, 2002).

Gene flow and volunteers: Not eradicating sugar beet bolters and groundkeepers before flowering may lead to gene flow of the HR trait and to seed dispersal, although at rates far below those encountered with oilseed rape, for example, which regularly flowers and produces seeds. HR volunteers may occur in subsequent rotations when seeds of bolters and groundkeepers fall to the ground (Bartsch *et al.*, 2003; Keller *et al.*, 1999). HR weedy relatives or interspecific hybrids (Arnaud *et al.*, 2003; Frese *et al.*, 2001) may evolve due to pollen-mediated gene flow from HR bolters and HR groundkeepers. HR volunteers may also evolve in non-HR sugar beet fields due to pollen-mediated gene flow from flowering HR bolters and HR groundkeepers to neighbouring sugar beet fields, and also due to neighbouring volunteers from sugar beet seed banks (Desplanque *et al.*, 2002; Viard *et al.*, 2002). When neighbouring sugar beets with other HR traits are cultivated, multiple HR may develop in weed beet; selective advantage then maintains this, as evident in North America with GMHR oilseed rape (Beckie *et al.*, 2006; Orson, 2002).

In sugar beet seed production areas, seed purity standards include minimum distances between fields to avoid cross-pollination (Märländer *et al.*, 2010). Nonetheless, outcrossing into wild relatives may occur, also because the amount of GMHR sugar beet pollen is likely to be higher than that of neighbouring wild relatives.

Tillage and rotation system: GMHR sugar beet facilitates the use of enhanced crop cover and no-tillage or reduced-tillage. This, in turn, minimizes weed pressure and soil compaction, prevents soil erosion and promotes soil bioactivity (Agronomy Guide, 2010; Thorbek and Bilde, 2004). If HR sugar beet weeds and volunteers in the follow crops develop as a result of flowering bolters and/or groundkeepers, the necessary control may trigger more intensive tillage and/or may require wider rotations or crops with other HR traits. Since seeds may persist for years in the soil, returning to a conventional sugar beet in the crop rotation may become difficult due to HR volunteers and their admixture in the harvest.

Coexistence: Avoiding GM material presence in non-GM crop production practice may require changes in GMHR sugar beet cultivation (European Commission, 2003). Normal farming practice involves preventing flowering and thus reducing vertical gene flow to neighbouring fields. Depending on various factors, flowering bolters and/or groundkeepers can sometimes be encountered; they act as pollen donators or acceptors, producing HR seeds. Despite the potentially low rate of gene flow to avoid contamination of non-GMHR sugar beet fields and potential HR seed production, this calls for coexistence measures such as isolating fields of GM sugar beet, introducing isolation distances, and sowing and harvesting at a modified time schedule, preferably using other varieties (Schiemann, 2003). In sugar beet seed production areas, flowering is necessary; to guarantee seed purity standards, seed companies have introduced various temporal and spatial isolation measures.

5 Direct and indirect effects on the agro-environment

The biological features, combined with the HR technology, as shown above, may entail direct, indirect, immediate, delayed and/or cumulative agro-environmental effects (Table 1 and 2).

Effects on the agro-environment may be induced by single or several different mechanisms; these may work singly or cumulatively (Graef, 2009). The agro-environmental effects may be detected at a single scale or at multiple levels.

Section 6 discusses whether the effects are considered to be adverse, positive, not relevant or relevant for monitoring, whether they require further studies for the ERA or raise the concern of an environmental risk leading to deny approval, or whether they may constitute an environmental damage (Bartz *et al.*, 2009) that merits withdrawal of approval.

Effects directly or indirectly linked to sugar beet biology: Bolting, formation of groundkeepers, along with the high rate and distance of pollen spread and cross-pollination (Drießen *et al.*, 2001; OECD, 2006) may trigger HR gene flow to neighbouring non-GM sugar beet, weed beets, wild beets and related wild species. The preconditions are spatial concurrence (Frese, 1998) and inefficient prevention of flowering by agricultural practice.

The potential increase of HR sugar beet in the seed banks, which may incorporate viable HR seeds over 10 years, may lead to persisting and invasive HR weed beets, wild beets and related wild species in fields and natural habitats. The preconditions: a selection advantage due to repeated glyphosate application, enhanced fitness parameters and genetic drift. Wilkinson *et al.* (2000) and Snow (2003) observed this for HR oilseed rape. Cultivated beet genes can persist in wild beet (Bartsch *et al.*, 1999; Sukopp *et al.*, 2005). If HR weed beets, wild beets and related wild species become invasive, this may variously impact habitats, food chains and biodiversity (Watkinson *et al.*, 2000; Züghart and Breckling, 2003).

A horizontal gene transfer of HR from plant residues to soil microorganisms is rare but possible, irrespective of the HR trait (Heinemann and Traavik, 2004; Nielsen and Townsend, 2004), but its environmental implications are hard to determine. Another general ecological concern is the potential for a) adverse combinatory effects when GMHR sugar beet hybridises with weed beets, wild beets and related wild species that potentially affect the hybrid's biology and/or herbivores and b) pleiotropic and epigenetic genome effects of the GM crop caused by the genetic modification procedure, as shown for instance with GM wheat (Zeller *et al.*, 2010). These unintended and unanticipated effects, for instance on non-target organisms (NTOs), must be considered, especially when performing the ERA (GMO Panel, 2010).

Effects directly or indirectly linked to the HR technology: More efficient weed suppression leads to less biomass, food and flowers for field organisms after spraying. This, in turn, entails lower abundances of various herbivores, pollinators and beneficial species (pest antagonists) (Bohan *et al.*, 2005; Heard *et al.*, 2003b), and may decrease agrobiodiversity (Watkinson *et al.*, 2000). A shift in weedy species (Heard *et al.*, 2003b,a) and an increase of perennial weeds due to minimum-till practice is likely (Frick and Thomas, 1992). Extending the HR technology may have various potential effects on field organisms and soil bio-geochemical cycles on the larger landscape scale (Benton *et al.*, 2002; Cerdeira and Duke, 2006). Another ecological concern is possible adverse indirect effects on migratory and mobile species, leaf litter quality, crop competitiveness, and insect resistance (Squire *et al.*, 2003).

Applying glyphosate formulations compared to other herbicides has adverse effects on fields and neighbouring habitats. These include increased mortality of amphibians (Relyea, 2005a,b,c) and mammals (Richard *et al.*, 2005; Benachour and Séralini, 2009) and, in combination with simultaneous exposure to parasites, reduced survival of freshwater fish (Kelly *et al.*, 2010). Adverse direct or indirect glyphosate effects were also reported for micronutrient uptake (Tesfamariam *et al.*, 2009), soil microflora and plant disease severity (Fernandez *et al.*, 2009; Heuer *et al.*, 2002; Johal and Huber, 2009; Kremer and Means, 2009). This can hamper soil functions or bio-geochemical cycles (Züghart and Breckling, 2003). Glyphosate formulations containing surfactants such as POEA (polyethoxylated tallow amine) are more toxic than the ai glyphosate alone (Benachour and Séralini, 2009), in particular for aquatic organisms (Brausch and Smith, 2007). Some studies also indicate less herbicide toxicity and persistency than other herbicides (Agronomy Guide, 2010; Cerdeira and Duke, 2006; Squire *et al.*, 2003).

Until post-emergent spraying, more biomass is available for feeding organisms (Werner *et al.*, 2000; Strandberg *et al.*, 2005). After spraying, however, biomass drops compared to conventional spraying. Furthermore, spraying is usually done before weed seed development (Dewar *et al.*, 2000), reducing the weed seed bank in the long term. Early band applications combined with late overall treatments may enhance biomass availability during the growing period (May *et al.*, 2005). Nonetheless, seed development and abundance in arable flora are also reduced in the long run. Post-emergent spraying may increase herbicide drift into the agro-environment, for example due to increased spraying height (Johnson, 2001). Post-emergent spraying also often entails a change in spray schedules of insecticides and fungicides, with potential implications for microbial and faunal activity (Champion *et al.*, 2003; Thorbek and Bilde, 2004).

To control possible HR sugar beet volunteers in follow crops, modified crop rotations may be necessary. This may require farmers to change the tillage system (Schütte *et al.*, 2004), affecting field organisms and soil bio-geochemical cycles (McLaughlin and Mineau, 1995; Orson, 2002). It may also require more ai, different types of herbicides or higher spraying frequency to control HR in weeds (Van Acker *et al.*, 2003). Again, this can impact agrobiodiversity. Coexistence measures to reduce gene flow may change agricultural practice and entail various environmentally relevant effects.

Table 2: Potential agro-enviromental effects across spatial scale levels linked to the HR technology and relevant to the ERA and PMEM (Graef, 2009, modified).

Practice changes	**Chain of potential agro-environmental effects**	**References**[1]			**Evidence**[2]
		laboratory or greenhouse experiments	field trials or observations	landscape-scale experiments or observations	
introduction of HR technology	increased weed suppression → less biomass, food, flowers and habitats for field organisms after spraying → lower abundance of various herbivores, pollinators and beneficial species (pest antagonists) → decrease in agrobiodiversity		20, 25	3, 6, 7, 9, 28, 33, 36, 37, 38	high
	development of herbicide tolerance in weeds			3, 4, 9, 16, 35, 44	high
	reduced crop rotation options			3, 9,	medium
	decrease and/or shift of weedy species and weed seedbank			4, 16, 28, 33, 35, 36, 37, 38, 44	high

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Table 2 – *Continued*

Practice changes	**Chain of potential agro-environmental effects**	**References**[1]			**Evidence**[2]
		laboratory or greenhouse experiments	field trials or observations	landscape-scale experiments or observations	
	little or no evidence: impact on migratory and mobile species, changed quality of plant parts, changed crop competitiveness, changed insect resistance, pleiotropic and epigenetic genome effects, impact on soil functions	46	20, 23	4, 21, 22, 36	low
→ reduced herbicide ai amount, reduced no. of spray rounds, use of glyphosate only	less negative impacts on field organisms and/or soil compaction		1, 23, 25	2, 3, 12, 13	high
→ higher herbicide & insecticide applications in formerly not cultivated areas	various adverse effects on field and aquatic organisms and/or soil bio-geochemical cycles	43		3, 22, 23, 29, 31	high
→ glyphosate use instead of other more persistent or toxic herbicides	less residual activity to followcrops, less adverse effects on field organisms		46	2, 4, 8	medium
→ glyphosate use instead of other less toxic herbicides	adverse effects on field organisms and/or aquatic communities in cropped fields and neighbouring habitats, higher glyphosate concentrations in surface and ground waters	26, 40		4, 22, 36, 41	high
	adverse effects on fungal communities, diseases and nutrient availability	10	10, 11, 15, 39	10, 45	high
→ post-emergent spraying	more biomass for feeding organisms until spraying		25,	5, 9, 28, 33, 36	high
	less erosion due to more weed biomass and residues			4, 8	medium
	increased drift and pollution due to higher late-season wind speeds and/or increased spraying height		6, 27	22	medium
→ change in spray schedules of insecticides and fungicides due to modified herbicide spraying	positive or negative implications for microbial and/or fauna activities			9, 13, 22, 37	medium
→ minimum till associated with HR sugar beet cultivation	increased competitiveness of perennial weeds			14, 32	high

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Table 2 – *Continued*

Practice changes	**Chain of potential agro-environmental effects**	**References**[1]			**Evidence**[2]
		laboratory or greenhouse experiments	field trials or observations	landscape-scale experiments or observations	
	less soil compaction, higher soil biodiversity		24, 34	4, 8, 9	high
control of HR sugar beet volunteers in followcrops	reduced crop rotation options (e.g., wider rotations or crops with other HR traits) → various positive or negative implications for field organisms and soil bio-geochemical cycles			3, 32	low
	changes in tillage system → positive or negative implications for soil degradation and erosion			3, 17, 32	medium
control of increased HR in weeds	increased ai amount, different types of herbicides, higher spraying frequency			3, 12, 18, 22, 42	high
	→ various adverse effects on field organisms and/or soil bio-geochemical cycles		25	3, 17, 29, 32	high
in case of increased yield potential → increased fertiliser use	increased nutrient leaching		8	30	medium
coexistence measures to reduce vertical gene flow	reduced crop rotation options, isolating fields of GMHR sugar beet			13, 19	medium
	→ various positive or negative implications for field organisms and/or soil bio-geochemical cycles			22, 26, 32	medium

[1]**References legend (E: Expert opinions; M: Models; R: Review; O: Original data):** 1 (Champion and May, 2004 O); 2 (Kleter *et al.*, 2007 R); 3 (Schütte *et al.*, 2004 E, R); 4 (Cerdeira and Duke, 2006 R); 5 (Bohan *et al.*, 2005 O); 6 (Owen, 1999 E); 7 (Krebs *et al.*, 1999 E, R); 8 (Agronomy Guide, 2010 E, O); 9 (Werner *et al.*, 2000 E, M, R); 10 (Johal and Huber, 2009 R); 11 (Fernandez *et al.*, 2009 O); 12 (Benbrook, 2009 R, O); 13 (Champion *et al.*, 2003 O); 14 (Frick and Thomas, 1992 O); 15 (Tesfamariam *et al.*, 2009 O); 16 (Beckie *et al.*, 2006 R, O); 17 (Van Acker *et al.*, 2003 E, R); 18 (Légère, 2005 E, R); 19 (Schiemann, 2003 E); 20 (Firbank and Forcella, 2000 E, R); 21 (Regal, 1994 E, R); 22 (Züghart and Breckling, 2003 R); 23 (Watkinson *et al.*, 2000 M, E); 24 (Jordan *et al.*, 2004 O); 25 (Strandberg *et al.*, 2005 O); 26 (Relyea, 2005a,b,c O); 27 (Johnson, 2001 E, O); 28 (Heard *et al.*, 2003b O); 29 (Robinson and Sutherland, 2002 R, O); 30 (Pacini *et al.*, 2003 O); 31 (Benton *et al.*, 2002 R, O); 32 (McLaughlin and Mineau, 1995); 33 (Heard *et al.*, 2003a O); 34 (Thorbek and Bilde, 2004 O); 35 (Owen and Zelaya, 2005 / O); 36 (Squire *et al.*, 2003 O); 37 (Hole *et al.*, 2005 R); 38 (Firbank *et al.*, 2006 O); 39 (Larson *et al.*, 2006 O); 40 (Benachour and Séralini, 2009 O); 41 (Popp *et al.*, 2008 O); 42 (Sanyal *et al.*, 2008 E,O); 43 (Kelly *et al.*, 2010 O); 44 (Johnson *et al.*, 2009 R); 45 (Kremer and Means, 2009 O); 46 (Zobiole *et al.*, 2010 / O)

[2]Evidence of effects among the references based on the data quality aspects a) how closely the measured or observed effects and indicators resemble the actual effects and indicators about which information is desired; b) quality, mode and accuracy of the methodological design and the degree to which empirical or expert observations have been used to produce the data; c) statistical design, number of replications, spatio-temporal representativeness (Graef, 2009)

6 Potential adverse agro-environmental effects across different spatial scale levels and implications for ERA and PMEM

As shown above, the combination of biological properties and the modified HR technology may entail various positive, neutral or adverse agro-environmental effects. These can be demonstrated to varying degrees by scientific observations and/or experiments, each of which were made on various scales of precision, space and time (Figure 3). Compiling this information helps in the overall assessment of the evidence for potential agro-environmental effects (Table 1 and 2). This information is collected and investigated for the ERA of the HR sugar beet, and conclusions can be drawn for the PMEM. If information on specific aspects of the HR sugar beet is considered insufficient, further observations and experiments may be required.

An important aspect of the ERA procedure is to evaluate effects, i.e., are they adverse and do they constitute environmental damage. This is a function of hazard and likelihood of occurrence. The mere occurrence of a GM crop, for instance in arable non-GM fields or other biotopes, is not considered damage, but rather as an indicator for potential damage. According to Bartz *et al.* (2009), an adverse effect can be defined as a reduction in valued attributes of one or more conservation resources. Moreover, environmental damage can be defined as a significant adverse effect on a biotic or abiotic conservation resource that has an impact a) on the environmental value of the conservation resource in whole or part, b) on the conservation resource as an ecosystem component, or c) on the sustainable use of the conservation resource or the ecosystem. This implies that components (composition, structure, functions) and scale levels (gene, species, ecosystem, landscape) of a conservation resource are considered in their entirety (Bartz *et al.*, 2009, Figure 3). Once an adverse effect is detected and sufficiently evidenced, its significance, which ultimately is an operational threshold value, must be assessed on a normative basis. It may then be considered as significant or not significant. If the significance is low or cannot yet be assessed, it can be allocated either to general surveillance (GS) or to a case-specific monitoring (CSM) (Figure 3).

Table 3 presents the level of evidence of effects considered to be adverse, based on available findings listed in Tables 1 and 2. We provide indications of evidence as to whether an effect requires more research on the greenhouse- or field-scale level and/or whether it should be part of the PMEM carried out on the landscape scale. The indication of relevant scale levels depends on the type of effect or indicator group to be investigated (Graef *et al.*, 2005b; Hilbeck *et al.*, 2008b). In general, the larger the scale of investigation, the more ecological relevance applies to an effect or indicator and the more challenging the risk management or control (Figure 1). Some adverse effects such as invasiveness can be detected, if ever, only at larger scales. Furthermore, the larger the scale of investigation, the more challenging the experimental design and the less likely that standardised detection methods exist. Findings on the laboratory or greenhouse scale are not easily reproduced and/or confirmed on the field scale. Reasons include a) their poor relevance at larger scales due to higher variations and multiple influences of environmental factors, b) a lack of suitable field detection methods, or c) a poor statistical design. Based on our findings in Sections 3, 4, and 5, we outline research shortcomings at three different scale levels.

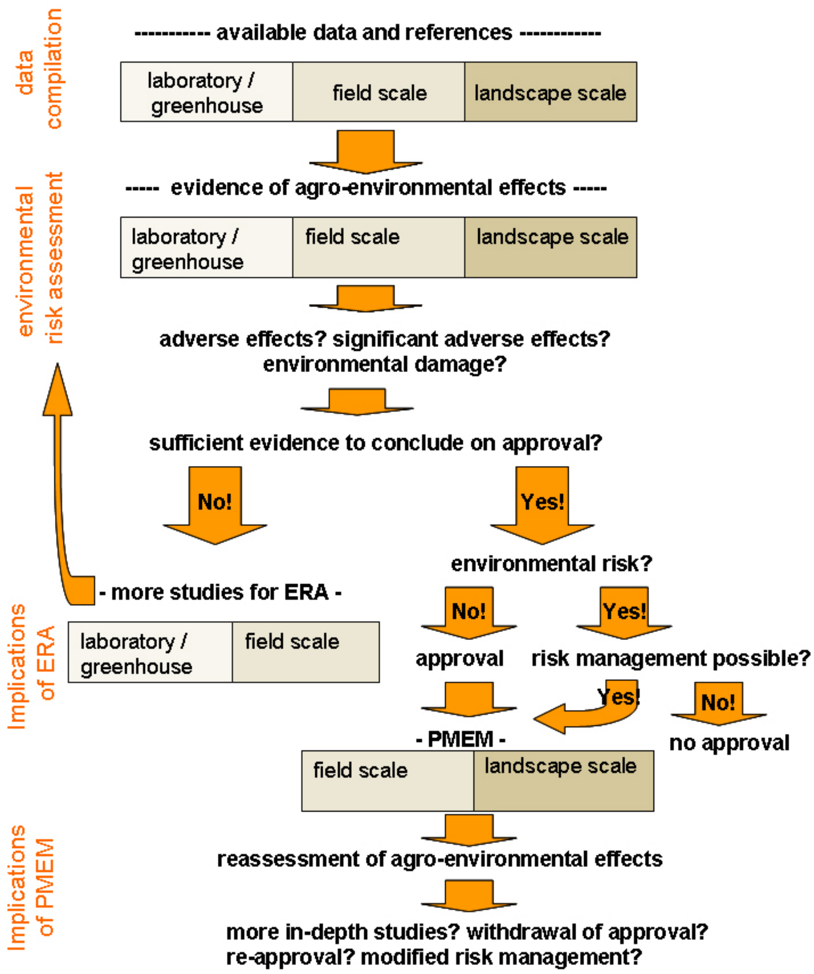

Figure 3: Pathways for ERA and PMEM and relevance of scales.

Table 3: Evidence of potential adverse agro-environmental effects and relevance for ERA and PMEM at different scale levels.

Evidence[1]	**Potential adverse agro-environmental effects**[1]	**Research and/or PMEM**
	Field scale (limited in time and scale)	
high	glyphosate use → adverse effects on field organisms and/or aquatic communities in cropped fields and neighbouring habitats	examine adverse effects on field organisms and aquatic communities / PMEM (CSM)
high	glyphosate use → adverse effects on soil fungal communities, diseases and nutrient availability	examine adverse effects on fungal communities / PMEM (CSM)
medium	change in spray schedules of insecticides and fungicides → implications for microbial and/or fauna activities	examine possible adverse effects for microbial and/or fauna activities / PMEM (GS)
medium	changes in tillage system → implications for soil degradation and erosion	examine possible adverse effects on soil degradation and erosion / PMEM (GS)
medium	transgene may be transferred via horizontal gene transfer to other species such as bacteria	examine likelihood and possible adverse effects of horizontal gene transfer
low	stable weed beet populations → unpredictable combinatory effects with cultivated and/or wild relatives	investigate possible adverse combinatory effects
low	pleiotropic and epigenetic genome effects of HR sugar beet	investigate possible adverse pleiotropic and epigenetic genome effects
low	changed quality of plant parts	investigate possible adverse changes / PMEM (GS)
low	effects on various soil functions	investigate possible adverse effects on soil functions

Continued on next page...

Table 3 – *Continued*

Evidence[1]	**Potential adverse agro-environmental effects**[1]	**Research and/or PMEM**
	Landscape scale	
high	glyphosate use → higher concentrations in surface and ground waters neighbouring fields treated with glyphosate	PMEM (CSM) of increased glyphosate concentrations in aquatic ecosystems
high	in case of bolters and groundkeepers flowering → HR seed production → stable HR seed bank	bolter and volunteer monitoring and eradication according to agric. practice
high	in case of bolters and groundkeepers flowering → hybridisation with neighbouring cultivated, feral and weed beets	PMEM (GS) of possibly persisting or invasive HR beets
high	in case of bolters and groundkeepers flowering → hybridisation with wild relatives	PMEM (GS) of possibly persisting or invasive wild HR relatives
high	increase of HR in sugar beet seed bank → hybridisation with neighbouring cultivated and/or wild relatives → in case of a selective advantage and/or genetic drift: HR sugar beet and related species may persist and become invasive	PMEM (GS) of possible increase of HR beet, hybridised HR species and/or transgene in the environment
high	development of HR in weeds	PMEM (CSM) of possibly increased HR in weeds
high	control of increased HR in weeds→ increased ai amount, different types of herbicides, higher spraying frequency → various adverse effects on field organisms and/or soil bio-geochemical cycles	PMEM (CSM) of herbicide application regime and adverse effects on field organisms and soil functions
high	increased weed suppression → less biomass, food, flowers and habitats for field organisms after spraying → lower abundance of various herbivores, pollinators and beneficial species (pest antagonists) → effects on depending organisms / food web → decrease in agrobiodiversity	PMEM (CSM) of possible decrease in agrobiodiversity
high	decrease and/or shift of weedy species and weed seed bank	PMEM (GS) of adverse effects of decrease of weedy species and weed seed bank
medium	reduced crop rotation options	PMEM (GS) of long-term sustainability of cropping systems
medium	post-emergent spraying → increased drift and pollution due to higher late-season wind speeds and/or increased spraying height	PMEM (GS) of increased herbicide drift and pollution
medium	coexistence measures to reduce vertical gene flow → reduced crop rotation options → various positive or negative implications for field organisms and/or soil bio-geochemical cycles	PMEM (GS) of long-term sustainability of cropping systems
low	possible impact on migratory and mobile species	PMEM (GS) of potential adverse effects on migratory and mobile species
low	control of HR sugar beet volunteers in followcrops → reduced crop rotation options (e.g., wider rotations or crops with other HR traits) → possible implications for field organisms and soil bio-geochemical cycles	PMEM (GS) of possible adverse effects on field organisms and soil functions
low	stable weed populations → adverse effects on neighbouring habitats such as ecosystem food chains; impacts on biodiversity	PMEM (GS) of ecosystem food chain effects and biodiversity

[1]References see Table 1 and 2

Laboratory or greenhouse scale: Lab or greenhouse experiments on the GM crop can involve survivability, genetic stability, as well as interactions with target and NTOs and the abiotic environment. Such experiments are a regular part of the GM crop development process. However, in general data on potential environmental effects of the GMHT sugar beet are few, inadequate or missing. Additionally, experimental results lack a sound statistical design and thus are not or hardly reproducible. For the acute toxicity tests in the risk assessment of NTOs we suggest to use the whole GM plant material instead of isolated microbially produced transgene products that are usually provided. We suggest prolonged ecotoxicity tests (Hilbeck *et al.*, 2008a), since in reality NTOs may be exposed over longer periods, for example one or several life stages or even over the whole life cycle. Moreover, they are exposed to the complete plant material of the GMP, not only to single substances. We also recommend incorporating realistic exposure pathways when testing NTOs (Römbke *et al.*, 2010). So far, the selection of test organisms is poorly founded. For instance, tests with insect pests of other crop species such as the European corn borer and the Colorado potato beetle are a questionable choice for investigating effects of a GMHR sugar beet on NTOs.

Field scale (limited in time and space): For the field scale, we suggest further experiments of adverse effects of a) the GMP and b) different glyphosate formulations on various trophic levels of field organisms, aquatic communities and soil microbial communities. The latter should focus on specific effects caused by the changed time frame of glyphosate spraying in GMHR sugar beet. To differentiate between herbicide effects and possible pleiotropic effects, part of the experiments should exclude pesticide application. Further research and more information for the ERA is also required on possible adverse effects for microbial and/or faunal activities and on soil degradation. Irrespective of the HR sugar beet, the relevance and potential adverse effects of horizontal gene transfer should be further clarified. Only little is known about possible combinatory effects after hybridisation and about pleiotropic and epigenetic genome effects of the HR sugar beet. More information is also required about potentially altered quality of plant parts after years of cultivation and about possible effects on various soil functions of the HR sugar beet.

Landscape scale: For the landscape scale, we suggest the CSM of possibly increased glyphosate formulation concentrations in soils and aquatic ecosystems as an indicator for adverse effects on field organisms and aquatic communities. Observation and eradication of sugar beet bolters and volunteers is usual agricultural practice. In some places and/or in some years, bolters or volunteers may be either overlooked or too frequent to overcome. This may lead to HR pollen flow, hybridisation with neighbouring beets and wild relatives, seed production and seed shed. We suggest the GS of possible persistence and invasiveness (triggered by a possible selective advantage and/or genetic drift) for HR cultivated, feral and weed beets, hybridised HR related species and/or the transgenes in the neighbouring environment. This applies especially to HR sugar beet seed production regions. A CSM is recommended for detecting the likely increase of HR in weeds and subsequently the likely increase and/or change in the herbicide application regime after several years of glyphosate application. A further recommendation is the CSM of the possible HR-related decrease in agrobiodiversity, including weed communities, herbivores, pollinators and beneficial species. GS should be done of the long-term sustainability of cropping systems and their rotations as well as of a potentially increased herbicide drift and pollution. Although current evidence is poor for potential adverse effects on migratory and mobile species, on soil functions and ecosystem food chain effects, and on larger scale biodiversity, literature findings do suggest GS of these issues.

7 A step-by-step scale approach for HR sugar beet?

The European Commission (2001) regulations require a step-by-step procedure for GM crop market approval that includes experimental data at the field scale to predict the outcome of larger landscape-scale release (Figure 1). Our review of available references on the one hand indicates various potential and demonstrated adverse environmental effects and, on the other, a lack of well-designed experimental data and/or other information with environmental relevance. The references screened refer to different spatial scales. They firstly enable an assessment of environmental hazards, and/or secondly should trigger further experimental field-scale activities, and/or thirdly should be the basis for a landscape scale-based PMEM, either CSM or GS (Figure 3).

In GM crop approval practice, as in society in general, the conclusions on adverse effects and on their significance vary. This also pertains to subsequent requirements such as further experimental research, CSM or GS. Ultimately, however, decisions should be science-based , implying the use of data with sound statistical design and power (European Commission, 2001). Lövei and Arpaia (2005) and Perry *et al.* (2009) state that field studies for the ERA often lack such statistical design and power. Studies with NTOs to check for unexpected direct and/or indirect effects as previewed also by GMO Panel (2010) are largely missing.

Test species for NTOs risk assessment should be selected using a systematic, transparent, scientifically based and stepwise methodology as developed by the GMO ERA Guideline Project (Hilbeck and Andow, 2004; Hilbeck *et al.*, 2006; Andow *et al.*, 2008) and as refined by Hilbeck *et al.* (2008a). Furthermore, field sites for NTO testing should be selected to be representative for the receiving environments relevant to the market release as developed by Jänsch *et al.* (2010). Field studies should use plant material from the GMP and be performed in the laboratory, greenhouse or at the semi-field level, and in the field.

To date, the ERA in the application dossiers usually relies on tests originally developed and standardised for chemicals. These tests frequently do not examine the whole GM plants but only specific transgene products. Although this ecotoxicological testing concept is widely used, it does not fulfil the requirements of the Directive 2001/18/EC. A harmonised concept for ecotoxicological testing considering the whole GM plant characteristics is recommended, for example as outlined by Römbke *et al.* (2010). Furthermore, we recommend a more in-depth examination as to whether experimental data on specific potential adverse effects of GMHR sugar beet cultivation are scientifically-based and statistically sufficient to be upscaled (Breckling *et al.*, 2009; Squire *et al.*, 2009).

A general barrier to premarket tests and field studies is the research and publication control by GM seed companies. Under the threat of litigation, user agreements explicitly exclude the use of the seeds for any independent research (Waltz, 2009; Scientific American eds., 2009), and experimental results must be approved by the companies before being published. Thus, experimental data exhibiting potential adverse effects may not be made public and cannot enter the ERA process.

If potential adverse effects identified in the ERA are selected for further PMEM (Figure 3), this also requires statistical multi-scale designs (Firbank *et al.*, 2003; Stein and Ettema, 2003); this includes determining the environmental baseline status (European Commission, 2002) to identify adverse effects in a CSM and in GS. Science-based PMEM approaches are available (Graef *et al.*, 2005a,b; Züghart *et al.*, 2008) and should be carried out in a coordinated and harmonised way (Finck *et al.*, 2006; Graef *et al.*, 2008). The PMEM requires both static and flexible elements because agricultural systems are dynamic. They need to be adaptive as new information emerges (Lindemayer and Likens, 2009) and should feed back on the ERA as monitoring data becomes available.

8 Conclusions

A stepwise increase of release scale, based on results from a gradually enhanced data base, is a precautionary principle required through Directive 2001/18/EC. The foundation to ensure this principle is to collect sufficient data during lab, greenhouse experiments and field trials. This is the only approach that enables a solid environmental risk assessment and the subsequent planning and design of the post-market environmental monitoring. It is crucial in helping to detect possible larger-scale and long-term effects and to avoid possible environmental damage.

For the GMHR sugar beet as a case study, we identified shortcomings in the presently available information for the environmental risk assessment at all levels of spatial scales: On the laboratory scale, data on potential environmental effects are few, inadequate or missing, and/or statistical designs of experiments are poor. Ecotoxicity tests for non-target organisms are too short-lived and do not use the real GMHR sugar beet material. Finally, the selection of test organisms is questionable. On the field scale, further experiments are required on adverse effects of GMHR sugar beet and different glyphosate formulations on various trophic levels of field organisms, aquatic communities and soil microbial communities. We therefore argue that more research experiments should be done initially to enable completing the environmental risk assessment.

Once approved, a case-specific monitoring should be carried out for a) possibly increased glyphosate formulation concentrations in soils and aquatic ecosystems, b) adverse effects on field organisms and aquatic communities, c) herbicide resistance in weeds, d) the possible increase and/or change in the herbicide application regime after years of glyphosate application, and e) a possible decrease in agrobiodiversity (weed communities, herbivores, pollinators and beneficial species) linked to the HR technology. General surveillance should be carried out on a) possible persistence and invasiveness of HR cultivated, feral and weed beets, and hybridised HR-related species in the agro-environment, b) sustainability of cropping systems and their rotations, c) potentially increased herbicide drift and pollution, and d) potential adverse effects on migratory and mobile species, soil functions, ecosystem food chain effects, and large-scale biodiversity.

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10 Acronyms

Ai	active ingredient
HR	herbicide-resistant / herbicide resistance
GM	genetically modified
GMO	genetically modified organism
GMHR	genetically modified herbicide-resistant
ERA	environmental risk assessment
PMEM	post market environmental monitoring
NTO	non-target organism

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