SCIENTIFIC OPINION

Scientific Opinion on an application (EFSA-GMO-NL-2005-24) for the placing on the market of the herbicide tolerant genetically modified soybean 40-3-2 for cultivation under Regulation (EC) No 1829/2003 from Monsanto[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This Scientific Opinion reports on an evaluation of a risk assessment for the placing on the market for cultivation of genetically modified soybean 40-3-2, and updates the previous EFSA GMO Panel Scientific Opinion on the renewal applications for the continued marketing of soybean 40-3-2. The EFSA GMO Panel considered that soybean 40-3-2 is unlikely to raise additional environmental safety concerns compared with conventional soybean, but that the management of its cultivation could result in environmental harm under certain conditions. The Panel therefore recommended managing the use of glyphosate on soybean 40-3-2 in ways that result in similar or reduced environmental impacts compared with conventional soybean cultivation. There is no evidence of adverse effects on non-target organisms (including pollinators) due to the expression of the CP4 EPSPS protein, and there are no indications of the occurrence of adverse effects on non-target predators, herbivores and decomposers due to potential unintended changes in soybean 40-3-2. Owing to the lack of event-specific data on plant-pollinator interactions, scientific uncertainty on the occurrence of adverse effects on pollinators, due to potential unintended changes in soybean 40-3-2, remains, and strategies for resolving this uncertainty are discussed. The Panel recommended the deployment of case-specific monitoring to address: (1) changes in weed community diversity; and (2) the evolution of resistance to glyphosate in weeds due to changes in herbicide and cutivation regimes. The Panel agreed with the general surveillance plan of the applicant, but requested that the Panel's proposals to strengthen general surveillance are implemented. The Panel concluded that the information available for soybean 40-3-2 addresses the scientific comments raised by Member States and that soybean 40-3-2, as described in this application, is as safe as its conventional counterpart and commercial non-GM soybean varieties with respect to

[1] On request from the Competent Authority of the Netherlands for an application (EFSA-GMO-NL-2005-24) submitted by Monsanto, Question No EFSA-Q-2005-251, adopted on 31 May 2012.

[2] Panel members: Hans Christer Andersson, Salvatore Arpaia, Detlef Bartsch, Josep Casacuberta, Howard Davies, Patrick du Jardin, Gerhard Flachowsky, Lieve Herman, Huw Jones, Sirpa Kärenlampi, Jozsef Kiss, Gijs Kleter, Harry Kuiper, Antoine Messéan, Kaare Magne Nielsen, Joe Perry, Annette Pöting, Jeremy Sweet, Christoph Tebbe, Atte Johannes von Wright and Jean-Michel Wal. One member of the Panel did not participate in the discussion on the subject referred to above because of potential conflicts of interest identified in accordance with the EFSA policy on declarations of interests. Correspondence: gmo@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Standing Working Groups on Molecular Characterisation, Food and Feed, and Environment on GMO applications, the external experts Paolo Bàrberi, Boet Glandorf and Niels Bohse Hendriksen, and the EFSA staff member Yann Devos for the preparatory work on this scientific opinion, as well as EFSA staff members Yann Devos (ENV), Zoltán Divéki (MC) and Claudia Paoletti (FF) for the support provided in the development of this EFSA scientific output.

Suggested citation: EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on an application (EFSA-GMO-NL-2005-24) for the placing on the market of the herbicide tolerant genetically modified soybean 40-3-2 for cultivation under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2012;10(6):2753. [110 pp.] doi:10.2903/j.efsa.2012.2753. Available online: www.efsa.europa.eu/efsajournal

potential adverse effects on human and animal health. If subjected to appropriate management measures, the cultivation of soybean 40-3-2 is unlikely to have environmental effects any more adverse than those associated with conventional soybean cultivation.

KEY WORDS

GMO, soybean (*Glycine max*), 40-3-2, herbicide tolerance, CP4 *epsps*, risk assessment, food and feed safety, environment, environmental safety, cultivation, Regulation (EC) No 1829/2003

SUMMARY

Following the submission of an application (Reference EFSA-GMO-NL-2005-24) under Regulation (EC) No 1829/2003 from Monsanto, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a Scientific Opinion on the safety of the herbicide tolerant genetically modified (GM) soybean (also known as soya bean) 40-3-2 (Unique Identifier MON-Ø4Ø32-6) for cultivation. Although the scope of this application covers only cultivation of soybean 40-3-2, this Scientific Opinion also updates the previous EFSA GMO Panel safety evaluation on the continued marketing of: (1) food containing, consisting of, or produced from soybean 40-3-2; (2) feed containing, consisting of, or produced from soybean 40-3-2; and of (3) other products containing or consisting of soybean 40-3-2.

In delivering its Scientific Opinion, the EFSA GMO Panel considered: the application EFSA-GMO-NL-2005-24; additional information supplied by the applicant; scientific comments submitted by Member States; the environmental risk assessment report of the German Competent Authority (DE CA); and relevant scientific publications.

Soybean 40-3-2 expresses the enzyme CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS), which is derived from the CP4 strain of *Agrobacterium tumefaciens* (updated scientific name: *Rhizobium radiobacter*), and renders soybean 40-3-2 tolerant to the herbicidal active substance glyphosate.

The EFSA GMO Panel evaluated soybean 40-3-2 with reference to its intended uses and the appropriate principles described in its guidelines for the following: the risk assessment of GM plants and derived food and feed; the environmental risk assessment of GM plants; the selection of comparators for the risk assessment of GM plants; and the post-market environmental monitoring of GM plants. The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and expression of the target protein. An evaluation of the comparative analyses of composition and agronomic and phenotypic characteristics was undertaken, and the safety of the new protein and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional quality. An evaluation of environmental impacts and the post-market environmental monitoring plan was undertaken.

The molecular characterisation data established that soybean 40-3-2 contains one functional insert expressing CP4 EPSPS and a non-functional insert consisting of a 72 bp fragment of the CP4 *epsps* coding sequence. No other parts of the plasmid used for transformation are present in the transformed plant. Bioinformatic analyses of the open reading frames spanning the junction site within the functional insert or between the inserts and genomic DNA did not indicate specific hazards. The stability of the inserted DNA and the herbicide tolerance trait were confirmed over several generations. Analyses of the levels of CP4 EPSPS in leaves and seed collected from field trials performed in Europe were considered sufficient.

The EFSA GMO Panel compared the composition and agronomic and phenotypic characteristics of soybean 40-3-2 and its conventional counterpart, assessed all statistical differences identified, and came to the conclusion that soybean 40-3-2 is compositionally equivalent to commercial non-GM soybean varieties, except for the newly expressed protein. The risk assessment included an analysis of data from analytical studies, bioinformatic analyses, and *in vitro* and *in vivo* studies. The EFSA GMO Panel concludes that the soybean 40-3-2 is as safe as its conventional counterpart and commercial non-GM soybean varieties and that the overall allergenicity of the whole plant is not changed.

As the scope of the current application covers cultivation, the environmental risk assessment considered the environmental impact of full-scale commercialisation of soybean 40-3-2.

The DE CA provided EFSA with its report on the environmental risk assessment of soybean 40-3-2 (dated 9 September 2008) on 3 October 2008 in line with Articles 6.3(c) and 18.3(c) of Regulation (EC) No 1829/2003. The report on the environmental risk assessment of the DE CA is provided in

Annex H of the EFSA Overall Opinion, and has been considered throughout this EFSA GMO Panel Scientific Opinion.

The EFSA GMO Panel considers that soybean 40-3-2 has no altered agronomic and phenotypic characteristics, except for the herbicide tolerance. The likelihood of unintended environmental effects due to the establishment, survival and spread of soybean 40-3-2 is considered to be extremely low, and will be no different from that of conventional soybean varieties.

It is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the environment or human and animal digestive tracts. In the rare but theoretically possible case of transfer of the CP4 *epsps* gene from soybean 40-3-2 to soil bacteria, no novel property would be introduced into the soil bacterial community and thus no positive selective advantage that would not have been conferred by natural gene transfer between bacteria would be provided.

Based on the evidence provided by the applicant and relevant scientific literature on soybean 40-3-2, the EFSA GMO Panel concludes that there are no indications of the occurrence of adverse effects on predators, herbivores and decomposers due to potential unintended changes in soybean 40-3-2, and therefore considers *trait*-specific information appropriate to assess whether soybean 40-3-2 poses a risk to non-target organisms. However, the EFSA GMO Panel that scientific uncertainty pertaining to the occurrence of adverse effects on pollinators due to potential unintended changes in soybean 40-3-2 remains, as no event-specific data on plant-pollinator interactions were provided by the applicant. The EFSA GMO Panel considered that this scientific uncertainty should be resolved by experiments in relevant receiving environments in Europe that are designed to compare the effects of soybean 40-3-2 and its conventional counterpart (and optionally reference, commercial non-GM soybean varieties, if appropriate) on adult honeybees, as long as the five conditions explicitly stated in this Scientific Opinion are met.

The studies, supplied or reviewed by the applicant, showed no adverse effects on different types of non-target organisms due to the expression of the CP4 EPSPS protein in glyphosate tolerant crops.

The EFSA GMO Panel does not expect potential adverse effects on biogeochemical processes and the abiotic environment due to the expression of CP4 EPSPS protein in soybean 40-3-2.

The EFSA GMO Panel is of the opinion that potential adverse environmental effects of the cultivation of soybean 40-3-2 are associated with the use of the complementary glyphosate-based herbicide regimes. These potential adverse environmental effects could, under certain conditions, comprise: (1) a reduction in farmland biodiversity; (2) changes in weed community diversity due to weed shifts; (3) the selection of glyphosate resistant weeds; and (4) changes in soil microbial communities. The potential harmful effects could occur at the level of arable weeds, farmland biodiversity, and food webs and the ecological functions they provide. The magnitude of these potential adverse environmental effects will depend on a series of factors, including the specific herbicide and cultivation management applied at the farm level, the crop rotation and the characteristics of the receiving environments.

The conclusions of the EFSA GMO Panel on the environmental safety of soybean 40-3-2 are consistent with those of the DE CA. The DE CA concluded that "*no adverse effects on human and animal health and the environment are to be expected from the cultivation of soybean 40-3-2*", but that "*glyphosate-containing herbicides can be applied after germination of the soybean plants and thus could have effects on the accompanying weed flora. Based on experience from using conventional plant protection products it is to be expected that sooner or later tolerance to the active ingredient of glyphosate-containing herbicides will develop in the weed flora*" (see section 6.5 of the environmental risk assessment report of the DE CA). In its evaluation, the DE CA noted that "*there is potentially also an indirect interaction between the use of glyphosate-containing herbicides and nitrogen-fixing symbiotic partners of the soybean (e.g. Bradyrhizobium japonicum, Moorman et al., 1992, King et al., 2001), which could lead to a reduction in harvest yield (King et al., 2001). To compensate, potential increased application of nitrogen fertilizer might be necessary with the cultivation of HT soybeans*" (see section 6.5 of the environmental risk assessment report of the DE CA). With regard to potential adverse effects on non-target organisms due to potential unintended changes in soybean 40-3-2, the DE CA recommended "*conducting an additional study to confirm the absence of unintended adverse effects on non-target organisms*" (see section 6.3 of the environmental risk assessment report of the DE CA).

The EFSA GMO Panel anticipated that the repeated use of glyphosate at recommended application rates on soybean 40-3-2 grown either in rotation with other glyphosate tolerant crops, or continuously may lead to a greater risk of reducing weed community diversity than the current practices applied in soybean cropping systems. This may therefore result in reductions in weed community diversity and/or weed density to a level that might adversely affect food chains and webs, but not necessarily biological control functions, at the field and landscape level. Such reductions in weed community diversity and consequential reductions in farmland biodiversity may be considered problematic by risk managers depending upon protection goals pertaining to their region, especially in receiving environments that sustain little farmland biodiversity or in environmentally sensitive areas. Under such situations, the EFSA GMO Panel recommends that risk mitigation measures are put in place to manage potential herbicide effects, in order to ensure that glyphosate is used on soybean 40-3-2 in ways that result in similar or reduced adverse effects on farmland biodiversity compared with conventional soybean cultivation. Possible risk mitigation measures include reduced tillage, crop rotation, less intense in-crop weed management, protecting adjacent habitats from herbicide drift, and (re)introduction and better management of field margins and other 'out of crop' measures.

Soybean 40-3-2 grown in rotation with other glyphosate tolerant crops or continuously, in conjunction with the repeated and/or exclusive application of glyphosate-based herbicides, will cause changes in the weed flora, and will favour the evolution and spread of glyphosate resistant weeds due to the selection pressure exerted by glyphosate. The EFSA GMO Panel recommends that risk mitigation measures are put in place to reduce the selection pressure and hence to delay the evolution of resistance. This can be achieved by crop rotation (i.e., rotating glyphosat tolerant crops with non-glyphosate tolerant crops, alternating autumn- and spring-sown crops), using variable rates and timing of herbicide application, applying a variety of herbicidal active substances with different modes of action, and using non-herbicide weed control tools such as pre- and post-emergence cultivation and cover crops. To be most effective, these methods should be used in combination. A clear advantage of increasing cropping system diversity is that it would increase or conserve farmland biodiversity as well as reducing the risk of weed shifts and the evolution of glyphosate resistant weed biotypes.

The EFSA GMO Panel considers that current management practices are sufficient to cope with potential adverse effects on symbiotic nitrogen fixation arising from the use of glyphosate on soybean 40-3-2, but advises that risk managers inform farmers of the possibility of the occurrence of such effects.

The conclusions of the EFSA GMO Panel on the environmental safety of soybean 40-3-2 are consistent with those of the DE CA. In its evaluation, the DE CA recommended that "*herbicide and cultivation management of soybean 40-3-2 should be adapted to minimize potential negative effects*" (section 6.5 of the environmental risk assessment report of the DE CA).

The EFSA GMO Panel gives its opinion and makes recommendations on the scientific quality of the post-market environmental monitoring plan proposed by the applicant. In order to assess the efficacy of risk mitigation measures put in place to reduce levels of risk and in order to reduce the remaining scientific uncertainty identified in the environmental risk assessment, the EFSA GMO Panel recommends case-specific monitoring to address: (1) changes in weed community diversity; and (2) evolution of resistance to glyphosate in weeds due to changes in herbicide and cultivation regimes. In addition, the EFSA GMO Panel considers that it would be proportionate to the risk for the post-market studies on the occurrence of adverse effects on pollinators due to potential unintended changes in soybean 40-3-2, proposed by the applicant, to be conducted as case-specific monitoring. No case-specific monitoring is required to assess changes in soil microbial communities, but the EFSA GMO

Panel recommends that the applicant establishes stewardship systems encouraging farmers to report problems that may be due to reduced symbiotic nitrogen fixation. General surveillance (including appropriately designed farmer questionnaires) offers an effective approach to detect and report early warning signs indicating that such effects occur. The EFSA GMO Panel considers that risk managers should adapt monitoring methodologies to their local receiving environments, management systems and the interplay between the legislation for GMOs and plant protection products.

The EFSA GMO Panel agrees with the general surveillance plan for the cultivation of soybean 40-3-2 proposed by the applicant: (1) to establish farmer questionnaires as a reporting format of any on-farm observations of effects associated with the cultivation of soybean 40-3-2; (2) to use existing monitoring networks that observe changes in biota and production practices from farm up to regional level to obtain data on environmental impacts in the landscape where soybean 40-3-2 is cultivated; (3) to review all new scientific, technical and other information pertaining to soybean 40-3-2; and (4) to develop stewardship programmes for the introduction, marketing, management and stewardship of soybean 40-3-2. However, the EFSA GMO Panel requests that its proposals and those made by the DE CA to strengthen general surveillance are implemented. The EFSA GMO Panel agrees with the reporting intervals and modalities proposed by the applicant. The general surveillance plan for the import and processing of soybean 40-3-2 has been previously evaluated by the EFSA GMO Panel.

The DE CA considered that "*based on the safety assessment of soybean 40-3-2, no specific cause-effect relationship for adverse environmental impacts has been identified that would necessitate a case specific monitoring by the applicant*". However, with regard to the occurrence of adverse effects on non-target organisms due to potential unintended changes in soybean 40-3-2, the DE CA recommended that "*the applicant shall carry out a field study to confirm the absence of unintended adverse effects on non-target organisms in the EU with placing soybean 40-3-2 on the market. The design of such a study should be of a quality to allow appropriate scientific assessment as proposed in the application*".

Further, the DE CA was of the opinion that "*the monitoring plan needs some clarifications (reporting monitoring annually, and delivery of more comprehensive overviews after six and nine years); and improvement of the questionnaires*". The DE CA recommended that "*monitoring of the herbicide use is conducted as part of the stewardship for the herbicides by the companies involved, and under the auspices of the pesticide regulatory systems operating in Member States, in order to record compliance with the approved uses of the herbicides on GMHT, levels of weed control, and development of resistant weeds. The German Competent Authority assumes that possible indirect effects of complementary herbicide application will be taken into account by the applicant in the context of a Stewardship Program harmonized with the pesticide assessment authorities. This should ensure that unexpected effects (in general surveillance) can be detected*" (see section 8 of the environmental risk assessment report of the DE CA).

In conclusion, the EFSA GMO Panel considers that the information available for soybean 40-3-2 addresses the scientific comments raised by Member States and that soybean 40-3-2, as described in this application, is as safe as its conventional counterpart and commercial non-GM soybean varieties with respect to potential adverse effects on human and animal health, in the context of its intended uses. The EFSA GMO Panel also concludes that soybean 40-3-2 is unlikely to raise additional environmental safety concerns compared with conventional soybean, but that management of its cultivation could result in environmental harm under certain conditions. The EFSA GMO Panel therefore recommends managing the use of glyphosate on soybean 40-3-2 in ways that result in similar or reduced environmental impacts compared with conventional soybean cultivation. The EFSA GMO Panel recommends the deployment of case-specific monitoring to address: (1) changes in weed community diversity; and (2) evolution of resistance to glyphosate in weeds due to changes in herbicide and cultivation regimes. In addition, the EFSA GMO Panel considers that it would be proportionate to the risk for the post-market studies on the occurrence of adverse effects on pollinators due to potential unintended changes in soybean 40-3-2, proposed by the applicant, to be conducted as case-specific monitoring, as long as the five conditions explicitly stated in this Scientific Opinion are met. If subjected to appropriate management measures, the cultivation of soybean 40-3-2 is unlikely to have environmental effects any more adverse than those associated with conventional soybean cultivation.

TABLE OF CONTENTS

BACKGROUND

On 4 November 2005, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands an application (Reference EFSA-GMO-NL-2005-24) for authorisation of the herbicide tolerant genetically modified (GM) soybean (also known as soya bean) 40-3-2 (Unique Identifier MON-Ø4Ø32-6), submitted by Monsanto under Regulation (EC) No 1829/2003. The scope of this application covers cultivation of soybean 40-3-2. Although the scope of this application only covers cultivation of soybean 40-3-2, this Scientific Opinion also updates the previous EFSA GMO Panel safety evaluation on the continued marketing of: (1) food containing, consisting of, or produced from soybean 40-3-2; (2) feed containing, consisting of, or produced from soybean 40-3-2; and of (3) other products containing or consisting of soybean 40-3-2 (EFSA, 2010f).

After receiving the application EFSA-GMO-NL-2005-24 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed both Member States and the European Commission, and made the summary of the application publicly available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 16 June 2006 and 27 July 2006, EFSA received additional information requested under completeness check (requested on 3 March 2006 and 25 July 2006). On 29 September 2006, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

On 24 May 2006, following a call for expression of interest among Competent Authorities under Directive 2001/18/EC and in accordance with Articles 6.3(c) and 18.3(c) of Regulation (EC) No 1829/2003, EFSA requested the German Competent Authority (DE CA) to evaluate the initial environmental risk assessment of application EFSA-GMO-NL-2005-24 for the placing on the market of soybean 40-3-2 for cultivation. This call was initiated by EFSA on 10 March 2006 and the DE CA gave its conformity on 24 May 2006.

EFSA made the valid application available to Member States and the European Commission, and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member States had three months after the date of acknowledgement of the valid application (29 December 2006) within which to make their opinion known.

The DE CA asked the applicant for additional information on soybean 40-3-2 on 7 December 2006 and 7 November 2007. The applicant provided the requested information on 20 June 2007 and 7 May 2008, respectively.

The DE CA provided EFSA with its report on the environmental risk assessment of soybean 40-3-2 (dated 9 September 2008) on 3 October 2008 in line with Articles 6.3(c) and 18.3(c) of Regulation (EC) No 1829/2003.

The Scientific Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) carried out an evaluation of the scientific risk assessment of the GM soybean 40-3-2 in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. When carrying out the safety evaluation, the EFSA GMO Panel took into account: the appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed (EFSA, 2006a, 2011b), the environmental risk assessment of GM plants (EFSA, 2010e), the selection of comparators for the risk assessment of GM plants (EFSA, 2011a), and for the post-market environmental monitoring of GM plants (EFSA, 2006b, 2011c); the scientific comments of Member States; the additional information provided by the applicant; the environmental risk assessment report from the DE CA; and relevant scientific publications.

The EFSA GMO Panel asked the applicant for additional information on soybean 40-3-2 on 5 February 2007, 10 October 2008, 16 February 2009 and 9 November 2009. The applicant provided the requested information on 14 March 2007, 23 December 2008 and 22 November 2010. After receipt and assessment of the full data package, the EFSA GMO Panel finalised its risk assessment evaluation of soybean 40-3-2.

In giving its Scientific Opinion on soybean 40-3-2 to the European Commission, Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of six months from the acknowledgement of the valid application. As additional information was requested by both the DE CA and the EFSA GMO Panel, the time limit of six months was extended accordingly, in line with Articles 6(1), 6(2), 18(1) and 18(2) of Regulation (EC) No 1829/2003.

According to Regulation (EC) No 1829/2003, this Scientific Opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation, and thus will be part of the EFSA Overall Opinion in accordance with Articles 6(5) and 18(5).

The EFSA GMO Panel has evaluated two applications for the renewal of the authorisation and hence the continued marketing of: (1) food containing, consisting of, or produced from soybean 40-3-2; (2) feed containing, consisting of, or produced from soybean 40-3-2; and of (3) other products containing or consisting of soybean 40-3-2 with the exception of cultivation (EFSA, 2010f). The scope of the two renewal applications covered the continued marketing of: (1) existing food containing, consisting of, or produced from soybean 40-3-2 (including food additives) (Reference EFSA-GMO-RX-40-3-2$_{[8\text{-}1a/20\text{-}1a]}$) that have been placed on the market in accordance with Part C of Directive 90/220/EC before the entry into force of Regulation (EC) No 258/97 and as food additives subject to Directive 89/107/EEC; (2) existing feed containing, consisting of, or produced from soybean 40-3-2 (Reference EFSA-GMO-RX-40-3-2$_{[8\text{-}1b/20\text{-}1b]}$) that have been placed on the market in accordance with Part C of Directive 90/220/EEC and as feed materials and feed additives subject to Directive 70/524/EEC; and (3) other products containing or consisting of soybean 40-3-2 with the exception of cultivation (EC, 1996).

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific risk assessment of soybean 40-3-2 for cultivation in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market environmental monitoring requirements based on the outcome of the risk assessment and, in case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)(e) of Regulation (EC) No 1829/2003.

The EFSA GMO Panel was not requested to give a Scientific Opinion on information required under Annex II of the Cartagena Protocol, nor on the proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

ASSESSMENT

1. INTRODUCTION

Soybean 40-3-2 was developed to provide tolerance to the herbicidal active substance glyphosate by the introduction of a gene coding for the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from the CP4 strain of *Agrobacterium tumefaciens* (updated scientific name: *Rhizobium radiobacter*). Glyphosate is normally phytotoxic to a broad range of plants. Its mode of action is to bind to and competitively inhibit the EPSPS protein, which is the key enzyme in the shikimate pathway that leads to the biosynthesis of the aromatic amino acids tyrosine, tryptophan and phenylalanine (Alibhai and Stallings, 2001; Dill, 2005; Duke and Powles, 2008b). The disruption of this pathway and the resulting inability to produce key amino acids prevents growth and ultimately leads to plant death. However, in case of soybean 40-3-2, a gene has been introduced that codes for the expression of the CP4 EPSPS protein, which is insensitive to inhibition by glyphosate. This protein is not inhibited by glyphosate, thus allowing the crop to tolerate recommended dosages of glyphosate (Green, 2009; Dill et al., 2010).

Soybean 40-3-2 was assessed with reference to its intended uses and the appropriate principles described in the EFSA GMO Panel guidelines for the following: the risk assessment of GM plants and derived food and feed (EFSA, 2006a, 2011b); the environmental risk assessment of GM plants (EFSA, 2010e); the selection of comparators for the risk assessment of GM plants (EFSA, 2011a); and for the post-market environmental monitoring of GM plants (EFSA, 2006b, 2011c). In delivering its Scientific Opinion, the EFSA GMO Panel considered the information provided by the applicant in its application EFSA-GMO-NL-2005-24, and also: (1) peer-reviewed scientific data on soybean 40-3-2; (2) information on areas and quantity of production, importation and use in Europe of soybean 40-3-2 and information on known and estimated human and animal exposure; (3) updated information on composition, toxicology and allergenicity; (4) updated information on environmental issues; (5) the post-market (environmental) monitoring plan; and (6) the additional information submitted by the applicant in reply to questions from both the EFSA GMO Panel and DE CA.

The risk assessment evaluation presented here is also based on the scientific comments submitted by Member States (Annex G), the environmental risk assessment report of the DE CA (Annex H), and relevant scientific publications.

2. ISSUES RAISED BY MEMBER STATES

The scientific comments raised by Member States are addressed in Annex G of the EFSA Overall Opinion[4], and have been considered throughout this EFSA GMO Panel Scientific Opinion.

3. MOLECULAR CHARACTERISATION

3.1. Evaluation of relevant scientific data

Although the scope of this application covers only cultivation of soybean 40-3-2, this Scientific Opinion also updates the previous EFSA GMO Panel safety evaluation on the continued marketing of: (1) food containing, consisting of, or produced from soybean 40-3-2; (2) feed containing, consisting of, or produced from soybean 40-3-2; and of (3) other products containing or consisting of soybean 40-3-2 (EFSA, 2010f). Unless specifically indicated, the information provided in this application, which is described in the following sections, has been evaluated previously by the EFSA GMO Panel (EFSA, 2010f).

3.1.1. *Transformation process and vector constructs*[5]

Soybean 40-3-2 was produced by particle bombardment introducing the plasmid PV-GMGT04. This plasmid contains: two CP4 *epsps* expression cassettes; the marker gene *uid*A coding for β-D-

4 http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2005-251
5 Technical dossier / Sections C1, C2, C3 and D1

glucuronidase (GUS); a neomycin phosphotransferase (*npt*II) gene conferring resistance to kanamycin and neomycin for selection in *Escherichia coli*; and the ColE1 origin of replication from *E. coli*.

The first CP4 *epsps* expression cassette consists of: an enhanced 35S promoter derived from *Cauliflower mosaic virus* (CaMV); the sequence encoding CTP4 *N*-terminal chloroplast transit peptide from the *epsps* gene of *Petunia hybrida*; the CP4 *epsps* coding sequence; and the 3′ *nos* terminator from *A. tumefaciens*. The second CP4 *epsps* expression cassette contains the same elements, except for the *fmv* promoter from the *Figwort mosaic virus*, which replaces the CaMV 35S promoter. The *uid*A gene is under control of the mannopine synthase promoter from *A. tumefaciens*.

3.1.2. Transgenic constructs in soybean 40-3-2[6]

Molecular characterisation data demonstrated the presence of two inserts: a functional and a non-functional one. Southern blot analysis also demonstrated the absence of the *fmv* promoter and the *uidA* gene in soybean 40-3-2. The ColE1 origin of replication and the *npt*II gene were not detected by polymerase chain reaction (PCR) analysis. Sequencing of the functional insert demonstrated that the first 354 bp of the CaMV 35S promoter are absent, thereby removing a duplicate portion of the 35S enhancer region. In addition, a 250 bp fragment of the CP4 *epsps* coding sequence was found adjacent to the 3′ *nos* terminator. With these exceptions, the nucleotide sequence of the insert is identical to the corresponding sequence of PV-GMGT04. Sequencing also demonstrated that the non-functional insert consists of a 72 bp fragment of the CP4 *epsps* coding sequence (EFSA, 2010f).

The sequences adjacent to the 3′ and 5′ ends of the inserts were determined. The 3′ flanking sequence of the functional insert has been shown to be rearranged soybean genomic DNA. Results of BLASTn and BLASTx analyses of the flanking sequences of functional and non-functional inserts did not indicate the disruption of known genes in soybean 40-3-2. Bioinformatic analyses did not show any biologically relevant similarity to known allergens or toxins for any of the putative peptides that might be produced from open reading frames spanning the junctions of fragments within the functional insert or between the inserts and genomic DNA (EFSA, 2010f).

3.1.3. Information on the expression of the insert[7]

The levels of the CP4 EPSPS protein in soybean 40-3-2 leaves and seed were analysed by enzyme-linked immunosorbent assay (ELISA).

Samples for analysis were collected from field trials conducted in the USA in 1992 and 1993 and in Europe (France and Italy) during 1998. The analyses of the samples collected from field trials performed in the USA have been previously assessed by the EFSA GMO Panel (EFSA, 2010f). For the European field trials, the plants were either treated or not treated with glyphosate-based herbicides. The CP4 EPSPS levels in treated plants were 0.32–0.62 µg/mg fresh weight (average: 0.5 µg/mg fresh weight) in leaves and 0.09–0.27 µg/mg fresh weight (average: 0.17 µg/mg fresh weight) in seed. No significant differences in CP4 EPSPS protein levels were observed between glyphosate-treated and untreated samples.

There was no evidence that fusion proteins would be produced as a result of read-through transcription (EFSA, 2010f). In the unlikely event that a fusion protein was produced, bioinformatic analysis indicated that such a protein would not show similarity to known allergens or toxins (EFSA, 2010f).

Southern blot analysis of soybean 40-3-2 and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations (EFSA, 2010f).

6 Technical dossier / Sections C1, C2, C3 and D1
7 Technical dossier / Sections D3 and D5

3.2. Conclusion

The molecular characterisation data establish that soybean 40-3-2 contains a functional and a small non-functional insert. Updated bioinformatic analyses of the open reading frames spanning the junction site within the functional insert or between the inserts and genomic DNA did not identify specific hazards. The stability of the inserted DNA and the herbicide tolerance trait were confirmed over several generations. The potential impacts of the CP4 EPSPS protein levels, quantified in field trials carried out in Europe, are assessed in the food/feed and environment sections (see sections 5 and 6).

4. COMPARATIVE ANALYSIS

4.1. Evaluation of relevant scientific data

Although the scope of this application covers only cultivation of soybean 40-3-2, this Scientific Opinion also updates the previous EFSA GMO Panel safety evaluation on the continued marketing of: (1) food containing, consisting of, or produced from soybean 40-3-2; (2) feed containing, consisting of, or produced from soybean 40-3-2; and of (3) other products containing or consisting of soybean 40-3-2 (EFSA, 2010f). Unless specifically indicated, the information provided in this application, which is described in the following sections, has been evaluated previously by the EFSA GMO Panel (EFSA, 2010f).

4.1.1. Choice of comparator and production of material for the compositional assessment[8]

The original application EFSA-GMO-NL-2005-24 for cultivation of soybean 40-3-2 within the European Union presented compositional data from material collected in field trials in France (1998), Italy (1998), Puerto Rico (1991–1992) and the USA (1992 and 1993). The design of these field trials with respect to choice of comparator, replication, herbicide spraying regime, materials collected for compositional analysis and compounds analysed varied considerably, and was not in accordance with the applicable EFSA GMO Panel guidance document (EFSA, 2006a). Following a request for a comprehensive assessment of these field trial data from the DE CA evaluating the environmental risk assessment, the applicant provided compositional data on soybean forage and seeds from additional field trials in Romania in 2005. These field trials were designed to compare the composition of soybean 40-3-2 with that of a conventional soybean variety having a comparable genetic background. The EFSA GMO Panel made a comprehensive comparative assessment of the compositional data in the application, but particularly focused on the data from the Romanian field trials (EFSA, 2010f).

In most compositional studies, soybean 40-3-2 was compared with the non-GM Asgrow variety A5403, which was the commercial non-GM soybean variety originally used when the soybean was transformed to establish transformation event 40-3-2. When event 40-3-2 had been bred into a soybean variety with another genetic background, the corresponding conventional counterpart (Dekabig) was used as control.

The Romanian field trials in 2005 were performed at five sites, and included soybean 40-3-2 (cultivar S2254), the conventional counterpart (Dekabig), and 11 reference soybean varieties (Harrigan et al., 2007). The reference soybean varieties were to provide data on the natural variation in composition of commercial non-GM soybean varieties. Four reference soybean varieties were grown at each field trial site. The reference soybean varieties were checked for natural contamination with soybean 40-3-2. Two of the eleven reference soybean varieties were contaminated and deemed unsuitable as comparators. At each field trial site, soybean 40-3-2, the conventional counterpart and the reference soybean varieties were planted following a randomised complete block design with three replicates per site. Whereas the conventional counterpart and the reference soybean varieties were treated with required maintenance pesticides, soybean 40-3-2 was in addition treated with a glyphosate-based herbicide.

8 Technical dossier / Section D7.1 // Additional information received on 20/06/2007

4.1.2. Compositional analysis[9]

Soybean seeds were harvested and analysed for proximates (protein, fat, ash, and moisture), fibre fractions, amino acids, fatty acids, vitamin E, anti-nutrients (i.e., phytic acid, trypsin inhibitor, lectins, stachyose and raffinose) and other secondary metabolites (isoflavones). Forage was analysed for proximates, including fibre fractions. In total 63 different compounds were analysed in the Romanian field trials, 56 in seeds and seven in forage, essentially those recommended by the Organization for Economic Cooperation and Development (OECD, 2001). Materials from the earlier field trials were analysed for fewer constituents. The data on each analyte were statistically analysed for potential differences in levels between soybean 40-3-2 and its conventional counterpart within site (replicated trials) and across sites (all sites of the trial combined). Fourteen of the fatty acids analysed were rare and frequently occurred at levels below the limit of quantification. When this occurred in more than 50 % of the samples, the analyte was omitted from the statistical analysis.

When the compositional data for forage and seed samples from the Romanian field trial were statistically evaluated across sites, a statistically significant difference between soybean 40-3-2 and its conventional counterpart was found for four of the 49 comparisons. These were acid detergent fibre in forage (31.93 % vs. 30.26 % dry weight), and isoleucine (1.69 % vs. 1.73 % dry weight), valine (1.80 % vs. 1.84 % dry weight) and genistein (1642 vs. 1717 µg/g dry weight) in seeds. However, when evaluated per site, the level of these constituents was significantly different at none or at only one of the five individual field trial sites. Differences were small and levels fell within the normal variation of soybean constituents demonstrated by the reference soybean varieties included in the study and data described in the International Life Sciences Institute database (ILSI, 2006) and the United States Department of Agriculture (USDA-ISO, 2006) isoflavone database. In addition to these differences at individual sites, additional statistically significant differences were found for other constituents in the per site analysis. Twenty of these were found at one site only, and four at two of the five sites. Also in these cases differences were small and inconsistent and levels fell within the normal variation established by the reference lines.

Of the earlier field trials, the European field trials in 1998 were non-replicated and performed at seven sites, four in France and three in Italy, all locations characteristic of the European soybean growing regions. At each European field trial site soybean 40-3-2 was grown side by side with a set of reference soybean varieties used as comparators. No control with a genetic background similar to soybean 40-3-2 (conventional counterpart) was included in these field trials. Although only a few reference soybean varieties were grown at each site, in total eleven different reference soybean varieties were used. Eight of these were used in France, six in Italy, and three in both countries. All plants were grown under normal agricultural conditions. Some soybean 40-3-2 plots were in addition sprayed with a glyphosate-based herbicide at the recommended commercial dose, whereas others were not sprayed with this herbicide.

In total, 48 different compounds were analysed in the material from the earlier European field trials. Of the 136 statistical comparisons performed between soybean 40-3-2 and the mean of the eleven reference soybean varieties, a statistically significant difference in the level of a constituent was observed for six compounds: five compounds in untreated soybean 40-3-2 (stearic, oleic and eicosenoic acid, total fat and carbohydrates) and two compounds in soybean 40-3-2 sprayed with glyphosate (eicosenoic acid and arginine). In all cases, differences were small and not consistently found. As the level of these constituents in soybean 40-3-2 was comparable to the levels commonly observed in reference soybean varieties, these statistically significant differences were concluded to be without biological relevance.

The data from the European field trials confirmed data from earlier published results from field trials in the USA in 1992 (nine sites) and 1993 (four sites) (Padgette et al., 1996; Taylor et al., 1999). The test materials, soybean 40-3-2 and the conventional counterpart A5403, were hand weeded and treated with maintenance pesticides in 1992, whereas in 1993 soybean 40-3-2 was treated with glyphosate-

[9] Technical dossier / Section D7.1 // Additional information received on 20/06/2007

based herbicides and soybean A5403 with maintenance herbicides. Seed and leaf material were collected for analytical studies. The analytical results from these studies demonstrated that levels of constituents in unsprayed and glyphosate-sprayed soybean 40-3-2 are comparable to the levels in the conventional counterpart A5403 and reference soybean varieties. Seed materials from the 1992 harvest were also used to analyse processed products. Defatted toasted meal was analysed for proximates, trypsin inhibitor, lectins, urease, isoflavones, stachyose, raffinose and phytate; non-toasted meal was analysed for proximates; urease was analysed for trypsin inhibitor; protein isolate and protein concentrate were analysed for proximates; lecithin was analysed for phosphorylated compounds; and refined, bleached, deodorised soybean oil was analysed for fatty acids. No difference was found between processed products from soybean 40-3-2 and soybean A5403.

The EFSA GMO Panel considered the total set of compositional data supplied and the statistically significant differences between soybean 40-3-2 and its comparators reported in the light of the field trial design, measured biological variation and level of the studied compounds in conventional soybean varieties, and concluded that no biologically relevant differences were identified in the compositional characteristics of soybean 40-3-2 in comparison with its comparators, and that its composition fell within the range of commercial non-GM soybean varieties, except for the newly expressed protein.

When giving its opinion on the renewal applications for the continued marketing of soybean 40-3-2 products in 2010, the EFSA GMO Panel reviewed the available scientific literature related to the composition of soybean 40-3-2, and concluded that several investigators have independently confirmed the compositional equivalence of soybean 40-3-2 and commercial non-GM soybean varieties with regard to the content of isoflavone isomers, saponins, phospholipids, trypsin inhibitors and lectins (EFSA, 2010f).

In a subsequently published investigation of the compositional stability of glyphosate-sprayed soybeans with event 40-3-2 compared with commercial non-GM soybean varieties, performed over several growing seasons (from 2000 to 2009) in multiple germplasms (112 unique commercial soybean 40-3-2 varieties) and under different environmental conditions in North America, the means and range values for the studied parameters (proximate, anti-nutrients and isoflavones) were each year similar in soybean 40-3-2 and the conventional comparators (Zhou et al., 2011).

The additional data considered support the conclusions of the EFSA GMO Panel.

4.1.3. Agronomic traits and GM phenotype[10]

The applicant performed comparative assessments of the phenotypic and agronomic characteristics, and of the reproduction, dissemination and survivability of soybean 40-3-2, the conventional counterpart and reference soybean varieties based on field trials in the USA and Puerto Rico (1991–1994), Argentina (1993–1994), Canada (1993–1994), France (1994) and Italy (1994, 1996 and 1997). Parameters studied included date of emergence, percent emergence, plant count, plant height, vigour and colour, morphological changes, date at 50 % flowering, susceptibility to insects, nodes per plant, pods per plant, percent lodging, percent leaf drop, yield and moisture content, reproduction, dissemination and survivability. No meaningful differences among soybean 40-3-2, its conventional counterpart and reference soybean varieties were identified, except for the newly expressed protein.

Following commercial introduction of soybean 40-3-2 in North America, several research groups have independently published phenotypic, agronomic and ecological data on yield, height and glyphosate tolerance, as well as data on susceptibility of soybean 40-3-2 to insect pests, nematodes and plant pathogens, including resistance to fungal pathogens (EFSA, 2010f). The slightly reduced yield in soybean 40-3-2 noted by Elmore et al. (2001a) is still within the yield range of commercial non-GM soybean varieties. On the basis of all information available at the time when the EFSA GMO Panel published its Scientific Opinion on the renewal applications for the continued marketing of soybean

[10] Technical dossier / Sections D4 and D7.4

40-3-2, the Panel concluded that "*soybean 40-3-2 is phenotypically and agronomically not different from conventional soybeans, except for expressing the introduced glyphosate tolerance trait*" (EFSA, 2010f).

The additional data considered support the previous conclusion of the EFSA GMO Panel that the characteristics of soybean 40-3-2 do not differ from those of commercial non-GM soybean varieties, except for the newly expressed protein.

4.2. Conclusion

The EFSA GMO Panel considered the total set of compositional and agronomic/phenotypic data and the statistically significant differences identified between soybean 40-3-2 and its comparators in light of the field trial design, the measured biological variation and the level of the studied compounds in conventional soybean varieties. The EFSA Panel concludes that soybean 40-3-2 is compositionally and agronomically not different from its conventional counterpart and commercial non-GM soybean varieties, except for the newly expressed protein. Furthermore, the EFSA GMO Panel found no indication that the genetic modification had induced unintended effects that would raise safety concerns.

5. FOOD/FEED SAFETY ASSESSMENT

5.1. Evaluation of relevant scientific data

Although the scope of this application covers only cultivation of soybean 40-3-2, this Scientific Opinion also updates the previous EFSA GMO Panel safety evaluation on the continued marketing of: (1) food containing, consisting of, or produced from soybean 40-3-2; (2) feed containing, consisting of, or produced from soybean 40-3-2; and of (3) other products containing or consisting of soybean 40-3-2 (EFSA, 2010f). Unless specifically indicated, the information provided in this application, which is described in the following sections, has been evaluated previously by the EFSA GMO Panel (EFSA, 2010f).

5.1.1. Product description and intended use

The harvested soybean 40-3-2 will be used as food or feed, or for the production of derived soybean products as any commercial non-GM soybean variety. The main product for human use is soybean oil. In addition, approximately 10 % of the defatted soybean meal goes to production of soybean products for human consumption, including flours, soybean protein concentrates and various textured products simulating meats, seafoods and cheeses. The rest of the defatted soybean meal goes to feed, in the EU mainly to poultry, pig and cattle (OECD, 2001). Whole soybeans are used to produce soy sprouts, baked soybeans, and roasted soybeans. There is also a limited direct use of soybeans as animal feeds.

The genetic modification in soybean 40-3-2 results in the expression of the CP4 EPSPS protein, which allows soybean 40-3-2 to grow normally in the presence of glyphosate-based herbicides. Thus, the genetic modification is intended to improve agronomic performance only and is not intended to influence the nutritional aspects, the processing characteristics and overall use of soybean as a crop.

5.1.2. Effect of processing[11]

Soybean 40-3-2 will be used for production and manufacturing of food and feed products as any other commercial non-GM soybean variety. Taking into account the compositional analysis providing no indication of relevant compositional changes, the EFSA GMO Panel has no reason to assume that the characteristics of soybean 40-3-2 and derived processed products would be different from those of the respective products derived from commercial non-GM soybean varieties. The influence of temperature on the activity of the CP4 EPSPS protein derived from a recombinant *Escherichia coli* strain (section 5.1.3.1) was studied *in vitro* by determining the specific activity after incubation of the enzyme at various temperatures. Intermediate temperatures (55°C) reduced the activity of the CP4

[11] Technical dossier / Section D7.6

EPSPS protein, whereas higher temperatures (65^{o} and 75^{o}C) completely inactivated the protein. The pH had less influence on the activity, only slightly lowering it at the low end of the pH range 4-11. Studies by Kim et al. (2006) showed that the CP4 EPSPS protein is degraded during preparation of foods such as tofu and soybean paste.

5.1.3. Toxicology

5.1.3.1. CP4 EPSPS protein used for safety assessment

Due to the low expression level of the CP4 EPSPS protein in soybean 40-3-2 and the very difficult task to isolate a sufficient quantity of purified protein from the GM soybean, the safety studies with the newly expressed protein were conducted with a CP4 EPSPS protein encoded by the CP4 *epsps* gene from *Agrobacterium* sp. strain CP4 and expressed in *E. coli*. The structural similarity and physicochemical and functional equivalence of the CP4 EPSPS protein produced by *E. coli* to that produced in soybean 40-3-2 was shown by N-terminal sequencing (Edman degradation), Western analysis, mobility in SDS-PAGE, MALDI-TOF mass spectrometry, glycosylation analysis and determination of CP4 EPSPS enzymatic activity. These studies comprehensively confirmed the equivalence of the bacterial and the plant CP4 EPSPS proteins. Based on the identified similarity in structure, and equivalence in physicochemistry and function between these proteins, the EFSA GMO Panel accepts the use of CP4 EPSPS test material derived from *E. coli* for the degradation studies and safety testing of the CP4 EPSPS protein present in soybean 40-3-2, as well as a reference standard in the enzyme-linked immunosorbent assay (ELISA) to estimate CP4 EPSPS expression levels in various tissues of soybean 40-3-2.

5.1.3.2. Toxicological assessment of expressed novel proteins soybean 40-3-2

The newly introduced gene in soybean 40-3-2 is derived from *Agrobacterium* sp. strain CP4. The gene codes for a protein, CP4 EPSPS, unknown to be toxic to humans and animals, and also not known to be an allergen. Humans and animals have a history of safe consumption of the endogenous plant protein EPSPS, and the CP4 EPSPS protein is functionally similar to the soybean EPSPS. Furthermore, CP4 EPSPS-expressing crops have now been consumed as food and feed over ten years without any adverse effects being linked to the consumption. For example, around 60 % of all soybeans consumed during the last years are estimated to contain this protein.

Bioinformatic analysis

Searches for amino acid sequence homology of the CP4 EPSPS protein expressed in soybean 40-3-2 with amino acid sequences of toxic and general proteins stored in data bases indicated significant homology only with other EPSPS and related proteins. No sequence homology with known toxic proteins was found.

Degradation in simulated digestive fluids

Degradation of the CP4 EPSPS protein produced in *E. coli* was studied *in vitro* by following the CP4 EPSPS enzymatic activity after incubation in simulated gastric fluid containing pepsin, and by identifying peptide fragments using SDS-PAGE colloidal blue gel staining and Western blot analysis (Harrison et al., 1996). The SDS-PAGE colloidal blue gel staining demonstrated that at least 98 % of the CP4 EPSPS protein was fully degraded by pepsin-containing simulated gastric fluid of pH 2 within 15 seconds. In agreement with this finding, Western blotting showed that most of the CP4 EPSPS protein was digested in simulated gastric fluid within the same time frame. Similarly, studies on the function of the CP4 EPSPS protein exposed to simulated gastric fluid revealed that the enzymatic activity was reduced by more than 90 % within 15 seconds. In studies with simulated intestinal fluid containing pancreatin, the CP4 EPSPS protein had a half-life of less then 10 minutes as demonstrated with Western blot analysis. At this point in time only 5 % of the enzymatic activity had been lost. After 4.5 hours of incubation more than 91 % of the enzymatic activity was lost.

A fast degradation of microbially-produced CP4 EPSPS protein, CP4 EPSPS extracted from soybean 40-3-2 and the protein as present in the plant when exposed to gastro-intestinal conditions has been confirmed also in studies by independent investigators (EFSA, 2010f).

Acute toxicity testing

The applicant provided a single dose toxicity study with ten male and ten female DC-1 mice per treatment group. A summary of the study has been published (Harrison et al., 1996). All animals survived and there were no indications of adverse effects up to the highest dose of the CP4 EPSPS protein tested (572 mg/kg body weight).

5.1.3.3. Toxicological assessment of new constituents other than proteins

No new constituent other than the CP4 EPSPS protein is expressed in soybean 40-3-2 and no relevant changes in the composition of soybean 40-3-2 were detected by the compositional analysis.

5.1.3.4. Toxicological assessment of the whole GM food/feed[12]

Although the chemical analysis showed soybean 40-3-2 to be compositionally not different to commercial non-GM soybean varieties (except for the newly expressed protein) and, therefore, no animal feeding study being required for the risk assessment according to the Guidance Document (EFSA, 2006a), the applicant referred to four rat feeding studies with the GM soybean. Two of these were over four weeks with processed and unprocessed soybean 40-3-2, respectively. The other two were over thirteen and fifteen weeks with processed and heat-treated soybean 40-3-2, respectively.

In the first of the two 28-day studies, CD rats of both sexes (ten animals/sex) were fed *ad libitum* a diet with 24.8 % processed (dehulled, defatted and toasted) soybean meal from either event 40-3-2 or the conventional counterpart. An additional group of animals were fed a commercial rat diet containing dehulled soybean meal. Test animals survived and appeared healthy. The test diet neither influenced feed consumption and body weights of the rats, nor had any significant influence on organ weights (only liver, testes, and kidneys measured). The few findings in the histopathological examinations at necropsy were randomly distributed among treatment groups and were commonly observed in control animals of this rat strain in the testing laboratory.

The second 28-day study had an experimental design very similar to the first study and also used CD rats of both sexes, but instead of feeding the animals processed soybean meal used unprocessed meal at inclusion rates of 5 % and 10 % of the diet. Such low inclusion rates might have been required as monogastric animals usually are not fed unprocessed soybeans due to the presence of anti-nutritive factors in the raw bean. Ruminants tolerate the raw material as the anti-nutrients are degraded by the rumen micro-flora. In this study test animals appeared healthy, and the diet neither influenced feed consumption, body weight and cumulative body weight gain, nor had any significant influence on absolute and relative organ weights (only liver, testes, and kidneys measured) in relation to the conventional counterpart. When soybean 40-3-2 fed rats were compared with rats fed the commercial rat feed, a slightly higher relative kidney weight was observed at a dose of 5 % soybean 40-3-2, but not at the higher dose. As there was no difference in relative kidney weight at the higher dose, the finding was considered incidental. Animals that received the higher dose unprocessed soybean frequently showed darker livers, possibly related to the inclusion rate of unprocessed soybean and not to the genetic modification. The few findings in the histopathological examinations at necropsy were randomly distributed among all groups as in the first experiment. Since unprocessed soybean meal contains trypsin inhibitors that can cause hypertrophy of the pancreas when soybeans are the sole protein source (Liener and Kakade, 1980), this organ was examined histologically for all animals in the study. No pathological lesions, but minimal to mild microscopic changes were observed in the pancreas of animals of all groups. Thus, this characteristic was not related to the treatment with soybean 40-3-2.

[12] Technical dossier / Section D7.8.4

The third study was a 90-day feeding study in Sprague-Dawley rats fed *ad libitum* diets with large quantities of processed soybean 40-3-2 meal or meal from a conventional soybean (Zhu et al., 2004). The test diets contained 30 %, 60 % or 90 % processed soybean 40-3-2 meal or 60 % conventional soybean meal. The only deviation in feed intake and body weight was observed during the first week in rats of both sexes fed 90 % soybean 40-3-2 meal, apparently due to the exposure to high protein levels and not to the exposure to soybean 40-3-2. Later on in the study, there was no influence on feed intake and body weight gain. There were no relevant differences between the test groups and the control group regarding gross necropsy findings, haematology or urinalysis parameters. No treatment-related adverse effects were observed in the study.

The fourth study was a 15-week rat feeding study with heat-treated soybean meal in female Brown Norway rats and female B10A mice, aiming to study potential effects on the immune system (Teshima et al., 2000). The heat-treated soybean meal was incorporated at a level of 30 % in the rat and mice feed. The meals were produced from soybean 40-3-2 for the test group and from a closely related commercial non-GM soybean for the control group. No treatment-related changes in growth, food consumption, liver and spleen weight between rats and mice fed diets with soybean 40-3-2 and animals fed the control soybean meal was observed. Based on presence of soybean-specific IgG and IgE in rodent sera and histological examinations of immune-related organs, it was concluded that soybean 40-3-2 was not more antigenic or immunogenic than commercial non-GM soybeans.

A few additional rodent feeding studies with diets containing soybean 40-3-2 are available in the peer-reviewed scientific literature and were referred to by the EFSA GMO Panel in its Scientific Opinion on the renewal applications for the continued marketing of soybean 40-3-2 (EFSA, 2010f). Studies accepted after a critical analysis of their design and the test material used supports the conclusion that soybean 40-3-2 is as safe as commercial non-GM soybean varieties. The EFSA GMO Panel concludes that available feeding studies on experimental animals confirm that soybean 40-3-2 and products thereof are as safe as commercial non-GM soybean varieties and their products.

5.1.4. *Allergenicity*[13]

The strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and on whether the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (CAC, 2003; EFSA, 2006a, 2010a, 2011b).

5.1.4.1. Assessment of allergenicity of the newly expressed proteins

The CP4 *epsps* gene originates from *Agrobacterium* sp. strain CP4, a soil-borne and plant-interacting microorganism that is not known to be allergenic. A bioinformatics-supported comparison of the amino acid sequence of the CP4 EPSPS protein with the sequences of known allergens, gliadins, and glutenins stored in an updated propriety database has been performed. This analysis included both overall sequence alignments using the FASTA algorithm and searches for short identical stretches of at least eight contiguous amino acids. No match of such short sequences of the CP4 EPSPS protein was found to similar-sized fragments of allergenic proteins. Neither was any identity larger than 35 % found between the entire CP4 EPSPS protein and known allergenic proteins.

As described in section 5.1.3.2., the CP4 EPSPS protein is rapidly degraded under simulated gastric and intestinal conditions.

Several researchers have independently confirmed that soybean and food allergic subjects from the EU and Asia do not express IgE that specifically bind the purified CP4 EPSPS protein (EFSA, 2010f).

[13] Technical dossier / Section D7.9 // Additional information received on 20/06/2007

Based on the available data, the EFSA GMO Panel considers that it has been confirmed that the newly expressed CP4 EPSPS protein is unlikely to be allergenic.

5.1.4.2. Assessment of allergenicity of the whole GM plant

Allergenicity of the whole crop could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, for example through qualitative or quantitative modifications of the pattern of expression of endogenous proteins. However, given that equivalence (with the exception of the introduced trait) to the conventional counterpart (A5403) was demonstrated on the basis of extensive compositional and agronomic analysis, no increased allergenicity is anticipated for soybean 40-3-2. Because soybean is a recognised allergenic food, the applicant performed a comparative study on the endogenous allergens in soybean 40-3-2, its conventional counterpart (soybean A5403), and three commercial non-GM soybean varieties, using extracts from these soybeans and sera from soybean allergic patients (Burks and Fuchs, 1995). The study revealed no quantitative and qualitative difference in immunoblotting reaction of sera to extract of soybean 40-3-2 and extracts of the different comparators. The EFSA GMO-Panel agrees with the applicant that these data indicate that the transformation generating soybean 40-3-2 has caused no change in the allergen repertoire of soybean.

The result of the initial pre-marketing studies referred to above have recently been confirmed by several independent investigators after the product has been on the market for some time (EFSA, 2010f). In addition, in a murine model (Balb/c mice) of IgE-mediated soybean sensitization induced by intragastric immunization (in the presence of Cholera Toxin) with soybean extracts, Gizzarelli et al. (2006) observed that extracts of soybean 40-3-2 induced an immunological response that was comparable with that induced by extracts of commercial non-GM soybean varieties. The EFSA GMO Panel concludes that the information presented confirms that the overall allergenicity of the whole soybean 40-3-2 plant is not changed.

5.1.5. Nutritional assessment of GM food/feed[14]

To substantiate that soybean 40-3-2 is as nutritious as commercial non-GM soybean varieties, as indicated by comparable chemical composition the applicant supplied short-term feeding studies with soybean 40-3-2 on the target animals broiler chicken, quail, swine, dairy cow and catfish (Hammond et al., 1996). The EFSA GMO Panel considered the feeding studies on broiler chickens, swine and catfish for the nutritional assessment of soybean 40-3-2 as compared with its conventional counterpart. The study with dairy cattle was not accepted by the EFSA GMO Panel because of a too short duration (three weeks only) and additional weaknesses in experimental design (Flachowsky and Aulrich, 1999). The feeding study in quails was not considered due to its short duration (five days only).

Broiler chickens were fed starter diets containing 32.9 % processed (dehulled, defatted and toasted) soybean meal (soybean 40-3-2 or its conventional counterpart) from day 0 to 21, and grower/finisher diets containing 26.6 % soybean meal from day 22 to 42, when the study was terminated (Hammond et al., 1996). In these 42 days, broilers reach a market weight of approximately 2 kg. The experimental diets had no influence on feed intake, weight gain, feed conversion, and livability (percent live birds; survival rate). There were also no significant difference in the performance parameters investigated (breast muscle weight and abdominal fat pad weight; in both cases total weight and percent of body weight) between broilers fed diets with soybean 40-3-2 and broilers fed its conventional counterpart. Additional information on broiler chickens is available from a small feeding study in which the birds were given a diet with 24–25 % soybean meal (Deaville and Maddison, 2005). The broilers fed soybean 40-3-2 had as high feed intake, growth and feed conversion ratio as broilers fed control soybean.

When giving its opinion on the renewal applications for the continued marketing of soybean 40-3-2, the EFSA GMO Panel reviewed additional information from feeding studies by independent

[14] Technical dossier / Section D7.10 // Additional information received on 20/06/2007

investigators in broiler chickens (EFSA, 2010f). More recently, Swiatkiewicz et al. (2010a) fed Ross 308 broiler chickens diets with 32–39 % soybean meal from soybean 40-3-2 or a commercial non-GM soybean variety. Feed intake, growth parameters, and mortality were not different in the two groups, and there were no statistically significant differences found in carcass parameters, organ weights, and chemical composition of the breast muscles analysed after slaughter. The pH and water holding capacity values of breast and thigh muscles indicated no statistically significant differences between broilers fed diets containing soybean 40-3-2 and birds fed diets with meal of the commercial non-GM soybean variety (Stadnik et al., 2011a). Furthermore, studies in Bovans Brown laying hens by the same investigators showed that the laying performance, digestibility of nutrients and egg quality did not differ between hens that received meal of soybean 40-3-2 and hens that received meal of commercial non-GM soybean varieties in the diet (Swiatkiewicz et al., 2010b). Recombinant DNA was not detected in internal organs, blood, muscles, excreta and eggs of examined birds.

One hundred cross-bred pigs of both sexes were for about 100 days fed diets containing about 14–24 % (depending on age of animals) dehulled soybean meal derived from either the event 40-3-2 or its conventional counterpart (Cromwell et al., 2002). During the feeding period the pigs grew in weight from about 24 kg to 111 kg. No difference between treatment groups were observed for feed intake, efficiency of feed utilisation and body weight gain, scanned backfat and longissimus area, and calculated carcass lean percentage. The sensory characteristics of the longissimus muscles were not influenced by treatment. The differences observed were not between pigs given the different feeds but those expected between sexes.

Recently, Polish researchers in a similar experiment on swine found diets containing 14–18 % meal of soybean 40-3-2 or the conventional counterpart to have no differential influence on feed utilization, body weight, and carcass yields. Type of soybean in the diet also did not influence the quality and chemical composition of the meat (Swiatkiewicz et al., 2011), including the pH of loin and neck muscles and water holding capacity (Stadnik et al., 2011b). The dietary treatments had no influence on colour parameters of the loin meat, whereas some differences were noted in the neck muscle, possibly due to the natural heterogeneity of this primal cut. DNA fragments specific for soybean 40-3-2 could be identified in the content of the stomach and duodenum but not further down in the gastrointestinal tract, and in various tissues and in blood (Swiatkiewicz et al., 2011).

The original fish feeding study was performed on 300 fingerling channel catfish (*Ictalurus punctatus*) of mixed sex. The study was over ten weeks with diets containing processed meal (45–47 % w/w) (Hammond et al., 1996). There was no statistically significant difference in survival, feed conversion ratio, and percentage weight gain between the groups receiving diets based on control soybean meal and soybean 40-3-2 meal. Although fish receiving the diet with soybean 40-3-2 meal consumed slightly less feed than fish fed a diet with the control soybean meal, this did not influence body composition data. There were no differences in moisture, protein, fat or ash content among fish regardless of dietary treatment.

In its opinion on the renewal applications for the continued marketing of soybean 40-3-2, the EFSA GMO Panel reviewed additional information from published feeding studies on Atlantic salmon and rainbow trout (EFSA, 2010f). Several research teams have recently reported on the dietary use of glyphosate tolerant soybeans (soybean meal of event 40-3-2) and the fate of the transgenic DNA in carp, Nile tilapia and Atlantic salmon. Japanese investigators found no significant difference in growth and feed efficiency of the common carp (*Cyprinus carpio*) and Nile tilapia (*Oreochromis niloticus*) raised on diets with the GM soybean or the corresponding conventional counterpart (Suharman et al., 2009, 2010). The investigator analysed the muscles and blood for the CaMV 35S promoter sequence of the event 40-3-2 and found no signal in muscles and blood of the carp (Suharman et al., 2010), whereas a small number of muscle samples from Nile tilapia gave a positive signal for the promoter fragment. However, the promoter fragment could not be identified the second day after switching from diet containing soybean 40-3-2 to a diet with meal of commercial non-GM soybean varieties (Suharman et al., 2009).

Sissener et al. (2009a,b) performed a 7-month feeding trial with diets containing 25 % meal of soybean 40-3-2 or its conventional counterpart in Atlantic salmon (*Salmo salar*) in order to study growth, body composition, organ development, intestinal changes, haematological parameters, clinical chemistry and lysozyme levels, and stress response. As the salmons went through the parr-smolt transformation during the feeding experiment, samples were collected both in the freshwater- and seawater-stages. Of the many parameters studied, only mid-intestine being smaller, plasma triacylglycerol levels being higher, and the mucosal fold height in the distal intestine (one sampling time of three) and mucosal fold fusion was more pronounced in fish supplied the diet with GM soybean. No other diet-related morphological differences were found in any organs, and there was no difference in stress response. Furthermore, proteomic profiling of liver cells from these salmons only identified minor differences in liver protein synthesis between fish fed GM and non-GM soybean (Sissener et al., 2010a). Sanden et al. (2011) followed the fait of soybean DNA in the intestinal tract of the salmon. Transgenic DNA was not detected in any of the analysed intestinal organs but the multi-copy rubisco gene was found in all segments of the intestine, for example in the vacuolar system of the distal intestine. Feed restriction gradually cleared DNA within five days. Re-feeding revealed DNA within two hours. Thus, it seems as feeding status regulates the appearance of DNA in various intestinal segments. The investigators concluded that it appears as inclusions levels of 25 % GM soybean in the fish diet does not cause any adverse effects of importance on organ morphology or stress response compared with non-GM soybean. The lack of consistency with previous studies (see, EFSA, 2010f) suggests that the minor differences observed might be caused by variations in the soybean variety rather than the genetic modification *per se*.

The EFSA GMO Panel concludes that soybean 40-3-2 is as nutritious as commercial non-GM soybean varieties.

5.1.6. *History of exposure to soybean 40-3-2 in Europe*[15]

Soybean 40-3-2 was first cultivated in the USA and Argentina in 1996, and subsequently commercialised in Canada, Uruguay, South Africa, Brazil, Romania and Paraguay. Thus, in Romania, soybean 40-3-2 was commercially produced between 1999 and 2006, prior to the accession to the EU in 2007. Production of soybean 40-3-2 was rapidly adopted in many markets, but most notably in the USA and Argentina, where current adoption rates exceed 90 % of total soybean production area. When soybean 40-3-2 production was discontinued in Romania in 2006, it was cultivated on 84 % of the area devoted to soybean cultivation in that country.

Based on data on import of soybean seed, soybean meal and soybean oil into the 27 countries of the EU from five soybean 40-3-2-producing countries (Argentina, Brazil, Canada, Paraguay and the USA) during the years 2003-2006, the applicant calculated that around 55 % of soybean seed, 61 % of soybean meal and 54 % of soybean oil used in the EU might be based on soybean 40-3-2. It should be noted, however, that the calculations of these figures are based on several assumptions. Because operators in the food and feed chain in some EU Member States have made efforts to preferentially source non-GM soybean products, the actual consumption of products derived from soybean 40-3-2 in food and feed may vary between Member States.

Based on FAO Statistics from 1997 to 2001, the human soybean oil consumption in the EU was calculated at 6.3-7.0 g/person/day. Assuming that 54 % of the soybean oil was derived from soybean 40-3-2, an estimated average exposure of the European consumer to products of soybean 40-3-2 would be in the range of 3.4-3.7 g/person/day.

Animal feed is the major end use of soybean meal. The applicant calculated, based on data from 2006, that the maximum inclusion levels (percent of the diet) of soybean 40-3-2 meal in the EU would be 21 % for broiler chickens, 18 % for pigs and 12 % for dairy cattle.

[15] Technical dossier / Sections D7.7 and D7.8.4 // Additional information received on 20/06/2007

5.1.7. *Post-market monitoring of GM food/feed*

The risk assessment concluded that no data have emerged to indicate that soybean 40-3-2 is any less safe than its conventional counterpart. In addition, soybean 40-3-2 is, from a nutritional point of view, equivalent to commercial non-GM soybean varieties. Therefore, and in line with the then applicable and more recent Guidance Documents (EFSA, 2006a, 2011b), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

5.2. Conclusion

The CP4 EPSPS protein is quickly degraded in simulated gastric and intestinal fluids without leaving stable peptide fragments. Bioinformatics analyses demonstrated that the CP4 EPSPS protein shows no homology to known toxic and allergenic proteins. The CP4 EPSPS protein induced no toxicity when administered orally to mice in an acute toxicity study.

A number of feeding studies of various lengths on laboratory rodents given processed and unprocessed soybean 40-3-2 in the diet indicated no toxicity related to the genetic modification. Whole-product testing with sera from soy-allergic patients shows that the overall allergenicity of soybean 40-3-2 is not different from that of the conventional counterpart. Feeding studies on broiler chickens, laying hens, rabbits, goat, swine, catfish, carp, Nile tilapia, Atlantic salmon and rainbow trout show that soybean 40-3-2 is as nutritious as the conventional counterpart.

The EFSA GMO Panel is of the opinion that soybean 40-3-2 is as safe as its conventional counterpart and commercial non-GM soybean varieties, and considers that no additional animal safety or nutritional studies are needed.

6. Environmental risk assessment and risk management strategies

6.1. Evaluation of relevant scientific data

The scope of application EFSA-GMO-NL-2005-24 covers cultivation of soybean 40-3-2. Considering the intended uses of soybean 40-3-2, the environmental risk assessment is concerned with potential direct and indirect environmental effects of the cultivation and the spread of the GM plant into non-cultivated environments. As this EFSA GMO Panel Scientific Opinion also updates its previous safety evaluation on the continued marketing of: (1) food containing, consisting of, or produced from soybean 40-3-2; (2) feed containing, consisting of, or produced from soybean 40-3-2; and of (3) other products containing or consisting of soybean 40-3-2 (EFSA, 2010f), indirect exposure through manure and faeces from animals fed soybean 40-3-2 is also considered.

The EFSA GMO Panel considered the following issues in the environmental risk assessment submitted by the applicant: (1) changes in plant fitness due to the genetic modification; (2) potential for gene transfer and its consequences; (3) interactions between the GM plant and target organisms; (4) interactions between the GM plant and non-target organisms; (5) effects on animal and human health; (6) interactions with biogeochemical processes and the abiotic environment; (7) impacts of the specific cultivation, management and harvesting techniques; and (8) risk management strategies (including post-market environmental monitoring).

The DE CA provided EFSA with its report on the environmental risk assessment of soybean 40-3-2 (dated 9 September 2008) on 3 October 2008 in line with Articles 6.3(c) and 18.3(c) of Regulation (EC) No 1829/2003. The report on the environmental risk assessment of the DE CA is provided in Annex H of the EFSA Overall Opinion, and has been considered throughout this EFSA GMO Panel Scientific Opinion.

6.2. Environmental risk assessment[16]

6.2.1. Changes in plant fitness due to the genetic modification[17]

A series of field trials with soybean 40-3-2 was conducted by the applicant at several locations in representative soybean growing areas in the USA and Puerto Rico (1991-1994), Argentina (1993-1994), Canada (1993-1994)[18] and in the EU (France (1994)[19], Italy (1994, 1996 and 1997)[20]) to compare the agronomic performance and field characteristics of soybean 40-3-2 with its comparators. Information on phenotypic and agronomic characteristics of soybean 40-3-2 and its comparators was generated to compare their growth habit, vegetative vigour and reproduction characteristics. Several endpoints related to growth habit, vegetative growth, reproduction, and yield and grain characteristics were measured. The EFSA GMO Panel evaluated this dataset previously (EFSA, 2010f), and concluded that "*no meaningful differences between soybean 40-3-2 and its conventional counterpart were identified, except the expected difference in tolerance to glyphosate herbicides*", and that the field trial data did not show "*changes in plant characteristics that indicate altered fitness and invasiveness of GM soybean 40-3-2 compared to its conventional counterpart, except in the presence of glyphosate herbicides*".

Following a request for clarification from the EFSA GMO Panel, the applicant provided additional data from agronomic and phenotypic field trials performed at four locations in representative soybean growing areas in Romania during the growing season of 2005.[21] A randomised complete block design with three replications was used in the field trials. The comparators used in the Romanian field trials consisted of the conventional counterpart Dekabig, a conventional soybean with a genetic background similar with soybean 40-3-2 with the exception of the glyphosate tolerance trait, and ten reference soybean varieties. Soybean 40-3-2 and the comparators received the same conventional herbicide treatments.

In the Romanian 2005 field trial data, a number of parameters (i.e., plant height, lodging, grain moisture, weight of 100 seeds) showed statistically significant differences in the across-location comparisons between soybean 40-3-2 and its conventional counterpart. These differences were not consistently observed in each location, and were not considered biologically meaningful with respect to persistence and invasiveness potential, as the range of values for agronomic and phenotypic characteristics fell within the range of values observed in the reference soybean varieties used in the 2005 field trials. No visually observable response to naturally occurring insects, diseases and/or abiotic stressors recorded during the growing season provided any indication of altered stress responses of soybean 40-3-2 as compared with its conventional counterpart.

After commercial introduction of soybean 40-3-2 in North America, various research groups have published data on yield, height and glyphosate tolerance (Delannay et al., 1995; Elmore et al., 2001a,b), as well as data on: susceptibility of soybean 40-3-2 to insect pests (Morjan and Pedigo, 2002; McPherson et al., 2003); nematode damage (Koennig, 2002; Yang et al., 2002); and diseases, including resistance to fungal pathogens (Lee et al., 2000; Sanogo et al., 2000, 2001; Harikrishnan and Yang, 2002; Mueller et al., 2003; Njiti et al., 2003). These data contribute to the conclusion that the characteristics of soybean 40-3-2 do not differ from those of conventional soybean varieties, except for soybean 40-3-2 giving a slightly reduced yield (Elmore et al., 2001a), but which is still within the range in yield of conventional soybean varieties, and being glyphosate tolerant as a consequence of the newly introduced trait.

It is considered very unlikely that the establishment, spread and survival of soybean 40-3-2 would be increased due to the herbicide tolerance trait. This trait can only be regarded as providing a potential

---

16 Technical dossier / Section D9
17 Technical dossier / Sections B2, B3, B4, D4, D9.1 and D9.2
18 Technical dossier / Section D.4 / Page 57 / Annex: Monsanto Company (1994)
19 Technical dossier / Section D.4 / Page 56 / Annex: Monsanto France (1994)
20 Technical dossier / Section D.4 / Page 56 / Annex: Monsanto Italy (1994, 1996 & 1997)
21 Additional information received on 20/06/2007 / Question 3 / Pages 4-5 / Confidential annex: Kendrick and Clark (2006)

selective advantage to soybean 40-3-2 when glyphosate-based herbicides are applied. Moreover, it is considered very unlikely that soybean 40-3-2 plants or their progeny will differ from conventional soybean varieties in their ability to survive as volunteers until subsequent seasons, or to establish feral populations under European environmental conditions (section 6.2.2.2). Soybean is highly domesticated and generally unable to survive in the environment without management intervention (Bagavathiannan and Van Acker, 2008). Seed-mediated establishment of soybean and its survival outside of cultivation is rare in spite of extensive cultivation in many countries and accidental seed dispersal. Neither the pods, nor the seeds, have morphological characteristics that would facilitate animal transport (OECD, 2000). Soybean seeds rarely display any dormancy characteristics and only under certain environmental conditions grow as volunteers in the year following cultivation (OECD, 2000; Yoshimura et al., 2011).

The survival of soybean is limited by a combination of: low competitiveness; absence of a dormancy phase; and susceptibility to plant pathogens, herbivores and cold climatic conditions. In soybean fields, seeds do not usually survive during the winter due to predation, rotting and germination resulting in death, or due to management practices prior to planting the subsequent crop (Owen, 2005). Soybean plants are only winter hardy in regions with mild winters, and in those situations soybean seeds remaining in the field after harvest can germinate and grow. In case they should establish, volunteers do not compete well with the following crop in the rotation, and can easily be controlled mechanically or chemically (OECD, 2000). While soybean 40-3-2 volunteers occurring in cultivated areas will be tolerant to glyphosate, they are normally controlled by current agricultural practices, including the use of selective herbicidal active ingredients and/or cultivation techniques (Owen, 2005).[22]

Despite cultivation for decades, soybean plants do not occur outside cultivated or in disturbed land in Europe. In addition to the data presented by the applicant, the EFSA GMO Panel is not aware of any scientific report of increased establishment and spread of soybean 40-3-2 and any change in survival (including over-wintering), persistence and invasiveness capacity. Because the general characteristics of soybean 40-3-2 are unchanged, herbicide tolerance is not likely to provide a selective advantage outside of cultivation in Europe.

Since soybean 40-3-2 has no altered agronomic and phenotypic characteristics, except for the herbicide tolerance, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects due to the establishment and survival of soybean 40-3-2 will be no different to that of conventional soybean varieties.

The conclusion of the EFSA GMO Panel is consistent with that of the DE CA. The DE CA considered that "*experience gained during the commercial cultivation of soybean 40-3-2 in numerous parts of the world supports the conclusion that soybean 40-3-2 is equivalent to traditional soybean in its phenotypic and agronomic characteristics and shows no evidence for any change with respect to the ability to survive, reproduce, and disperse*". Therefore, the DE CA concluded that "*the potential of persistence, dispersal, and invasiveness of the genetically modified plants is not different from traditionally bred soybeans*" (sections 5.3.5 and 6.1 of the environmental risk assessment report of the DE CA).

6.2.2. Gene transfer

The EFSA GMO Panel evaluated the potential for horizontal and vertical gene flow of soybean 40-3-2, as well as the potential environmental consequences of such gene transfer. A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of deoxyribonucleic acid (DNA), or vertical gene flow via the dispersal of pollen and seed.

[22] Additional information received on 20/06/2007 / Question 4 / Pages 6-8

6.2.2.1. Plant to bacteria gene transfer and its consequences[23]

Bacteria are capable of exchanging genetic material directly between each other and even across species boundaries using different mechanisms such as conjugation, transduction or natural transformation. DNA from plants, including fragments or full sequences of the recombinant genes of GM plants, could hypothetically be acquired by bacteria through horizontal gene transfer. After initial horizontal gene transfer from plants to bacteria, the acquired genes may be further spread to other bacterial strains or species.

Current scientific evidence indicates that the transfer of genes derived from GM plants into bacteria and their stable integration, either does not occur or, if it has occurred, it has been below the limit of detection in all the studies performed (see Keese, 2008; EFSA, 2009a and references therein; Brigulla and Wackernagel, 2010; Ma et al., 2011; Townsend et al., 2012). The main barriers for horizontal gene transfer from plants to bacteria are the lack of efficient mechanisms of integration of unrelated chromosomal DNA and the limited potential for positive directional selection of the acquired recombinant gene-encoded traits.

The exposure of bacteria to the recombinant DNA fraction of soybean 40-3-2, the barriers limiting horizontal gene transfer, and the impact of hypothetical horizontal gene transfer in receiving environments are described below.

The probability and frequency of horizontal transfer of plant DNA (including the recombinant DNA fraction) to exposed bacteria is determined by: (1) the concentration and quality of plant DNA accessible to bacteria in receiving environments; (2) the presence of bacteria with a capacity to develop competence for natural transformation, i.e., to take up extracellular DNA; (3) the ability for genetic recombination by which the plant DNA can be incorporated and thus stabilised in the bacterial genome (including chromosomes or plasmids); (4) the expression and the function of the protein in the bacterial recipient; and by (5) the selective advantage provided by the acquired recombinant gene-encoded traits.

The release and low-level temporal persistence of gene-sized plant DNA fragments is expected in environments where crops are grown and in gastrointestinal systems after consumption (EFSA, 2009a; Rizzi et al., 2012). The scope of this application is for cultivation. Therefore, the main exposure to DNA would occur in agricultural soils. As this EFSA GMO Panel Scientific Opinion also updates its previous safety evaluation on the continued marketing of: (1) food containing, consisting of, or produced from soybean 40-3-2; (2) feed containing, consisting of, or produced from soybean 40-3-2; and of (3) other products containing or consisting of soybean 40-3-2 (EFSA, 2010f), exposure to DNA in gastrointestinal systems is also considered.

Bacteria in the digestive tract of humans, domesticated animals, and other animals feeding on soybean 40-3-2 will be exposed to low levels of fragmented products of the ingested DNA, including the recombinant genes (section 5.1.3). DNA is a component of many food and feed products derived from soybean, but becomes substantially degraded during food/feed processing, and in the process of digestion in the mammalian gastrointestinal tracts (Jonas et al., 2001; van den Eede et al., 2004; Ramessar et al., 2007). Due to its substantial degradation in the digestive tract, full-length genes from plants will rarely be detectable in the large intestine or in faeces of mammals (EFSA, 2009a and references therein).

Bacteria in soil environments will be exposed to extracellular DNA released from plant cells throughout and after the growing season (reviewed by Levy-Booth et al., 2007). During plant growth, free plant DNA may originate from sloughed off root cap cells (Hawes, 1990; de Vries et al., 2003) or necrotic root tissue infected by pathogens (Polverari et al., 2000; Kay et al., 2002). Pollen release at anthesis falling onto the soil surface (de Vries et al., 2003; Webster et al., 2008) and DNA release from decomposing plant residue remaining in agricultural areas after harvest, especially when

[23] Technical dossier / Section D6

incorporated into the soil during tillage operations (Widmer et al., 1997; Ceccherini et al., 2003; Stotzky, 2004), can also contribute to the presence of plant DNA in soil later during the growing season. However, the vast majority of plant DNA is expected to be degraded shortly after harvest by plant and microbial DNases in the soil environment. Therefore, plant DNA is considered a transient component of the total DNA pool in soil (Levy-Booth et al., 2007; Gulden et al., 2008). For instance, Gulden et al. (2008) did not observe accumulation of the *epsps* gene in the soil environment upon repeated cultivation of Roundup Ready maize (event 39T67). While adsorption to soil particles, particularly clay, can slow down DNA degradation, the vast majority will be degraded shortly after harvest. It can therefore be concluded that the presence of extracellular DNA fragments (including the CP4 *epsps* gene of soybean 40-3-2) in gastrointestinal tracts, soil or other environments is time-limited and that it is mainly present as short fragments at relative low concentrations.

Several bacterial species with the potential to develop competence for natural transformation (take up and recombine with extracellular DNA) belong to the common gut microbial community (Rizzi et al., 2008, 2012; EFSA, 2009a). However, competence development and transformation of such bacteria with genomic DNA of plants has not been observed in the lower gastrointestinal tract even with optimised model systems providing a selective advantage (Nordgård et al., 2007; EFSA, 2009a; Rizzi et al., 2012). In contrast, some studies have shown that introduced bacteria can be naturally transformed in the oral cavity of humans and animals (Duggan et al., 2000, 2003; Mercer et al., 1999a,b, 2001; Rizzi et al., 2012). Once the recombinant DNA is taken up, it must integrate into the recipient genome to persist during host replication. The likelihood of gene integration is influenced by the context (i.e., the surrounding/neighbouring sequences) of the recombinant gene(s) in the plant genome (EFSA, 2009a).

Homologous recombination efficiently facilitates integration of non-mobile, chromosomal DNA fragments into bacterial genomes (see EFSA, 2009a and references therein). This process depends on the presence of stretches of identical DNA sequences between the recombining DNA molecules. In addition to substitutive recombination events, where only the homologous genes are replaced, homologous recombination can also facilitate the insertion of non-homologous DNA sequences into bacterial genomes (additive recombination) if the flanking regions share sufficient sequence similarity.

The CP4 *epsps* gene in soybean 40-3-2 is derived from *Agrobacterium* strain CP4, and theoretically contains sufficient DNA similarity for homologous recombination to take place in bacterial genomes already containing similar genes. In addition, the *nos* terminator sequence in soybean 40-3-2, which has been derived from *A. tumefaciens*, theoretically contains sufficient DNA similarity for single homologous recombination to take place in bacteria carrying the *nos* gene.

In addition to homology-based recombination processes, non-homologous recombination events, that do not require the presence of DNA similarity between the recombining DNA molecules, are also theoretically possible. Non-homologous recombination has rarely been described in bacteria. In one study, the transformation rates for non-homologous recombination-based gene acquisitions were 10^{10}-fold lower than for homologous recombination-based gene acquisitions (de Vries et al., 2004; Hülter and Wackernagel, 2008; EFSA 2009b). Non-homologous recombination events have not been detected in studies that have exposed bacteria to high concentrations of DNA from GM plants (see EFSA 2009b). Non-homologous recombination scenarios for the CP4 *epsps* gene in soybean 40-3-2 are therefore not further considered here.

Expression of the acquired DNA is considered a prerequisite to produce a risk-relevant change in the phenotype of the transformed bacteria. If the CP4 *epsps* cassette from soybean 40-3-2 is transferred to bacterial cells, the expression of the CP4 *epsps* gene cannot be excluded because the P-e35S promoter (section 3.1.1) has been shown to be functional in some bacteria (Assaad and Signer, 1990; Lewin et al., 1998; Jacob et al., 2002). Therefore, the EFSA GMO Panel takes into account that expression might occur in some bacterial cells.

Bacterial communities are continually exposed to a high diversity of DNA sources in the environment. Therefore, a positive directional selection is considered to be required for rare horizontal gene transfer events to become biologically meaningful in the risk assessment.

The horizontal gene transfer event hypothesised above is not likely to be maintained in bacterial populations in the gastrointestinal tract of mammals due to the lack of selective advantage for rare bacterial recipients, provided they would be able to express the hypothetically acquired CP4 *epsps* gene. The overall exposure level of environmental bacterial communities to the soybean 40-3-2 CP4 *epsps* gene must be seen in the context of the natural occurrence and level of exposure to other sources of similar genes to which bacterial communities are continually exposed. The use of glyphosate could generate transiently selective conditions to favour growth of soil bacteria with glyphosate tolerant EPSPS. *Epsps* like-genes encoding for glyphosate tolerant enzymes are already present in various bacterial genera and species in the environment (e.g., *Agrobacterium*, *Ochrobactrum*, *Pseudomonas putida*). Therefore, no novel selective advantage that could not be conferred by natural bacteria is anticipated for hypothetical bacterial recipients expressing the CP4 *epsps* gene.

The unlikelihood of functional gene acquisition by double homologous recombination, the wide environmental presence of genetically diverse natural variants of the *epsps* genes, and the absence of an identified plausible selective advantage, except in the presence of glyphosate suggest that the recombinant DNA of soybean 40-3-2 will not transfer and establish in the genome of bacteria in the human and animal digestive tract or in the environment. In the theoretical case of horizontal transfer of the CP4 *epsps* gene to bacteria, no novel property would be introduced into soil bacterial communities as *epsps*-like genes would already be present.

In its evaluation, the EFSA GMO Panel did not identify properties with the inserted DNA in soybean 40-3-2 that would change its likelihood of horizontal transfer compared with other plant genes. A plausible and novel selective advantage of hypothesised horizontal transfer of the recombinant gene (CP4 *epsps*) to bacteria has not been identified. Therefore, the EFSA GMO Panel concludes that the recombinant DNA in soybean 40-3-2 does not represent an environmental risk in relation to its potential for horizontal transfer to bacteria.

The conclusion of the EFSA GMO Panel is consistent with that of the DE CA. The DE CA concluded that "*the possibility of gene transfer from the genetically modified soybean 40-3-2 to microorganisms and its consequences is not regarded as a safety concern*" (section 6.2.2 of the environmental risk assessment report of the DE CA).

6.2.2.2. Plant to plant gene transfer and its consequences[24]

Soybean is an annual almost completely self-pollinating crop in the field which has a percentage of cross-pollination usually lower than 1 % (Weber and Hanson, 1961; Caviness, 1966; Ahrent and Caviness, 1994; Ray et al., 2003; Lu, 2005; Yoshimura et al., 2006; Abud et al., 2007).[25] Soybean pollen dispersal is limited because the anthers mature in the bud and directly pollinate the stigma of the same flower (Caviness, 1966; OECD, 2000). Pollination and fertilisation are usually accomplished before the flower opens. However, cross-pollination rates as high as 6.3 % have been reported for closely spaced plants (Ray et al., 2003), suggesting the potential of some within-crop gene flow in soybean. Data indicated that cross-pollination rates vary significantly depending upon: the soybean variety; flower synchrony; environmental conditions; experimental design; and presence of pollinators (Abrams et al., 1978; Gumisiriza and Rubaihayo, 1978; Kikuchi et al., 1993; Ahrent and Caviness, 1994; Ray et al., 2003; Lu, 2005). Pollinators such as honeybees are thought to mediate pollination, though soybeans are not as attractive to insects as many other plants (Jaycox, 1970; Erickson, 1975a,b, 1984; Abrams et al., 1978; Erickson et al., 1978; Ortiz-Perez et al., 2006, 2008).[26] Based on field trial data and a wind tunnel experiment, Yoshimura (2011) concluded that wind-mediated pollination

[24] Technical dossier / Sections B2, B3, B4, D4, D6 and D9.3
[25] Technical dossier / Section B2 // Additional information received on 06/07/2006 / Question 1 / Pages 1-3 // Additional information received on 20/06/2007 / Question 5 / Pages 9-11
[26] Additional information received on 20/06/2007 / Question 5 / Pages 9-11

appears to be negligible, as little airborne pollen was observed in and around a soybean field. Most cross-pollination events occur within a few meters of the pollen source, and decrease rapidly with increasing distance from the pollen source (Caviness, 1966; Yoshimura et al., 2006; Abud et al., 2007).

The EFSA GMO Panel does not consider pollen dispersal and consequent cross-pollination as environmental hazards in themselves, and is primarily concerned with assessing the environmental consequences of transgene flow on ecosystems by considering the spread and fitness of hybrids and backcross progeny, as well as exposure to non-target organisms.

The genus *Glycine* is divided into two distinct subgenera: *Glycine* and *Soja*. Soybean belongs to the subgenus *Soja*. The subgenus *Glycine* contains 16 perennial wild species, whereas the cultivated soybean, *Glycine max*, and its wild and semi-wild annual relatives, *Glycine soja* and *Glycine gracilis*, are classified as members of the subgenus *Soja* (OECD, 2000). Due to the low level of genomic similarity among species of the genus *Glycine*, *Glycine max* can only cross with other members of *Glycine* subgenus *Soja*: species of the subgenus *Soja* are capable of hybridising and the hybrid seed that is produced can germinate normally and produce plants with fertile pollen and seed (Hymowitz et al., 1998; Abe et al., 1999; Nakayama and Yamaguchi, 2002; Lu, 2005; Mizuguti et al., 2009, 2010). *Glycine soja* and *Glycine gracilis* are indigenous to China, Taiwan, Korea, Japan, Far East Region of Russia, Australia, the Philippines and South Pacific, and they have not been reported in other parts of the world where the cultivated soybean is grown (Dorokhov et al., 2004; Lu, 2005).

Theoretically, seeds originating from the cross-pollination of certain sexually compatible wild relatives can mediate the potential spread and establishment of hybrid and backcross progeny (Wilkinson et al., 2003; Morales and Traveset, 2008; Devos et al., 2009a). However, in the EU, there are no sexually cross-compatible wild relatives with which soybean can hybridise and form backcross progeny (OECD, 2000). The only recipient plants that can be cross-fertilised by soybean are other conventional soybean varieties. Therefore, the plant to plant gene transfer from soybean is restricted to cultivated soybean in the EU. Since the molecular analysis and food/feed safety evaluation did not raise safety concerns (sections 3 to 5; EFSA, 2010f), the EFSA GMO Panel does not consider cross-pollination in soybean an environmental risk, but an agricultural management and coexistence issue (Devos et al., 2009b) that is not within its remit.

Seed-mediated establishment of soybean and its survival outside of cultivation is rare in spite of extensive cultivation in many countries and accidental seed dispersal. Neither the pods, nor the seeds, have morphological characteristics that would facilitate animal transport (OECD, 2000). Soybean seeds rarely display any dormancy characteristics and only under certain environmental conditions grow as volunteers in the year following cultivation (OECD, 2000; Yoshimura et al., 2011). Even in soybean fields, seeds usually do not survive during the winter due to predation, rotting, germination resulting in death, or due to management practices prior to planting the subsequent crop (Owen, 2005). The occurrence of GM soybean plants outside cropped areas has not been observed (i.e., Badea et al., 2006; Kim et al., 2006; Lee et al., 2009). The survival of soybean outside cultivation in Europe is limited by a combination of: low competitiveness; absence of a dormancy phase; and susceptibility to plant pathogens, herbivores and cold climatic conditions. Furthermore, since these general characteristics are unchanged in soybean 40-3-2, it is considered very unlikely that soybean 40-3-2 or its progeny will differ from conventional soybean varieties in their ability to persist as volunteers, or to establish feral populations under European environmental conditions. The herbicide tolerance trait is not likely to provide selective advantages outside cultivation or other areas where glyphosate-based herbicides could be applied in Europe. Therefore, as for any other conventional soybean varieties, GM soybean plants are not likely to establish feral populations under European environmental conditions.

In conclusion, as discussed in section 6.2.1, soybean 40-3-2 has no altered agronomic and phenotypic characteristics that would lead to enhanced survival, establishment or invasiveness in natural, semi-natural or cultivated environments, except in the presence of glyphosate-based herbicide. Therefore, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from soybean 40-3-2 is considered to be extremely low.

The conclusion of the EFSA GMO Panel is consistent with that of the DE CA on soybean 40-3-2. The DE CA concluded that "*the possibility of gene transfer from the genetically modified soybean 40-3-2 is assessed as not being any different from traditionally bred soybeans*" (section 6.2.2 of the environmental risk assessment report of the DE CA).

6.2.3. Interactions of the GM plant with target organisms[27]

Potential effects on target organisms due to the expression of the CP4 EPSPS protein were not considered an issue by the EFSA GMO Panel, nor by the DE CA and most Member States, because the protein does not interact with any specific target organisms. The CP4 EPSPS protein renders soybean 40-3-2 tolerant to the herbicidal active substance glyphosate, allowing direct application of glyphosate-based herbicides during cultivation. Glyphosate has a broad spectrum of target plant species, and potential impacts of the specific cultivation, management and harvesting techniques are considered in section 6.2.7.

6.2.4. Interactions of the GM plant with non-target organisms[28]

The potential of soybean 40-3-2 to have direct or indirect adverse effects on non-target organisms, their ecological functions (and related ecosystem services), such as pollination, biological control or decomposition (Sanvido et al., 2009; Arpaia, 2010), was evaluated by the EFSA GMO Panel. This evaluation covers the assessment of potential adverse environmental effects on non-target organisms due to intended and unintended changes in the GM plant (e.g., Hjältén et al., 2007; Desneux et al., 2010; Garcia-Alonso, 2010; Raybould et al., 2010; Arpaia et al., 2011). Intended changes in the GM plant are those that fulfil the original objectives of the genetic modification, whereas unintended changes are defined as consistent differences between the GM plant and its appropriate comparator, which go beyond the primary intended changes of introducing the transgene(s) (EFSA, 2010d,e). These changes may have consequences for the environment, and it is the potential adverse nature of these consequences that requires assessment. The EFSA GMO Panel follows two distinct yet complementary approaches for the risk assessment of potential adverse effects on non-target organisms (EFSA, 2010d,e).

6.2.4.1. Adverse effects on non-target organisms due to potential unintended changes in soybean 40-3-2

Based on the phenotypic characterisation of soybean 40-3-2 and compositional and nutritional analyses, the applicant considered that the genetic modification does not have any unanticipated effects on characteristics of soybean 40-3-2 that might impact non-target organisms.

The molecular characterisation of the DNA insert and flanking regions of soybean 40-3-2 did not indicate unintended changes due to the insertion (section 3). Moreover, no biologically relevant differences in the composition of key analytes or agronomic and phenotypic characteristics were identified between soybean 40-3-2 and its conventional counterpart (EFSA, 2010f).

In order to conclude reliably on the occurrence of adverse effects on non-target organisms due to unintended changes in a GM plant, applicants should consider and collate all the information available from a number of sources, using a weight-of-evidence approach. Data sources relevant to plant-environment interactions are always necessary to support the possible exclusion of unintended changes. The EFSA GMO Panel considers that data on plant-non-target organism interactions provide an additional indication on the occurrence of unintended changes in the GM plant (EFSA, 2010d,e). For soybean 40-3-2, the EFSA GMO Panel therefore requested the applicant to provide *in planta*

[27] Technical dossier / Section D9.4
[28] Technical dossier / Section D9.5

[event-specific] data on the main functional groups of non-target organisms (predators, pollinators and herbivores) that are exposed either directly or indirectly to soybean.

Following the request of the EFSA GMO Panel, the applicant reviewed higher-tier studies conducted with soybean 40-3-2 in the EU (Badea et al., 2006) and the USA (Buckelew et al. 2000; Bitzer et al., 2002; Jasinski et al., 2003; McPherson et al., 2003; Jackson and Pitre, 2004b,c).[29] Based on these studies, the applicant concluded that there are no indications of altered interactions between soybean 40-3-2 and predators and herbivores.

The studies on non-target organisms, supplied by the applicant, showed no adverse effects of soybean 40-3-2 on predators, herbivores and decomposers. The EFSA GMO Panel notes that the studies provided or reviewed by the applicant differ in quality in terms of experimental design and statistical power, and therefore are not equally informative to the evaluation of the environmental risk assessment of soybean 40-3-2. Based on the evidence provided by the applicant and relevant scientific literature on soybean 40-3-2, the EFSA GMO Panel concludes that there are no indications of the occurrence of adverse effects on non-target predators, herbivores and decomposers due to potential unintended changes in soybean 40-3-2.

With regard to pollinators, the original application contained one greenhouse study in which the interaction between the honeybee species *Apis mellifera*[30] and a different GM crop expressing a similar trait (maize NK603) was assessed. *A. mellifera* was selected as representative of potentially exposed pollinators. Both the DE CA and EFSA GMO Panel considered the honeybee study was of inadequate quality and provided no useful evidence on which to conclude. Further, the EFSA GMO Panel reiterates that, since unintended effects are to a large extent event specific, data from other events or from similar events in other plant species carry little weight in supporting an application (EFSA, 2010d,e). The DE CA and the EFSA GMO Panel therefore requested further information and/or *in planta* data on four separate occasions, in order to be able to conclude on the occurrence of adverse effects on pollinators due to potential unintended changes in soybean 40-3-2.

In response, the applicant noted: (1) that exposure of honeybees to pollen from soybean flowers is limited; and that (2) the extensive cultivation of soybean 40-3-2 outside Europe for 15 years had established a 'history of safe use'. Regarding exposure, the EFSA GMO Panel concurs that soybean flowers attract relatively few honeybees and that the level of exposure of honeybees to soybean 40-3-2 will be low. Soybean anthers are enclosed within the flower, so that the potential for pollination by honeybees is reduced. The attractiveness of soybean flowers to honeybees, the quantity of nectar produced and the level of cleistogamy of soybean flowers are highly variable, and depend upon the plant variety. Factors such as climate, soil characteristics and the proximity of apiaries to the cultivated field, also influence the attractiveness and/or receptiveness of soybean flowers to honeybees, as well as the frequency of honeybee visits. However, the EFSA GMO Panel considers that some exposure is possible, despite the low attractiveness of soybean plants compared with many other plants. Evidence indicates that seed set can be improved significantly when honeybees visit soybean flowers (Jaycox, 1970; Erickson, 1975a,b, 1984; Abrams et al., 1978; Erickson et al., 1978; Chiari et al., 2005; Ortiz-Perez et al., 2006, 2008). Chiari et al. (2011) recently confirmed that Africanised honeybees can increase gene flow from GM soybean to conventional soybean varieties significantly (up to 1.6 %) under field plot conditions.

During the extensive cultivation of soybean 40-3-2 for several years in different locations worldwide, there have been no reports of adverse effects on pollinators, and so the EFSA GMO Panel considers the likelihood of such unintended effects potentially resulting from unintended changes of soybean 40-3-2 as very low. However, no event-specific data on plant-pollinator interactions were provided by the applicant. The EFSA GMO Panel considers that these data are essential for the environmental risk

[29] Technical dossier / Section D9.8 / Pages 122-125 / Annex: Goldstein (2003) // Additional information received on 21/11/2010 / Question 1 / Pages 5-12

[30] Technical dossier / Section D9.5 / Pages 108-118 / Annex: Boonkrit et al. (2002) // Additional information received on 21/11/2010 / Question 1 / Page 2 / Annexes: Richards (2008a,b)

assessment, and therefore scientific uncertainties pertaining to the occurrence of adverse effects on pollinators due to potential unintended changes in soybean 40-3-2 remains. The EFSA GMO Panel notes the proposal of the applicant to conduct post-market studies during which exposure of honeybees to soybean 40-3-2 will be observed for a period of two years (see section 6.3).

In summary, since there are no indications of altered interactions between soybean 40-3-2 and predators, herbivores and decomposers, the EFSA GMO Panel considers *trait*-specific information appropriate to assess whether soybean 40-3-2 poses a risk to non-target organisms. The assessment of potential adverse effects on non-target organisms due to the expression of the CP4 EPSPS protein is described in section 6.2.4.2. However, the EFSA GMO Panel notes that scientific uncertainty pertaining to the occurrence of adverse effects on pollinators due to potential unintended changes in soybean 40-3-2 remains, as no event-specific data on plant-pollinator interactions were provided by the applicant.

The conclusion of the EFSA GMO Panel is consistent with the evaluation carried out by the DE CA. The DE CA recommended that "*the applicant shall carry out a field study to confirm the absence of unintended effects on non-target organisms in the EU with placing soybean 40-3-2 on the market. The design of such a study should be of a quality to allow appropriate scientific assessment as proposed in the application*" (section 8 of the environmental risk assessment report of the DE CA).

6.2.4.2. Adverse effects on non-target organisms due to the expression of the CP4 EPSPS protein

Based on the mode of action of the CP4 EPSPS protein and the history of safe use of soybean 40-3-2 and other glyphosate tolerant crops, it is unlikely that the expression of this protein in glyphosate tolerant crops will cause direct adverse effects on non-target organisms (see e.g., EFSA, 2009b for maize NK603; CERA, 2010; EFSA, 2011e for maize MON 88017). The CP4 EPSPS protein shares no significant homology with known toxic proteins (section 4; EFSA, 2010f) and is homologous with the wild-type CP4 EPSPS protein, which is ubiquitous in plants and microorganisms (CaJacob et al., 2004; CERA, 2010).

The applicant noted that the probability of direct adverse effects of soybean 40-3-2 on non-target organisms due to the expression of the CP4 EPSPS protein is very low, as no biologically relevant differences in the composition of key analytes or agronomic characteristics were identified between soybean 40-3-2 and its conventional counterpart, and because the molecular characterisation of the DNA insert and flanking regions of soybean 40-3-2 did not raise safety concerns (section 4; EFSA, 2010f).

The applicant provided and referred to several lower-tier studies performed either with the purified CP4 EPSPS protein or with CP4 EPSPS-containing plant material, as well as higher-tier studies, in order to confirm the lack of acute toxicity of the CP4 EPSPS protein to: predators such as the green lacewing *Chrysoperla carnea*[31] and the big-eyed bug *Geocoris punctipes* (Jackson and Pitre, 2004a); pollinators such as the honeybee species *A. mellifera*[32]; decomposers such as the springtail species *Folsomia candida*[33], the earthworm species *Eisenia fetida*[34]; and herbivores such as aphids[35], the green cloverworm *Hypena scabra* (Morjan and Pedigo, 2002), the Colorado potato beetle[36] and the European corn borer.[37] Harrison et al. (1996) did not report adverse effects on mice after exposure to CP4 EPSPS-containing diet. In its application, the applicant also discussed higher-tier studies conducted with soybean 40-3-2 in the EU (Badea et al., 2006) and the USA (Buckelew et al. 2000; Bitzer et al.,

[31] Technical dossier / Section D9.5 / Pages 108-118 / Annex: Chamornman et al. (2002)
[32] Technical dossier / Section D9.5 / Pages 108-118 / Annex: Boonkrit et al. (2002) // Additional information received on 21/11/2010 / Question 1 / Page 2 / Annexes: Richards (2008a,b)
[33] Technical dossier / Section D9.8 / Pages 122-125 / Annex: Goldstein (2003) // Additional information received on 21/11/2010 / Question 1 / Pages 5-12
[34] Technical dossier / Section D9.8 / Pages 122-125 / Annex: Levine (2004) // Additional information received on 13/05/2008 / Question 1 / Pages 2-4 / Annex: Sindermann et al. (2004)
[35] Technical dossier / Section D9.5 / Pages 108-118 / Annex: Chamornman et al. (2002)
[36] Additional information received on 13/05/2008 / Question 1 / Pages 2-4 / Annex: Levine and Uffman (2007)
[37] Additional information received on 13/05/2008 / Question 1 / Pages 2-4 / Annex: Uffman and Levine (2007)

2002; Jasinski et al., 2003; McPherson et al., 2003; Jackson and Pitre, 2004b,c), or with maize NK603 (Rosca, 2004; Reyes, 2005; Rodriguez et al., 2006; Schier, 2006) to confirm that the exposure of several non-target organisms to CP4 EPSPS-expressing crops poses no potential hazard, supporting conclusions of lower-tier studies. The EFSA GMO Panel notes that the studies provided or reviewed by the applicant differ in quality in terms of experimental design, statistical power, representativeness of test species, and therefore are not equally informative to the evaluation of the environmental risk assessment of soybean 40-3-2.

Available evidence indicated no adverse effects on different types of non-target organisms due to the expression of the CP4 EPSPS protein in glyphosate tolerant crops. For instance, in their recent lower-tier study, Hendriksma et al. (2012) confirmed the lack of insecticidal effects of the CP4 EPSPS protein on developing honeybee larvae: even at a test concentration of 6.4 μg/10 μL of diet, survival rates remained unaffected. The EFSA GMO Panel notes that soybean 40-3-2 and other glyphosate tolerant crops have been cultivated extensively in Argentina, Brazil, Canada, Mexico, Paraguay, USA and elsewhere for several years, and is not aware of any reports of direct effects on non-target organisms due to the expression of the CP4 EPSPS protein. Recent publications confirmed that there is no evidence that glyphosate tolerant crops have a direct effect on biological diversity or species abundance within cropped fields due to the expression of the CP4 EPSPS protein (Firbank et al., 2003a; Cerdeira and Duke, 2006, 2007, 2010; Albajes et al., 2008, 2009, 2010; Owen, 2008; CERA, 2010; Hendriksma et al., 2012).

Potential adverse environmental effects of the cultivation of soybean 40-3-2 and the use of glyphosate are considered in section 6.2.7.

The EFSA GMO Panel concludes that there are no indications of adverse effects of soybean 40-3-2 to non-target organisms due to the expression of the CP4 EPSPS protein. This conclusion is consistent with the DE CA conclusion that "*potential adverse effects on non-target invertebrates are unlikely. No adverse cause and effect interactions could be identified that would necessitate a case specific monitoring*". However, the DE CA noted that "*several of the studies provided by the applicant show poor experimental design, including an insufficient exposure of non-target arthropods, an inadequate number of replications or an insufficient statistical evaluation. Many of the tested organisms do not represent organisms that occur in the biocoenosis of growing soybeans or do not occur in Europe. Furthermore, not all relevant trophic levels have been tested with conclusive studies. In some of the studies the applicant used crop plants other than soybeans e.g. genetically modified maize or wheat with glyphosate tolerance traits*" (section 6.3 of the environmental risk assessment report of the DE CA).

6.2.5. *Effects on human and animal health*[38]

The molecular analysis and the food and feed safety assessment of soybean 40-3-2 did not raise safety concerns for human and animal health (sections 3 to 5; EFSA, 2010f). In its previous Scientific Opinion on the continued marketing of: (1) food containing, consisting of, or produced from soybean 40-3-2; (2) feed containing, consisting of, or produced from soybean 40-3-2; and of (3) other products containing or consisting of soybean 40-3-2 (EFSA, 2010f), the EFSA GMO Panel concluded that "*soybean 40-3-2 is as safe as its conventional counterpart with respect to potential effects on human and animal health*", and that "*soybean 40-3-2 is unlikely to have any adverse effect on human and animal health, in the context of its intended uses*".

The conclusion of the EFSA GMO Panel on the absence of adverse effects of soybean 40-3-2 on human and animal health is consistent with that of the DE CA. The DE CA considered that "*based on the lack of toxicity and allergenicity of the CP4 EPSPS protein and based on the results of the comparative assessment as well as of the feeding studies, unintended effects of soybean 40-3-2 on animal and human health due to incidental consumption are comparable to conventional soybean. No*

[38] Technical dossier / Sections D9.6 and D9.7

negative effects from handling and storage of the soybean seeds or whole beans are expected" (section 6.4 of the environmental risk assessment report of the DE CA).

6.2.6. Interactions with biogeochemical processes and the abiotic environment[39]

The CP4 EPSPS protein expressed in soybean 40-3-2 might be transferred during its cultivation into the soil through physical damage to plant tissues, decomposition of shed root cells, possibly root exudation, and after harvest through decomposing plant residues remaining in fields, which might be incorporated into the soil during tillage operations (Stotzky, 2004). This results in exposure of non-target soil organisms to the CP4 EPSPS protein. In this EFSA GMO Panel Scientific Opinion, the indirect route of exposure through manure and faeces from animals fed soybean 40-3-2 has also been considered, although most of the CP4 EPSPS protein would be degraded by enzymatic activity in the intestinal tract and subsequently by microbial processes in the faeces and the manure.

No direct effects on biogeochemical processes and the abiotic environment of soybean 40-3-2 due to the expression of the CP4 EPSPS protein have been reported by the applicant. The CP4 EPSPS protein was shown not to alter key soil microbial processes, such as carbon and nitrogen mineralisation, via lower- and higher-tier studies performed with GMHT soybean and maize in the USA (Liphadzi et al., 2005), maize NK603 in France (Philippot et al., 2006), or with GMHT maize in Canada (Hart et al., 2009). A lower-tier study with the purified CP4 EPSPS protein did not reveal adverse effects on microorganisms or microbial-mediated carbon and nitrogen mineralisation processes in the soil.[40] Because the CP4 EPSPS protein of soybean 40-3-2 is homologous to the EPSPS proteins found in plants and microorganisms (CERA, 2010), it is unlikely that it will affect the microbial community and hence biogeochemical processes adversely. Likewise, the expression of the newly introduced trait is not expected to alter the natural interactions of soybean plants with the abiotic environment. Once released into soil, the CP4 EPSPS protein will serve the soil microbial community as a source of energy and nutrients. Its contribution to the total amount of proteins introduced by plants, however, is negligible and thus no considerable direct effects on biogeochemical processes by the EPSPS protein as an additional substrate are anticipated. The EFSA GMO Panel is not aware of any reports of effects on biogeochemical processes and the abiotic environment due to the introduced trait (Dunfield and Germida, 2004; Cerdeira and Duke, 2006; Powell et al., 2007; CERA, 2010).

Potential adverse environmental effects of the cultivation of soybean 40-3-2 and the use of glyphosate are considered in section 6.2.7.

In its environmental risk assessment report, the DE CA did not consider potential interactions of soybean 40-3-2 with biogeochemical processes and the abiotic environment "*as relevant for the assessment of potential adverse effects of the soybean 40-3-2 on the environment*" (section 6 of the environmental risk assessment report of the DE CA).

6.2.7. Impacts of the specific cultivation, management and harvesting techniques[41]

The applicant concluded that "*the in-crop use of glyphosate should not be considered as a novel agronomic or management technique specific to soybean 40-3-2, but merely a flexible, additional herbicide option for weed control in the crop*". The EFSA GMO Panel disagrees with the applicant, and considers that the use of glyphosate, a broad-spectrum, non-selective herbicide associated with the cultivation of genetically modified herbicide tolerant (GMHT) soybean 40-3-2 in cropping systems is a substantial change in the cultivation and management of this soybean compared with conventional soybean. Currently, the control of weeds in soybean is mostly achieved by using pre-emergence soil acting residual herbicides and/or post-emergence selective herbicides in Europe, though mechanical weed control is also used in some situations (Brookes, 2005).

[39] Technical dossier / Sections D9.8 and D9.10

[40] Technical dossier / Section D9.8 / Pages 122-125 / Annex: Carson et al. (2004)

[41] Technical dossier / Section D9.9 // Additional information received on 20/06/2007 / Question 8 / Pages 36-40 // Additional information received on 13/05/2008 / Question 3 / Pages 8-12 // Additional information received on 22/11/2010 / Questions 1 & 2 / Pages 13-24

In the EU, glyphosate is currently used in conventional cropping by some farmers as a pre-sowing or pre-emergence herbicide (Monsanto, 2010). Glyphosate use prior to soybean sowing is especially common where perennial weeds (e.g., *Convolvulus arvensis*, *Rumex* spp., *Sorghum halepense*) are present. In some situations, glyphosate is applied in an emerged crop as a band application between crop rows with the herbicide application being directed away from crop foliage to avoid crop injury, or it is used through weed wipers when weeds (particularly perennials) are taller than the crop.[42] In addition, glyphosate is sometimes used as a crop dessicant, which will control late emerged weeds in the crop, and is also applied at various times post-harvest to remove weed seedlings and volunteers that emerge during the period between crop cultivation, prior to seedbed preparation. However, GMHT crops will allow the use of glyphosate 'over the top of the crop', which is considered by the EFSA GMO Panel as a substantial change in the cultivation and management of GMHT crops compared with conventional crops.

6.2.7.1. Interplay between the legislation for GMOs and plant protection products

Directives 2001/18/EC and 91/414/EEC (which was repealed by Regulation (EC) No 1107/2009 on 14 June 2011) are both relevant for the risk assessment of GMHT crops and their associated weed control management practices (EC, 2008; EFSA, 2008a; Ehlers, 2011). The registration and use of herbicidal active substances in formulations in the EU was covered by Directive 91/414/EEC (which is now replaced by Regulation (EC) No 1107/2009) as operated by individual Member States. Since GMHT plants rely on specific herbicides as an integral part of a weed management strategy, an environmental risk assessment must also consider their potential impact on biodiversity under Directive 2001/18/EC. In the current legislation governing the registration of plant protection products in Europe, the environmental risk assessment of pesticides includes an assessment of impacts on certain non-target organisms (such as fish, *Daphnia*, algae, birds, mammals, earthworms, bees and beneficial arthropods and non-target plants) and studies of residual activities in soil and water (cf., environmental fate) (Streloke, 2011). On the basis of environmental impact indices, a large number of authors have claimed that some of the herbicidal active substances used on GMHT crops (e.g., glyphosate) have reduced environmental impacts compared with those applied on their conventional counterparts (Nelson and Bullock, 2003; Peterson and Hulting, 2004; Brimner et al., 2005; Brookes and Barfoot, 2006; Leroux et al., 2006; Kleter et al., 2007; Bonny, 2008, 2011; Devos et al., 2008; Arregui et al., 2010; Mamy et al., 2010; Stewart et al., 2011). However, the environmental impact indices used for these calculations are generally based on residuality, persistence and ecotoxicity characteristics, and do not relate to efficacy (e.g., van der Werf, 1996; Reus et al., 2002). Indeed, the environmental risk assessment under Directive 91/414/EEC did not include studies of impacts on biodiversity within crops and changes in agro-ecosystems, which are required under Directive 2001/18/EC in relation to GM crops. Due to these different legal requirements, a herbicide used on a GMHT crop is currently assessed differently from the same herbicide used on non-GM HT crops (e.g., imidazolinone tolerant crops) and conventional crops. The assessment of GMHT crops regimes includes evaluating potential effects on farmland biodiversity, while this is not a requirement for non-GM crop herbicide regimes (ACRE, 2007; Morris, 2007; Sanvido et al., 2007, 2011a,b; Ehlers, 2011). Even though an assessment of indirect effects of herbicidal active substances on biodiversity was not required for the risk assessment of pesticides under Directive 91/414/EEC, the new Regulation (EC) No 1107/2009, concerning the placing of plant protection products on the market, explicitly mentions biodiversity as a protection goal (Streloke, 2011). Moreover, Directive 2009/128/EC aims to strike a new balance between food security and the support of biodiversity by promoting the sustainable use of pesticides.

6.2.7.2. Environmental impact of herbicide regimes used in GMHT cropping systems

The EFSA GMO Panel considers that the introduction of glyphosate-based cropping systems may have different environmental impacts to the existing systems. Therefore, the EFSA GMO Panel does not agree with the applicant's assessment for environmental impact of the specific cultivation, management and harvesting techniques, which was limited to statements that "*in comparison to any*

[42] http://www.monsanto-ag.co.uk/content.output/181/181/Roundup/Application%20Information/Weed%20wipers.mspx

other soybean, no typical characteristics of the genetically modified plant could be identified, which may cause adverse effects on the environment through a need to change management practices. Therefore, the environmental impact of farming practices to grow soybean 40-3-2 in the E.U. is considered no different from any other soybean".

It has long been recognised that the widespread use of herbicides in agriculture has resulted in serious declines in both plant and animal diversity in many farming areas (Krebs et al, 1999; Chamberlain et al., 2000; Donald et al., 2001; Marshall et al., 2001, 2003; Stoate et al., 2001; Robinson et al., 2002; Fried et al., 2009; Geiger et al., 2010; Storkey et al., 2011). Concern has been expressed that GMHT crops, through the in-crop repeated use of very effective broad-spectrum herbicides, will further deplete biodiversity in farmland (Marshall et al., 2001). It is expected that the long-term persistence of arable weeds in the soil seedbank will decline in less-weedy fields, while invertebrates, small mammals and seed-eating birds might be threatened by reduced food resources and/or foraging and nesting habitats (Watkinson et al., 2000; Gibbons et al., 2006; Butler et al., 2007). Arable weeds play an important role in supporting biological diversity and have numerous interactions with other organisms that depend on them for food and shelter, and some of these interactions can have direct effects on the functioning of the agro-ecosystem (Clergue et al., 2005; Moonen and Bàrberi, 2008; Bàrberi et al., 2010; Petit et al., 2011). Herbivores, predators and parasitoids associated to arable weeds may in turn mediate essential processes through the functioning of arable food webs (Norris and Kogan, 2000; Hawes et al., 2003, 2009; Marshall et al., 2003; Gibbons et al., 2006; Taylor et al., 2006; Hilbeck et al., 2008). For example, granivorous and omnivorous carabid beetles interact closely with the arable weed seedbank (Lundgren, 2009; Bohan et al., 2011). The role of post-dispersal seed predators and consumers, including generalist vertebrates (birds, rodents) and invertebrates (Coleoptera, Hymenoptera, earthworms, molluscs, etc.) on the regulation of weed populations is being increasingly recognised (Tooley and Brust, 2002; Bàrberi et al., 2010). Westerman et al. (2005) showed that predation by opportunist invertebrates can substantially reduce the surface weed seed stock ('biological weed control' service). The regulation and control of arthropod pest populations resulting from the activity of natural enemies is also an important ecological function in arable systems (Losey and Vaughan, 2006; Macfadyen et al., 2009; Sanvido et al., 2009).

There is extensive literature on the range of effects of the use of glyphosate and its associated weed control management practices in glyphosate tolerant crops (reviewed by Cerdeira and Duke, 2006, 2007, 2010; Dewar, 2010). Beneficial effects (e.g., increase in collembolans, reduction of soil erosion, reduction in virus infection) due to the retention of weed cover on the soil surface during the early growth of the crop have been reported (Brooks et al., 2003; Dewar et al., 2003; May et al, 2005). Furthermore, the use of glyphosate allows greater adoption of no- or reduced-tillage systems (Locke et al., 2008; Givens et al., 2009b), which is expected to increase in glyphosate tolerant soybean compared with its conventional counterparts, as it has been shown in the USA (Cerdeira and Duke, 2006). The effects of these tillage systems depend much on the local context. They contribute variously to reductions in soil erosion, fossil fuel use, carbon dioxide emissions, nitrogen and pesticide leaching, and in loss of soil moisture, and to improved soil structure (Baylis, 2000; Cerdeira and Duke, 2006, 2007, 2010; Dewar, 2010; Basso et al., 2011; Carpenter, 2011). The abundance of soil-dwelling carabid beetles and spiders has been shown to increase in no- or reduced-tillage systems, as weeds provide a more favourable habitat for predators, such as carabids or spiders, or because more abundant prey, such as Collembola, are available (Witmer et al., 2003; Hough-Goldstein et al., 2004; Rodríguez et al., 2006; Schier, 2006). A life-cycle assessment in which the risks of conventional sugar beet agricultural practices were compared with those that might be expected if GMHT sugar beet was grown, suggested that growing GMHT sugar beet would be less environmentally harmful than its conventional counterpart (Bennett et al., 2004). Glyphosate has also been shown to be more environmentally and toxicologically benign than many of the herbicidal active substances that it replaces (reviewed by Cerdeira and Duke, 2006, 2007, 2010; Carpenter, 2011; see also[43]).

[43] Giesy et al., 2000; Wauchope et al., 2002; Nelson and Bullock, 2003; Solomon and Thompson, 2003; Peterson and Hulting, 2004; Brimner et al., 2005; Brookes and Barfoot, 2005; Mamy et al., 2005; Screpanti et al., 2005; Vereecken, 2005; Leroux et al., 2006; Kleter et al., 2007, 2008; Borggaard and Gimsing, 2008; Bonny, 2008, 2011; Devos et al.,

On the negative side, there is evidence that, depending upon the specific herbicide regimes applied at the farm level, the cultivation of GMHT crops may: (1) reduce farmland biodiversity; (2) induce changes in weed community diversity due to weed shifts; (3) select for glyphosate resistant weeds; and (4) affect soil microbial communities (see also EFSA, 2009b, 2011e). These potential adverse indirect environmental effects of the cultivation of soybean 40-3-2 are discussed below.

Impact on farmland biodiversity

With the exception of the monitoring study conducted by Badea et al. (2006), the EFSA GMO Panel is not aware of any scientific studies having considered the impact of GMHT soybean systems and their associated herbicide regimes on farmland biodiversity under European environmental conditions. Several studies, however, have assessed the impact of glyphosate-based herbicide regimes used in GMHT soybean in Argentina (Tuesca et al., 2001; Vitta et al., 2004; De la Fuente et al., 2006; Scursoni et al., 2007; Scursoni and Satorre, 2010), Canada (Simard et al., 2011), Japan (Imura et al., 2010) and the USA (Buckelew et al. 2000; Bitzer et al., 2002; Jasinski et al., 2003; McPherson et al., 2003; Jackson and Pitre, 2004b,c; Scursoni et al., 2006). In the EU, research projects such as: the project on Botanical and Rotational Implications of Genetically modified Herbicide Tolerance in winter oilseed rape and sugar beet (BRIGHT) (Sweet et al., 2004; Lutman et al., 2008); the Farm Scale Evaluations (FSEs) (Firbank et al., 2003a,b) in the United Kingdom; and the study of the National Environmental Research Institute (NERI) in Denmark (e.g., Strandberg and Pedersen, 2002) have considered the impact of more general GMHT cropping systems and their associated herbicide regimes on farmland biodiversity. Further, there are some other studies on herbicide tolerant crops in EU countries that have compared the environmental impact on farmland biodiversity of conventional production systems with that of GMHT cropping systems (Madsen and Jensen, 1995; Bückmann et al., 2000; Coyette et al., 2002; Firbank et al., 2003a,b; Soukup et al., 2008; Verschwele and Mülleder, 2008; Albajes et al., 2008, 2009, 2010, 2011; Szekeres et al., 2008; Thieme, 2010; Verschwele, 2011; Pálinkás et al., 2012).

The above studies confirmed that effects on arable weed populations, and hence farmland biodiversity, are highly dependent on the management of the herbicides in the GMHT and conventional crop production systems. The extent and direction of the effects of weed management on weeds and invertebrates are dependent on the relative efficacy of the existing conventional regimes and the forthcoming GMHT herbicide regimes. Extensive research has shown that impacts on biodiversity, as well as depending on the management of individual crops, are also determined by factors such as rotations, and on the provision of forage and habitat resources across the entire farmed landscape (Firbank et al., 2003b). Here, crop management includes the dose applied, the time and the frequency of applications both of the specific non-selective and of other herbicides (Champion et al., 2003). Timing of application is particularly important, since with broad-spectrum herbicides, application is often delayed until a later plant growth stage than is the case with the more selective herbicides associated with conventional crops. The higher mortality of larger (reproductive) individual weeds caused by the later herbicide application in GMHT crops (Heard et al., 2003b) tends to reduce the persistence of plant populations in the farmed landscape and reduce seed and seedbank densities and in turn emerged plants. This loss of food resources is likely to cause reductions in the abundance of key invertebrate groups (Hawes et al., 2003) and of species at higher trophic levels, such as farmland birds.

Long-term effects of change in the timing of herbicide applications are difficult to predict, since they depend much on the local context of weather, soil, management and current biodiversity. All of the factors above vary from region to region, from Member State to Member State, from season to season, and from biodiversity component to biodiversity component. For these reasons, whilst meaningful conclusions can be drawn from general principles, the EFSA GMO Panel acknowledges that there are

2008; Duke and Powles, 2008b; Gardner and Nelson, 2008; Klier et al., 2008; Shipitalo et al., 2008; Struger et al., 2008; Arregui et al., 2010; Dewar, 2010; Mamy et al., 2010; Stewart et al., 2011 considerable challenges to making accurate predictions on the environmental consequences of the use of herbicides in different GMHT cropping systems. Predictions from models would need to consider all the issues detailed above, over the full range of possible parameters that may be varied in the management of the GMHT crops, and the full range of receiving environments within Europe. Large-scale experimentation to determine the impacts of all the herbicide programmes incorporating glyphosate that are likely to be adopted by farmers in the different farming regions of each Member State cultivating soybean 40-3-2 is deemed infeasible for reasons of practicability and cost (e.g., Perry et al., 2003; Squire et al., 2003; Qi et al. 2008). Therefore, modelling may be attempted (e.g., Holst et al., 2007; Caron-Lormier et al., 2009, 2011), particularly to assess regional-scale (Firbank et al., 2003a) and long-term effects (Lutman et al., 2008) of possible changes in agricultural practice over the course of many rotations. However, present models do not provide a robust means of predicting outcomes, because of their critical dependence on underlying assumptions. Different models of the same system may give very different predictions and therefore caution must be exercised in reviewing the output of models. As an illustration, consider four models that were built around the GMHT cropping systems studied in the FSEs. In an initial assessment, Heard et al. (2003a,b) used long-term data from the decline in UK weed soil seedbanks and compounded this with the reduction in soil seedbank density found for dicotyledons in GMHT crops (sugar/fodder beet and oilseed rape). They predicted a worst-case decline in soil seedbanks of 7 % per annum for a five-course cereal rotation with a break crop grown every five years. By contrast, they believed that it was quite possible that, under rotations including glufosinate-ammonium tolerant maize, weed populations would in the long-term be stable or increase. Heard et al. (2005) later revised and refined their earlier opinion for GMHT beet and oilseed rape, after taking into account density dependence of the weeds that integrated both population dynamics and grower response to weeds, within a seven-course, 4-year rotational framework. Gibbons et al. (2006) calculated the quantitative effects of changes in seed rain on the dietary requirements of 17 granivorous farmland bird species, although they declined to predict effects on individual bird species. They concluded that should beet, spring and winter oilseed rape in the UK be largely replaced by GMHT crops and managed as in the FSEs, this would markedly reduce important food resources for farmland birds, many of which had already suffered decline during the last 30 years. Butler et al. (2007) used a semi-qualitative approach and concluded that of 39 susceptible farmland bird species, even under nationwide introduction of the GMHT beet and oilseed rape systems studied in the FSE regimes, only one species would be re-classified to a less favourable conservation status due to the implementation of such systems. Grower uptake was predicted to have only a limited effect on Farmland Bird Indices. Further guidance on the need to upscale experimental results spatially and temporally, from field and season scales to region and decadal, multi-rotational scales was given by EFSA (EFSA, 2008b; see also Castellazzi et al., 2007, 2008).

Evidence indicates that the response of arthropods to altered weed abundance and composition is variable, being dependent on life-history characteristics (Brooks et al., 2003). For instance, the lower density of arable weeds on maize plots treated with glyphosate did not necessarily alter the biological control functions provided by natural enemies or lead to more insect pests (Albajes et al., 2008, 2009, 2011). This can be attributed to the complexity of arable ecosystems in which changes in arthropod composition may be influenced by: the effect of functional redundancy in the system (Johnson, 2000); the crop itself (if it provides resources for arthropods); arthropod dispersal (Haughton and Bohan, 2008; Smith et al., 2008b); habitat heterogeneity (Benton et al., 2003); and interactions between habitat structure, land use and arthropod species ecology (Haenke et al., 2009; Goulson et al., 2010). In their paper, Albajes et al. (2009) reported that leafhoppers and aphids were more abundant and phytophagous thrips were less abundant in glyphosate-treated plots, compared with untreated plots. Among predators, *Orius* spp., spiders, and trombidids were more abundant on glyphosate-treated plots, whereas nabids and carabids were more abundant in untreated plots; the same case was found for carabids and spiders caught in pitfall traps. Among parasitoids, ichneumonids were more abundant in untreated plots and mymarids in the glyphosate-treated plots. The higher abundance of on-crop plant predators such as *Orius* spp. in treated plots was the result of more prey (e.g., leafhoppers and to a lesser extent aphids) in less-weedy maize fields. For *Nabis* sp., which was more abundant on untreated plots, no relation to any of the herbivores tested was shown (Albajes et al., 2011). In a continuation of the study by Albajes et al. (2009), where the impact of glyphosate-based herbicide regimes on non-target arthropods through the food web was compared with that of currently applied herbicide regimes, it was observed that populations of arthropod herbivores and natural enemies are not greatly affected, unless weed abundance is drastically altered (Albajes et al., 2010, 2011). This indicates that differences in weed abundance, induced by the adoption of different herbicide regimes, are not necessarily ecologically relevant (in terms of functionality) (e.g., Bohan et al., 2007; Smith et al., 2008b).

In conclusion, the different effects on weed flora associated with the use of the complementary glyphosate-based herbicide regimes and non-chemical control methods have the potential to cause adverse impacts on farmland biodiversity. The magnitude of this reduction in farmland biodiversity is dependent upon a series of factors (Table MF), which include the efficacy of the applied herbicide regimes in controlling weeds, crop rotations, and the level of farmland biodiversity sustained in receiving environments.

Table MF. Major factors affecting the risk of reducing weed community diversity based on expert judgment and historical experience

Management factors	Risk of reducing weed community diversity		
	Low	Moderate	High
Crop rotation	> four years, presence of functionally distinct crops (e.g., cereals and legumes) and seasonally distinct crops (winter vs. spring-summer)	Limited duration (two/three to four years) with reduced presence of functionally distinct and/or seasonally distinct crops	No rotation (continuous cropping)
Tillage system	Alternation between ploughing and minimum/no-till systems	Only minimum tillage or no-till	Only ploughing
Weed management intensity in cropping systems	Low (e.g., integrated weed management controlling only emerged and visible weeds involving a mix of cultural, mechanical and chemical approaches)	Medium (e.g., management using a mix of mechanical and chemical approaches as required)	High (e.g., routine prophylactic treatments using pre- and post-emergence herbicides)
Landscape features (other regionally relevant factors)	Highly mixed crops on many small fields	Moderately mixed crops on medium-sized fields	Mostly one type of crop on large fields
Conservation headlands and/or uncultivated field margins	Present	Limited presence	Not present

Weed shifts and the selection of weed communities composed of more tolerant or resistant species

The sole usage of a single herbicide over a wide cropping area for an extended period is known to potentially cause changes in weed flora, and to increase the selection of communities dominated by tolerant weed species, or of resistant weed biotypes (Gressel, 2009; Dewar, 2010; Reddy and Norsworthy, 2010; Owen, 2011; Green and Owen, 2011). Direct evidence for such effects has been found for systems comprising the use of glyphosate with no or reduced tillage (Fernandez-Cornejo et al., 2002; Tingle and Chandler, 2004; Johnson et al., 2009; Kruger et al., 2009; Powles, 2008, 2010; Gressel, 2009; NRC, 2010; Powles and Yu, 2010; Waltz, 2010; Webster and Sosnoskie, 2010; Beckie, 2011; Owen et al., 2011; Heap, 2012; Shaner et al., 2012). The lack of residual activity of glyphosate may result in two to four applications of this herbicidal active substance per growing season, depending on weed seedling emergence patterns. In addition, no- or reduced-tillage systems, enabled by the use of glyphosate (Locke et al., 2008; Givens et al., 2009b), may further increase the selection pressure on weeds and weed density (Cardina et al., 2002). Because mechanical pre-plant weed control is reduced or completely replaced by the use of glyphosate in no- or reduced-tillage systems, herbicide applications become more important (Givens et al., 2009a). Moreover, no- or reduced tillage systems have a tendency to concentrate weed seeds close to the soil surface (Bàrberi and Lo Cascio, 2001; Moonen and Bàrberi, 2004; Sosnoskie et al., 2006, 2009; Vasileiadis et al., 2007) from which they can more easily emerge, giving rise to increased in-field weed densities, which require more frequent glyphosate applications.

The increased selection pressure imparted by glyphosate may cause changes in abundance of selected weed populations and in species relative abundances (and consequently in weed community diversity). Weed shifts occur because of differential natural tolerance of glyphosate between species in a weed community, and/or because of the spread of herbicide resistant biotypes (Norsworthy et al., 2001; Soukup et al., 2008; Reddy and Norsworthy, 2010). Glyphosate avoidance (non-exposure) is achieved either by very early weed emergence and rapid maturation, in case of late post-emergence application, or by late season weed emergence, in case of early post-emergence application (Scursoni et al., 2007; Scursoni and Satorre, 2010). Weeds emerging after a glyphosate application can fill niches vacated by the weeds that were effectively controlled by glyphosate. Moreover, elimination of competition from early-season weeds create a favourable environment for late-season weeds (Owen, 2008; Reddy and Norsworthy, 2010).

A survey of twelve weed scientists from eleven states across the USA, to assess weed shifts in GMHT cotton, maize and soybean, revealed that weed shifts were observed in GMHT soybean, with various winter annuals, lambsquarter (*Chenopodium album*) and waterhemp (*Amaranthus rudis*) becoming more problematic (Culpepper, 2006). Vitta et al. (2004) showed that weed community diversity either decreased or remained stable early in the season before herbicide application, whereas it increased at harvest in GMHT soybean. Weed communities changed after glyphosate application due to the emergence of new weed species. In contrast, other species that were abundant disappeared over time. By decreasing the density of the most abundant weeds, glyphosate reduced competition among weeds and allowed for new species to appear in cultivated areas. Puricelli and Tuesca (2005) reported that the density and diversity of early emerging broadleaved and grassy annuals decreased in response to continuous and exclusive applications of glyphosate in systems planted to GMHT crops over five years, regardless of the cropping sequence considered in their study (soybean monoculture, wheat-soybean and soybean-maize). Other studies also reported or predicted shifts in weed populations due the increased frequency and rate of glyphosate use in GMHT cropping systems (e.g., Shaner, 2000; Owen, 2000, 2008; Hilgenfeld et al., 2004; Duke, 2005; Owen and Zelaya, 2005; Culpepper, 2006; De la Fuente et al., 2006; Scursoni et al., 2006, 2007; Wilson et al., 2007; Scursoni and Satorre, 2010). Weed shifts may exacerbate weed problems and reduce the effectiveness of weed control (Young, 2006).

Some studies reported that risks for weed population shifts from GMHT crops are no greater than those associated with other herbicides and non-GMHT crops. In their 6-year field study, Westra et al. (2008) showed no difference in overall weed control comparing a diverse crop rotation with continuous maize, or comparing rotating herbicide mode of action with using only glyphosate. Further, no resistant weeds evolved, but certain weed populations increased when glyphosate was used at reduced application rates. In a 6-year field study, Verschwele and Mülleder (2008) considered potential changes in weed communities due to the use of glyphosate in a continuous maize NK603 rotation at three sites in Germany, and did not observe statistically significant differences between local standard herbicide treatments and the glyphosate-based treatments on the mean values of seedbank, species richness, species diversity and dominance (see also Verschwele, 2011). The variation in weed seedbank size and composition was mainly attributed to site and year effects. Hartzler et al. (2006) compared five weed management systems, including a glyphosate-only treatment, with tank mixtures of herbicides with and without glyphosate in a soybean-maize rotation over a 4-year period. The weed management systems did not influence weed population density or composition over the experimental period, and no differences in yield were observed in three of the four years. However, this study was conducted using small plot areas and may not represent potential changes that might occur on a field scale. Gulden et al. (2009) compared conventional with GMHT cropping systems for weed control, diversity and yield. Overall, the 6-year study showed higher levels of weed control in systems using glyphosate than in systems using conventional herbicides. There were some differences in mid-season weed population density between treatments, which were influenced more by initial weed population density than by treatment. Wilson et al. (2011) and Owen (2011) noted that none of the above mentioned studies has been conducted over a wide range of cropping systems and environments, and therefore a need exists to confirm the results in a broader geography that relates to the diversity and scale of GMHT cropping systems.

The use of glyphosate and its potential effects on the environment are also assessed under Regulation (EC) No 1107/2009. Questions related to the evolution of herbicide resistance to glyphosate in weeds are addressed by each Member State on receipt of the biological assessment dossier contained within the chemical market registration dossier. As part of the biological assessment dossier, applicants assess the likelihood of weed resistance evolving as a result of the use of glyphosate on GMHT crops, and provide a weed resistance management plan to delay this process. The assessment of the likelihood of weed resistance evolving is in line with the European guidelines PP 1/213 of the European and Mediterranean Plant Protection Organization (EPPO, 2003; Ulber et al., 2012). These guidelines propose a resistance risk analysis of two-stages, composed of resistance risk assessment, in which the probability of evolution of resistance and its likely impact are evaluated, and resistance risk management where, if necessary, possible strategies for avoiding or delaying the appearance of resistance are considered and suitable conditions of use are chosen and implemented. In resistance risk assessment, the inherent risk is first assessed using the characteristics of the pest and the product; the unmodified risk is then evaluated from the inherent risk when the product is applied under unrestricted conditions of use. In resistance risk management, the decision is made whether the unmodified risk is acceptable; if it is, the process can stop. If the unmodified risk is not acceptable, possible modifiers are then analysed to determine whether they can be used to mitigate the risk. If suitable modifiers exist, the conclusion of the resistance risk analysis will be a resistance management strategy (comprising one or more modifiers) that can be applied when the product is used commercially (EPPO, 2003).

Despite the low inherent risk of resistance evolution in weed species attributed to the biochemical, chemical and biological properties of glyphosate in plants and soil (Bradshaw et al., 1997), instances of weeds evolving resistance to glyphosate under field situations have been reported since 1996 (e.g., Powles et al., 1998; Pratley et al., 1999). Since then, there have been increasing instances of evolved glyphosate resistance in some weed species, particularly following the advent of GMHT crop cultivations (Owen and Zelaya, 2005; Sanderman, 2006; Powles, 2008; Beckie, 2011; Green and Owen, 2011; Owen et al., 2011; Heap, 2012), contradicting the initial speculations that the evolution of glyphosate resistant weeds was unlikely (Bradshaw et al., 1997). Currently, 21 weed species have evolved glyphosate resistant populations globally and 12 glyphosate resistant weed species (such as *Amaranthus palmeri*, *A. rudis*, *A. tuberculatus*, *Ambrosia artemisiifolia*, *A. trifida*, and various *Conyza* and *Lolium* spp.) have been identified in the USA , most of which evolved resistance to glyphosate in GMHT cropping systems (Beckie, 2011; Heap, 2012). Likewise, in cultivation areas of GMHT crops in Argentina and Brazil, glyphosate resistant populations of *Sorghum halepense* and *Euphorbia heterophylla* have been reported, respectively (Vidal et al., 2007, but see [44]; Powles et al., 2008). The basis for resistance has been attributed to altered EPSPS target site, reduced translocation or cellular transport to the symplast, and sequestration in the vacuole (reviewed by Powles, 2008; Powles and Yu, 2010; Beckie, 2011; Shaner et al., 2012; Vila-Aiub et al., 2012). The problem of glyphosate resistant

[44] http://www.weedscience.org/paper/Euphorbia_English.pdf

weeds is exacerbated by the fact that new resistance mechanisms such as gene amplification are being found (e.g., Gaines et al., 2010; Salas et al., 2012). Moreover, the evolution of multiple and cross-resistances to herbicides is becoming increasingly more common (Heap, 2012). The overreliance on glyphosate to control non-glyphosate herbicide resistant weeds contributed to the evolution of multiple resistances in populations (i.e., two or more resistance mechanisms) as a consequence of sequential selection or pollen flow, such as in glyphosate resistant *Lolium* spp. in Australia and South Africa (Neve et al., 2004; Yu et al., 2007; Preston et al., 2009; Preston, 2010) and in *A. palmeri* in cotton fields in southern USA (Culpepper et al., 2010). Multiple resistances to ALS-inhibiting herbicides and glyphosate are reported in horseweed (*Conyza canadensis*) (Davis et al., 2009).

It is important to note that glyphosate does not 'cause' weeds to evolve resistance *per se*, but rather how it is used that leads weeds to evolve resistance (Owen et al., 2011; Wilson et al., 2011). Evidence from the USA confirms that, where there is very intense glyphosate selection (i.e., the cultivation of glyphosate tolerant crops on continuous or rotational ground), little diversity in weed control practices and no mandated herbicide resistance programmes (Waltz, 2010), glyphosate resistant weeds may evolve and spread rapidly (e.g., Dauer et al., 2009; Owen et al., 2011). This in turn may induce modification of farmers' weed management practices through intensification of herbicide use and use of herbicides with less benign environmental profile, with consequent adverse environmental effects (Johnson et al., 2009; Kruger et al., 2009; Shaw et al., 2009; Webster and Sosnovski, 2010). In regions where glyphosate resistant weeds have to be controlled, farmers might exacerbate this phenomenon by increasing rates of glyphosate applied, which may further increase the selection pressure on weeds and lead to more instances of resistance (Duke, 2005; Pline-Srnic, 2005; Neve, 2008; Owen et al., 2011).

While the scale of glyphosate resistant weed occurrences has remained relatively small so far, a concern is that glyphosate resistant weeds would become more widespread in the near future (Service, 2007), as this would represent a significant threat to the sustainability of the herbicide and trait (Duke and Powles, 2008a; Powles, 2010; Owen et al., 2011; Ronald, 2011).

Impact on soil microbial communities

Soil microbial communities are the basis of important ecosystem services, and factors affecting the maintenance of these microbial processes can reduce the functional sustainability of soils (Smit et al., 2012). While no direct adverse effects of the CP4 EPSPS protein have been reported on non-target organisms, biogeochemical processes, or the abiotic environment (sections 6.2.4.2 and 6.2.6), the herbicide management associated with the cultivation of soybean 40-3-2 may, under certain conditions, have adverse effects on the biotic environment and biogeochemical processes. Glyphosate can enter the soil in pre-plant use, during early growth stages of GMHT crops, in post-harvest applications, and from runoff or leaching of the herbicide from vegetation. Further, plants do not metabolise glyphosate, but translocate it to actively growing regions, including roots, from which it can enter the soil (Hetherington et al., 1999; Feng et al., 2003; Kremer et al., 2005; Cakmak et al., 2009). The exudation of glyphosate can cause direct exposure of microorganisms in the rhizosphere, including beneficial plant growth promoting microorganisms such as mycorrhiza and nitrogen fixing symbiotic bacteria (Feng et al., 2005; Kremer et al., 2005).

Glyphosate can affect soil microbial communities by being a source of energy and nutrients (carbon, nitrogen and phosphorus) to microorganisms that can metabolise glyphosate (Borggaard and Gimsing, 2008; Mijangos et al., 2009; Zabaloy et al., 2012), or by inhibiting growth of glyphosate susceptible microorganisms (Gimsing et al., 2004). Microorganisms with glyphosate resistant forms of EPSPS (Class II) can metabolise glyphosate. Glyphosate resistant forms of EPSPS (Class II) have been found in several different bacteria, among those occurring in soil, such as *Agrobacterium*, *Pseudomonas putida* and *Ochrobacterum*. However, like in glyphosate susceptible plants, glyphosate can inhibit the shikimic acid pathway in microorganisms with glyphosate sensitive forms of EPSPS (Class I), and therefore decrease or fully inhibit the synthesis of aromatic amino acids (Busse et al., 2001; CaJacob et al., 2004; Zablotowicz and Reddy, 2004). Further, glyphosate can alter the concentration of carbohydrates and amino acids in root exudates, which may affect the composition and activity of the microbial community (Kremer et al., 2005).

Potential consequences of frequent glyphosate applications in GMHT cropping systems comprise alterations in the microbial community and microbial-mediated processes carried out in the crop rhizosphere, and may encompass effects on potential phytopathogen antagonist interactions, and interference with plant-growth-promoting rhizobacteria under worst-case conditions (Busse et al., 2001; Zablotowicz and Reddy, 2004, 2007; Lupwayi et al., 2007, 2009; Means et al., 2007; Hart et al., 2009; Kremer and Means, 2009; Powell et al., 2009a). Further, Powell et al. (2009b) reported that, depending upon the location of litter placement, glyphosate use can significantly reduce plant-litter decomposition. Impacts of glyphosate on microbial communities, however, are thought to be limited, because the majority of soil microorganisms appear not to be affected by glyphosate due to their tolerance to or their ability to metabolise glyphosate (Zabaloy et al., 2012), and due to the instability of glyphosate in soil, which decreases exposure (Locke et al., 2008). A proportion of glyphosate is actually sorbed by soil particles, limiting its activity and degradability, while glyphosate in soil-water is degraded by microorganisms (Haney et al., 2002; Powell et al., 2009a). Since sorbed glyphosate will be in equilibrium with the water-dissolved molecules, it is also expected to be degraded and decline in concentration over time (Locke et al., 2008). Sorption to soil particles will, however, slow down glyphosate degradation rates in soil and these rates will depend on the soil properties or agricultural management practices. In fact, it was recently shown that fertilisers may increase the mobility of glyphosate in soil due to competition for binding sites at the surface of the soil particles with phosphate (Bott et al., 2011). Such a mobilisation will also enhance the bioavailability of glyphosate, increasing its degradation in soil.

In bulk soil or rhizosphere soils, Weaver et al. (2007) did not observe any significant effects of glyphosate on soil microbial communities and mineralisation, even at concentrations well above recommended field application rates. In contrast, the repeated application of glyphosate at a rate of 49 μg ai g^{-1} soil to the soil surface, representing the concentration of glyphosate present to a depth of 2 mm, was shown to induce shifts in soil microbial communities, and indicated that repeated applications of glyphosate appear to have a greater impact on soil microorganisms than single applications (Lancaster et al., 2009). The addition of glyphosate as a technical compound such as Roundup Ultra resulted in increased microbial biomass accompanied by higher soil respiratory activity (Haney et al., 2000). In relation to beneficial microorganisms, no effects of glyphosate were found on the entomopathogenic fungi *Metarhizium anisopliae* (Mochi et al., 2005), or *Beauveria bassiana*, *M. anisopliae*, *Nomuraea rileyi* and *Neozygites floridana* (Morjan et al. (2002)), which might be used as microbial pest control agents. Glyphosate applied at recommended field application rates had no effect on the colonisation of soybean roots or leaf tissues by the arbuscular mycorrhizal fungi such as *Glomus intraradices* (Powell et al., 2007, 2009a; Savin et al., 2009).

In a field experiment, Liphadzi et al. (2005) found that the use of glyphosate on GMHT maize did not affect soil respiration or the diversity of the dominant soil bacteria. Likewise, it was found that neither the diversity of denitrifying bacteria, nor that of root-colonising fungi was affected by the application of glyphosate on GMHT maize (event DKC3551) (Hart et al., 2009). Barriuso et al. (2011) showed that glyphosate treatments applied on GMHT maize during the 3-year period of seasonal cultivation in two different fields did not significantly change the maize rhizobacterial communities when compared with those of the untreated soil. These and other studies indicate that one single application of glyphosate has only small and transient effects on the soil microbial community (Motavalli et al., 2004; Cerdeira et al., 2007; Gomez et al., 2009; Barriuso et al., 2010, 2011; Barriuso and Mellado, 2012), if they occur, and that its repeated use may favour soil microorganisms capable of metabolising or which are tolerant to glyphosate (Lancaster et al., 2009; Lane et al., 2011; Zabaloy et al., 2012).

Glyphosate released into the rhizosphere of GMHT soybean, combined with the release of high concentrations of carbohydrates and/or amino acids may favour increased fungal root colonisation and growth, including that of fungal soil borne plant pathogens, either directly or indirectly by suppressing bacterial antagonists (Johal and Huber, 2009). A number of studies have shown that glyphosate stimulates the growth of pathogenic fungi such as *Fusarium*, *Pythium*, *Phytopthora*, *Corynespora* and *Sclerotinia*, and can inhibit beneficial fungi. Responses of individual fungal species varied depending on their sensitivity to glyphosate; some species express glyphosate sensitive forms of EPSPS and may not metabolise glyphosate (Morjan et al., 2002), whilst others may readily metabolise glyphosate (Castro et al., 2007). In a laboratory study, growth of the plant pathogens *Pythium ultimum* and *Fusarium solani* could be stimulated or inhibited, depending on glyphosate concentration (Kawate et al., 1992). Kremer and Means (2009) reported that *Fusarium* spp. colonisation levels of roots of GMHT soybean receiving glyphosate were two to five times higher, compared with soybean receiving no herbicides, or a conventional herbicide, indicating that glyphosate induces fungal colonisation of soybean rhizospheres and hence affects the ability of plants to suppress potential pathogen colonisation and root infection. Such effects have also been observed under controlled conditions, but in a recent review, Powell and Swanton (2008) argued that experimental field trials, investigating the link between glyphosate and crop diseases associated with *Fusarium* spp., are not representative of the interactions that occur under actual farming conditions. Further, not all *Fusarium* strains responded in the same way; some strains did not show enhanced growth in glyphosate-based plant exudates (Kremer et al., 2005). A negative relationship between the population size of culturable fluorescent pseudomonads and root colonisation by *Fusarium* spp. was shown in GMHT soybean (Kremer and Means, 2009). Fluorescent pseudomonads include some important plant growth promoting bacteria capable of producing relevant secondary metabolites in the rhizosphere, and they can be transiently reduced in the rhizosphere of GMHT soybean due to their sensitivity to glyphosate that is exuded from the roots (Zobiole et al., 2011). Evidence also suggests that glyphosate may increase disease severity in plants by modifying soil microbial communities in ways that affect the availability and uptake of specific plant nutrients involved in disease resistance, or by the immobilisation of such nutrients (Johal and Huber, 2009; Yamada et al., 2009).

There are some indications that glyphosate-based cropping systems can cause nutrient deficiencies. Glyphosate can also interfere with root enzymes involved in mineral uptake from the soil, immobilise nutrients, or may transform nutrients to plant unavailable forms by stimulating certain microorganisms (Cakmak et al., 2009; Johal and Huber, 2009; Kremer and Means, 2009). Glyphosate applied to foliage of plants, including GMHT soybean, has been reported to decrease manganese-reducing rhizobacteria, and lead to manganese deficiencies (Gordon, 2007) due to the decreased ratio of potential manganese reducers/manganese oxidizers in the rhizosphere (Kremer and Means, 2009; Zobiolo et al., 2011). Frequent applications of glyphosate may thus directly or indirectly induce deficiencies in micro- and macro-nutrients (Bott et al., 2008, 2011; Zobiolo et al., 2010b), requiring altered fertiliser applications. However, Lane et al. (2011) reported that glyphosate applications did not reduce the plant available potassium (soil exchangeable or plant tissue potassium) in GMHT soybean.

In GMHT soybean, glyphosate has been shown to be translocated to metabolically active growing plant compartments, including root-nodules, where nitrogen fixing symbionts (bacteroids), such as *Bradyrhizobium japonicum* (Rhizobiaceae) (Salvucci et al., 2012), can be exposed to glyphosate. Reddy and Zablotowicz (2003) reported glyphosate concentrations in glyphosate tolerant soybean nodules ranging from 39 to 147 ng/g nodule dry weight, following exposure to up to two applications of glyphosate. Because *B. japonicum* possesses a glyphosate sensitive shikimic acid pathway, exposure to glyphosate may be accompanied with growth inhibition and/or death of *B. japonicum*, depending upon the glyphosate concentration (Cerdeira and Duke, 2006). Under culture conditions, glyphosate concentrations of less than 1 mM inhibited *B. japonicum* growth, and concentrations greater than 5 mM were lethal to *B. japonicum* (Moorman et al., 1992). Hernandez et al. (1999) revealed a differential growth inhibition by glyphosate among different *B. japonicum* strains. In their lower-tier studies, Dos Santos et al. (2005) also observed a direct effect of glyphosate formulations on the growth of *Bradyrhizobium*. Glyphosate plus the surfactant in the formulant affected *Bradyrhizobium* much more than glyphosate alone. Upon exposure to glyphosate, bacteroids may also accumulate high concentrations of shikimate and several hydroxybenzoic acids that can inhibit plant growth. The combination of *B. japonicum* sensitivity and potential accumulation of glyphosate or benzoic acids could interfere with nodule development and nitrogen fixation in glyphosate tolerant soybean compared with conventional soybean, in contrast to other herbicides (Zablotowicz and Reddy, 2004, 2007). The consequences of this could be that glyphosate applications reduce populations of *Bradyrhizobium* with glyphosate sensitive forms of EPSPS, and thus reduce the potential to form symbiotic relationships with soybean (Reddy et al., 2000; King et al., 2001; Reddy and Zablotowicz, 2003; Bohm et al., 2009; Zobiole et al., 2010a). For example, King et al. (2001) reported reduced nitrogen fixation activity after multiple applications of glyphosate on glyphosate tolerant soybean under growth chamber conditions. In cropping systems, this could reduce the nodulation of the crop and increase the need for additional nitrogen fertilisers in nitrogen-depleted soils (Bohm et al., 2009). However, due to the lack of bacterial symbionts for soybean in European agricultural soils, inoculation with selected *Bradyrizhobium* strains can be used to take advantage of symbiotic nitrogen fixation for soybean cultivation. Instead of bacterial inoculation, nitrogen can be supplied in agricultural practice as organic or inorganic fertiliser. The success of bacterial inoculation depends greatly on particular environmental conditions (climate, geographical region, soil properties) and is hard to predict. If supplemental bacterial cultures would be used as an alternative to fertilisation in soybean 40-3-2 cultivations, then results of Hernandez et al. (1999) suggest that the use of glyphosate tolerant strains may help to overcome potential glyphosate interference with soybean symbiosis. Furthermore, agricultural practice shows that nodulation of host-plants by rhizobia can be inconsistent, varying unpredictably with amongst othersfactors such as the rate and timing of glyphosate application (Cerdeira and Duke, 2006; Powell et al., 2009a). In higher-tier field studies, performed in the USA in soils with naturally occurring *Bradyrhizobium*, Zablotowicz and Reddy (2004, 2007) reported transient negative effects on nitrogen metabolism in GMHT soybean due to exposure to glyphosate, but crop yield was unaffected. Other studies performed under a wide range of environments indicated no yield reductions due to glyphosate applications on glyphosate tolerant soybean, suggesting that soybean has the potential to recover from glyphosate stress (Delannay et al., 1995; Reddy and Whiting, 2000; Elmore et al., 2001a; Krausz and Young, 2001; Nelson and Renner, 2001; Reddy and Zablotowicz, 2003). Further, Powell et al. (2009a) reported that nitrogen fixation was greater in GMHT soybean treated with glyphosate than in untreated plants when glyphosate was applied at the first trifoliate soybean growth stage.

6.2.7.3. Environmental impact of herbicide regimes used in soybean 40-3-2 cropping systems

As outlined in section 6.2.7.2, the introduction of GM plants for cultivation may require specific cropping and management practices and lead to additional changes in cropping systems. In the EU, current cropping systems are diverse, covering a wide range of cropping and management practices which, in addition, are continuously evolving under external drivers (e.g., regulation on pesticides, common agricultural policies, market requirements, agricultural innovations). Changes in cropping and management practices due to the introduction of GM plants and their potential environmental impacts are therefore to be seen in the context of the already existing and evolving range of current practices, as well as their environmental impacts.

Compared with current EU soybean cropping systems, an array of changes in cropping and management practices is to be expected in soybean 40-3-2 cropping systems, ranging from no change at all to the increased cultivation of soybean in short crop rotations under no- or reduced-tillage systems reliant on the exclusive and repeated use of glyphosate to control weeds. In the following sections, the range of different cropping and management practices potentially applied in cropping systems including soybean 40-3-2 are described and compared with those in cropping systems including conventional soybean. Depending on the cropping and management practices adopted by farmers in soybean 40-3-2 cropping systems, lower, similar or higher environmental impacts are expected, compared with those caused by the cropping and management practices currently applied in soybean cropping systems. Since the comparative environmental impacts of the different cropping and management practices will vary according to the receiving environment(s), intensity of crop production, rotational systems and a range of other factors, the EFSA GMO Panel assessed under what circumstances the specific cropping and management practices adopted under soybean 40-3-2 cropping systems may lead to greater, similar or lower adverse environmental effects than the current practices applied in soybean cropping systems they are likely to replace (EFSA, 2010e).

Typology of cropping and management practices in conventional soybean cropping systems

Crop rotations: In 2011, the EU cultivation area of soybean (including Croatia) was approximately 475,000 ha, with the highest share being grown in Italy (35 %), followed by Romania (16 %), Croatia (12 %), France and Hungary (9 %), Austria (8 %), Slovakia (4 %) and the Czech Republic (2 %).[45] Soybean is mostly grown as a break crop within a crop rotation, providing enhanced soil nitrogen to the following crop (Brookes, 2005). The purposes of growing soybean in rotation are: to improve yield and profitability of crops over time; decrease the need for nitrogen fertiliser on the crop following soybean; mitigate or break diseases; limit insect damage; break weed cycles; reduce soil erosion; and to increase soil organic matter. The rotation choice available to farmers, following soybean cultivation, typically includes maize or other cereals (mostly wheat). Crop rotations for soybean in the EU range from a 2-year crop rotation (soybean-wheat/maize) to a 5-year crop rotation (soybean-wheat-wheat/barley-oilseed rape-maize). Typical crop rotations for soybean are:

- a 2-year crop rotation (soybean-wheat/maize) in France and Italy;
- a 3-year crop rotation (soybean-wheat-maize) in Romania;
- a 4-year crop rotation (soybean-maize/sugar beet/sorghum-wheat-barley) in France, Hungary and Italy, or (soybean-wheat-oilseed rape-maize) in Romania.

Legumes (such as peas, lentils) or sunflower can also be grown in rotation with soybean in some EU regions.

Continuous soybean cultivation is not a common practice, but soybean is occasionally cropped continuously for up to three consecutive years in some EU regions (e.g., NE Italy). Evidence from the USA indicates that a small fraction of soybean (approximately 10 %) is sometimes grown for at least two consecutive years on certain fields (2006 USDA/ERS ARMS survey of soybean producers; Kruger et al., 2009; Prince et al., 2011a,b; Shaw et al., 2011).

Herbicide usage and regimes: The sensitivity of soybean to early weed competition is well-understood and the need for efficient weed control in the early soybean growth (unifoliate to 1st- to 3rd-trifoliate) stages often requires herbicide use with soil (residual) activity. Soybean is very delicate in its early growth stages; it is very susceptible to competition for resources such as water, nutrients and light. Therefore, it is important to protect the early growth stages from weed interference, also because crop canopy may not reach complete closure, especially when soybean is grown at larger inter-row distances (e.g., 75 cm).

Three different herbicidal weed management strategies are applied in conventional soybean for the control of weeds in the EU (Badea et al., 2006; Beckie et al., 2006; Young, 2006; Stewart et al., 2011; Wilson et al., 2011):

- application(s) pre-emergence of the crop;
- application(s) post-emergence. Imazamox applied post-emergence is frequently used as sole herbicide weed management option;
- sequential applications, where a combination of herbicides with soil (residual) activity is applied pre-emergence followed by post-emergence herbicides with foliar activity. Pre- and post-emergence applications can also include herbicide mixtures, typically between dicotiledonicides (e.g., linuron, chlomazone, metribuzin, oxadiazon) and graminicides (e.g., S-metolachlor, flufenacet, petoxamide, pendimethalin), especially in the presence of species that are difficult to control (Rapparini et al., 2011).

[45] Eurostat: http://epp.eurostat.ec.europa.eu/portal/page/portal/statistics/search_database; apro_cpp_crop

These herbicide regimes are effective against a range of weed species, but there are gaps in the spectrum of weeds that they control. Furthermore, they can provide poor control under some environmental and soil conditions (e.g., dry soil, limited of rainfall). The recommended herbicide regimes in soybean include typically one to three applications (one pre-emergence spray, and/or one to two post-emergence sprays), in order to control different weeds emerging at different times. Not all farmers use full rate applications of herbicides, especially when they rely on sequential post-emergence applications to target late emerging weeds. The choice of herbicides, applied alone or in tank mixtures, full or reduced rate, split application, row treatment, band application, etc. is driven by the need to manage the spectrum of weeds expected to occur at different stages of crop growth, which can vary greatly according to receiving environments (climate, soil type, season, field history, rotation, weed life cycles, IPM programmes and cultivation practices).

Typology of cropping and management practices in GMHT soybean cropping systems

Glyphosate-based herbicide usage and regimes: Glyphosate-based herbicides can be applied post-emergence with little or no injury to the GMHT crop. In contrast to selective herbicides that need to be applied when weeds are still in a young development stage, weed management strategies relying on glyphosate enable growers to delay the post-emergence application of a broad-spectrum herbicide until after full weed emergence and establishment (Gianessi, 2005; Cerdeira and Duke, 2006). The efficacy of glyphosate at controlling weeds is less dependent on weed phenology, so that glyphosate can be used up to a later growth stage for weeds, offering a greater flexibility in timing of weed management (Gianessi, 2008; Sartorato et al., 2011). However, the control of larger and perennial weeds will often require higher application rates or sequential treatments. It is also expected that the introduction of glyphosate in GMHT soybean will replace or reduce the use of other herbicidal active substances used pre-emergence or post-emergence of the crop (Beckie et al., 2006).

Several strategies have been proposed for controlling weeds in GMHT soybean depending upon the spectrum and density of weeds present (Reddy, 2001).[46]

- A single application or sequential applications of a glyphosate-based herbicide alone, with no use of pre-emergence herbicides. In narrow-row glyphosate tolerant soybean, a well-timed single glyphosate application can prevent yield loss (Ateh and Harvey, 1999; Mulugeta and Boerboom, 2000; Payne and Oliver, 2000; Vangessel et al., 2000; Young et al., 2001; Ivany, 2004; Owen et al., 2010; Stewart et al., 2010; Sartorato et al., 2011). However, field trials have shown that the use of a single post-emergence application of glyphosate alone at the recommended application rates can be inadequate to control all the weeds present throughout a full growing season (Corrigan and Harvey, 2000; Payne and Oliver, 2000; Vangessel et al., 2000; Ivany, 2004). Further, the achievement of acceptable levels of herbicide efficacy depends upon the correct timing of glyphosate application (Van Acker et al., 1993; Mulugeta and Boerboom, 2000; Knezevic et al., 2003; Dalley et al., 2004a,b; Hilgenfeld et al., 2004; Beckie et al., 2006; Scursoni et al., 2007; Sartorato et al., 2011). If the first treatment is applied too early, then weeds emerging after the application will remain unaffected. These weeds can not only reduce crop yield by competing for resources, but can shed seed and therefore increase weed pressure in subsequent years.

  Sequential applications of glyphosate have provided greater and more consistent weed control than single applications (Wait et al., 1999; Payne and Oliver, 2000; Reddy and Whiting, 2000; Wiesbrook et al., 2001; Knezevic et al., 2009; Owen et al., 2010; Stewart et al., 2010; Sartorato et al., 2011). For example, Swanton et al. (2000), found that sequential applications of glyphosate (pre-plant to unifoliate stage followed by a second application at the 1st- to 3rd-trifoliate stage) most consistently minimised competition from early- or late-emerging weeds, and optimised yields and net economic returns for soybean planted in wide rows. In its label proposals, the applicant recommended the use of glyphosate at dose rates ranging between 720 and 1,080 g/ha in two applications. The recommendation consists in applying glyphosate post-emergence

[46] Additional information received on 22/11/2010 / Questions 1 & 2 / Pages 13-24 / Appendix 1

(1,080 g/ha) at the 2nd- and 3rd-trifoliate stage, or when the weeds reach 10-15 cm in height, followed by a second application of glyphosate (720-1,080 g/ha) before the flowering stage and before weeds reach 10-15 cm in height. The increased frequency of glyphosate use, however, could be a more important factor than glyphosate rate in favouring selection for glyphosate resistance, as shown by Preston et al. (2009) in *Lolium rigidum*.

- Application of a glyphosate-based herbicide in combination with other herbicides. A delay in the first glyphosate application can lead to yield reductions if there is an extended period of early weed competition. To limit such early-season competition and avoid soybean yield losses, another strategy involves the use of other herbicides, particularly residual herbicides applied pre-emergence (Gonzini et al., 1999; Corrigan and Harvey, 2000; Reddy and Whiting, 2000; Reddy, 2000, 2001; Ellis and Griffin, 2002; Shaw and Arnold, 2002; Arregui et al., 2006; Beckie et al., 2006; Grichar, 2006; Nurse et al., 2007; Knezevic et al., 2009; Legleiter et al., 2009; Owen et al., 2010; Johnson et al., 2011). Glyphosate is a contact herbicidal active substance with little or no soil acting (residual) activity, and it strongly binds to soil particles. Moreover, glyphosate uptake by the plant roots is minimal. If glyphosate is applied pre-sowing, then there would be no long-term exposure of weeds to herbicidal activity, and weeds germinating subsequent to application would remain unaffected. Therefore, specific suggestions for this second strategy are for the use of pre-emergence residual conventional herbicides followed by a single delayed post-emergence application of glyphosate, which may eliminate the need for a second application. In this situation, the application rates of glyphosate proposed by the applicant in its label proposals are within the range of 720 to 1,080 g/ha.

- A single application of a glyphosate-based herbicide in combination with other compatible post-emergence herbicides with residual activity (Gonzini et al., 1999; Reddy, 2000, 2001; Vangessel et al., 2000; Shaw and Arnold, 2002; Grichar, 2006; Knezevic et al., 2009; Legleiter et al., 2009; Owen et al., 2010). This strategy has been recommended by Gianessi (2008) specifically for receiving environments in which early post-emergence herbicides are predominantly used instead of pre-emergence herbicides. If applied sufficiently early, this strategy can eliminate early-season weed competition and facilitate the control of weeds that are less susceptible to glyphosate (e.g., Norsworthy et al., 2001; Soukup et al., 2008).

- Several applications of broad-spectrum herbicides, including glyphosate. In cases of high weed pressure, other chemical-containing weed control strategies suggested include the sequential application of glyphosate in conjunction with crop compatible residual herbicides applied pre-emergence or early post-emergence; or the use of other crop compatible broad-spectrum herbicides in combination with glyphosate (Green and Castle, 2010; Green, 2011; Green and Owen, 2011).

In conclusion, pre- or post-emergence crop compatible residual herbicides could be used in combination with the post-emergence application of glyphosate around the unifoliate to 1st- to 3rd-trifoliate stages of soybean, in order to give optimum control and yield protection during the most vulnerable soybean growth stages.

Based on the soybean 40-3-2 cultivation experience from Romania (i.e., Badea et al., 2006) and elsewhere, the applicant provided provisional recommendations on the application rates of glyphosate on soybean 40-3-2 in its application. The applicant recommended herbicide regimes where the maximum total application rate of glyphosate is 2,160 g/ha per year, administered in up to two applications per crop, and clarified that higher application rates (up to 1,350 g/ha), are expected to provide effective control of difficult to control perennial weeds, whilst lower doses (generally 720 to 1,080 g/ha) might be sufficient to give effective control of annual weeds in soybean. These proposed application rates will be reviewed by the applicant in relation to the chemical market dossier according to Annex III of Directive 91/414/EEC (which was repealed by Regulation (EC) No 1107/2009 on 14 June 2011), and are indicative of the range of application rates, mixtures and systems that might be applied in the future. Hence, the applicant suggested that an appropriate recommended rate of glyphosate should be determined by: the weed species, density and growth stages; mixture patterns (according to local good agricultural practices); and production practices (e.g., planting density or row width).

Potential changes in cropping and management practices in soybean cropping systems in response to the introduction of soybean 40-3-2

An array of changes in cropping and management practices is to be expected when soybean 40-3-2-is cultivated as compared with current EU soybean cropping systems. These could include:

Adoption of altered cropping practices in GM soybean-based cropping systems: Soybean 40-3-2 may be introduced into farming systems as a substitute for non-GM soybean causing little change to cropping (including rotational) and management practices apart from the use of glyphosate as a substitute for the herbicides currently applied to soybean. Soybean 40-3-2 is anticipated to be used by farmers as any other soybean appearing in rotational-planning adapted to each specific geographic region. Thus, soybean 40-3-2 may be included within the range of the typical EU crop rotations for soybean, covering for example both short (2-year) and long (4-year) crop rotations.

However, evidence suggests that the annual area of arable land cropped to glyphosate tolerant soybean may increase over time at a landscape level in some regions (Bindraban et al., 2009). Moreover, the frequency of soybean cultivation may increase at the field level, with a shift from longer (4-year) to shorter (2-year) crop rotations. In these shorter crop rotations, other glyphosate tolerant crops (mainly maize), may be grown continuously with glyphosate tolerant soybean (see maize-soybean cropping systems in the USA). This could lead to shorter rotations of glyphosate tolerant crops. Bindraban et al. (2009) also reported a slight trend to an increased share of continuous glyphosate tolerant soybean cultivation in Latin America, though the continuous cultivation of soybean is not unique to GMHT systems.

Adoption of altered management practices such as glyphosate-based herbicide regimes in GM soybean-based cropping systems: Farmers are likely to adopt glyphosate-based herbicide regimes that represent a mixture of strategies involving different numbers of applications (single vs. sequential), doses, timing of application, use of other herbicides (including soil acting residuals), and non-chemical control methods in association with glyphosate. Variation between locally-adopted herbicide regimes for glyphosate tolerant soybean is expected in response to crop row spacing, weed species and biology, meteorological and agro-environmental conditions, farming systems, integrated pest and crop management, economics, growing season and farmers' behaviour. Despite this variability, three key strategies (outlined above) are likely to be applied for controlling weeds in glyphosate tolerant soybean:

- a single application or sequential (usually two) applications of glyphosate alone, with no use of pre-emergence herbicides;
- application of pre-emergence residual conventional herbicides followed by glyphosate;
- a single application of glyphosate in combination with other compatible post-emergence herbicides with residual activity.

Glyphosate-based herbicide regimes applied on soybean 40-3-2 could replace, partially or fully, the current post-emergence herbicides used, without any further modification of the cropping or management practices. The initial trend in herbicide regimes for GMHT crops has been a movement towards glyphosate-only systems, notably devoid of residual herbicides (Young, 2006; Johnson et al., 2007; Hurley et al., 2009; Prince et al., 2011a), but currently more diversity in herbicide regimes is implemented by farmers (Givens et al., 2009a; Prince et al., 2011a). Farmers may thus exploit the flexibility of the GMHT technology fully, and switch from conventional herbicide regimes to the exclusive use of post-emergence herbicide regimes, in which glyphosate-based herbicides can be applied alone or in conjuction with other post-emergence herbicides with residual activity (e.g., imazamox).

Adoption of altered management practices such as no- or reduced tillage systems in GM soybean-based cropping systems: Experience from Argentina (Bindraban et al., 2009), Brazil (Cerdeira et al., 2011) and the USA (Bonny, 2008, 2011) indicates that the introduction of soybean 40-3-2, is likely to be accompanied by changes in soil tillage practices and crop rotation. The use of glyphosate allows greater adoption of no- or reduced-tillage systems (Locke et al., 2008; Givens et al., 2009b), which is expected to increase in glyphosate tolerant soybean compared with its conventional counterparts (Cerdeira and Duke, 2006).

Comparative assessment of environmental impacts of different cropping and management practices in soybean cropping systems in response to the introduction of soybean 40-3-2

In this section, the EFSA GMO Panel compared the environmental impacts of several different possible management practices, which, as outlined above, may accompany the introduction of soybean 40-3-2. Consistent with EFSA (2010e) this is done by developing various scenarios. One is a 'substitution' scenario, in which the conventional soybean (with its specific management practices) is substituted by soybean 40-3-2 with its specific herbicide management, but without any other changes in other management practices. Another scenario is a "worst-case" one, which describes the effects on receiving environments of repeated, large-scale, and intensive management using soybean 40-3-2 and its adapted management practices, including the adoption of no- or reduced-tillage systems. A third scenario is a "best-case" scenario in which soybean 40-3-2 is cultivated using an approach which embeds its management into practices consistent with the goals of sustainable agriculture (as defined in, for example, Directive 2009/128/EC). The elaboration of each scenario and its use to develop general recommendations to aid risk assessment and risk management, as outlined here, is tentative. It should be viewed as the initial stage of a process requiring refinement. Therefore, no absolute estimates of risk are derived; such predictions might be overly sensitive to the particular assumptions made. However, it is believed that robust estimates of relative risk can be derived, based on the scientific literature concerning management practices, as discussed above. Since the six-step approach of EFSA (2010e) applies to each hazard identified, it is reasonable for separate scenarios to be generated for the two potential adverse effects: reductions in weed community diversity and resistance evolution to glyphsate in weeds. Indeed, Member States may have different protection goals relating to these two risks, depending on their perceived balance between agricultural production and the protection of farmland biodiversity. Therefore, separate scenarios were developed for both risks. However, it is recognised that the loss of weed community diversity represents a major driver for agricultural policy, and therefore the outcomes of the scenarios developed for this risk should also be assessed with regard to the risk of weed resistance.

Impact on farmland biodiversity: The factors considered by the EFSA GMO Panel to assess under what circumstances the specific cropping and management practices adopted under soybean 40-3-2 cropping systems may lead to greater, similar or lower adverse environmental effects on weed community biodiversity than the current practices applied in soybean cropping systems they are likely to replace encompassed three broad issues: crop rotation, herbicide usage and tillage. The categorisation was done on the basis of a linear model of a number of parameters, each weighted according to its perceived importance. For crop rotation the specific factors considered included the number of different crops in the rotation, the degree to which the growing season changed within the rotation and the competitive ability of crops with regard to weeds. For herbicide usage the specific factors considered included the use of glyphosate compared with conventional herbicides applied pre- and post-emergence, in terms of their efficacy and duration of weed control. For tillage the factors included the type (conventional inversion, reduced-tillage and no-tillage) and the diversity of tillage systems employed during a rotation. Estimated relative risks were categorised as: LL (considerably lower risk,); L (lower risk); S (similar risk); H (higher risk); and HH (considerably higher risk).

Three crop rotations representative of conventional cropping systems including soybean in the typical EU receiving environments were considered for each scenario (EFSA, 2010e): a 2-year soybean-maize crop rotation; a 3-year soybean-maize-wheat crop rotation; and a 4-year soybean-maize-oilseed-wheat crop rotation. In all cases, the following cropping and management practices were assumed:

- for soybean, ploughing (referred to in the tables as inversion) and spring-sown soybean treated with a sequential application of conventional pre-emergence herbicides followed by conventional post-emergence herbicides (referred to hereafter as treated with 1 × PRE + 1 × POST);

- for maize, ploughing and spring-sown maize treated with 1 × PRE + 1 × POST;

- for oilseed rape, ploughing and winter-sown oilseed rape treated with two applications of conventional post-emergence herbicides (referred to hereafter as treated with 2 × POST);

- for wheat, ploughing and winter-sown wheat treated with 2 × POST.

For each of the above conventional soybean cropping systems, several scenarios involving a soybean 40-3-2 cropping system were generated, representing 'best-case', 'substitution' and 'worst-case' scenarios (Table WD). In all scenarios, soybean 40-3-2 replaced conventional soybean with the adoption of glyphosate-based herbicide regimes, and in some cases also the adoption of no- or reduced-tillage systems:

- 'Best-case' scenario: soybean 40-3-2 treated with a single application of glyphosate (referred to hereafter as 1 × GLY) in combination with the adoption of no-or reduced-tillage systems;

- 'Substitution' scenario: ploughing and soybean 40-3-2 treated with an application of conventional pre-emergence herbicides and two applications of glyphosate (referred to hereafter as 1 × PRE + 1 × GLY);

- 'Worst-case' scenario: ploughing and soybean 40-3-2 treated with an application of conventional pre-emergence herbicides and two applications of glyphosate (referred to hereafter as 1 × PRE + 2 × GLY).

For simplicity the cropping and management practices applied to the other conventional crops of the soybean 40-3-2 cropping systems were kept identical to those of the conventional soybean cropping systems.

In addition, a 'very-worst-case' scenario consisting of ploughing and continuous soybean 40-3-2 (no crop rotation) treated with 1 × PRE + 2 × GLY was considered.

Clearly, the estimates will be somewhat sensitive to the form of the linear model and the weights allocated, and there are many ways in which the model may be formulated. However, the conclusions drawn here are not considered to be unduly sensitive to this uncertainty. The objective is to give information on relative risks to risk managers rather than to make specific predictions of weed community diversity.

Depending on the duration of the crop rotation, the best-case scenario in which soybean 40-3-2 is grown in a crop rotation with the same duration as that of the conventional soybean cropping system was likely to have a lower relative risk to reduce weed community diversity than the conventional soybean cropping system (Table WD). The relative risk to weed community diversity under the substitution and worst-case soybean 40-3-2 scenarios is likely similar to and greater than those of the conventional cropping system, respectively. The very-worst-case scenario, which involved the continuous cultivation of soybean 40-3-2 for two consecutive years along with a single application of conventional pre-emergence herbicides followed by the repeated application of glyphosate, may lead to the highest relative risks. The very-worst-case soybean 40-3-2 scenario was estimated to have a considerably higher risk than the 2-year conventional soybean cropping system in particular, and, a higher risk than all the other cropping systems considered.

Table WD. Comparison of relative risks of reductions in weed community diversity within various soybean and soybean 40-3-2 cropping systems. Relative risks compared with appropriate baseline are defined as follows: LL (considerably lower risk than baseline), L (lower risk), S (similar risk), H (higher risk), and HH (considerably higher risk). Comparisons should be made within columns.

Scenarios		**Crop rotations**		
		2-year (soybean -maize*)	**3-year** (soybean-maize-wheat*)	**4-year** (soybean-maize-oilseed rape-wheat*)
Conventional soybean cropping systems		Baseline$_2$	Baseline$_3$	Baseline$_4$
Soybean 40-3-2 cropping systems				
Best-case WD	Substituting with RR soybean, GLY$_{[1x]}$, no- or reduced-till	LL	L	L
Substitution WD	Substituting with RR soybean, PRE$_{[1x]}$ + GLY$_{[1x]}$, inversion	S	S	S
Worst-case WD	Substituting with RR soybean, PRE$_{[1x]}$ + GLY$_{[2x]}$, inversion	H	H	S
Very-worst-case WD	Replacing with continuous RR soybean, PRE$_{[1x]}$ + GLY$_{[2x]}$, inversion	HH	HH	HH

Abbreviations: GLY = glyphosate; PRE = conventional pre-emergence herbicides; POST = post-emergence herbicides; [x] = number of applications; WD = weed community diversity

* In all cases, the following cropping and management practices were assumed: for soybean, spring-sown / inversion / treated with 1 × PRE + 1 × POST; for maize, spring-sown / inversion / treated with 1 × PRE + 1 × POST; for oilseed rape, winter - sown / inversion / treated with 2 × POST; and for wheat, winter-sown / inversion / treated with 2 × POST

In each case described above, the relative risks are likely to be higher in the receiving environments with 2-year soybean cropping systems compared with those having a 3- or 4-year cropping systems (not shown in Table WD, in which comparisons are made solely within columns). In situations where farmers increase the frequency of soybean 40-3-2 in crop rotations, there are likely to be more adverse effects on weed community diversity. This is because crop rotation favours a more diverse composition of weed communities, and allows alternative weed control strategies to be used, thereby diversifying the selection pressure on weed populations and making it more difficult for one weed species to dominate a weed community.

In conclusion, the EFSA GMO Panel considers that the repeated use of glyphosate-based herbicides at recommended application rates on soybean 40-3-2 grown either in rotation with other glyphosate crops or continuously in conjuction with inversion tillage systems may lead to greater risk to reduce weed community diversity than the current practices applied in soybean cropping systems. This may therefore result in reductions in weed community diversity and/or weed density to a level that might adversely affect food chains and webs, but not necessarily biological control functions, at the field and landscape level. In addition glyphosate used in combination with other broad spectrum pre-emergent herbicides can also suppress weed communities to a greater degree than many conventional weed management programmes. Such reductions in weed community diversity and consequential reductions in farmland biodiversity may be considered problematic by risk managers depending upon protection goals pertaining to their region, especially in receiving environments that sustain little farmland biodiversity or in environmentally sensitive areas. However, under other conditions outlined above, soybean 40-3-2 cropping systems may have a similar or lower risk to reduce weed community diversity.

The EFSA GMO Panel conclusion on potential impacts of specific cultivation, management and harvesting techniques associated with the cultivation of soybean 40-3-2 is consistent with that of the DE CA. In its evaluation, the DE CA identified potential adverse effects of the herbicide used on soybean 40-3-2 on the environment, and they considered that "*it cannot be excluded that cultivation and planting management will change through the use of GM soybeans. Glyphosate-containing herbicides can be applied after germination of the soybean plants and thus could have effects on the accompanying weed flora*" (section 6.5 of the environmental risk assessment report of the DE CA).

Weed shifts and the selection of weed communities composed of more tolerant or resistant species: For simplicity the EFSA GMO Panel used the frequency of glyphosate-based applications and other weed management options over the rotation, including non-chemical ones, to estimate the potential risk of weeds evolving resistance to glyphosate and used this as an indicator of the selection pressure exerted by glyphosate. The relative risk of weed resistance was estimated using a function of the ratio of the number of glyphosate applications and the total number of weed management options. Estimated relative risks were categorised as: Z (zero risk); LL (very low risk); L (low risk); M (moderate risk); H (high risk); and HH (very high risk).

For each of the conventional soybean cropping systems described above, several scenarios involving a soybean 40-3-2 cropping system were selected, representing 'best-case', 'substitution' and 'worst-case' scenarios (Table WR). In all scenarios, soybean 40-3-2 replaced conventional soybean with the adoption of glyphosate-based herbicide regimes, and in some cases also the adoption of no- or reduced-tillage systems:

- 'Best-case' scenario: ploughing and soybean 40-3-2 treated with 1 × PRE + 1 × GLY or with the application of glyphosate tank mixed with conventional post-emergence herbicides (referred to herafter as 1 × GLY + 1 × POST);

- 'Substitution' scenario: ploughing and soybean 40-3-2 treated with 2 × GLY;

- 'Worst-case' scenario: soybean 40-3-2 treated with 2 × GLY in combination with the adoption of no- or reduced-tillage systems.

For simplicity the cropping and management practices applied to the other conventional crops in the soybean 40-3-2 cropping systems were kept identical to those in the conventional soybean cropping systems. Note that in these scenarios there is the assumption that glyphosate is not used in the intervening periods between conventional crop harvest and emergence or as a crop dessicant or inter-row treatment in conventional crops.

In addition, the two following 'very-worst-case' scenarios were considered:

- 'Very-worst-case' scenario 1: continuous soybean 40-3-2 treated with 2 × GLY in combination with the adoption of no- or reduced-tillage systems;

- 'Very-worst-case' scenario 2: soybean-maize rotation with soybean 40-3-2 and glyphosate tolerant maize treated with 2 × GLY in rotation with the adoption of no- or reduced-tillage systems in soybean 40-3-2 only.

Clearly, the estimates will be somewhat sensitive to the form of the linear model and the weights allocated, and there are many ways in which the model may be formulated. However, the conclusions drawn here are not considered to be unduly sensitive to this uncertainty. The objective is to give information on relative risks to risk managers rather than to make specific predictions of durations until the onset of resistance.

Overall, the estimated relative risks of weeds evolving resistance to glyphosate are likely to be the highest in short (2-year) soybean 40-3-2 cropping systems where glyphosate is used repeatedly and exclusively (Table WR). For 2-year crop rotations the estimated relative risk for all the soybean 40-3-2 cropping systems studied was either high or very high, except for the best-case scenario.

The adoption of no- or reduced-tillage systems is likely to increase the relative risk of weed resistance further; this is due not only to the additional use of glyphosate which usually accompanies such systems and which increases the selection pressure on weeds, but also because no-tillage systems select a particular spectrum of weed species with ecological characteristics favoured by lack of soil disturbance, and which are at an increased risk of evolving resistance.

Within glyphosate-based systems, the replacement of a second glyphosate application by a conventional herbicide with residual activity, applied either pre-emergence or post-emergence, is likely to greatly reduce the relative risk of weed resistance evolution. Also, in glyphosate-based systems where only one glyphosate application is employed, the additional application of conventional herbicides may slightly reduce the relative risk of resistance. Hence, combining a single application of glyphosate with herbicides with residual activity, applied either pre- or post-emergence, may reduce the relative risk of resistance compared with glyphosate-only systems devoid of residual herbicides.

Table WR. Comparison of relative risks of glyphosate resistance evolving in weeds between various soybean and soybean 40-3-2 cropping systems. Relative risks are defined as follows: Z (zero risk), LL (very low risk), L (low risk), M (moderate risk), H (high risk) and HH (very high risk). Comparisons should be made within columns.

Scenarios		**Crop rotations**		
		2-year (soybean -maize*)	**3-year** (soybean-maize-wheat*)	**4-year** (soybean-maize-oilseed rape-wheat*)
Conventional soybean cropping systems		Z	Z	Z
Soybean 40-3-2 cropping systems				
Best-case WR	Substituting with RR soybean, PRE + $GLY_{[1x]}$, inversion	L	L	LL
Substitution WR	Substituting with RR soybean, $GLY_{[2x]}$, inversion	H	M	L
Worst-case WR	Substituting with RR soybean, $GLY_{[2x]}$, no- or reduced-till	HH	H	M
Very-worst-case WR1	Replacing with continuous RR soybean -RR maize crop rotation, $GLY_{[2x]}$, no- or reduced-till in RR soybean, inversion in RR maize	HH	HH	HH
Very-worst-case WR2	Replacing with continuous RR soybean, $GLY_{[2x]}$, no- or reduced-till	HH	HH	HH

Abbreviations: GLY = glyphosate; PRE = conventional pre-emergence herbicides; POST = post-emergence herbicides; [x] = number of applications; WR = weed resistance

* In all cases, the following cropping and management practices were assumed: for soybean, spring-sown / inversion / treated with 1 × PRE + 1 × POST; for maize, spring-sown / inversion / treated with 1 × PRE + 1 × POST; for oilseed rape, winter-sown / inversion / treated with 2 × POST; and for wheat, winter-sown / inversion / treated with 2 × POST

The estimated relative risks of weeds evolving resistance to glyphosate are likely to decrease with the duration of the crop rotation. In most scenarios considered, the lowest risk values were estimated in the longer (4-year) cropping systems than in the shorter (2-year) ones due to the reduced frequency of exposure to glyphosate in longer crop rotations compared with shorter ones. For the best-case scenario and typical scenario with soybean 40-3-2 grown in 3- and 4-year crop rotations the relative risks are likely to be low or very low. In contrast, the repeated use of glyphosate-based herbicides at recommended application rates on soybean 40-3-2 grown either in rotation with other glyphosate tolerant crops or continuously may lead to very high relative risks, as predicted for the two very-worst-case scenarios.

In conclusion, the EFSA GMO Panel considers that soybean 40-3-2 grown in rotation with other glyphosate tolerant crops in repeated short (2-year) crop rotations or continuously, in conjunction with the repeated and/or exclusive application of glyphosate-based herbicides, will cause changes in the weed flora, and will favour the evolution and spread of glyphosate resistant weeds due to the selection pressure exerted by glyphosate. The adoption of no- or reduced-tillage systems, enabled by the use of glyphosate, may further increase the selection pressure on some weeds and hence increase the rate at which resistance evolves in weeds. However, where there is more diversity in cropping and management practices, and where mandated herbicide resistance programmes are put in place, the selection pressure of glyphosate on weeds will be reduced, significantly decreasing the selection of more tolerant or resistant weeds.

The EFSA GMO Panel conclusion on potential impacts of specific cultivation, management and harvesting techniques associated with the cultivation of soybean 40-3-2 is consistent with that of the DE CA. In its evaluation, the DE CA considered that "*it cannot be excluded that cultivation and planting management will change through the use of GM soybeans. Glyphosate-containing herbicides can be applied after germination of the soybean plants and thus could have effects on the accompanying weed flora. Based on experience from using conventional plant protection products it is to be expected that sooner or later tolerance to the active ingredient of glyphosate-containing herbicides will develop in the weed flora*" (section 6.5 of the environmental risk assessment report of the DE CA).

Impact on soil microbial communities: Whilst glyphosate can affect some soil bacteria, mycorrhizal fungi and *Bradyrhizobium* and other rhizobia, impacts of glyphosate on microbial communities, however, are thought to be limited, because the majority of soil microorganisms appear not to be affected by glyphosate due to their tolerance to or their ability to metabolise glyphosate, and due to the instability of glyphosate in soil, which decreases exposure. Temporal rearrangements of soil microbial communities and their structural diversity occur frequently in the agricultural environment. They are typically associated with several sources of variation such as natural variability (e.g., soil heterogeneity, weather conditions) and impacts of agricultural practices (e.g., soil tillage, crop rotation, irrigation measures, pesticide and fertiliser applications), and are thus not necessarily an indication of environmental harm (Kowalchuk et al., 2003). The magnitude and direction of responses of the soil microbial community to glyphosate application depend on the microorganisms investigated, herbicide dose, the soil type, and the ecological interactions, including whether the studies are conducted under laboratory or field conditions (Wardle and Parkinson, 1990, 1992; Gorlach-Lira et al., 1997; Busse et al., 2001; Motavalli et al., 2004; Powell and Swanton, 2008; Savin et al., 2009). Transient shifts in soil microbial communities and their structural diversity may occur after the application of glyphosate, but to the knowledge of the EFSA GMO Panel, a persistent effect of glyphosate on soil microbial communities has not been reported.

The EFSA GMO Panel considers that the use of glyphosate-based herbicides at recommended field application rates of glyphosate on soybean 40-3-2 is unlikely to cause adverse effects to the majority of members of soil microbial communities compared with currently used herbicide programmes. Adverse effects of glyphosate may occur to soil microorganisms harboring sensitive forms of the target enzyme EPSPS, including bacterial symbionts of soybean (such as *Bradyrhizobium*). The consequences of this could be that glyphosate applications will reduce rhizobial populations, at least temporarily, which, in turn, could reduce the nodulation of the crop and lead to additional use of nitrogen fertilisers. However, under field conditions, there is also some evidence indicating that soybean yield and performance is not affected by glyphosate (see above literature review) and that soybean has the potential to recover from glyphosate stress. Therefore, potential adverse effects are likely to be transient in most situations.

The EFSA GMO Panel concludes that a hazard to some members of microbial communities has been identified, but that scientific uncertainty remains as to whether a change in the diversity of soil microbial communities leads to adverse environmental effects under field conditions.

In its evaluation, the DE CA noted that "*there is potentially also an indirect interaction between the use of glyphosate-containing herbicides and nitrogen-fixing symbiotic partners of the soybean (e.g. Bradyrhizobium japonicum, Moorman et al., 1992, King et al., 2001), which could lead to a reduction in harvest yield (King et al., 2001). To compensate, potential increased application of nitrogen fertilizer might be necessary with the cultivation of HT soybeans. The German Competent Authority recommends that herbicide and cultivation management of soybean 40-3-2 should be adapted to minimize potential negative effects*" (section 6.5 of the environmental risk assessment report of the DE CA).

6.2.8. Conclusion on the environmental risk assessment

As the scope of the current application covers cultivation, the environmental risk assessment considered the environmental impact of full-scale commercialisation of soybean 40-3-2.

The DE CA provided EFSA with its report on the environmental risk assessment of soybean 40-3-2 (dated 9 September 2008) on 3 October 2008 in line with Articles 6.3(c) and 18.3(c) of Regulation (EC) No 1829/2003. The report on the environmental risk assessment of the DE CA is provided in Annex H of the EFSA Overall Opinion, and has been considered throughout this EFSA GMO Panel Scientific Opinion.

The EFSA GMO Panel considers that soybean 40-3-2 has no altered agronomic and phenotypic characteristics, except for the herbicide tolerance. The likelihood of unintended environmental effects due to the establishment, survival and spread of soybean 40-3-2 is considered to be extremely low, and will be no different from that of conventional soybean varieties.

It is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the environment or human and animal digestive tracts. In the rare but theoretically possible case of transfer of the CP4 *epsps* gene from soybean 40-3-2 to soil bacteria, no novel property would be introduced into the soil bacterial community and thus no positive selective advantage that would not have been conferred by natural gene transfer between bacteria would be provided.

Based on the evidence provided by the applicant and relevant scientific literature on soybean 40-3-2, the EFSA GMO Panel concludes that there are no indications of the occurrence of adverse effects on non-target predators, herbivores and decomposers due to potential unintended changes in soybean 40-3-2, and therefore considers *trait*-specific information appropriate to assess whether soybean 40-3-2 poses a risk to non-target organisms.

The EFSA GMO Panel notes that scientific uncertainty pertaining to the occurrence of adverse effects on pollinators due to potential unintended changes in soybean 40-3-2 remains, as no event-specific data on plant-pollinator interactions were provided by the applicant. As specified in EFSA (2010e), the remaining scientific uncertainty concerning the presence of these effects and the conditions under which this may be resolved both require examination. The EFSA GMO Panel considers that the absence of biologically relevant differences in the composition of key analytes or agronomic and phenotypic characteristics identified between soybean 40-3-2 and its conventional counterpart, the restricted exposure of honeybees to pollen from soybean flowers and the information from the extensive cultivation of soybean 40-3-2 outside Europe for a considerable number of years combine, under the weight-of-evidence approach, to make it unlikely that unintended adverse effects on pollinators will occur. This likelihood, however, cannot be easily estimated. This uncertainty should be resolved by experiments designed to compare the effects of soybean 40-3-2 and its conventional counterpart (and optionally reference, commercial non-GM soybean varieties, if appropriate) on adult honeybees in relevant receiving environments in Europe. The EFSA GMO Panel considers that the following five conditions must be met in these experiments: (1) honeybees should be observed over a period of at least two years; (2) beehives should be added to the experiments to ensure that honeybees are present in sufficient quantity to enable potential effects to be detected; (3) the experiments should be replicated spatially over at least three sites; (4) the same weed control regime treatments should be applied across all plots; and (5) data on all relevant measurement endpoints should be obtained (abundance, foraging behaviour, survival, etc.).

The studies, supplied or reviewed by the applicant, showed no adverse effects on different types of non-target organisms due to the expression of the CP4 EPSPS protein in glyphosate tolerant crops.

The EFSA GMO Panel does not expect potential adverse effects on biogeochemical processes and the abiotic environment due to the expression of the CP4 EPSPS protein in soybean 40-3-2.

The EFSA GMO Panel is of the opinion that potential adverse environmental effects of the cultivation of soybean 40-3-2 are associated with the use of the complementary glyphosate-based herbicide regimes. These potential adverse environmental effects could, under certain conditions, comprise: (1) a reduction in farmland biodiversity; (2) changes in weed community diversity due to weed shifts; (3) the selection of glyphosate resistant weeds; and (4) changes in soil microbial communities. The potential harmful effects could occur at the level of arable weeds, farmland biodiversity, and food webs and the ecological functions they provide. The magnitude of these potential adverse environmental effects will depend on a series of factors, including the specific herbicide and cultivation management applied at the farm level, the crop rotation and the characteristics of the receiving environments.

The conclusions of the EFSA GMO Panel on the environmental safety of soybean 40-3-2 are consistent with those of the DE CA. The DE CA concluded that "*no adverse effects on human and animal health and the environment are to be expected from the cultivation of soybean 40-3-2*", but that "*glyphosate-containing herbicides can be applied after germination of the soybean plants and thus could have effects on the accompanying weed flora. Based on experience from using conventional plant protection products it is to be expected that sooner or later tolerance to the active ingredient of glyphosate-containing herbicides will develop in the weed flora*" (see section 6.5 of the environmental risk assessment report of the DE CA). In its evaluation, the DE CA noted that "*there is potentially also an indirect interaction between the use of glyphosate-containing herbicides and nitrogen-fixing symbiotic partners of the soybean (e.g. Bradyrhizobium japonicum, Moorman et al., 1992, King et al., 2001), which could lead to a reduction in harvest yield (King et al., 2001). To compensate, potential increased application of nitrogen fertilizer might be necessary with the cultivation of HT soybeans*" (see section 6.5 of the environmental risk assessment report of the DE CA). With regard to the occurrence of adverse effects on non-target organisms due to potential unintended changes in soybean 40-3-2, the DE CA recommended "*conducting an additional study to confirm the absence of unintended adverse effects on non-target organisms*" (see section 6.3 of the environmental risk assessment report of the DE CA).

6.3. Risk management strategies (including post-market environmental monitoring)

6.3.1. Risk mitigation measures

6.3.1.1. General aspects of mitigation

According to the EFSA GMO Panel guidelines on the environmental risk assessment of GM plants (EFSA, 2010e) and in line with Annex II of the Directive 2001/18/EC, the risk assessment can identify risks that require management and propose risk mitigation measures to reduce the levels of risk. In order to reduce the identified risks associated with the GM plant deployment to a level of no concern, both the DE CA and the EFSA GMO Panel evaluated the scientific quality of the risk mitigation measures proposed by the applicant, as well as their adequacy and efficacy. Risk mitigation should be proportionate to the results of the different risk scenarios studied, the specific protection goals in the receiving environments, and to the levels of scientific uncertainty and risk identified in the environmental risk assessment (EFSA, 2011c).

6.3.1.2. Interplay between environmental risk assessment and mitigation

Based on the evidence provided by the applicant and relevant scientific literature on soybean 40-3-2, the EFSA GMO Panel concluded that there are no indications of the occurrence of adverse effects on predators, herbivores and decomposers due to potential unintended changes in soybean 40-3-2. As no event-specific data on plant-pollinator interactions were provided by the applicant, scientific uncertainy pertaining to the occurrence of adverse effects on pollinators due to potential unintended changes in soybean 40-3-2 remains.

In addition to these conclusions, the EFSA GMO Panel concluded that the cultivation of soybean 40-3-2 may result in adverse environmental effects due to the use of the complementary glyphosate-based herbicides. These potential adverse environmental effects could, under certain conditions, comprise: (1) a reduction in farmland biodiversity; (2) changes in weed community diversity due to weed shifts; (3) the selection of glyphosate resistant weeds; and (4) changes in soil microbial communities. As the magnitude of these potential adverse environmental effects will depend upon a series of factors, the EFSA GMO Panel recommends that risk mitigation measures are put in place to ensure that glyphosate on soybean 40-3-2 will be used in ways that result in similar or reduced environmental impacts compared with conventional soybean cultivation.

The specific risks, identified in section 6.2.8 (conclusion on the environmental risk assessment), requiring mitigation are: (1) a reduction in farmland biodiversity; (2) changes in weed community diversity due to weed shifts; (3) the selection of glyphosate resistant weeds; and (4) changes in soil microbial communities. The EFSA GMO Panel notes that the risks (1-4) are related to the use of the complementary herbicide, and judges that risk mitigation measures could equally well be put in place either under the legislation for plant protection products (Regulation (EC) No 1107/2009, which replaced Directive 91/414/EEC on 14 June 2011, and Directive 2009/128/EC), or under the legislation for GMOs (Directive 2001/18/EC). In reaching this view, the EFSA GMO Panel considered: the interplay between the legislation for GMOs and plant protection products (section 6.2.7.1); the fact that some herbicide tolerant systems on the market are non-GM; and the fact that protection goals are set at Member State level. However, since the remit of the EFSA GMO Panel to propose risk mitigation measures is linked inextricably to Directive 2001/18/EC, subsequent recommendations in this section are based on GMO legislation.

Possible risk mitigation measures, which can be put in place to reduce levels of risk and remaining scientific uncertainty, and their efficacy, were evaluated by the EFSA GMO Panel, and this evaluation is described below.

6.3.1.3. Risk mitigation measures to reduce adverse effects due to changes in herbicide regimes

The applicant indicated it will develop a Technology Use Guide for the EU soybean 40-3-2 markets. This guide will provide detailed weed control recommendations on soybean 40-3-2 to ensure that farmers adhere to good agricultural practices. It will therefore cover recommendations on minimum and maximum application dose rates, herbicide mixtures and herbicide rotation in cropping systems.

Depending upon protection goals set at Member State level (e.g., EFSA, 2010d,e) and in situations where potential adverse herbicide effects are likely, risk managers should consider putting risk mitigation measures in place to manage potential herbicide effects and to ensure the implementation of good agricultural practices, including integrated pest management. Such measures should ensure that biodiversity is maintained at least at current levels, and that potential adverse effects on weed community diversity, farmland biodiversity, food webs and the ecological functions they provide are limited to the levels currently found in non-GMHT soybean. Likewise, whenever relevant, risk managers should recommend putting specific risk mitigation measures in place to reduce the selection of more tolerant or resistant weeds.

Impact on farmland biodiversity

In line with protection goals set at Member State level by relevant legislations and according to the legal provisions of Directive 2001/18/EC (e.g., EFSA, 2010c,d,e), appropriate measures should be put in place to mitigate potential adverse environmental effects on biodiversity by targeting their main drivers (Butler et al., 2007). Member States may recommend using glyphosate on soybean 40-3-2 only in regimes that have similar or reduced environmental impacts compared with conventional soybean cultivation, and that do not interfere with biological functions currently supported by soybean cropping systems. The EFSA GMO Panel also notes that the new legislations for the assessment and use of plant production products, introduced biodiversity more explicitly as a protection goal. Regulation (EC) No 1107/2009 mentions that plant protection products shall have no unacceptable effects on the environment, especially on biodiversity and the ecosystem, whereas the use of herbicides will have to adhere to the principles of integrated pest management and be consistent with good plant protection practice in order to ensure high levels of protection of human and animal health and the environment. In addition, Member States will describe in their national action plans how they will ensure that the general principles of integrated pest management, as set out in Annex III of Directive 2009/128/EC on the sustainable use of pesticides, are implemented by all professional users by 1 January 2014.

The EFSA GMO Panel considers that the delivery of both food production and biodiversity conservation should be reconciled at the field and landscape level (Firbank, 2005; Benton, 2007; EFSA, 2008b; Sutherland et al., 2009; Godfray et al., 2010). Soybean has been shown to have low levels of biodiversity, while maize has less biodiversity than oilseed rape and beet under EU conditions (Firbank et al., 2003b; Dewar et al., 2005; Bohan et al., 2007; Smith et al., 2008b). Further, the repeated use of glyphosate at recommended application rates on soybean 40-3-2 grown either in rotation with other glyphosate tolerant crops or continuously may result in reductions in weed community diversity and/or weed density to a level that might adversely affect food chains and webs, but not necessarily biological control functions, at the field and landscape level. In addition, plant communities in cropped and uncropped areas of the farm differ; it is therefore questionable whether providing plant resources on uncropped land only will be sufficient to reverse the declining trends in farmland biodiversity. Beneficial weed species adapted to the cropped area of the field can be distinct from the flora found in uncropped land, so sustaining their populations would increase the overall functional diversity of the farm ecosystem (Storkey, 2006). Besides plant communities, also the scale of cropped and uncropped areas of the farm differs, with the uncropped land usually representing a small percentage of the total area of the farm. In addition, Roschewitz et al. (2005) established that plant species diversity in agricultural landscapes is not only affected by management of single fields, but also by the heterogeneity of the landscape. It also remains debatable whether increases in crop yield will reduce the extent to which semi-natural and natural habitats are converted into arable land in EU countries (i.e., Balmford et al., 2005; Mooney et al., 2005; Matson and Vitousek, 2006; Ewers et al., 2009; Godfray et al., 2010). Therefore, the EFSA GMO Panel recommends that risk mitigation measures are put in place that can provide considerable benefits for biodiversity at the cost of no or only small reductions in crop yield (Dewar et al., 2003; May et al., 2005; Pidgeon et al., 2007).

A number of options for risk mitigation measures are possible, and can be divided into those that target uncropped land such as field margins and set-aside, or cropped areas (Marshall and Moonen, 2002; Kleijn and Sutherland, 2003; Kleijn et al., 2006, 2011; Storkey and Westbury, 2007; Andreasen and Andresen, 2011; Whittingham, 2011). Possible risk mitigation measures for uncropped land include protecting adjacent habitats from herbicide effects. For example, in Germany, the approval for the application of glyphosate on glyphosate tolerant crops incorporates recommendations for not spraying within 20 m from certain sensitive areas and measures for the protection of water courses, in order to limit potential adverse effects due to herbicide drift (Streloke, 2011).

Impacts on biodiversity may also be mitigated by better field margin management or other ‘out of crop’ measures, which are increasingly applied in conventional cropping systems to deliver desired ecological benefits (Marshall, 1989; Wilson and Aebischer, 1995; Thomas and Marshall, 1999; Norris and Kogan, 2000; Marshall and Moonen, 2002; Meek et al., 2002; Roschewitz et al., 2005; Moonen et al., 2006; Butler et al., 2007; Clarke et al., 2007; Walker et al., 2007; Smith et al., 2008a; Dewar, 2009; Fried et al., 2009; Andreasen and Andresen, 2011; Cordeau et al., 2011). Headlands and/or field margins (Greaves and Marshall, 1987) are strips of land lying between crops and the field boundary, and extending for a limited distance into the crop (Marshall and Moonen, 2002). These margins fall into two broad categories (1) uncropped, either sown (with grass or grass and wildflower seed mixtures) or left to regenerate naturally (including naturally regenerated or sown [temporary or long-term] set-aside margins), and (2) cropped, sown with crops usually under modified management, such as conservation headlands, with wild bird cover crops and/or with plant mixtures providing pollen and nectar. Cropped and uncropped margins can be managed in a range of ways particularly in terms of cutting and/or cultivation (reviewed by Vickery et al., 2009). Sensitive management of field margins can increase species density in agro-ecosystems, provide habitats for rare or endangered species, and enhance ecosystem services (Marshall and Moonen, 2002; Moonen et al., 2006; Vickery et al., 2009). Conservation headlands have less intensive management with reduced fertiliser and pesticide inputs to field edges and margins (Sotherton, 1991; Kleijn and Snoeijing, 1997; Kleijn and Van der Voort, 1997), and can be supplemented with unsprayed field margin strips or semi-permanent beetle banks (Thomas et al., 2001; Fried et al., 2009). Field margins can also include boundary features such as hedgerows and ditches which are an extremely valuable habitat for invertebrates and birds, providing food, shelter and nest cover (Jobin et al., 2001; Fuller et al., 2004; Pywell et al., 2005a,b). These conservation areas would help to maintain a relatively high degree of botanical diversity at field edges, which is expected to be higher in more structurally complex margins (Moonen et al., 2006).

In cropped areas, delayed or less intense in-crop weed management can promote arable weed communities, and thereby deliver benefits for farmland biodiversity. Heard et al. (2005) believed that growers might learn to tolerate higher weed densities at certain periods of the growing cycle, provided that these weeds do not cause economic loss. For fodder beet treated with glyphosate, Strandberg and Pedersen (2002) reported that with careful management according to label recommendations or with further delays to applications, there may be significant improvements in weed flora and arthropod fauna, but that weed seed production was reduced. Less intense in-crop weed management with glyphosate applied to a proportion of the field or crop can also maintain desired levels of biodiversity. In GMHT sugar beet, this can be achieved either by over-the-row band spraying to allow early season weed growth between, but not within, crop rows, or by overall spraying early only to allow some later emerging weeds. Weeds occurring between rows after an early over-the-row band spraying could be controlled by a later overall spray (Dewar et al., 2000, 2003; May et al., 2005; Pidgeon et al., 2007). Results showed that some weeds can be left for a longer period between the crop rows without yield loss (Dewar et al., 2003). These weeds can increase the number of beneficial invertebrates during the early to mid-season (Dewar et al., 2003), and produce seed in the autumn as food for birds (May et al., 2005). The use of similar management measures should also be considered for applications of glyphosate to glyphosate tolerant soybean.

The choice of crop sequences can also be an important tool for manipulating weed communities and to prevent the build up of particular weed species and herbicide resistance evolution (Squire et al., 2000; Anderson, 2009). Rotating soybean with other crops that require different weed management strategies would also be a good strategy to prevent the selection of more tolerant or resistant weeds (see below).

In-crop risk mitigation measures can be more difficult to implement than managing uncropped land and field margins for biodiversity (Squire et al., 2000; Hawes et al., 2010). Managing weeds within the crop to support biodiversity involves the risk of reducing crop yield (Oerke, 2006) and the long-term build-up of problem weed communities (Storkey, 2006); there is an inevitable challenge in maintaining effective control of problem weeds, while sustaining beneficial weed species at economically acceptable levels (Storkey, 2006; Storkey and Cussans, 2007). It has been argued that more robust tools are required for assessing beneficial weed communities in terms of the ecological functions they provide to the ecosystem and their effect on crop yield, and ultimately to identify the appropriate threshold level of these weeds that is economically acceptable and ecologically significant (i.e., Bastiaans et al., 2000; Storkey, 2006; Storkey and Cussans, 2007). Another difficulty is that protection goals are not always clearly defined, as reaching agreement on what needs to be protected from harm (i.e., protection goals) presents challenges (Nienstedt et al., 2012; Sanvido et al., 2011a, 2012). So far, risk managers have failed to define clearly 'how many weeds' or 'what type of weeds' are desired in arable fields (Sanvido et al., 2011b), hampering the choice of risk mitigation measures that are proportionate to the specific protection goals in the receiving environments.

In its evaluation, the DE CA recommended that "*herbicide and cultivation management of soybean 40-3-2 should be adapted to minimize potential negative effects*". The DE CA noted that "*the herbicide registration is currently performed on Member State level and thus allows to set herbicide application conditions (such as used amount, intensity of weed control or management to prevent resistance developing against glyphosate-containing herbicides) to be adapted to the regional requirements of the European Union*" (section 6.5 of the environmental risk assessment report of the DE CA).

Weed shifts and the selection of weed communities composed of more tolerant or resistant species

Based on the specific biochemical, chemical and biological properties of glyphosate in plants and soil, the applicant argued that the inherent risk of weed resistance to glyphosate may be considered low to medium, depending upon the weed species. Despite the low to medium inherent risk of weed resistance to glyphosate, tolerant and resistant weeds are evolving in countries with extensive and repeated use of glyphosate, especially on GMHT crops (e.g., USA and Argentina) (reviewed by Beckie, 2011; Owen, 2011; Heap, 2012). Current soybean management in the EU differs from region to region depending on the levels of adoption of certain agricultural practices including crop rotations, mechanical weed control, herbicide mixtures and herbicide use throughout the cropping systems. However, in some parts of the EU, soybean 40-3-2 may be grown in rotation with other glyphosate tolerant crops or continuously, so there is a potentially high risk of resistance evolution where glyphosate is repeatedly used on glyphosate tolerant crops (section 6.2.7). The applicant has stated that the use of herbicides with different modes of action and compliance to best practices, such as scouting for weeds and the use of crop rotations will be applied in line with the stewardship guidelines for herbicide labels made by the Herbicide Resistance Action Committee (HRAC) industry group.[47] The applicant indicated that "*in Roundup Ready soybean, the most common practices for proactively managing resistance are to use residual herbicides at or near to planting followed by glyphosate. In this way, different modes of action are applied, reducing the probability of development of resistance. Applications of different modes of actions are also possible when planting Roundup Ready soybean following or preceding other crops where non-glyphosate herbicides are used for in-crop weed management. In addition, some growers may choose to also use mechanical cultivation of the soil as a weed management tool to complement the use of glyphosate*".

The widespread adoption of GMHT soybean in the USA has reduced the use of residual herbicides before or at planting, the use of tank mixtures with other herbicides, and the use of alternative herbicides in rotation with glyphosate (Johnson and Gibson, 2006; Young, 2006; Johnson et al., 2009). However, recently the use of residual herbicides along with glyphosate has increased in GMHT soybean and maize (Owen et al., 2011; Wilson et al., 2011). The EFSA GMO Panel considers it essential that diversified weed management systems are maintained, and agrees with the applicant that integrated weed management programmes that aim at improved diversity in crop management and weed control practices can enable the mitigation of weed shifts and can delay weed resistance evolution (reviewed by Beckie, 2006, 2011; Wilson et al., 2011; Shaner et al., 2012; Norsworthy et al., 2012; Vencill et al., 2012). Such measures can be put in place under Regulation (EC) No 1107/2009 or Directive 2009/128/EC to ensure compliance with regulatory requirements, operating in Member States, for the use of plant protection products. Such measures can ensure the appropriate management of glyphosate on GMHT soybean, so that the evolution of resistant weeds is delayed. Scientific evidence showed that the selection pressure on weeds can be reduced by crop rotation (e.g., rotating glyphosate tolerant crops with non-glyphosate tolerant crops), using variable application rates and timing, applying a variety of herbicidal active substances with different modes of action, and by using

[47] Additional information received on 22/11/2010 / Question 2 / Pages 13-24 / Appendix 1

non-herbicide weed control tools such as post-emergence cultivation and cover crops (Gressel and Segel, 1990; Liebman and Dyck, 1993; Gardner et al., 1998; Doucet et al., 1999; Cardina et al., 2002; Neve et al., 2003a,b; Nazarko et al., 2005; Beckie et al., 2006; Culpepper, 2006; Sammons et al., 2007; Gustafson, 2008; Owen, 2008; Werth et al., 2008, 2010; Beckie and Reboud, 2009; Busi and Powles, 2009; Gressel, 2009; Gulden et al., 2009; Shaw et al., 2009; Meissle et al., 2010; NRC, 2010; Beckie, 2011; Owen et al., 2011; Wilson et al., 2011). Using combinations of different weed management practices in integrated and diverse systems will reduce the selection pressure of any single practice or product (Sammons et al., 2007; Green and Owen, 2011; Shaner et al., 2012).

In contrast to monocultures, crop rotation favours a more diverse composition of weed communities, instead of communities that are dominated by one or few weed species. Crop rotation allows alternative weed control strategies to be used and enables alteration of patterns and timings of soil disturbance, light transmission through the crop canopy and natural enemies living in the crop, thereby diversifying the selection pressure on weed populations and making it more difficult for one weed species to dominate a weed community. Wilson et al. (2011) reported that crop rotation reduced weed population density in GMHT crops. The authors indicated that, compared with continuous GMHT soybean, rotating GMHT soybean with another GMHT crop, or rotating GMHT soybean with a non-GMHT crop, reduced weed seed population density in the seedbank, emerged weed population before crop planting, and reduced emerged weed population density at harvest. Similarly, rotating GMHT maize with another crop resulted in a reduction in weed population density after crop emergence and at harvest. Sosnoskie et al. (2009) observed that the weed seedbank diversity in a maize-oat-hay rotational system was higher than the weed communities associated with the continuous maize and maize-soybean rotational systems. Further, species analyses indicated that the number of significant indicator species for the seedbank and weed communities was generally greater in the three-year crop rotation as compared with the continuous maize and maize-soybean rotations. It remains, however, difficult to isolate the effects of crop rotation on weed communities from the weed control strategies that are used for the production of the crop (Sosnoskie et al., 2009). In some studies, the cropping sequence has been reported to be the dominant factor affecting the soil seedbank (Cardina et al., 2002), whereas, in others, crop rotation did not affect weed densities (e.g., Ball, 1992; Doucet et al., 1999). Moreover, crop rotation and weed control strategies often interact (Cardina et al., 2002). As such, the cropping system (which includes both the crops grown in rotation and the associated cultural practices) is the best reference term to frame risk mitigation measures for the use of GMHT crops.

The efficacy of herbicides to protect crop production from weed competition can be optimised within a cropping system (Wilson et al., 2011) by a range of practices including scouting for weeds, integrating knowledge of weed biology and ecology, and using appropriate application technologies (Nazarko et al., 2005; Parker et al., 2006). There is also the possibility to redesign cropping systems in a manner that reduces the size and interference capacity of weeds (Nazarko et al., 2005). The EFSA GMO Panel acknowledges that the transition to integrated weed management will depend upon a wide range of technical, economic and socio-economic factors (Meissle et al., 2010). A clear advantage of focussing on increased cropping system and management diversification is that it would increase or conserve farmland biodiversity, and reduce the risk of weed shifts and the evolution of glyphosate resistant weed biotypes.

The EFSA GMO Panel pinpoints the importance of providing farmers with sufficient information so that they understand the reasons for adopting integrated weed management programmes and the need to utilise best management practices, especially in those situations where weed resistance is most likely to evolve (Table MF). It is also advisable that weed resistance management is considered for the implementation of integrated pest management principles, as foreseen under Directive 2009/128/EC. Product stewardship programmes, technical guides and label recommendations as proposed by the applicant can help educate farmers to effectively manage the evolution of glyphosate resistant weeds and to develop sustainable long-term management strategies (Sammons et al., 2007; Owen et al., 2011; Shaner et al., 2012).

In its evaluation, the DE CA recommended that "*herbicide and cultivation management of soybean 40-3-2 should be adapted to minimize potential negative effects*". The DE CA noted that "*the herbicide registration is currently performed on Member State level and thus allows to set herbicide application conditions (such as used amount, intensity of weed control or management to prevent resistance developing against glyphosate-containing herbicides) to be adapted to the regional requirements of the European Union*" (section 6.5 of the environmental risk assessment report of the DE CA).

Impact on soil microbial communities

Adverse effects of glyphosate may occur to soil microorganisms harboring sensitive forms of the target enzyme EPSPS, including bacterial symbionts of soybean (such as *Bradyrhizobium*). Scientific uncertainty remains as whether this could lead to environmental impacts such as reduction of nitrogen fixing or crop yields under European conditions. Due to the lack of bacterial symbionts for soybean in European agricultural soils, inoculation with selected *Bradyrizhobium* strains is used to take advantage of symbiotic nitrogen fixation for soybean cultivation. The success of bacterial inoculation depends greatly on particular environmental conditions (climate, geographical region, soil properties) and is hard to predict. Farmers already thoroughly monitor soybean and, whenever necessary, apply nitrogen fertilisers.

The EFSA GMO Panel considers that current management practices are sufficient to cope with potential adverse effects on symbiotic nitrogen fixation arising from the use of glyphosate on soybean 40-3-2, but advises that risk managers inform farmers of the possibility of the occurrence of such effects.

In its evaluation, the DE CA noted that "*there is potentially also an indirect interaction between the use of glyphosate-containing herbicides and nitrogen-fixing symbiotic partners of the soybean (e.g. Bradyrhizobium japonicum, Moorman et al., 1992, King et al., 2001), which could lead to a reduction in harvest yield (King et al., 2001). To compensate, potential increased application of nitrogen fertilizer might be necessary with the cultivation of HT soybeans. The German Competent Authority recommends that herbicide and cultivation management of soybean 40-3-2 should be adapted to minimize potential negative effects*" (section 6.5 of the environmental risk assessment report of the DE CA).

6.3.1.4. Conclusion on risk mitigation measures

The EFSA GMO Panel considered several risk mitigation measures that can be put in place to reduce the risks that the cultivation of soybean 40-3-2 may pose to the environment (section 6.3.1.3 and Table MF). In practice, it is the responsibility of risk managers to decide upon risk mitigation measures that are consistent with the environmental protection goals and biodiversity action plans pertaining to their regions, and that are proportionate to the levels of risk and scientific uncertainty identified in the environmental risk assessment.

Farming and crop cultivation practices (e.g., crop rotation, mechanical weed control, herbicide regimes, etc.) including the use of glyphosate in GMHT crops may increase or decrease weed community diversity. The EFSA GMO Panel anticipated that the use of glyphosate as a post emergence herbicide alone or in combination with other herbicides on soybean 40-3-2 grown either in rotation with other glyphosate tolerant crops, or continuously may lead to greater risk to reduce weed community diversity than the current practices applied in soybean cropping systems. This may therefore result in reductions in weed community diversity and/or weed density to a level that might adversely affect food chains and webs, but not necessarily biological control functions, at the field and landscape level. Such reductions in weed community diversity and consequential reductions in farmland biodiversity may be considered problematic by risk managers depending upon protection goals pertaining to their region, especially in receiving environments that sustain little farmland biodiversity or in environmentally sensitive areas. Under such situations, the EFSA GMO Panel recommends that risk mitigation measures are put in place to manage potential herbicide effects, in order to ensure that glyphosate is used on soybean 40-3-2 in ways that result in similar or reduced adverse effects on farmland biodiversity compared with conventional soybean cultivation. Possible risk mitigation measures include tillage diversification, crop rotations, less intense in-crop weed management, protecting adjacent habitats from herbicide drift, (re)introduction and better management of field margins and other 'out of crop' measures.Soybean 40-3-2 grown in rotation with other glyphosate tolerant crops or continuously, in conjunction with the repeated and/or exclusive application of glyphosate-based herbicides, will cause changes in the weed flora, and will favour the evolution and spread of glyphosate resistant weeds due to the selection pressure exerted by glyphosate. The selection pressure on weeds can be reduced by crop rotations (i.e., rotating glyphosate tolerant crops with non-glyphosate tolerant crops), using variable application rates and timing, applying a variety of herbicidal active substances with different modes of action, and by using non-herbicide weed control tools such as cultivation and cover crops. To be most effective, these methods should be used in combination. A clear advantage of increasing cropping system diversification is that it would increase or conserve farmland biodiversity, as well as reducing the risk of weed shifts and the evolution of glyphosate resistant weed biotypes.

The EFSA GMO Panel considers that current management practices are sufficient to cope with potential adverse effects on symbiotic nitrogen fixation arising from the use of glyphosate on soybean 40-3-2, but advises that risk managers inform farmers of the possibility of the occurrence of such effects.

In its evaluation, the DE CA recommended that "*herbicide and cultivation management of soybean 40-3-2 should be adapted to minimize potential negative effects*" (section 6.5 of the environmental risk assessment report of the DE CA).

6.3.2. *Post-market environmental monitoring*[48]

6.3.2.1. General aspects of post-market environmental monitoring

Directive 2001/18/EC introduces an obligation for applicants to implement monitoring plans, in order to trace and identify any direct or indirect, immediate, delayed or unanticipated effects on human health or the environment of GMOs as or in products after they have been placed on the market. Monitoring plans should be designed according to Annex VII of the Directive. According to Annex VII, the objectives of an environmental monitoring plan are: (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO or its use in the environmental risk assessment are correct; and (2) to identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the environmental risk assessment.

Post-market environmental monitoring is composed of case-specific monitoring and general surveillance (EFSA, 2006b, 2011c). Case-specific monitoring is not obligatory, but may be required to verify assumptions and conclusions of the environmental risk assessment, and to inform the environmental risk assessment further when significant levels of critical scientific uncertainty (EFSA 2011c) linked to the GM plant and its management have been identified. By contrast, general surveillance is mandatory, in order to take account of general or unspecified scientific uncertainty and any unanticipated adverse effects associated with the release and management of a GM plant. Due to different objectives between case-specific monitoring and general surveillance, their underlying concepts differ (Sanvido et al., 2005). Case-specific monitoring should enable the determination of whether and to what extent adverse effects anticipated in the environmental risk assessment occur during the commercial use of a GM plant, and thus to relate observed changes to specific risks. It is triggered by scientific uncertainty that was identified in the environmental risk assessment. As a consequence, a hypothesis can be established that can be tested on the basis of newly-collected monitoring data.

48 Technical dossier / Section D11 / Appendices 29 and 30 // Additional information received on 13/07/2009 / Appendix 9

The objective of general surveillance is to identify unanticipated adverse effects of the GM plant or its use on human health and the environment that were not predicted or specifically identified during the environmental risk assessment. In contrast to case-specific monitoring, the general status of the environment that is associated with the use of the GM plant is monitored without any preconceived hypothesis, in order to detect any possible effects that were not anticipated in the environmental risk assessment, or that are long-term and cumulative. Should any such effects be observed, they are studied in more detail to determine whether the effect is adverse and whether it is associated with the use of the GM plant (Sanvido et al., 2005, 2009, 2011a,b; EFSA, 2006b, 2011c).

6.3.2.2. Interplay between environmental risk assessment, mitigation and post-market environmental monitoring

Based on the evidence provided by the applicant and relevant scientific literature on soybean 40-3-2, the EFSA GMO Panel concluded that there are no indications of the occurrence of adverse effects on predators, herbivores and decomposers due to potential unintended changes in soybean 40-3-2. As no event-specific data on plant-pollinator interactions were provided by the applicant, the EFSA GMO Panel considers that scientific uncertainty pertaining to the occurrence of adverse effects on pollinators due to potential unintended changes in soybean 40-3-2 remains. The EFSA GMO Panel concluded that the cultivation of soybean 40-3-2 may result in adverse environmental effects due to the use of the complementary glyphosate-based herbicides. These potential adverse environmental effects could, under certain conditions, comprise: (1) a reduction in farmland biodiversity; (2) changes in weed community diversity due to weed shifts; (3) the selection of glyphosate resistant weeds; and (4) changes in soil microbial communities. The magnitude of these potential adverse environmental effects depends upon a range of environmental and management factors and the EFSA GMO Panel proposed several risk mitigation measures to reduce environmental impacts to those of comparable conventional soybean cultivation, or to meet the protection goals of different farming regions. It is anticipated that the practicality and implementation of these measures will vary according to local conditions, so there is scientific uncertainty as to whether they will achieve the desired goals.

6.3.2.3. Case-specific monitoring[49]

When potential adverse effects or important gaps in scientific information or significant levels of critical uncertainty linked to the GM plant and its management have been identified in the environmental risk assessment, then case-specific monitoring should be carried out after placing on the market, in order to confirm assumptions made in the environmental risk assessment and to further inform the environmental risk assessment. Case-specific monitoring should be targeted at assessment endpoints and environmental protection goals identified as being at risk during the environmental risk assessment, or where levels of critical uncertainty were identified in relation to potential risks associated with the GM plant (EFSA, 2011c).

The specific risks identified in section 6.2.8 (conclusion on the environmental risk assessment) are: (1) a reduction in farmland biodiversity; (2) changes in weed community diversity due to weed shifts; (3) the selection of glyphosate resistant weeds; and (4) changes in soil microbial communities. The remaining scientific uncertainty identified in section 6.2.8 relates to the occurrence of potential adverse effects n pollinators due to potential unintended changes in soybean 40-3-2 (see below). The EFSA GMO Panel notes that the risks (1-4) are related to the use of the complementary herbicide, and judges that monitoring could equally well be put in place either under the legislation for plant protection products (Regulation (EC) No 1107/2009, which replaced Directive 91/414/EEC on 14 June 2011, and Directive 2009/128/EC), or under the legislation for GMOs (Directive 2001/18/EC). In reaching this view, the EFSA GMO Panel considered: the interplay between the legislation for GMOs and plant protection products (section 6.2.7.1); the fact that some herbicide tolerant systems on the market are non-GM; and the fact that protection goals are set at Member State level. However, since the remit of the EFSA GMO Panel to propose monitoring is linked inextricably

[49] Technical dossier / Section D11.3

to Directive 2001/18/EC, subsequent recommendations in this section are based on GMO legislation; the terminology 'case-specific monitoring' is therefore used in that context.

In considering the form that case-specific monitoring should take, the EFSA GMO Panel reiterates the considerable challenges it identified previously (EFSA, 2009b, 2011e) to the drawing of meaningful conclusions on the environmental consequences of the use of herbicides from large-scale multi-site experiments, such as the FSEs, which seek to compare HT with conventional herbicide management (Squire et al., 2003, 2009). On the grounds of scientific practicability (e.g., Perry et al., 2003) and of cost (e.g., Qi et al., 2008), and the fact that glyphosate is already extensively used in a wide range of crops, such studies are considered disproportionate to the identified risks.

In order to assess the efficacy of risk mitigation measures put in place to reduce levels of risk and in order to reduce the remaining scientific uncertainty, the EFSA GMO Panel recommends case-specific monitoring to address: (1) changes in weed community diversity; and (2) resistance evolution to glyphosate in weeds due to changes in herbicide and cultivation regimes. No case-specific monitoring is required to assess changes in soil microbial communities, but the EFSA GMO Panel recommends that the applicant establishes stewardship systems encouraging farmers to report problems that may be due to potentially reduced symbiotic nitrogen fixation. The EFSA GMO Panel considers that general surveillance (including appropriately designed farmer questionnaires) offers an effective approach to detect and report early warning signs indicating that such effects occur.

Monitoring the occurrence of adverse effects on pollinators due to potential unintended changes in soybean 40-3-2

No event-specific data on plant-pollinator interactions were provided by the applicant. Therefore, the EFSA GMO Panel noted that scientific uncertainty pertaining to the occurrence of adverse effects on pollinators due to potential unintended changes in soybean 40-3-2 remains, as no event-specific data on plant-pollinator interactions were provided by the applicant.

The EFSA GMO supports the applicant's proposal to conduct post-market studies during which exposure of honeybees to soybean 40-3-2 and potential changes in interactions between soybean 40-3-2 and honeybees will be observed for a period of at least two years. The EFSA GMO Panel notes that such a study would only deliver useful results under the conditions specified explicitly above in section 6.2.8.

The EFSA GMO Panel considers that in the initial stages of commercialisation of soybean 40-3-2, when uptake is limited, the potential exposure of pollinators is likely to be low, and therefore the risk to pollinators is extremely low. Hence, the EFSA GMO Panel considers that it would be proportionate for the post-market studies proposed by the applicant to be conducted under case-specific monitoring in the first years of commercialisation.

The conclusion of the EFSA GMO Panel is not inconsistent with that of the DE CA. The DE CA recommended that "*the applicant shall carry out a field study to confirm the absence of unintended effects on non-target organisms in the EU with placing soybean 40-3-2 on the market. The design of such a study should be of a quality to allow appropriate scientific assessment as proposed in the application*" (section 8 of the environmental risk assessment report of the DE CA).

Monitoring changes in weed community diversity due to changes in herbicide and cultivation regimes

The EFSA GMO Panel recommends that the applicant proposes a detailed stewardship scheme to farmers for the use of glyphosate on soybean 40-3-2. This scheme should recommend detailed herbicide/cropping regimes that are environmentally sustainable and no more harmful to weed community diversity than the current conventional management practices within each receiving environment, according to local environmental protection goals and taking into account the use of glyphosate throughout the cropping system including usage on other crops in rotation with soybean 40-3-2 (both before and during the crop growing cycle). The applicant should provide explicit justification based on data from field trials/experiments and from on-farm demonstrations concerning the efficacy of these regimes compared with the baseline, for each receiving environment. At early stages of commercialisation, the justification for the applicant's proposed regimes may be supported by field trials and farmer demonstrations that usually accompany the introduction of new agrochemicals and new technology into agriculture. Local experimental or demonstration sites are already in place in several Member States to assess the impact of various crop protection programmes, including integrated pest management strategies (Fried et al., 2009; Cordeau et al., 2011), and could also be used to assess the impact of glyphosate-based regimes on the level of weed community diversity.

The EFSA GMO Panel recommends that monitoring be put in place to assess that the proposed herbicide/cropping regimes recommended by the applicant are implemented satisfactorily for soybean 40-3-2 and that they restrict adverse effects on biodiversity to levels no greater than those caused by conventional management. This may be achieved during the cultivation of soybean 40-3-2 through a combination of collection of additional information from the farmer questionnaires (section 6.3.2.4) on herbicide and crop management practices and on weed populations, and from a strictly limited number of more specific and focussed multi-annual scientific studies at sites where adequate baselines have already been established. In addition, case-specific monitoring is recommended to monitor the efficacy of any of those measures discussed generally in section 6.3.1 and specified in section 6.3.1.4 above adopted to mitigate harm to biodiversity.

The responsibility for the generation of strategies to manage adverse environmental effects and of a monitoring methodology for determining the efficacy of such regimes rests properly with the applicant. However, the EFSA GMO Panel recommends that: (1) whatever monitoring methodology is chosen, it is likely that it would benefit from a close collaboration between the applicant and the research community. Scientists with relevant expertise in this area (e.g., ecologists, weed scientists) should be consulted; (2) variation amongst local protection goals implies that Member State involvement in planning is essential; (3) adequate baselines be established prior to the introduction of the GMHT cropping systems, to enable changes to be detected; (4) impacts at a landscape scale should be considered; (5) conclusions should be drawn not only for a single season but also at the temporal scale of complete rotations; and (6) measurement endpoints should be selected to confirm the preservation of functional biodiversity sufficient to guarantee the quality of agro-ecosystems systems and ensure their sustainability (Storkey et al., 2008; EFSA, 2010d,e).

In its evaluation report, the DE CA considered that "*based on the safety assessment of soybean 40-3-2, no specific cause-effect relationship for adverse environmental impacts has been identified that would necessitate a case specific monitoring by the applicant*". The DE CA assumed that "*possible indirect effects of complementary herbicide application will be taken into account by the applicant in the context of a Stewardship Program harmonized with the pesticide assessment authorities. This should ensure that unexpected effects (in general surveillance) can be detected*" (sections 7 and 8 of the environmental risk assessment report of the DE CA).

Monitoring resistance evolution to glyphosate in weeds due to changes in herbicide and cultivation regimes

Since glyphosate is a widely used herbicide and managing resistance evolution is a condition of its registration as a pesticide (section 6.2.7.3), the EFSA GMO Panel advises that the use of glyphosate on GMHT crops, including soybean 40-3-2, is integrated in the monitoring conducted by the applicant in relation to all uses of glyphosate within Member States.

The EFSA GMO Panel recommends that applicants establish stewardship systems which encourage farmers to report weed control failures to them as required under Regulation (EC) No 1107/2009. Applicants will need to liaise with other providers of glyphosate-based herbicides and also with the producers of other glyphosate tolerant crops. Such observations may reveal the occurrence of localised resistance before it spreads, and may serve as a trigger for further investigations (Shaner, 2010).

In addition, risk managers should consider additional routine monitoring for weed resistance in areas where the risk of resistance evolution is highest, i.e., in areas of continuous or repeated use of glyphosate (not solely on soybean 40-3-2) as described in section 6.2.7.3.

The EFSA GMO Panel considers that farmer questionnaires provide an opportunity for farmers to report weed control failures or declines in the efficacy of glyphosate. In addition, farmers will indicate their herbicide regimes, and so it will be possible to determine whether they are implementing resistance management strategies and following stewardship guidelines (section 6.3.2.4).

Weed resistance should be reported to each Member State on an annual basis, and these national reports can then be submitted to organisations such as the European Weed Research Society (EWRS), that has Working Groups monitoring weed resistance and developing integrated weed management strategies aimed also to delay or manage weed resistance to herbicides.

In its evaluation report, the DE CA considered that "*based on the safety assessment of soybean 40-3-2, no specific cause-effect relationship for adverse environmental impacts has been identified that would necessitate a case specific monitoring by the applicant*". The DE CA assumed that "*possible indirect effects of complementary herbicide application will be taken into account by the applicant in the context of a Stewardship Program harmonized with the pesticide assessment authorities. This should ensure that unexpected effects (in general surveillance) can be detected*" (sections 7 and 8 of the environmental risk assessment report of the DE CA).

6.3.2.4. General surveillance[50]

According to Directive 2001/18/EC, the objective of general surveillance is to detect any unanticipated adverse effects on protected and valued entities of the environment that may be due to the cultivation of GM plants, including biodiversity and ecosystem services (EFSA, 2011c).

The applicant proposed to conduct general surveillance for soybean 40-3-2 throughout the period of validity of the authorisation. The general surveillance will take into consideration and be proportionate to the extent of cultivation of soybean 40-3-2 in the EU Member States. The applicant proposed to build its general surveillance on four approaches: (1) the use of annual farmer questionnaires; (2) the review of scientific information provided by existing monitoring networks; (3) the monitoring and review of ongoing research and development, as well as scientific literature; and (4) the implementation of industry stewardship programmes, in order to identify potential adverse effects associated with the intended uses of soybean 40-3-2.

Farmer questionnaires[51]

The EFSA GMO Panel agrees with the general surveillance approach of the applicant to establish farmer questionnaires as a reporting format that provides relevant information. The questionnaires to farmers exposed to or using GM plants are regarded by the EFSA GMO Panel as an adequate tool for addressing several aspects of general surveillance (EFSA, 2006b, 2011c). The EFSA GMO Panel is of the opinion that farmer questionnaires enable the reporting of any on-farm observations of effects associated with the cultivation of soybean 40-3-2, as this approach uses first-hand observations and rely on farmers' knowledge and experience of their local agricultural environments, comparative crop performance and other factors that may influence events on their land (Schmidt et al., 2008; Wilhelm et al., 2010). Some of the questions link directly to assessment endpoints or give indirect indications of effects on assessment endpoints (EFSA, 2011c).

Farmer questionnaires should be designed to determine whether the farmer/manager/worker has noticed any differences between the GM plant and its management and that of similar non-GM plants growing on the farm, nearby or previously (EFSA, 2011c). The applicant and risk managers are advised to consider the new EFSA GMO Panel guidelines on post-market environmental monitoring

[50] Technical dossier / Section D11.4 / Appendices 29 and 30
[51] Additional information received on 27/10/2009 / Question 4 / Pages 10-12 / Appendix 2

(EFSA, 2011c) and the specific recommendations on the annual post-market environmental monitoring report of maize MON 810 cultivation in 2009 (EFSA, 2011d) when finalising or evaluating their monitoring plans.

While the EFSA GMO Panel considers the format and contents of the farmer questionnaire, as provided by the applicant, comprehensive, it proposes the following modifications:

- to add questions on the possible occurrence and observation of (GM) volunteer soybean in subsequent crops and feral soybean plants (if any) in field margins for the consideration of unanticipated effects on the persistence and invasiveness potential of soybean 40-3-2;
- to add questions on the occurrence and observation of new crop volunteers in soybean 40-3-2;
- in addition to the relative impact on main weeds, the farmer questionnaire should specifically request information on the active substances (or commercial product names), dosage and timing of herbicide applications, as well as on the use of non-chemical direct weed control methods used throughout the season including pre-sowing and post-harvest. This will deliver relevant information that allows checking adoption of national/regional measures to mitigate the potential reduction of weed community diversity due to use of glyphosate on soybean 40-3-2. If herbicides containing glyphosate are used in that field at any point in the crop rotation, either in-crop or inter-crop, this should also be recorded (Castellazzi et al., 2007, 2008);
- to add questions on the adoption of weed resistance management strategies by farmers as requested by the applicant in stewardship recommendations;
- to consider potential (un)expected effects on beneficial ecological functions provided by soil microbial communities (such as symbiotic nitrogen fixation) due to the specific use of glyphosate-based herbicides during the growing season of soybean 40-3-2 or other glyphosate tolerant plants, as well during the growing season of subsequent crops in the rotation (see Smit et al., 2012 for indirect indicators in terms of yield, fertiliser use, quality, etc.).

In line with the general recommendations on the farmer questionnaire set in its 2011 Scientific Opinion on post-market environmental monitoring (EFSA, 2011c), the EFSA GMO Panel advises farmer questionnaires:

- are designed to ensure the appropriate statistical validity and representativeness of the collected data, including the proportion of fields growing soybean 40-3-2 in a region and a minimum percentage or number of questionnaires required to achieve statistical power in the data collected;
- are designed to generate data on the agronomic management of soybean 40-3-2, as well as data on impacts on farming systems and the farm environment;
- use a field or group of fields growing soybean 40-3-2 as the basic unit for monitoring in representative farming regions and for representative cropping systems within the country. The precise fields should be identified, so that their locations can be subsequently retrieved from registers of GM plant sites;
- clearly identify the comparator (e.g., variety, location) and whether it is being grown adjacent to soybean 40-3-2, on the same farm or in another location. If no comparators are being grown spatially or temporally close to soybean 40-3-2, then the rationale for selecting another comparator (e.g., historical data) should be fully described;
- where appropriate, observe the field/fields in subsequent years for any unusual residual effects;
- provide information on other GM plant events being grown at the same sites and farms;
- are user friendly but also information rich;

- are constructed to encourage independent and objective responses from farmers, land managers and others involved in the deployment of soybean 40-3-2 or its derived products;
- are audited to ensure the independence and integrity of all monitoring data.

In addition to the general recommendations on the farmer questionnaire (EFSA, 2011c) and in line with its 2011 Scientific Opinion on the annual post-market environmental monitoring report on maize MON 810 cultivation in 2009 (EFSA, 2011d), the EFSA GMO Panel advises the applicant to take into account the following points:

- the sampling frame should be comprehensive and a stratification should be applied consistently in each country. Adequate sampling should be carried out from the previous stratification exercise;
- the cultivation areas, with high uptake of soybean 40-3-2 and where soybean 40-3-2 has been continuously grown in previous years, should be over-represented in the sampling scheme;
- the number of farmers not participating in the survey and the reasons thereof should be documented;
- impartial and standardised interviews should be carried out by independent parties and effective quality and auditing procedures should be considered;
- additional questions to the farmer questionnaire should be considered to better describe the cultivation of soybean in the local area and/or the previous years, the receiving environments and the management systems in which soybean 40-3-2 is being grown;
- relevant data as from other sources of information (e.g., official statistics on crop management practices) should/could be considered for validity check of the questionnaires (e.g., consistency, representativeness);
- the raw data, programmes, logs and output files related to the statistical analysis of the farmer questionnaires should be provided. Confidence intervals for the analysis of the monitoring characteristics should be included in the statistical report;
- appropriate statistical procedures should be used based on using a distribution for appropriate outcomes;
- the use of a standard default effect size of 5 % is not relevant for all assessment endpoints and, where scientifically justified, different default effect sizes should be considered for some assessment endpoints;
- data should be pooled and statistically analysed over years. At the end of the ten years of general surveillance, the applicant should conduct a statistical analysis with all pooled data;
- a codification for farmers repeatedly surveyed over years should be set up. These farmers should be particularly monitored;
- the number of years the surveyed farmer has grown soybean 40-3-2 and other GM plants should be indicated.

The DE CA considered that "*the questionnaires should be improved, at least regarding the following points:*

- *reconsideration whether the alternative response "don't know" or similar ones should be added to the answering options to prevent false answers;*

- *questions should be added on the occurrence/observation of (GM) feral plants and/or (GM) volunteers in previous and current seasons (for the consideration of persistence or selection);*

- *questions should allow to reflect the change of the amount of pesticides used (for the consideration of effects on pests etc., non-targets, sustainability by changed practice);*

- *questions should allow to reflect the change of the amount of fertiliser used (for the consideration of effects on soil quality, sustainability by changed practice);*

- *independent from the occurrence/abundance of wildlife an open question/answer should address "unexpected observations" (... "if – please specify") (for the consideration of effects on non-target organisms)*" (section 7 of the environmental risk assessment report of the DE CA).

Existing monitoring networks

Since farmer questionnaires focus mainly on the cultivation area of the GM plant and its surroundings, the EFSA GMO Panel supports the consideration of additional information sources for general surveillance (EFSA, 2006b, 2011c). In this respect, Directive 2001/18/EC proposed to make use of established routine surveillance networks, in order to obtain data on environmental impacts in the landscape where GMOs are cultivated from a range of existing monitoring networks which observe changes in biota and production practices from farm up to regional level. EU Member States have various networks in place – some of which have a long history of data collection – that may be helpful in the context of general surveillance of GM plant cultivations. Existing monitoring networks involved in routine surveillance offer recognised expertise in a specific domain and have the tools to capture information on important environmental aspects over a large geographical area. However, the EFSA GMO Panel recognises that existing monitoring networks fully meeting all the needs of the monitoring of GM plant cultivations can be limited (Bühler, 2006; Mönkemeyer et al., 2006; Schmidtke and Schmidt, 2007; Graef et al., 2008; Smit et al., 2012). The development of harmonised criteria for the systematic identification, specification and analysis of existing surveillance networks across the EU is therefore considered important (EFSA, 2011c).

The EFSA GMO Panel agrees with the proposal of the applicant to describe the generic approaches for using existing monitoring networks. The applicant has also given consideration to the use of any future surveys of conservation goals as defined in the Directive 2004/35/EC on environmental liability (EC, 2004) in farming regions where soybean 40-3-2 will be cultivated and intends to investigate their suitability for providing data on potential changes in biota.

Knowing the limitations of existing monitoring networks, it is important to describe the processes and criteria that will be used for selecting and evaluating existing monitoring networks for supplying data related to the unanticipated adverse effects of GM plants in general surveillance. Therefore, the applicant, in consultation with Member States, should:

- consider the protection goals, the assessment endpoints and their indicators that could be monitored through existing monitoring programmes;

- identify the type of existing monitoring networks that would be appropriate to survey the protection goals considered to be at risk in the countries where soybean 40-3-2 will be grown;

- describe the generic approach and develop more detailed criteria to evaluate existing monitoring networks and how appropriate networks will be selected (considering the hereunder list of points);

- identify what changes need to be made to these monitoring networks and describe how these might be implemented, and identify gaps in information that could be filled by additional surveys;

- encourage these networks to adopt the proposed modifications and describe how data from these networks will be integrated and assessed.

In addition, when selecting existing monitoring networks to be part of general surveillance, the applicant is recommended to consider the following points for assessing the suitability of these existing networks to supply relevant general surveillance data:

- the relevance of protection goals and their indicators monitored through existing monitoring networks;

- the type (e.g., raw data) and quality of the data recorded;

- the statistical power and the effect sizes detected by monitoring networks, where appropriate;

- the ease of access to the data collected by existing monitoring networks (e.g., availability of data via Internet, free access to data or access subject to a fee, protected data of ongoing research projects);

- the track record and past performance of existing monitoring networks;

- the methodology used by existing monitoring networks (e.g., sampling and statistical approach) including: (1) the spatial scale of data collection (e.g., local, regional, national, zonal): existing monitoring networks focusing on agricultural areas cultivated with GM plants or with conventional plants like maize and potato (for which GM are also available and grown) should be preferred; (2) temporal scale of data collection: appropriate frequency of data collection and reporting (e.g., short-term vs. long-term data sets, regularity of data collection); and (3) other parameters such as the language of the reports and impartiality.

Furthermore, the EFSA GMO Panel recommends that the applicant describes arrangements with any third parties participating in its general surveillance plan. It is recommended to consider how arrangements for collecting, collating and analysing data will be made, and to describe how formal agreements, procedures and communication will be established with the European Commission and Member States or other third parties, although detailed arrangements may not have been agreed at the time of the application.

The EFSA GMO Panel also recommends to include in the sources of information that support general surveillance of soybean 40-3-2, existing monitoring networks that monitor herbicide usage, weed community diversity on farms and weed resistance evolution, so that the scientific requirements for the detection of any unforeseen environmental effects due to altered farm management practices associated with soybean 40-3-2 cultivation are met.

The DE CA considered that "*the monitoring plan still needs some improvement:*

- *the role and interplay of all intended actors on behalf of recording, analysis, evaluation and reporting of monitoring data should be specified and clarified transparently;*

- *the current monitoring plan describes the distribution and analysis of farmer questionnaires by the applicant. The obligation for further data collection and analysis is assigned to third parties and the Competent Authorities, although an agreement on the procedure is seemingly not achieved with these institutions nor is it clear which kind of data will be collected to allow further assessment. Stating to evaluate some annual reports from third parties provides no insight what is actually intended. The applicant should clarify this aspect*" (section 7 of the environmental risk assessment report of the DE CA).

Monitoring and review of ongoing research and development, as well as scientific literature

An additional approach to support general surveillance is to review all new scientific, technical and other information pertaining to soybean 40-3-2, including information on GM plants with similar traits or characteristics, which has emerged during the reporting period. This will include reviewing of results from ongoing research and development studies (e.g., variety registration trials) and all publications including peer-reviewed journal articles, conference proceedings, review papers and any additional studies or other sources of information relevant to the cultivation of the plant/trait combination for which the report is being drafted (EFSA, 2011c).

The EFSA GMO Panel recommends the applicant:

- to cover all relevant peer-reviewed publications, including peer-reviewed journal articles, conference proceedings, review papers and any additional studies or other sources of information relevant to the cultivation of the plant/trait combination for which the report is being drafted;

- to describe the criteria for selecting and evaluating the scientific reliability of publications;

- to adhere to systematic literature review methodology to select relevant papers (EFSA, 2010b).

Industry stewardship programmes

The EFSA GMO Panel welcomes the applicant's proposal to develop stewardship programmes for the introduction, marketing, management and stewardship of soybean 40-3-2, but advises that these programmes should be made available well in advance of the time of commercialisation so as to allow risk managers to validate the implementation of proportional risk management measures and detailed monitoring plans.

6.3.2.5. Reporting results of post-market environmental monitoring[52]

The applicant will submit a report on an annual basis covering case-specific monitoring and general surveillance. In case of adverse effects altering the conclusions of the environmental risk assessment, the applicant will immediately inform the European Commission and Member States. The EFSA GMO Panel agrees with the proposal made by the applicant on reporting intervals. The EFSA GMO Panel recommends that effective reporting procedures are established with the Competent Authorities of Member States and the European Commission as required under the Council Decision 2002/811/EC on monitoring.

The results of post-market environmental monitoring should be presented in accordance with the standard reporting formats established by the 2009/770/EC Commission Decision on standard reporting formats. In addition, the applicant is recommended to provide raw data, in order to allow different analyses and interrogation of the data and to allow scientific exchange and co-operation between Member States, the European Commission and EFSA. The EFSA GMO Panel recommends that the applicant describes whether the post-market environmental monitoring reports contain cumulative analyses of data with previous years' results.

6.3.2.6. Conclusion on post-market environmental monitoring

The EFSA GMO Panel gives its opinion and makes recommendations on the scientific quality of the post-market environmental monitoring plan proposed by the applicant. In order to assess the efficacy of risk mitigation measures put in place to reduce levels of risk, and in order to reduce the remaining scientific uncertainty identified in the environmental risk assessment, the EFSA GMO Panel recommends case-specific monitoring to address: (1) changes in weed community diversity; and (2) resistance evolution to glyphosate in weeds due to changes in herbicide and cultivation regimes. In addition, the EFSA GMO Panel considers that it would be proportionate to the risk for the post-market

52 Technical dossier / Section D11.5

studies on the occurrence of adverse effects on pollinators due to potential unintended changes in soybean 40-3-2, proposed by the applicant, to be conducted as case-specific monitoring, as long as the five conditions explicitly stated in this Scientific Opinion are metNo case-specific monitoring is required to assess changes in soil microbial communities, but the EFSA GMO Panel recommends that the applicant establishes stewardship systems encouraging farmers to report problems that may be due to potentially reduced symbiotic nitrogen fixation. General surveillance (including appropriately designed farmer questionnaires) offers an effective approach to detect and report early warning signs indicating that such effects occur. . The EFSA GMO Panel considers that risk managers should adapt monitoring methodologies to their local receiving environments and management systems.

The EFSA GMO Panel agrees with the general surveillance plan for the cultivation of soybean 40-3-2 proposed by the applicant: (1) to establish farmer questionnaires as a reporting format of any on-farm observations of effects associated with the cultivation of soybean 40-3-2; (2) to use existing monitoring networks that observe changes in biota and production practices from farm up to regional level to obtain data on environmental impacts in the landscape where soybean 40-3-2 is cultivated; (3) to review all new scientific, technical and other information pertaining to soybean 40-3-2; and (4) to develop stewardship programmes for the introduction, marketing, management and stewardship of soybean 40-3-2. However, the EFSA GMO Panel requests that its proposals and those made by the DE CA to strengthen general surveillance are implemented. The EFSA GMO Panel agrees with the reporting intervals and modalities proposed by the applicant. The general surveillance plan for the import and processing of soybean 40-3-2 has been previously evaluated by the EFSA GMO Panel.

The conclusions of the EFSA GMO Panel regarding post-market environmental monitoring are consistent with those of the DE CA. The DE CA considered that "*based on the safety assessment of soybean 40-3-2, no specific cause-effect relationship for adverse environmental impacts has been identified that would necessitate a case specific monitoring by the applicant*". However, with regard to the occurrence of adverse effects on non-target organisms due to potential unintended changes in soybean 40-3-2, the DE CA recommended that "*the applicant shall carry out a field study to confirm the absence of unintended adverse effects on non-target organisms in the EU with placing soybean 40-3-2 on the market. The design of such a study should be of a quality to allow appropriate scientific assessment as proposed in the application*".

Further, the DE CA was of the opinion that "*the monitoring plan needs some clarifications (reporting monitoring annually, and delivery of more comprehensive overviews after six and nine years); and improvement of the questionnaires*". The DE CA recommended that "*monitoring of the herbicide use is conducted as part of the stewardship for the herbicides by the companies involved, and under the auspices of the pesticide regulatory systems operating in Member States, in order to record compliance with the approved uses of the herbicides on GMHT, levels of weed control, and development of resistant weeds. The German Competent Authority assumes that possible indirect effects of complementary herbicide application will be taken into account by the applicant in the context of a Stewardship Program harmonized with the pesticide assessment authorities. This should ensure that unexpected effects (in general surveillance) can be detected*" (see section 8 of the environmental risk assessment report of the DE CA).

Overall Conclusions and Recommendations

Following the submission of an application (Reference EFSA-GMO-NL-2005-24) under Regulation (EC) No 1829/2003 from Monsanto, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a Scientific Opinion on the safety of the herbicide tolerant genetically modified (GM) soybean (also known as soya bean) 40-3-2 (Unique Identifier MON-Ø4Ø32-6) for cultivation. Although the scope of this application covers only cultivation of soybean 40-3-2, this Scientific Opinion also updates the previous EFSA GMO Panel safety evaluation on the continued marketing of: (1) food containing, consisting of, or produced from soybean 40-3-2; (2) feed containing, consisting of, or produced from soybean 40-3-2; and of (3) other products containing or consisting of soybean 40-3-2.

In delivering its Scientific Opinion, the EFSA GMO Panel considered: the application EFSA-GMO-NL-2005-24; additional information supplied by the applicant; scientific comments submitted by Member States; the environmental risk assessment report of the German Competent Authority (DE CA); and relevant scientific publications.

Soybean 40-3-2 expresses the enzyme CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS), which is derived from the CP4 strain of *Agrobacterium tumefaciens* (updated scientific name: *Rhizobium radiobacter*), and renders soybean 40-3-2 tolerant to the herbicidal active substance glyphosate.

The EFSA GMO Panel evaluated soybean 40-3-2 with reference to its intended uses and the appropriate principles described in its guidelines for the following: the risk assessment of GM plants and derived food and feed; the environmental risk assessment of GM plants; the selection of comparators for the risk assessment of GM plants; and the post-market environmental monitoring of GM plants. The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and expression of the target protein. An evaluation of the comparative analyses of composition and agronomic and phenotypic characteristics was undertaken, and the safety of the new protein and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional quality. An evaluation of environmental impacts and the post-market environmental monitoring plan was undertaken.

The molecular characterisation data established that soybean 40-3-2 contains one functional insert expressing CP4 EPSPS and a non-functional insert consisting of a 72 bp fragment of the CP4 *epsps* coding sequence. No other parts of the plasmid used for transformation are present in the transformed plant. Bioinformatic analyses of the open reading frames spanning the junction site within the functional insert or between the inserts and genomic DNA did not indicate specific hazards. The stability of the inserted DNA and the herbicide tolerance trait were confirmed over several generations. Analyses of the levels of CP4 EPSPS in leaves and seed collected from field trials performed in Europe were considered sufficient.

The EFSA GMO Panel compared the composition and agronomic and phenotypic characteristics of soybean 40-3-2 and its conventional counterpart, assessed all statistical differences identified, and came to the conclusion that soybean 40-3-2 is compositionally equivalent to commercial non-GM soybean varieties, except for the newly expressed protein. The risk assessment included an analysis of data from analytical studies, bioinformatic analyses, and *in vitro* and *in vivo* studies. The EFSA GMO Panel concludes that the soybean 40-3-2 is as safe as its conventional counterpart and commercial non-GM soybean varieties and that the overall allergenicity of the whole plant is not changed.

As the scope of the current application covers cultivation, the environmental risk assessment considered the environmental impact of full-scale commercialisation of soybean 40-3-2.

The DE CA provided EFSA with its report on the environmental risk assessment of soybean 40-3-2 (dated 9 September 2008) on 3 October 2008 in line with Articles 6.3(c) and 18.3(c) of Regulation (EC) No 1829/2003. The report on the environmental risk assessment of the DE CA is provided in Annex H of the EFSA Overall Opinion, and has been considered throughout this EFSA GMO Panel Scientific Opinion.

The EFSA GMO Panel considers that soybean 40-3-2 has no altered agronomic and phenotypic characteristics, except for the herbicide tolerance. The likelihood of unintended environmental effects due to the establishment, survival and spread of soybean 40-3-2 is considered to be extremely low, and will be no different from that of conventional soybean varieties.

It is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the environment or human and animal digestive tracts. In the rare but theoretically possible case of transfer of the CP4 *epsps* gene from soybean 40-3-2 to soil bacteria, no novel property would be introduced into the soil bacterial community and thus no positive selective advantage that would not have been conferred by natural gene transfer between bacteria would be provided.

Based on the evidence provided by the applicant and relevant scientific literature on soybean 40-3-2, the EFSA GMO Panel concludes that there are no indications of the occurrence of adverse effects on predators, herbivores and decomposers due to potential unintended changes in soybean 40-3-2, and therefore considers *trait*-specific information appropriate to assess whether soybean 40-3-2 poses a risk to non-target organisms. However, the EFSA GMO Panel that scientific uncertainty pertaining to the occurrence of adverse effects on pollinators due to potential unintended changes in soybean 40-3-2 remains, as no event-specific data on plant-pollinator interactions were provided by the applicant. The EFSA GMO Panel considered that this scientific uncertainty should be resolved by experiments in relevant receiving environments in Europe that are designed to compare the effects of soybean 40-3-2 and its conventional counterpart (and optionally reference, commercial non-GM soybean varieties, if appropriate) on adult honeybees, as long as the five conditions explicitly stated in this Scientific Opinion are met.

The studies, supplied or reviewed by the applicant, showed no adverse effects on different types of non-target organisms due to the expression of the CP4 EPSPS protein in glyphosate tolerant crops.

The EFSA GMO Panel does not expect potential adverse effects on biogeochemical processes and the abiotic environment due to the expression of CP4 EPSPS protein in soybean 40-3-2.

The EFSA GMO Panel is of the opinion that potential adverse environmental effects of the cultivation of soybean 40-3-2 are associated with the use of the complementary glyphosate-based herbicide regimes. These potential adverse environmental effects could, under certain conditions, comprise: (1) a reduction in farmland biodiversity; (2) changes in weed community diversity due to weed shifts; (3) the selection of glyphosate resistant weeds; and (4) changes in soil microbial communities. The potential harmful effects could occur at the level of arable weeds, farmland biodiversity, and food webs and the ecological functions they provide. The magnitude of these potential adverse environmental effects will depend on a series of factors, including the specific herbicide and cultivation management applied at the farm level, the crop rotation and the characteristics of the receiving environments.

The conclusions of the EFSA GMO Panel on the environmental safety of soybean 40-3-2 are consistent with those of the DE CA. The DE CA concluded that "*no adverse effects on human and animal health and the environment are to be expected from the cultivation of soybean 40-3-2*", but that "*glyphosate-containing herbicides can be applied after germination of the soybean plants and thus could have effects on the accompanying weed flora. Based on experience from using conventional plant protection products it is to be expected that sooner or later tolerance to the active ingredient of glyphosate-containing herbicides will develop in the weed flora*" (see section 6.5 of the environmental risk assessment report of the DE CA). In its evaluation, the DE CA noted that "*there is potentially also an indirect interaction between the use of glyphosate-containing herbicides and nitrogen-fixing symbiotic partners of the soybean (e.g. Bradyrhizobium japonicum, Moorman et al., 1992, King et al., 2001), which could lead to a reduction in harvest yield (King et al., 2001). To compensate, potential increased application of nitrogen fertilizer might be necessary with the cultivation of HT soybeans*" (see section 6.5 of the environmental risk assessment report of the DE CA). With regard to potential adverse effects on non-target organisms due to potential unintended changes in soybean 40-3-2, the DE CA recommended "*conducting an additional study to confirm the absence of unintended adverse effects on non-target organisms*" (see section 6.3 of the environmental risk assessment report of the DE CA).

The EFSA GMO Panel anticipated that the repeated use of glyphosate at recommended application rates on soybean 40-3-2 grown either in rotation with other glyphosate tolerant crops, or continuously may lead to a greater risk of reducing weed community diversity than the current practices applied in soybean cropping systems. This may therefore result in reductions in weed community diversity and/or weed density to a level that might adversely affect food chains and webs, but not necessarily biological control functions, at the field and landscape level. Such reductions in weed community diversity and consequential reductions in farmland biodiversity may be considered problematic by risk managers depending upon protection goals pertaining to their region, especially in receiving environments that sustain little farmland biodiversity or in environmentally sensitive areas. Under such situations, the EFSA GMO Panel recommends that risk mitigation measures are put in place to manage potential herbicide effects, in order to ensure that glyphosate is used on soybean 40-3-2 in ways that result in similar or reduced adverse effects on farmland biodiversity compared with conventional soybean cultivation. Possible risk mitigation measures include reduced tillage, crop rotation, less intense in-crop weed management, protecting adjacent habitats from herbicide drift, and (re)introduction and better management of field margins and other ‘out of crop’ measures.

Soybean 40-3-2 grown in rotation with other glyphosate tolerant crops or continuously, in conjunction with the repeated and/or exclusive application of glyphosate-based herbicides, will cause changes in the weed flora, and will favour the evolution and spread of glyphosate resistant weeds due to the selection pressure exerted by glyphosate. The EFSA GMO Panel recommends that risk mitigation measures are put in place to reduce the selection pressure and hence to delay the evolution of resistance. This can be achieved by crop rotation (i.e., rotating glyphosat tolerant crops with non-glyphosate tolerant crops, alternating autumn- and spring-sown crops), using variable rates and timing of herbicide application, applying a variety of herbicidal active substances with different modes of action, and using non-herbicide weed control tools such as pre- and post-emergence cultivation and cover crops. To be most effective, these methods should be used in combination. A clear advantage of increasing cropping system diversity is that it would increase or conserve farmland biodiversity as well as reducing the risk of weed shifts and the evolution of glyphosate resistant weed biotypes.

The EFSA GMO Panel considers that current management practices are sufficient to cope with potential adverse effects on symbiotic nitrogen fixation arising from the use of glyphosate on soybean 40-3-2, but advises that risk managers inform farmers of the possibility of the occurrence of such effects.

The conclusions of the EFSA GMO Panel on the environmental safety of soybean 40-3-2 are consistent with those of the DE CA. In its evaluation, the DE CA recommended that “*herbicide and cultivation management of soybean 40-3-2 should be adapted to minimize potential negative effects*” (section 6.5 of the environmental risk assessment report of the DE CA).

The EFSA GMO Panel gives its opinion and makes recommendations on the scientific quality of the post-market environmental monitoring plan proposed by the applicant. In order to assess the efficacy of risk mitigation measures put in place to reduce levels of risk and in order to reduce the remaining scientific uncertainty identified in the environmental risk assessment, the EFSA GMO Panel recommends case-specific monitoring to address: (1) changes in weed community diversity; and (2) evolution of resistance to glyphosate in weeds due to changes in herbicide and cultivation regimes. In addition, the EFSA GMO Panel considers that it would be proportionate to the risk for the post-market studies on the occurrence of adverse effects on pollinators due to potential unintended changes in soybean 40-3-2, proposed by the applicant, to be conducted as case-specific monitoring. No case-specific monitoring is required to assess changes in soil microbial communities, but the EFSA GMO Panel recommends that the applicant establishes stewardship systems encouraging farmers to report problems that may be due to reduced symbiotic nitrogen fixation. General surveillance (including appropriately designed farmer questionnaires) offers an effective approach to detect and report early warning signs indicating that such effects occur. The EFSA GMO Panel considers that risk managers should adapt monitoring methodologies to their local receiving environments, management systems and the interplay between the legislation for GMOs and plant protection products.

The EFSA GMO Panel agrees with the general surveillance plan for the cultivation of soybean 40-3-2 proposed by the applicant: (1) to establish farmer questionnaires as a reporting format of any on-farm observations of effects associated with the cultivation of soybean 40-3-2; (2) to use existing monitoring networks that observe changes in biota and production practices from farm up to regional level to obtain data on environmental impacts in the landscape where soybean 40-3-2 is cultivated; (3) to review all new scientific, technical and other information pertaining to soybean 40-3-2; and (4) to develop stewardship programmes for the introduction, marketing, management and stewardship of soybean 40-3-2. However, the EFSA GMO Panel requests that its proposals and those made by the DE CA to strengthen general surveillance are implemented. The EFSA GMO Panel agrees with the reporting intervals and modalities proposed by the applicant. The general surveillance plan for the import and processing of soybean 40-3-2 has been previously evaluated by the EFSA GMO Panel.

The DE CA considered that "*based on the safety assessment of soybean 40-3-2, no specific cause-effect relationship for adverse environmental impacts has been identified that would necessitate a case specific monitoring by the applicant*". However, with regard to the occurrence of adverse effects on non-target organisms due to potential unintended changes in soybean 40-3-2, the DE CA recommended that "*the applicant shall carry out a field study to confirm the absence of unintended adverse effects on non-target organisms in the EU with placing soybean 40-3-2 on the market. The design of such a study should be of a quality to allow appropriate scientific assessment as proposed in the application*".

Further, the DE CA was of the opinion that "*the monitoring plan needs some clarifications (reporting monitoring annually, and delivery of more comprehensive overviews after six and nine years); and improvement of the questionnaires*". The DE CA recommended that "*monitoring of the herbicide use is conducted as part of the stewardship for the herbicides by the companies involved, and under the auspices of the pesticide regulatory systems operating in Member States, in order to record compliance with the approved uses of the herbicides on GMHT, levels of weed control, and development of resistant weeds. The German Competent Authority assumes that possible indirect effects of complementary herbicide application will be taken into account by the applicant in the context of a Stewardship Program harmonized with the pesticide assessment authorities. This should ensure that unexpected effects (in general surveillance) can be detected*" (see section 8 of the environmental risk assessment report of the DE CA).

In conclusion, the EFSA GMO Panel considers that the information available for soybean 40-3-2 addresses the scientific comments raised by Member States and that soybean 40-3-2, as described in this application, is as safe as its conventional counterpart and commercial non-GM soybean varieties with respect to potential adverse effects on human and animal health, in the context of its intended uses. The EFSA GMO Panel also concludes that soybean 40-3-2 is unlikely to raise additional environmental safety concerns compared with conventional soybean, but that management of its cultivation could result in environmental harm under certain conditions. The EFSA GMO Panel therefore recommends managing the use of glyphosate on soybean 40-3-2 in ways that result in similar or reduced environmental impacts compared with conventional soybean cultivation. The EFSA GMO Panel recommends the deployment of case-specific monitoring to address: (1) changes in weed community diversity; and (2) evolution of resistance to glyphosate in weeds due to changes in herbicide and cultivation regimes. In addition, the EFSA GMO Panel considers that it would be proportionate to the risk for the post-market studies on the occurrence of adverse effects on pollinators due to potential unintended changes in soybean 40-3-2, proposed by the applicant, to be conducted as case-specific monitoring, as long as the five conditions explicitly stated in this Scientific Opinion are met. If subjected to appropriate management measures, the cultivation of soybean 40-3-2 is unlikely to have environmental effects any more adverse than those associated with conventional soybean cultivation.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the Netherlands, received on 4 November 2005, concerning a request for placing on the market of soybean 40-3-2 submitted in accordance with Regulation (EC) No 1829/2003 by Monsanto.

2. Acknowledgement letter, dated 28 November 2005, from EFSA to the Competent Authority of the Netherlands.

3. Letter from EFSA to the applicant, dated 3 March 2006, requesting additional information during the completeness check of the application.

4. Letter from the applicant to EFSA, received on 6 July 2006, providing additional information requested by EFSA during the completeness check of the application.

5. Letter from the applicant to EFSA, received on 14 July 2006, clarifying the scope of the application.

6. Letter from EFSA to the applicant, dated 25 July 2006, requesting additional information during the completeness check of the application.

7. Letter from the applicant to EFSA, received on 27 July 2006, providing the additional information requested by EFSA during the completeness check of the application.

8. Letter from EFSA to the applicant, dated 29 September 2006, delivering the 'Statement of Validity' for application EFSA-GMO-NL-2005-24 (placing on the market of soybean 40-3-2) submitted in accordance with Regulation (EC) No 1829/2003 by Monsanto.

9. Letter from EFSA (DE CA) to the applicant, dated 7 December 2006, requesting additional information and stopping the clock.

10. Letter from EFSA to the applicant, dated 5 February 2007, requesting additional information and maintaining the clock stopped.

11. Letter from applicant to EFSA, received on 14 March 2007, providing additional information requested.

12. Letter from applicant to EFSA (DE CA), received on 20 June 2007, providing additional information requested.

13. Letter from EFSA (DE CA) to the applicant, dated 7 November 2007, requesting additional information and maintaining the clock stopped (see also *corrigendum* letter dated 8 November 2007).

14. Letter from applicant to EFSA (DE CA), received on 13 May 2008, providing additional information requested.

15. Letter from EFSA (DE CA) to the applicant, dated 7 July 2008, restarting the clock.

16. Letter from EFSA to the applicant, dated 10 October 2008, requesting additional information and stopping the clock.

17. Letter from the applicant to EFSA, received on 23 December 2008, providing additional information requested.

18. Letter from EFSA to the applicant, dated 16 February 2009, requesting additional information and maintaining the clock stopped.

19. Letter from EFSA to the applicant, dated 9 November 2009, requesting additional information and maintaining the clock stopped.

20. Letter from the applicant to EFSA, received on 22 November 2010, providing the additional information requested.

21. Letter from EFSA to the applicant, dated 27 April 2011, restarting the clock.

REFERENCES

Abe J, Hasegawa A, Fukushi H, Mikami T, Ohara M, Shimamoto Y, 1999. Introgression between wild and cultivated soybeans of Japan revealed by RFLP analysis for chloroplast DNAs. Economic Botany 53, 285–291.

Abrams RI, Edwards CR, Harris T, 1978. Yields and cross-pollination of soybeans as affected by honey bees and alfalfa leafcutting bees. American Bee Journal 118, 555–556, 558.

Abud S, de Souza PIM, Vianna GR, Leonardecz E, Moreira CT, Faleiro FG, Júnior JN, Monteiro PMFO, Rech EL, Aragão FJL, 2007. Gene flow from transgenic to nontransgenic soybean plants in the Cerrado region of Brazil. Genetics and Molecular Research 6, 445–452.

ACRE, 2007. Managing the footprint of agriculture: towards a comparative assessment of risks and benefits for novel agricultural systems. DEFRA, London, http://www.defra.gov.uk/environment/acre/fsewiderissues/pdf/acre-wi-final.pdf.

Ahrent DK, Caviness CE, 1994. Natural cross-pollination of 12 soybean cultivars in Arkansas. Crop Science 34, 376–378.

Albajes R, Eizaguirre M, Casado D, Pérez M, López C, Lumbierres B, Pons X, 2008. Impact of glyphosate use on arthropods in transgenic herbicide-tolerant maize; preliminary results from studies in Spain. IOBC/wprs Bulletin 33, 23–29.

Albajes R, Lumbierres B, Pons X, 2009. Responsiveness of arthropod herbivores and their natural enemies to modified weed management in corn. Environmental Entomology 38, 944–954.

Albajes R, Lumbierres B, Pons X, 2010. Managing weeds in herbicide-tolerant GM maize for biological control enhancement. IOBC/wprs Bulletin 52, 1–8.

Albajes R, Lumbierres B, Pons X, 2011. Two heteropteran predators in relation to weed management in herbicide-tolerant corn. Biological Control, DOI:10.1016/j.biocontrol.2011.03.008 (in press).

Alibhai MF, Stallings WC, 2001. Closing down on glyphosate inhibition – with a new structure for drug discovery. Proceedings of the National Academy of Sciences of the United States of America 98, 2944–2946.

Anderson RL, 2009. Impact of preceding crop and cultural practices on rye growth in winter wheat. Weed Technology 23, 564–568.

Andreasen C, Andresen LC, 2011. Managing farmland flora to promote biodiversity in Europe. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 6, 047, 1–11.

Arpaia S, 2010. Genetically modified plants and “non-target” organisms: analysing the functioning of the agro-ecosystem. Collection of Biosafety Reviews 5, 12–80.

Arpaia S, De Cristofaro A, Guerrieri E, Bossi S, Cellini F, Di Leo GM, Germinara GS, Iodice L, Maffei ME, Petrozza A, Sasso R, Vitagliano S, 2011. Foraging activity of bumblebees (*Bombus terrestris* L.) on Bt-expressing eggplants. Arthropod-Plant Interactions 5, 255–261.

Arregui MC, Scotta RR, Sánchez D, 2006. Improved weed control with broadleaved herbicides in glyphosate-tolerant soybean (*Glycine max*). Crop Protection 25, 653–656.

Arregui MC, Sánchez D, Althaus R, Scotta RR, Bertolaccini I, 2010. Assessing the risk of pesticide environmental impact in several Argentinian cropping systems with a fuzzy expert indicator. Pest Management Science 66, 736–740.

Assaad FF, Signer ER, 1990. Cauliflower mosaic virus p35Spromoter activity in *Escherichia coli*. Molecular and General Genetics 223, 517–520.

Ateh CM, Harvey RG 1999. Annual weed control by glyphosate in glyphosate-resistant soybean (*Glycine max*). Weed Technology 13, 394–398.

Badea E, Rosca I, Sabau I, Ciocazanu I, 2006. Monitoring of Roundup Ready soybean in Romania. IOBC/wprs Bulletin 29, 27–37.

Bagavathiannan MV, Van Acker RC, 2008. Crop ferality: Implications for novel trait confinement. Agriculture, Ecosystems & Environment 127, 1–6.

Ball DA, 1992. Weed seedbank response to tillage, herbicides, and crop rotation sequence. Weed Science 40, 654–659.

Balmford A, Green RE, Scharlemann JPW, 2005. Sparing land for nature: exploring the potential impact of changes in agricultural yield on the area needed for crop production. Global Change Biology 11, 1594–1605.

Bàrberi P, Lo Cascio B, 2001. Long-term tillage and crop rotation effects on weed seedbank size and composition. Weed Research 41, 325–340.

Bàrberi P, Burgio G, Dinelli G, Moonen AC, Otto S, Vazzana C, Zanin G, 2010. Functional biodiversity in the agricultural landscape: relationships between weeds and arthropod fauna. Weed Research 50, 388–401.

Barriuso J, Marín S, Mellado RP, 2010. Effect of the herbicide glyphosate on glyphosate-tolerant maize rhizobacterial communities: a comparison with pre-emergency applied herbicide consisting of a combination of acetochlor and terbuthylazine. Environmental Microbiology 12, 1021–1030.

Barriuso J, Marín S, Mellado RP, 2011. Potential accumulative effect of the herbicide glyphosate on glyphosate-tolerant maize rhizobacterial communities over a three-year cultivation period. PLoS ONE 6, e27558.

Barriuso J, Mellado RP, 2012. Glyphosate affects the rhizobacterial communities in glyphosate-tolerant cotton. Applied Soil Ecology 55, 20–26.

Basso B, Sartori L, Bertocco M, Cammarano D, Martin EC, Grace PR, 2011. Economic and environmental evaluation of site-specific tillage in a maize crop in NE Italy. European Journal of Agronomy 35, 83–92.

Bastiaans L, Kropff MJ, Goudriaan J, van Laar HH, 2000. Design of weed management systems with a reduced reliance on herbicides poses new challenges and prerequisites for modeling crop-weed interactions. Field Crops Research 67, 161–179.

Baylis AD, 2000. Why glyphosate is a global herbicide: strengths, weaknesses and prospects. Pest Management Science 56, 299–308.

Beckie HJ, 2006. Herbicide-resistant weeds: management tactics and practices. Weed Technology 20, 793–814.

Beckie HJ, 2011. Herbicide-resistant weed management: focus on glyphosate. Pest Management Science 67, 1037–1048.

Beckie HJ, Reboud X, 2009. Selecting for weed resistance: herbicide rotation and mixture. Weed Technology 23, 363–370.

Beckie HJ, Harker KN, Hall LM, Warwick SI, Légère A, Sikkema PH, Clayton GW, Thomas AG, Leeson JY, Seguin-Swartz G, Simard MJ, 2006. A decade of herbicide-resistant crops in Canada. Canadian Journal of Plant Science 86, 1243–1264.

Bennett R, Phipps R, Strange A, Grey P, 2004. Environmental and human health impacts of growing genetically modified herbicide-tolerant sugar beet: a life-cycle assessment. Plant Biotechnology Journal 2, 273–278.

Benton TG, 2007. Managing farming's footprint on biodiversity. Science 315, 341–342.

Benton TG, Vickery JA, Wilson JD, 2003. Farmland biodiversity: is habitat heterogeneity the key? Trends in Ecology and Evolution 18, 182–188.

Bindraban PS, Franke AC, Ferraro DO, Ghersa CM, Lotz LAP, Nepomuceno A, Smulders MJM, van de Wiel CCM, 2009. GM-related sustainability: agro-ecological impacts, risks and opportunities of soy production in Argentina and Brazil. Report Plant Research International, Wageningen, http://edepot.wur.nl/7954

Bitzer RJ, Buckelew LD, Pedigo LP, 2002. Effects of transgenic herbicide-resistant soybean varieties and systems on surface-active springtails (Entognatha: Collembola). Environmental Entomology 31, 449–461.

Bohan DA, Hawes C, Haughton AJ, Denholm I, Champion GT, Perry JN, Clark SJ, 2007. Statistical models to evaluate invertebrate-plant trophic interactions in arable systems. Bulletin of Entomological Research 97, 265–280.

Bohan DA, Boursault A, Brooks DR, Petit S, 2011. National-scale regulation of weed seedbank by carabid predators. Journal of Applied Ecology 48, 888–898.

Bohm GM, Alves BJR, Urquiaga S, Boddey RM, Xavier GR, Hax F, Rombaldi CV, 2009. Glyphosate- and imazethapyr-induced effects on yield, nodule mass and biological nitrogen fixation in field-grown glyphosate-resistant soybean. Soil Biology & Biochemistry 41, 420–422.

Bonny S, 2008. Genetically modified glyphosate-tolerant soybean in the USA: adoption factors, impacts and prospects. A review. Agronomy for Sustainable Development 28, 21–32.

Bonny S, 2011. Herbicide-tolerant transgenic soybean over 15 years of cultivation: pesticide use, weed resistance, and some economic issues. The case of the USA. Sustainability 3, 1302–1322.

Borggaard OK, Gimsing AL, 2008. Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. Pest Management Science 64, 441–456.

Bott S, Tesfamariam T, Candan H, Cakmak I, Römheld V, Neumann G, 2008. Glyphosate-induced impairment of plant growth and micronutrient status in glyphosate-resistant soybean (*Glycine max* L.). Plant Soil 312, 185–194.

Bott S, Tesfamariam T, Kania A, Eman B, Aslan N, Römheld V, Neumann G, 2011. Phytotoxicity of glyphosate soil residues re-mobilised by phosphate fertilisation. Plant Soil 342, 249–263.

Bradshaw LD, Padgette SR, Kimball SL, Wells BH, 1997. Perspectives on glyphosate resistance. Weed Technology 11, 189–198.

Brigulla M, Wackernagel W, 2010. Molecular aspects of gene transfer and foreign DNA acquisition in prokaryotes with regard to safety issues. Applied Microbiology and Biotechnology 86, 1027–1041.

Brimner TA, Gallivan GJ, Stephenson GR, 2005. Influence of herbicide-resistant canola on the environmental impact of weed management. Pest Management Science 61, 47–52.

Brookes G, 2005. The farm-level impact of herbicide-tolerant soybeans in Romania. AgBioForum 8, 235–241.

Brookes G, Barfoot P, 2005. Global impact of biotech crops: socio-economic and environmental effects in the first ten years of commercial use. AgBioForum 9, 139–151.

Brooks DR, Bohan DA, Champion GT, Haughton AJ, Hawes C, Heard MS, Clark SJ, Dewar AM, Firbank LG, Perry JN, Rothery P, Scott RJ, Woiwod IP, Birchall C, Skellern MP, Walker JH, Baker P, Bell D, Browne EL, Dewar AJG, Fairfax CM, Garner BH, Haylock LA, Horne SL, Hulmes SE, Mason NS, Norton LR, Nuttall P, Randle Z, Rossall MJ, Sands RJN, Singer EJ, Walker MJ, 2003. Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. I. Soil-surface-active invertebrates. Philosophical Transactions of the Royal Society B: Biological Sciences 358, 1847–1862.

Buckelew LD, Pedigo LP, Mero HM, Owen MDK, Tylka GL, 2000. Effects of weed management systems on canopy insects in herbicide-resistant soybeans. Journal of Economic Entomology 93, 1437–1443.

Bückmann H, Petersen J, Schlinker G, Märländer B, 2000. Weed control in genetically modified sugar beet – Two years experiences of a field trial series in Germany. Journal of Plant Diseases and Protection XVII, 353–362.

Bühler C, 2006. Biodiversity monitoring in Switzerland: what can we learn for general surveillance of GM crops? Journal of Consumer Protection and Food Safety 1, 37–41.

Burks AW, Fuchs RL, 1995. Assessment of the endogenous allergens in glyphosate-tolerant and commercial soybean varieties. Journal of Allergy and Clinical Immunology 96, 1008–1010.

Busi R, Powles SB, 2009. Evolution of glyphosate resistance in a *Lolium rigidum* population by glyphosate selection at sublethal doses. Heredity 103, 318–325.

Busse MD, Ratcliff AW, Shestack CJ, Powers RF, 2001. Glyphosate toxicity and the effects of long term vegetation control on soil microbial communities. Soil Biology & Biochemistry 33, 1777–1789.

Butler SJ, Vickery JA, Norris K, 2007. Farmland biodiversity and the footprint of agriculture. Science 315, 381–384.

CAC, 2003. Codex principles and guidelines on foods derived from biotechnology. Joint FAO/WHO Food Standards Programme, Food and Agriculture Organisation, Rome, http://www.bfr.bund.de/cm/208/codex_principles_and_guidelines_on_foods_derived_from_biotechnology.pdf.

CaJacob CA, Feng PCC, Heck GR, Alibhai MF, Sammons RD, Padgette SR, 2004. Engineering resistance to herbicides. In: Christou P, Klee H (Eds), *Handook of Plant Biotechnology*, John Wiley & Sons Ltd, pp 353–372.

Cakmak I, Yazici A, Tutus Y, Ozturk L, 2009. Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean. European Journal of Agronomy 31, 114–119.

Cardina J, Herms CP, Doohan DJ, 2002. Crop rotation and tillage system effects on weed seedbanks. Weed Science 50, 448–460.

Caron-Lormier G, Bohan DA, Hawes C, Raybould A, Haughton AJ, Humphry RW, 2009. How might we model an ecosystem? Ecological Modeling 220, 1935–1949.

Caron-Lormier G, Bohan DA, Dye R, Hawes C, Humphrey RW, Raybould A, 2011. Modelling an ecosystem: the example of agroecosystems. Ecological Modeling 222, 1163–1173.

Carpenter JE, 2011. Impacts of GM crops on biodiversity. GM Crops 2, 1–17.

Castellazzi MS, Perry JN, Colbach N, Monod H, Adamczyk K, Viaud V, Conrad KF, 2007. New measures and tests of temporal and spatial pattern of crops in agricultural landscapes. Agriculture, Ecosystems & Environment 118, 339–349.

Castellazzi MS, Wood GA, Burgess PJ, Morris J, Conrad MF, Perry JN, 2008. A systematic representation of crop rotations. Agricultural Systems 97, 26–33.

Castro JV, Peralba MCR, Ayub MAZ, 2007. Biodegradation of the herbicide glyphosate by filamentous fungi in platform shaker and batch bioreactor. Journal of Environmental Science and Health – Part B 42, 883–886.

Caviness CE, 1966. Estimates of natural cross-pollination in Jackson soybeans in Arkansas. Crop Science 6, 211–212.

Ceccherini MT, Poté J, Kay E, Van VT, Marechal J, Pietramellara G, Nannipieri P, Vogel TM, Simonet P, 2003. Degradation and transformability of DNA from transgenic leaves. Applied and Environmental Microbiology 69, 673–678.

CERA, 2010. A review of the environmental safety of the CP4 EPSPS protein, ILSI Research Foundation, Washington D.C., http://cera-gmc.org/docs/cera_publications/pub_01_2010.pdf.

Cerdeira AL, Duke SO, 2006. The current status and environmental impacts of glyphosate-resistant crops: A review. Journal of Environmental Quality 35, 1633–1658.

Cerdeira AL, Duke SO, 2007. Environmental impacts of transgenic herbicide-resistant crops. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 2, 1–14.

Cerdeira AL, Duke SO, 2010. Effects of glyphosate-resistant crop cultivation on soil and water quality. GM crops 1, 1–9.

Cerdeira AL, Gazziero DLP, Duke SO, Matallo MB, Spadotto CA, 2007. Review of potential environmental impact of transgenic glyphosate-resistant soybean in Brazil. Journal of Environmental Science and Health – Part B 42, 539–549.

Cerdeira AL, Gazziero DLP, Duke SO, Matallo MB, 2011. Agricultural impacts of glyphosate-resistant soybean cultivation in South America. Journal of Agricultural and Food Chemistry 59, 5799–5807.

Chamberlain DE, Fuller RJ, Bunce RGH, Duckworth JC, Shrubb M, 2000. Changes in the abundance of farmland birds in relation to the timing of agricultural intensification in England and Wales. Journal of Applied Ecology 37, 771–788.

Champion GT, May MJ, Bennett S, Brooks DR, Clark SJ, Daniels RE, Firbank LG, Haughton AJ, Hawes C, Heard MS, Perry JN, Randle Z, Rossall MJ, Rothery P, Skellern MP, Scott RJ, Squire GR, Thomas MR, 2003. Crop management and agronomic context of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. Philosophical Transactions of the Royal Society B: Biological Sciences 358, 1801–1818.

Chiari WC, de Toledo VDA, Ruvolo-Takasusuki MCC, de Oliveira AJB, Sakaguti ES, Attencia VM, Costa FM, Mitsui MH, 2005. Pollination of soybean (*Glycine max* L. Merril) by honeybees (*Apis mellifera* L.). Brazilian Archives of Biology and Technology 48, 31–36.

Chiari WC, Ruvolo-Takasusuki MCC, Chambó ED, Arias CA, Hoffmann-Campo CB, de Toledo VDA, 2011. Gene flow between conventional and transgenic soybean pollinated by honeybees. In Abd MN and Hasaneenh EG (Eds), *Herbicides - Mechanisms and Mode of Action*, InTech Open Access Publisher.

Clarke JH, Cook SK, Harris D, Wiltshire JJJ, Henderson IG, Jones NE, Boatman ND, Potts SG, Westbury DB, Woodcock BA, Ramsay AJ, Pywell RF, Goldsworthy PE, Holland JM, Smith BM, Tipples J, Morris AJ, Chapman P, Edwards P, 2007. The SAFFIE project report. ADAS, Boxworth, UK, http://www.hgca.com/document.aspx?fn=load&media_id=3567&publicationId=3919.

Clergue B, Amiaud B, Pervanchon F, Lasserre-Joulin F, Plantureux S, 2005. Biodiversity: function and assessment in agricultural areas. A review. Agronomy for Sustainable Development 25, 1–15.

Cordeau S, Reboud X, Chauvel B, 2011. Relative importance of farming practices and landscape context on the weed flora of sown grass strips. Agriculture, Ecosystems & Environment 139, 595–602.

Corrigan KA, Harvey RG, 2000. Glyphosate with and without residual herbicides in no till glyphosate-resistant soybean (*Glycine max*). Weed Technology 14, 569–577.

Coyette B, Tencalla F, Brants I, Fichet Y, Rouchouze D, 2002. Effect of introducing glyphosate-tolerant sugar beet on pesticide usage in Europe. Pesticide Outlook 13, 219–223.

Cromwell GL, Lindemann MD, Randolph JH, Parker GR. Coffey RD, Laurent KM, Armstrong CL, Mikel WB, Stanisiewski EP, Hartnell GF, 2002. Soybean meal from roundup ready or conventional soybeans in diets for growing-finishing swine. Journal of Animal Sciences 80, 708–715.

Culpepper SA, 2006. Glyphosate-induced weed shifts. Weed Technology 20, 277–281.

Culpepper AS, Webster TM, Sosnoskie LM, York AC, 2010. Glyphosate resistant *Palmer amaranth* in the United States. In: Nandula VK (Ed), *Glyphosate Resistance in Crops and Weeds: History, Development, and Management*, John Wiley & Sons, Inc., New York, pp 195–212.

Dalley CD, Kells JJ, Renner KA, 2004a. Effect of glyphosate application timing and row spacing on corn (*Zea mays*) and soybean (*Glycine max*) yields. Weed Technology 18, 165–176.

Dalley CD, Kells JJ, Renner KA, 2004b. Effect of glyphosate application timing and row spacing on weed growth in corn (*Zea mays*) and soybean (*Glycine max*). Weed Technology 18, 177–182.

Dauer JT, Luschei EC, Mortensen DA, 2009. Effects of landscape composition on spread of an herbicide-resistant weed. Landscape Ecology 24, 735–747.

Davis VM, Kruger GR, Stachler JM, Loux MM, Johnson WG, 2009. Growth and seed production of horseweed (*Conyza canadensis*) populations resistant to glyphosate, ALS-inhibiting, and multiple (glyphosate + ALS-inhibiting) herbicides. Weed Science 57, 494–504.

De la Fuente EB, Suarez SA, Ghersa CM, 2006. Soybean weed community composition and richness between 1995 and 2003 in the Rolling Pampas (Argentina). Agriculture, Ecosystems & Environment 115, 229–236.

de Vries J, Heine M, Harms K, Wackernagel W, 2003. Spread of recombinant DNA by roots and pollen of transgenic potato plants, identified by highly specific biomonitoring using natural transformation of an *Acinetobacter* sp. Applied and Environmental Microbiology 69, 4455–4462.

de Vries J, Herzfeld T, Wackernagel W, 2004. Transfer of plastid DNA from tobacco to the soil bacterium *Acinetobacter* sp. by natural transformation. Molecular Microbiology 53, 323–334.

Deaville ER, Maddison BC, 2005. Detection of transgenic and endogenous plant DNA fragments in the blood, tissues, and digesta of broilers. Journal of Agricultural and Food Chemistry 53, 10268–10275.

Delannay X, Bauman TT, Beighley DH, Buettner MJ, Coble HD, DeFelice MS, Derting CW, Diedrick TJ, Friffin JL, Hagood ES, Hancock FG, Hart SE, LaVallee BJ, Loux MM, Lueschen WE, Matson KW, Moots CK, Murdock E, Nickell AD, Owen MDK, Paschal II EH, Prochaska LM, Raymond PJ, Reynolds DB, Rhodes WK, Roeth FW, Sprankle PL, Tarochione LJ, Tinius CN, Walker RH, Wax LM, Weigelt HD, Padgette SR, 1995. Yield evaluation of a glyphosate-tolerant soybean line after treatment with glyphosate. Crop Science 35, 1461–1467.

Desneux N, Ramírez-Romero R, Bokonon-Ganta AH, Bernal JS, 2010. Attraction of the parasitoid *Cotesia marginiventris* to host (*Spodoptera frugiperla*) frass is affected by transgenic maize. Ecotoxicology 19, 1183–1192.

Devos Y, Cougnon M, Vergucht S, Bulcke R, Haesaert G, Steurbaut W, Reheul D, 2008. Environmental impact of herbicide regimes used with genetically modified herbicide-resistant maize. Transgenic Research 17, 1059–1077 (Erratum: 18, 315–316).

Devos Y, De Schrijver A, Reheul D, 2009a. Quantifying the introgressive hybridisation propensity between transgenic oilseed rape and its wild/weedy relatives. Environmental Monitoring and Assessment 149, 303–322.

Devos Y, Demont M, Dillen K, Reheul D, Kaiser M, Sanvido O, 2009b. Coexistence of genetically modified (GM) and non-GM crops in the European Union. A review. Agronomy for Sustainable Development 29, 11–30.

Dewar AM, 2009. Weed control in glyphosate-tolerant maize in Europe. Pest Management Science 10, 1047–1058.

Dewar AM, 2010. GM glyphosate-tolerant maize in Europe can help alleviate the global food shortage. Outlooks on Pest Management 21, 55–63.

Dewar AM, Haylock LA, Bean KM, May MJ, 2000. Delayed control of weeds in glyphosate-tolerant sugar beet and consequences on aphid infestation and yield. Pest Management Science 56, 345–350.

Dewar AM, May MJ, Woiwod IP, Haylock LA, Champion GT, Garner BH, Sands RJN, Qi A, Pidgeon JD, 2003. A novel approach to the use of genetically modified herbicide tolerant crops for environmental benefit. Proceedings of the Royal Society B: Biological Sciences 270, 335–340.

Dewar AM, Champion GT, May MJ, Pidgeon JD, 2005. The UK Farm Scale Evaluations of GM crops – a post script. Outlooks on Pest Management 16, 164–173

Dill GM, 2005. Glyphosate-resistant crops: history, status and future. Pest Management Science 61, 219–224.

Dill GM, Sammons RD, Feng PCC, Kohn F, Kretzmer K, Mehrsheikh A, Bleeke M, Honegger JL, Farmer D, Wright D, Haupfear EA, 2010. Glyphosate: discovery, development, applications and properties. In: Nandula VK (Ed), *Glyphosate Resistance in Crops and Weeds: History, Development, and Management*, John Wiley & Sons, Inc., New York, pp 1–33.

Donald PF, Green RE, Heath MF, 2001. Agricultural intensification and the collapse of Europe's farmland bird populations. Proceedings of the Royal Society B: Biological Sciences 268, 25–29.

Dorokhov D, Ignatov A, Deineko E, Serjapin A, Ala A, Skryabin K, 2004. In: den Nijs HCM, Bartsch D, Sweet J (Eds), *Introgression from Genetically Modified Plants into Wild Relatives*, CAB International, pp 151–161.

Doucet C, Weaver SE, Hamill AS, Zhang J, 1999. Separating the effects of crop rotation from weed management on weed density and diversity. Weed Science 47, 729–735.

Dos Santos JB, Ferreira EA, Kasuya MCM, DaSilva AA, Procopio SO, 2005. Tolerance of *Bradyrhizobium* strains to glyphosate formulations. Crop Protection 24, 543–547.

Duggan PS, Chambers, PA, Heritage, J, and Forbes, JM, 2000. Survival of free DNA encoding antibiotic resistance from transgenic maize and the transformation activity of DNA in ovine saliva, ovine rumen fluid and silage effluent. FEMS Microbiology Letters 191, 71–77.

Duggan PS, Chambers, PA, Heritage, J, and Forbes, JM, 2003. Fate of genetically modified maize DNA in the oral cavity and rumen of sheep. British Journal of Nutrition 89, 159–166.

Duke SO, 2005. Taking stock of herbicide-resistant crops ten years after introduction. Pest Management Science 61, 211–218.

Duke SO, Powles SB, 2008a. Glyphosate-resistant weeds and crops. Pest Management Science 64, 317–318.

Duke SO, Powles SB, 2008b. Glyphosate: a once-in-a-century herbicide. Pest Management Science 64, 319–325.

Dunfield KE, Germida JJ, 2004. Impact of genetically modified crops on soil- and plant-associated microbial communities. Journal of Environmental Quality 33, 806–815.

EC, 1996. Commission Decision (96/281/EC) of 3 April 1996 concerning the placing on the market of genetically modified soya beans (*Glycine max* L.) with increased tolerance to the herbicide glyphosate, pursuant to Council Directive 90/220/EEC. Official Journal of the European Communities L107, 10–11, http://www.biosafety.be/GB/Dir.Eur.GB/Market/96_281/96_281.html.

EC, 2004. Directive 2004/35/CE of the European Parliament and of the council of 21 April 2004 on environmental liability with regard to the prevention and remedying of environmental damage. Official Journal of the European Union L143, 56–75.

EC, 2008. Letter from the European Commission to EFSA on the environmental risk assessment of herbicide tolerant plants – interplay between Directive 2001/18/EC and Directive 91/414/EEC (Ref ENV/B3/AA/JH/YK/gm D(2008)ARES(2008)25125), http://www.efsa.europa.eu/cs/BlobServer/DocumentSet/gmo_response_european_commission_en.pdf.

EFSA, 2006a. Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal 99, 1–100, http://www.efsa.europa.eu/cs/BlobServer/Scientific_Document/gmo_guidance_gm_plants_en.pdf.

EFSA, 2006b. Opinion of the Scientific Panel on Genetically Modified Organisms on the Post Market Environmental Monitoring (PMEM) of genetically modified plants. The EFSA Journal 319, 1–27, http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/gmo_op_ej319_pmem_en,0.pdf.

EFSA, 2008a. Working document of the GMO Panel on the interplay between Directive 2001/18/EC (GMOs) and Directive 91/414/EEC (Plant Protection Products), http://www.efsa.europa.eu/cs/BlobServer/DocumentSet/gmo_working_document_en.pdf.

EFSA, 2008b. Environmental risk assessment of genetically modified plants -- challenges and approaches. EFSA Scientific Colloquium Series 8, June 2007. European Food Safety Authority, Brussels, http://www.efsa.europa.eu/en/colloquiareports/colloquiagmoera.htm.http://www.efsa.europa.eu/cs/BlobServer/Event_Meeting/sci_coll_8_summary_report.pdf

EFSA, 2009a. Statement of EFSA on the consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "use of antibiotic resistance genes as marker genes in genetically modified plants" and the Scientific Opinion of the GMO Panel on "consequences of the opinion on the use of antibiotic resistance genes as marker genes in genetically modified plants on previous EFSA assessments of individual GM plants". The EFSA Journal 1108, 1–8, http://www.efsa.europa.eu/EFSA/ScientificPanels/GMO/efsa_locale-1178620753812_Statements456.htm.

EFSA, 2009b. Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMO-NL-2005–22 and EFSA-GMO-RX-NK603) for the placing on the market of the genetically modified glyphosate tolerant maize NK603 for cultivation, food and feed uses and import and processing, and for renewal of the authorisation of maize NK603 as existing product. The EFSA Journal 1137, 1–50, http://www.efsa.europa.eu/en/scdocs/doc/1137.pdf.

EFSA, 2010a. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. The EFSA Journal 1700, 1–168, http://www.efsa.europa.eu/en/efsajournal/doc/1700.pdf.

EFSA, 2010b. Guidance on the application of systematic review methodology to food and feed safety assessments to support decision making. The EFSA Journal 1637, 1–90, http://www.efsa.europa.eu/en/scdocs/doc/1637.pdf.

EFSA, 2010c. Scientific Opinion on the development of specific protection goal options for environmental risk assessment of pesticides, in particular in relation to the revision of the Guidance Documents on Aquatic and Terrestrial Ecotoxicology (SANCO/3268/2001 and SANCO/10329/2002). The EFSA Journal 1821, 1–55, http://www.efsa.europa.eu/en/scdocs/doc/1821.pdf.

EFSA, 2010d. Scientific Opinion on the assessment of potential impacts of genetically modified plants on non-target organisms. The EFSA Journal 1877, 1–72, http://www.efsa.europa.eu/en/efsajournal/doc/1877.pdf.

EFSA, 2010e. Guidance on the environmental risk assessment of genetically modified plants. The EFSA Journal 1879, 1–111, http://www.efsa.europa.eu/en/efsajournal/doc/1879.pdf.

EFSA, 2010f. Scientific Opinion on applications (EFSA-GMO-RX-40–3-2) for the renewal of authorisation for the continued marketing of (1) food containing, consisting of, or produced from genetically modified soybean 40–3-2; (2) feed containing, consisting of, or produced from soybean 40–3-2; and (3) other products containing or consisting of soybean 40–3-2 with the exception of cultivation, all under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 1908, 1–38, http://www.efsa.europa.eu/en/efsajournal/doc/1908.pdf.

EFSA, 2011a. Guidance on selection of comparators for the risk assessment of genetically modified plants. The EFSA Journal 2150, 1–37, http://www.efsa.europa.eu/en/efsajournal/doc/2150.pdf.

EFSA, 2011b. Guidance for the risk assessment of food and feed from genetically modified plants. The EFSA Journal 2193, 1–54, http://www.efsa.europa.eu/en/efsajournal/doc/2193.pdf.

EFSA, 2011c. Guidance on the post-market environmental monitoring (PMEM) of genetically modified plants. The EFSA Journal 2316, 1–40, http://www.efsa.europa.eu/en/efsajournal/doc/2316.pdf.

EFSA, 2011d. Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON810 in 2009. The EFSA Journal 2376, 1–66, http://www.efsa.europa.eu/en/efsajournal/doc/2376.pdf.

EFSA, 2011e. Scientific Opinion on application (EFSA-GMO-CZ-2008–54) for placing on the market of genetically modified insect resistant and herbicide tolerant maize MON 88017 for cultivation under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 2428, 1–152, http://www.efsa.europa.eu/en/efsajournal/doc/2428.pdf.

EFSA, 2011f. Scientific Committee; Statistical significance and biological relevance. The EFSA Journal 2372, 1–17, http://www.efsa.europa.eu/en/efsajournal/doc/2372.pdf.

Ehlers U, 2011. Interplay between GMO regulation and pesticide regulation in the EU. Journal of Consumer Protection and Food Safety 6, 61–64.

Elmore RW, Roeth FW, Nelson LA, Shapiro CA, Klein RN, Knezevic SZ, Martin A, 2001a. Glyphosate-resistant soybean cultivar yields compared with sister lines. Agronomy Journal 93, 408–412.

Elmore RW, Roeth FW, Nelson LA, Klein RN, Knezevic SZ, Martin A, Nelson A, Shapiro CA, 2001b. Glyphosate-resistant soybean cultivar response to glyphosate. Agronomy Journal 93, 404–407.

EPPO, 2003. Efficacy evolution of plant protection products. Resistance risk analysis. EPPO Bulletin 33, 37–63.

Ellis JM, Griffin JL, 2002. Benefits of soil-applied herbicides in glyphosate-resistant soybean (*Glycine max*). Weed Technology 16, 541–547.

Erickson EH, 1975a. Variability of floral characteristics influence honeybee visitation to soybean flowers. Crop Science 15, 767–771.

Erickson EH, 1975b. Honey bees and soybeans. American Bee Journal 115, 351–353, 372.

Erickson EH, 1984. Soybean pollination and honey production: A research project report. American Bee Journal 124, 775–779.

Erickson EH, Berger GA, Shannon JG, Robbins JM, 1978. Honey bee pollination increases soybean yields in the Mississippi Delta region of Arkansas and Missouri. Journal of Economic Entomology 71, 601–603.

Ewers RM, Scharlemann JPW, Balmford A, Green RE, 2009. Do increases in agricultural yield spare land for nature? Global Change Biology 15, 1716–1726.

Feng PCC, Chiu T, Douglas Sammons R, 2003. Glyphosate efficacy is contributed by its tissue concentration and sensitivity in velvetleaf (*Abutilon theophrasti*). Pestic. Biochem. Physiol. 77, 83–91.

Feng PCC, Baley GJ, Clinton WP, Bunkers GJ, Alibhai MF, Paulitz TC, Kidwell KK, 2005 Glyphosate inhibits rust diseases in glyphosate-resistant wheat and soybean. Proceedings of the National Academy of Sciences of the United States of America 102, 17290–17295.

Fernandez-Cornejo J, Klotz-Ingram C, Jans S, 2002. Farm-level effects of adopting herbicide-tolerant soybeans in the USA. Journal of Agricultural and Applied Economics 34, 149–163.

Firbank LG, 2005. Striking a new balance between agricultural production and biodiversity. Annals of Applied Biology 146, 163–175.

Firbank LG, Heard MS, Woiwod IP, Hawes C, Haughton AJ, Champion GT, Scott RJ, Hill MO, Dewar AM, Squire GR, May MJ, Brooks DR, Bohan DA, Daniels RE, Osborne JL, Roy DB, Black HIJ, Rothery P, Perry JN, 2003a. An introduction to the Farm-Scale Evaluations of genetically modified herbicide-tolerant crops. Journal of Applied Ecology 40, 2–16.

Firbank LG, Perry JN, Squire GR, Bohan DA, Brooks DR, Champion GT, Clark SJ, Daniels RE, Dewar AM, Haughton AJ, Hawes C, Heard MS, Hill MO, May MJ, Osborne JL, Rothery P, Roy DB, Scott RJ, Woiwod IP, 2003b. The implications of spring-sown genetically modified herbicide-tolerant crops for farmland biodiversity: a commentary on the farm scale evaluations of spring sown crops, http://webarchive.nationalarchives.gov.uk/20080306073937/http://www.defra.gov.uk/environment/gm/fse/results/fse-commentary.pdf.

Flachowsky G, Aulrich K, 1999. Animal nutrition and genetic modified organism (GMO). Landbauforschung Volkenrode 49, 13–20.

Fried G, Petit S, Dessaint F, Reboud X, 2009. Arable weed decline in Northern France: crop edges as refuges for weed conservation? Biological Conservation 142, 238–243.

Fuller RJ, Hinsley SA, Swetnam RD, 2004. The relevance of non-farmland habitats, uncropped areas and habitat diversity to the conservation of farmland birds. Ibis 146, 22–31.

Gaines TA , Zhang W, Wang D, Bukun B, Chisholm ST, Shaner DL, Nissen SJ, Patzoldt WL, Tranel PJ, Culpepper AS, Grey TL, Webster TM, Vencill WK, Sammons RD, Jian J, Preston C, Leach JE, Westra P, 2010. Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. Proceedings of the National Academy of Sciences of the United States of America 107, 1029–1034.

Garcia-Alonso M, 2010. Current challenges in environmental risk assessment: The assessment of unintended effects of GM crops on non-target organisms. IOBC/wprs Bulletin 52, 57–63.

Gardner JG, Nelson GC, 2008. Herbicides, glyphosate resistance and acute mammalian toxicity: simulating an environmental effects of glyphosate-resistant weeds in the USA. Pest Management Science 64, 470–478.

Gardner JG, Gressel J, Mangel M, 1998. A revolving dose strategy to delay the evolution of both quantitative vs. major monogene resistances to pesticides and drugs. International Journal of Pest Management 44, 161–180.

Geiger F, Bengtsson J, Berendse F, Weisser WW, Emmerson M, Morales MB, Ceryngier P, Liira J, Tscharntke T, Winqvist C, Eggers S, Bommarco R, Pärt T, Bretagnolle V, Plantegenest V, Clement LW, Dennis C, Palmer C, Onate JJ, Guerrero I, Hawro V, Aavik T, Thies C, Flohre A, Hänke S, Fischer C, Goedhart PW, Inchausti P, 2010. Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. Basic and Applied Ecology 11, 97–105.

Gianessi LP, 2005. Economic and herbicide use impacts of glyphosate-resistant crops. Pest Management Science 61, 241–245.

Gianessi LP, 2008. Economic impacts of glyphosate-resistant crops. Pest Management Science 64, 346–352.

Gibbons DW, Bohan DA, Rothery P, Stuart RC, Haughton AJ, Scott RJ, Wilson JD, Perry JN, Clark SJ, Dawson RJG, Firbank LG, 2006. Weed seed resources for birds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. Proceedings of the Royal Society B: Biological Sciences 273, 921–1928.

Giesy JP, Dobson S, Solomon KR, 2000. Ecotoxicological risk assessment for roundup herbicide. Reviews of Environmental Contamination and Toxicology 167, 35–120.

Gimsing AL, Borggaard OK, Jacobsen OS, Aamand J, Sørensen J, 2004. Chemical and microbiological soil characteristics controlling glyphosate mineralization in Danish surface soils. Applied Soil Ecology 27, 233–242.

Givens WA, Shaw DR, Johnson WG, Weller SC, Young BG, Wilson RG, Owen MDK, Jordan D, 2009a. A grower survey of herbicide use patterns in glyphosate-resistant cropping systems. Weed Technology 23, 156–161.

Givens WA, Shaw DR, Kruger GR, Johnson WG, Weller SC, Young BG, Wilson RG, Owen MDK, Jordan D, 2009b. Survey of tillage trends following the adoption of glyphosate-resistant crops. Weed Technology 23, 150–155.

Gizzarelli F, Corinti S, Barletta B, Iacovacci P, Brunetto B, Butteroni C, Afferni C, Onori R, Miraglia M, Panzini G, Di Felice G, Tinghino R, 2006. Evaluation of allergenicity of genetically modified soybean protein extract in a murine model of oral allergen-specific sensitization. Clinical and Experimental Allergy 36, 238–248.

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C, 2010. Food security: the challenge of feeding 9 billion people. Science 327, 812–819.

Gomez E, Ferreras L, Lovotti L, Fernandez E, 2009. Impact of glyphosate application on microbial biomass and metabolic activity in a Vertic Argiudoll from Argentina. European Journal of Soil Biology 45, 163–167.

Gonzini LC, Hart SE, Wax LM, 1999. Herbicide combinations for weed management in glyphosate-resistant soybean (*Glycine max*). Weed Technology 13, 354–360.

Gordon B, 2007. Manganese nutrition of glyphosate-resistant and conventional soybeans. Better Crops 91, 12–13.

Gorlach-Lira K, Stefaniak O, Slizak W, Owedyk I, 1997. The response of forest soil microflora to the herbicide formulations Fusilade and Roundup. Microbiology Research 152, 319–329.

Goulson D, Lepais O, O'Connor S, Osborne JL, Sanderson RA, Cussans J, Goffe L, Darvill B, 2010. Effects of land use at a landscape scale on bumblebee nest density and survival. Journal of Applied Ecology 47, 1207–1215.

Graef F, De Schrijver A, Murray A, 2008. GMO monitoring data coordination and harmonisation at EU level – outcomes of the European Commission Working Group on Guidance Notes supplementing Annex VII of Directive 2001/18/EC. Journal of Consumer Protection and Food Safety 3, 17–20.

Greaves MP, Marshall EJP, 1987. Field margins: definitions and statistics. In: Way JM, Greig-Smith PJ (Eds), *Field Margins*, Monograph No 35, British Crop Protection Council, Thornton Heath, Surrey, pp 3–10.

Green JM, 2009. Evolution of glyphosate-resistant crop technology. Weed Science 57, 108–117.

Green JM, 2011. Outlookk on weed management in herbicide-resistant crops: need for diversification. Outlooks on Pest Management 22, 100–104.

Green JM, Castle LA, 2010. Transitioning from single to multiple herbicide-resistant crops. In: Nandula VK (Ed), *Glyphosate Resistance in Crops and Weeds: History, Development, and Management*, John Wiley & Sons, Inc., New York, pp 67–91.

Green JM, Owen MDK, 2011. Herbicide-resistant crops: utilities and limitations for herbicide-resistant weed management. Journal of Agricultural and Food Chemistry 59, 5819–5829.

Gressel J, 2009. Evolving understanding of the evolution of herbicide resistance. Pest Management Science 65, 1164–1173.

Gressel J, Segel LA, 1990. Modeling the effectiveness of herbicide rotation and mixtures as a strategy to delay or preclude resistance. Weed Technology 4, 186–198.

Grichar WJ, 2006. Using soil-applied herbicides in glyphosate-resistant soybeans along the Texas gulf coast. Weed Technology 20, 633–639.

Gulden RH, Lerat S, Blackshaw RE, Powell JR, Levy-Booth DJ, Dunfield KE, Trevors JT, Pauls KP, Klironomos JN, Swanton CJ, 2008. Factors affecting the presence and persistence of plant DNA in the soil environment in corn and soybean rotation. Weed Science 56, 767–774.

Gulden RH, Sikkema PH, Hamill AS, Tardif F, Swanton CJ, 2009. Conventional vs. glyphosate-resistant cropping systems in Ontario: weed control, diversity, and yield. Weed Science 57, 665–672.

Gumisiriza G, Rubaihayo PR, 1978. Factors that influence outcrossing in soybean. Journal of Agronomy and Crop Science 147, 129–133.

Gustafson DI, 2008. Sustainable use of glyphosate in North American cropping systems. Pest Management Science 64, 409–416.

Haenke S, Scheid B, Schaefer M, Tscharntke T, Thies C, 2009. Increasing syrphid fly diversity and density in sown flower strips within simple vs. complex landscapes. Journal of Applied Ecology 46, 1106–1114.

Hammond BG, Vicini JL, Hartnell GF, Naylor MW, Knight CD, Robinson EH, Fuchs RL, Padgette SR, 1996. The feeding value of soybeans fed to rats, chickens, catfish and dairy cattle is not altered by genetic incorporation of glyphosate tolerance. Journal of Nutrition 126, 717–727.

Haney RL, Senseman SA, Hons FM, Zuberer DA, 2000. Effect of glyphosate on soil microbial activity and biomass. Weed Science 48, 89–93.

Haney RL, Senseman SA, Hons FM, 2002. Effect of Roundup Ultra on microbial activity and biomass from selection soils. Journal of Environmental Quality 31, 730–735.

Harikrishnan R, Yang XB, 2002. Effects of herbicides on root rot and damping-off caused by *Rhizoctonia solani* in glyphosate-tolerant soybean. Plant Disease 86, 1369–1373.

Harrigan GG, Ridley WP, Riordan SG, Nemeth MA, Sorbet R, Trujillo WA, Breeze ML, Schneider RW, 2007. Chemical composition of glyphosate-tolerant soybean 40–3-2 grown in Europe remains equivalent with that of conventional soybean (*Glycine max* L.). Journal of Agricultural and Food Chemistry 55, 6160–6168.

Harrison LA, Bailey MR. Naylor MW, Ream JE, Hammond BG, Nida DL, Burnette BL, Nickson TE, Mitsky TA, Taylor ML. Fuchs RL, Padgette SR, 1996. The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. The Journal of Nutrition 126, 728–740.

Hart MM, Powell JR, Gulden RH, Dunfield KE, Pauls KP, Swanton CJ, Klironomos JN, Antunes PM, Koch AM, Trevors JT, 2009. Separating the effect of crop from herbicide on soil microbial communities in glyphosate resistant corn. Pedobiologia 52, 253–262.

Hartzler RG, Singer JW, Kohler KA, Buhler DD, 2006. Effect of repeated glyphosate use on weed communities in a soybean–corn rotation. Crop Management, DOI:10.1094/CM-2006–0308–01-RS.

Haughton AJ, Bohan DA, 2008. The impacts of novel management on ecosystem dynamics; tales from the UK Farm Scale Evaluations of GMHT crops. IOBC/wprs Bulletin 33, 7–13.

Hawes C, Haughton AJ, Osborne JL, Roy DB, Clark SJ, Perry JN, Rothery P, Bohan DA, Brooks DR, Champion GT, Dewar AM, Heard MS, Woiwod IP, Daniels RE, Young MW, Parish AM, Scott RJ, Firbank LG, Squire GR, 2003. Responses of plants and invertebrate trophic groups to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. Philosophical Transactions of the Royal Society B: Biological Sciences 358, 1899–1915.

Hawes C, Haughton AJ, Bohan DA, Squire GR, 2009. Functional approaches for assessing plant and invertebrate abundance patterns in arable systems. Basic and Applied Ecology 10, 34–47.

Hawes C, Squire GR, Hallett PD, Watson CA, Young M, 2010. Arable plant communities as indicators of farming practice. Agriculture, Ecosystems & Environment 138, 17–26.

Hawes MC, 1990. Living plant cells released from the root cap: a regulator of microbial populations in the rhizosphere? Plant Soil 129, 19–27.

Heap I, 2012. The international survey of herbicide resistant weeds, http///www.weedscience.com.

Heard MS, Hawes C, Champion GT, Clark SJ, Firbank LG, Haughton AJ, Parish A, Perry JN, Rothery P, Scott RJ, Skellern M, Squire GR, Hill MO, 2003a. Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. 1. Effects on abundance and diversity. Philosophical Transactions of the Royal Society B: Biological Sciences 358, 1819–1832.

Heard MS, Hawes C, Champion GT, Clark SJ, Firbank LG, Haughton AJ, Parish AM, Perry JN, Rothery P, Roy DB, Scott RJ, Skellern MP, Squire GR, Hill MO, 2003b. Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. 2. The effects on individual species. Philosophical Transactions of the Royal Society B: Biological Sciences 358, 1833–1846.

Heard MS, Rothery P, Perry JN, Firbank LG, 2005. Predicting long-term changes in weed populations under GMHT crop management. Weed Research 45, 331–338.

Hendriksma HP, Härtel S, Babendreier D, von der Ohe W, Steffan-Dewenter I, 2012. Effects of multiple Bt proteins and GNA lectin on *in vitro*-reared honey bee larvae. Apidologie, DOI:10.1007/s13592–012–0123–3 (in press).

Hernandez A, Garcia-Plazzola JI, Becerril JM, 1999. Glyphosate effects on phenolic metabolism of nodulated soybean (*Glycine max* L. Merr.). Journal of Agricultural and Food Chemistry 47, 2920–2925.

Hetherington PR, Reynolds TL, Marshall G, Kirkwood RC, 1999. The absorption, translocation and distribution of the herbicide glyphosate in maize expressing the CP-4 transgene. Journal of Experimental Botany 50, 1567–1576.

Hilbeck A, Meier M, Benzler A, 2008. Identifying indicator species for post-release monitoring of genetically modified, herbicide resistant crops. Euphytica 164, 903–912.

Hilgenfeld KL, Martin AR, Mortensen DA, Mason SC, 2004. Weed management in a glyphosate resistant soybean system: weed species shifts. Weed Technology 18, 284–291.

Hjältén J, Lindau A, Wennström A, Blomberg P, Witzell J, Hurry V, Ericson L, 2007. Unintentional changes of defence traits in GM trees can influence plant–herbivore interactions. Basic Applied Ecology 8, 434–443.

Holst N, Rasmussen IA, Bastiaans L, 2007. Field weed population dynamics: a review of model approaches and applications. Weed Research 47, 1–14.

Hough-Goldstein JA, Vangessel J, Wilson AP, 2004. Manipulation of weed communities to enhance ground-dwelling arthropod populations in herbicide-resistant field corn. Environmental Entomology 33, 577–586.

Hülter N, Wackernagel W, 2008. Double illegitimate recombination events integrate DNA segments through two different mechanisms during natural transformation of *Acinetobacter baylyi*. Molecular Microbiology 67, 984–995.

Hurley TM, Mitchell PD, Frisvold GB, 2009. Weed management costs, weed best management practices, and the Roundup Ready® weed management program. AgBioForum 12, 281–290.

Hymowitz T, Singh RJ, Kollipara KP, 1998. The genomes of the *Glycine*. Plant Breeding Reviews 16, 289–317.

ILSI, 2006. International Life Sciences Institute Crop Composition Database Version 3.0. http://www.cropcomposition.org.

Imura O, Shi K, Imura K, Takamizo T, 2010. Assessing the effects of cultivating genetically modified glyphosate-tolerant varieties of soybeans (*Glycine may* (L.) Merr.) on populations of field arthropods. Environmental Biosafety Research 9, 101–112.

Ivany JA, 2004. Comparison of weed control strategies in glyphosate-resistant soybean [*Glycine max* (L.) Merr.] in Atlantic Canada. Canadian Journal of Plant Science 84, 1199–1204.

Jackson RE, Pitre HN, 2004a. Influence of Roundup Ready soybean and Roundup Ultra herbicide on *Geocoris punctipes* (Say) (Heteroptera: Lygaeidae) in the laboratory. Journal of Entomological Science 39, 56–61.

Jackson RE, Pitre HN, 2004b. Influence of Roundup Ready soybean production systems and glyphosate application on pest and beneficial insects in narrow-row soybean. Journal of Entomological Science 39, 62–70.

Jackson RE, Pitre HN, 2004c. Influence of Roundup Ready soybean production systems and glyphosate application on pest and beneficial insects in wide-row soybean. Journal of Agricultural and Urban Entomology 21, 61–70.

Jacob D, Lewin A, Meister B, Appel B, 2002. Plant-specific promoter sequences carry elements that are recognised by the eubacterial transcription machinery. Transgenic Research 11, 291–303.

Jasinski JR, Eisley JB, Young CE, Kovach J, Willson H, 2003. Select nontarget arthropod abundance in transgenic and nontransgenic field crops in Ohio. Environmental Entomology 32, 407–413.

Jaycox ER, 1970. Ecological relationships between honey bees and soybean. American Bee Journal 110, 306–307, 343–345, and 383–385.

Johal GS, Huber DM, 2009. Glyphosate effects on diseases of plants. European Journal of Agronomy 31, 144–152.

Johnson GA, Breitenbach F, Behnken L, Miller R, Hoverstad T, Gunsolus J, 2011. Comparison of herbicide tactics to minimize species shifts and selection pressure in glyphosate-resistant soybean. Weed Technology, DOI:abs/10.1614/WT-D-11–00106.1 (in press).

Johnson KH, 2000. Trophic-dynamic considerations in relating species diversity to ecosystem resilience. Biological Reviews 75, 347–376.

Johnson WG, Gibson KD, 2006. Glyphosate-resistant weeds and resistance management strategies: an Indiana grower perspective. Weed Technology 20, 768–772.

Johnson WG, Gibson KD, Cowley SP, 2007. Does weed size matter? An Indiana grower perspective about weed control timing. Weed Technology 21, 542–546.

Johnson WG, Davis VM, Kruger GR, Weller SC, 2009. Influence of glyphosate-resistant cropping systems on weed species shifts and glyphosate-resistant weed populations. European Journal of Agronomy 31, 162–172.

Jobin B, Choiniere L, Belanger L, 2001. Bird use of three types of field margins in relation to intensive agriculture in Quebec, Canada. Agriculture, Ecosystems & Environment 84, 131–143.

Jonas DA, Elmadfa I, Engel KH, Heller KJ, Kozianowski G, König A, Müller D, Narbonne JF, Wackernagel W, Kleiner J, 2001. Safety considerations of DNA in food. Annals of Nutrition and Metabolism 45, 235–254.

Kawate MK, Kawate SC, Ogg AG, Kraft JM, 1992. Response of *Fusarium solani* f. sp. *pisi* and *Pythium ultimum* to glyphostae. Weed Science 40, 497–502.

Kay E, Vogel TM, Bertolla F, Nalin R, Simonet P, 2002. *In situ* transfer of antibiotic resistance genes from transgenic (transplastomic) tobacco plants to bacteria. Applied and Environmental Microbiology 68, 3345–3351.

Keese P, 2008. Risks from GMOs due to horizontal gene transfer. Environmental Biosafety Research 7, 123–149.

Kikuchi A, Murata K, Tabuchi K, Sakai S, 1993. Inheritance of seed embryo color and investigation of degree of natural cross-pollination in soybeans. Breeding Science 43, 112.

Kim CG, Yi H, Park S, Yeon JE, Kim DY, Kim DI, Lee K-H, Lee TC, Paek IS, Yoon WK, Jeong S-C, Kim HM, 2006. Monitoring the occurrence of genetically modified soybean and maize around cultivated fields and at a grain receiving port in Korea. Journal of Plant Biology 49, 218–298.

King AC, Purcell LC, Vories ED, 2001. Plant growth and nitrogenase activity of glyphosate-tolerant soybean in response to glyphosate applications. Agronomy Journal 93, 179–186.

Kleijn D, Snoeijing GIJ, 1997. Field boundary vegetation and the effects of agrochemical drift: botanical change caused by low levels of herbicide and fertilizer. Journal of Applied Ecology 34, 1413–1425.

Kleijn D, Van der Voort LAC, 1997. Conservation headlands for rare arable weeds: the effects of fertiliser application and light penetration on plant growth. Biological Conservation 81, 57–67.

Kleijn D, Sutherland WJ, 2003. How effective are agrienvironment schemes in maintaining and conserving biodiversity? Journal of Applied Ecology 40, 947–969.

Kleijn D, Baquero RA, Clough Y, Diaz M, De Esteban J, Fernandez F, Gabriel D, Herzog F, Holzschuh A, Johl R, Knop E, Kreuss A, Marshall EJP, Steffan-Dewenter I, Tscharntke T, Verhulst J, West TM, Yela JL, 2006. Mixed biodiversity benefits of agri-environment schemes in five European countries. Ecology letters 9, 243–254.

Kleijn D, Rundlöf M, Scheper J, Smith HG, Tscharntke T, 2011. Does conservation on farmland contribute to halting the biodiversity decline? Trends in Ecology and Evolution 26, 474–481.

Kleter GA, Bhula R, Bodnaruk K, Carazo E, Felsot AS, Harris CA, Katayama A, Kuiper HA, Racke KD, Rubin B, Shevah Y, Stephenson GR, Tanaka K, Unsworth J, Wauchoppe RD, Wong S-S, 2007. Altered pesticide use on transgenic crops and the associated general impact from an environmental perspective. Pest Management Science 63, 1107–1115.

Kleter GA, Harris C, Stephenson G, Unsworth J, 2008. Comparison of herbicide regimes and the associated potential environmental effects of glyphosate-resistant crops versus what they replace in Europe. Pest Management Science 64, 479–488.

Klier C, Grundmann S, Gayler S, Priesack E, 2008. Modelling the environmental fate of the herbicide glyphosate in soil lysimeters. Water, Air and Soil Pollution: Focus 8, 187–207.

Knezevic SZ, Evans SP, Mainz M, 2003. Row spacing influences the critical timing for weed removal in soybean (*Glycine max*). Weed Technology 17, 666–673.

Knezevic SZ, Datta A, Scott J, Klein RN, Golus J, 2009. Problem weed control in glyphosate-resistant soybean with glyphosate tank mixes and soil-applied herbicides. Weed Technology 23, 507–512.

Koennig SR, 2002. Tolerance to *Hoplolaimus Columbus* in glyphosate-resistant, transgenic soybean cultivars. Journal of Nematology 34, 370–373.

Kowalchuk GA, Bruinsma M, Van Veen JA, 2003. Assessing responses of soil microorganisms to GM plants. Trends in Ecology and Evolution 18, 403–410.

Krausz RF, Young BG, 2001. Response of glyphosate-resistant soybean (*Glycine max*) to trimethylsulfonium and isopropylamine salts of glyphosate. Weed Technology 15, 745–749.

Krebs JR, Wilson JD, Bradbury RD, Sirwardena GM, 1999. The second Silent Spring? Nature 400, 611–612.

Kremer RJ, Means NE, Kim SJ, 2005. Glyphosate affects soybean root exudation and rhizosphere microorganisms. International Journal of Analytical Environmental Chemistry 85, 1165–1174.

Kremer RJ, Means NE, 2009. Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. European Journal of Agronomy 31, 153–161.

Kruger GR, Johnson WG, Weller SC, Owen MDK, Shaw DR, Wilcut JW, Jordan DL, Wilson RG, Bernards ML, Young BG, 2009. US grower views on problematic weeds and changes in weed pressure in glyphosate-resistant corn, cotton, and soybean cropping systems. Weed Technology 23, 162–166.

Lancaster SH, Hollister EB, Senseman SA, Gentry TJ, 2009. Effects of repeated glyphosate applications on soil microbial community composition and the mineralization of glyphosate. Pest Management Science 66, 59–64.

Lane M, Lorenz N, Saxena J, Ramsier C, Dick RP, 2011. Microbial activity, community structure and potassium dynamics in rhizosphere soil of soybean plants treated with glyphosate. Pedobiologia, DOI:10.1016/j.pedobi.2011.12.005 (in press).

Lee CD, Penner D, Hammerschmidt R, 2000. Influence of formulated glyphosate and activator adjuvants on *Sclerotinia sclerotiorum* in glyphosate-resistant and susceptible *Glycine max*. Weed Science 48, 710–715.

Lee B, Kim CG, Park JY, Park KW, Kim HJ, Yi H, Jeong CC, Yoon WK, Kim HM, 2009. Monitoring the occurrence of genetically modified soybean and maize in cultivated fields and along the transportation routes of the Incheon Port in South Korea. Food Control 20, 250–254.

Legleiter TR, Bradley KW, Massey RE, 2009. Glyphosate-resistant waterhemp control and economic returns with herbicide treatments in soybean. Weed Technology 23, 54–61.

Leroux GD, Chouinard N, Nadeau M, Buhler S, 2006. Volet 3 – Aspects agroenvironnementaux. 1. Les cultures tolérantes aux herbicides. In: Michaud D et collaborateurs (Eds), *Impact environnemental des cultures transgéniques cultivées au Québec*, Ministère du Développement durable, de l'Environnement et des Parcs du Québec, Québec City, Canada, pp 107–128.

Levy-Booth DJ, Campbell RG, Gulden RH, Hart MM, Powell JR, Klironomos JN, Pauls KP, Swanton CJ, Trevors JT, Dunfield KE, 2007. Cycling of extracellular DNA in the soil environment. Soil Biology & Biochemistry 39, 2977–2991.

Lewin A, Jacob D, Freytag B, Appel B, 1998. Gene expression in bacteria directed by plant-specific regulatory sequences. Transgenic Research 7, 403–411.

Liebman M, Dyck E, 1993. Crop rotation and intercropping strategies for weed management. Ecological Applications 3, 92–122.

Liener IE, Kakade ML, 1980. Protease inhibitors, In: Liener IE (Ed), Toxic Constituents of Plant Foodstuffs, Academic Press, New York, pp 7–57.

Liphadzi KB, Al-Khatib K, Bensch CN, Stahlman PW, Dille JA, Todd T, Rice CW, Horak MJ, Head G, 2005. Soil microbial and nematode communities as affected by glyphosate and tillage practices in a glyphosateresistant cropping system. Weed Science 53, 536–545.

Locke MA, Zablotowicz RM, Reddy KN, 2008. Integrating soil conservation practices and glyphosate-resistant crops: impacts on soil. Pest Management Science 64, 457–469.

Losey JE, Vaughan M, 2006. The economic value of ecological services provided by insects. BioScience 56, 311–323.

Lu BR, 2005. Multidirectional gene flow among wild, weedy, and cultivated soybeans. In: Gressel J (Ed), Crop Ferality and Volunteerism, OECD + Taylor & Francis, pp 137–147.

Lundgren JG, 2009. Relationships of natural enemies and non-prey foods. Springer Science + Business Media B.V.

Lupwayi NZ, Hanson KG, Harker KN, Clayton GW, Blackshaw RE, O'Donovan JT, Johnson EN, Gan Y, Irvine RB, Monreal MA, 2007. Soil microbial biomass, functional diversity and enzyme activity in glyphosate-resistant wheat-canola rotations under low-disturbance direct seeding and conventional tillage. Soil Biology & Biochemistry 39, 1418–1427.

Lupwayi NZ, Harker KN, Clayton GW, O'Donovan JT, Blackshaw RE, 2009. Soil microbial response to herbicides applied to glyphosate-resistant canola. Agriculture, Ecosystems & Environment 129, 171–176.

Lutman PJW, Sweet J, Berry K, Law J, Payne R, Simpson E, Walker K, Wightman P, 2008. Weed control in conventional and herbicide tolerant winter oilseed rape (*Brassica napus*) grown in rotations with winter cereals in the UK. Weed Research 48, 408–419.

Ma BL, Blackshaw RE, Roy J, He T, 2011. Investigation on gene transfer from genetically modified corn (*Zea mays* L.) plants to soil bacteria. Journal of Environmental Science and Health, Part B-Pesticides Food Contaminants and Agricultural Wastes 46, 590–599.

Macfadyen S, Gibson R, Plaszek A, Morris R, Craze P, Planque R, Symondson WOC, Memmott J, 2009. Do differences in food web structure between organic and conventional farms affect the ecosystem service of pest control? Ecology Letters 12, 229–238.

Madsen KH, Jensen JE, 1995. Weed control in glyphosate-tolerant sugar-beet (*Beta vulgaris* L.). Weed Research 35, 105–111.

Mamy L, Barriuso E, Gabrielle B, 2005. Environmental fate of herbicides trifluralin, metazachlor, metamitron and sulcotrione compared with that of glyphosate, a substitute broad spectrum herbicide for different glyphosate-resistant crops. Pest Management Science 61, 905–916.

Mamy L, Gabrielle B, Barriuso E, 2010. Comparative environmental impacts of glyphosate and conventional herbicides when used with glyphosate-tolerant and non-tolerant crops. Environmental Pollution 158, 3172–3178.

Marshall EJP, 1989. Distribution patterns of plants associated with arable field edges. Journal of Applied Ecology 26, 247–257.

Marshall EJP, Moonen AC, 2002. Field margins in northern Europe: their functions and interactions with agriculture. Agriculture, Ecosystems & Environment 89, 5–21.

Marshall EJP, Brown VK, Boatman ND, Lutman PJW, Squire GR, 2001. The impact of herbicides on weed abundance and biodiversity. Defra PN0940. A report for the UK Pesticides Safety Directorate. Bristol: IACR Long Ashton Research Station, http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/PSD/Research_PN0940.pdf.

Marshall EJP, Brown VK, Boatman ND, Lutman PJW, Squire GR, Ward LK, 2003. The role of weeds in supporting biological diversity within crop fields. Weed Research 43, 77–89.

Matson PA, Vitousek PM, 2006. Agricultural intensification: will land spared from farming be land spared for nature? Conservation Biology 20, 709–710.

May MJ, Champion GT, Dewar AM, Qi A, Pidgeon JD, 2005. Management of genetically modified herbicide tolerant sugar beet for spring and autumn environmental benefit. Proceedings of the Royal Society B: Biological Sciences 272, 111–119.

McPherson RM, Johnson WC, Millinix BG, Mills WA, Peeples FS, 2003. Influence of herbicide-tolerant soybean production systems on insect pest populations and pest-induced crop damage. Journal of Economic Entomology 96, 690–698.

Means NE, Kremer RJ, Ramsier C, 2007. Effects of glyphosate and foliar amendments on activity of microorganisms in the soybean rhizosphere. Journal of Environmental Science and Health, Part B-Pesticides Food Contaminants and Agricultural Wastes 42, 125–132.

Meek B, Loxton D, Sparks T, Pywell R, Pickett H, Nowakowski M, 2002. The effect of arable field margin composition on invertebrate biodiversity. Biological Conservation 106, 259–271.

Meissle M, Mouron P, Musa T, Bigler F, Pons X, Vasileiadis VP, Otto S, Antichi D, Kiss J, Pálinkás Z, Dorner Z, van der Weide R, Groten J, Czembor E, Adamczyk J, Thibord JB, Melander B, Cordsen Nielsen G, Poulsen RT, Zimmermann O, Verschwele A, Oldenburg E, 2010. Pests, pesticide use and alternative options in European maize production: current status and future prospects. Journal of Applied Entomology 134, 357–375.

Mercer DK, Melville CM, Scott KP, Flint HJ, 1999a. Natural genetic transformation in the rumen bacterium *Streptococcus bovis* JB1. FEMS Microbiology Letters 179, 485–490.

Mercer DK, Scott KP, Bruce-Johnson WA, Glover LA, Flint HJ, 1999b. Fate of free DNA and transformation of the oral bacterium *Streptococcus gordonii* DL1 by plasmid DNA in human saliva. Applied and Environmental Microbiology 65, 6–10.

Mercer DK, Scott KP, Melville CM, Glover LA, Flint HJ, 2001. Transformation of an oral bacterium via chromosomal integration of free DNA in the presence of human saliva. FEMS Microbiology Letters 200, 163–167.

Mijangos I, Becerril JM, Albizu I, Epelde L, Garbisu C, 2009. Effects of glyphosate on rhizosphere soil microbial communities under two different plant compositions by cultivation-dependent and -independent methodologies. Soil Biology & Biochemistry 41, 505–513.

Mizuguti A, Yoshimura Y, Matsuo K, 2009. Flowering phenologies and natural hybridization of genetically modified and wild soybean under field conditions. Weed Biology and Management 9, 93)96.

Mizuguti A, Ohigashi K, Yoshimura Y, Kaga A, Kuroda Y, Matsuo K, 2010. Hybridization between GM soybean (*Glycine max* (L.) Merr.) and wild soybean (*Glycine soja* Sieb. et Zucc.) under field conditions in Japan. Environmental Biosafety Research 9, 13–23.

Mochi DA, Monteiro AC, Barbosa JC, 2005. Action of pesticides to *Metarhizium anisopliae* in soil. Neotropical Entomology 34, 961–971.

Mönkemeyer W, Schmidt K, Beiβner L, Schiemann J, Wilhelm R, 2006. A critical examination of the potentials of existing German network for GMO-monitoring. Journal of Consumer Protection and Food Safety 1, 67–71

Monsanto, 2010. The agronomic benefits of glyphosate in Europe – review of the benefits of glyphosate per market use, http://www.monsanto.com/products/Documents/glyphosate-background-materials/Agronomic%20benefits%20of%20glyphosate%20in%20Europe.pdf.

Moonen AC, Bàrberi P, 2004. Size and composition of the weed seedbank after 7 years of different cover-crop-maize management systems. Weed Research 44, 163–177.

Moonen AC, Bàrberi P, 2008. Functional biodiversity: An agroecosystem approach. Agriculture, Ecosystems & Environment 127, 7–21.

Moonen AC, Castro Rodas N, Bàrberi P, Petacchi R, 2006. Field margin structure and vegetation composition effects on beneficial insect diversity at farm scale: a case study on an organic farm near Pisa. IOBC/wprs Bulletin 29, 77–80.

Mooney HA, Cropper A, Reid WV, 2005. Confronting the human dilemma. Nature 434, 561–562.

Moorman TB, Becerril JM, Lydon J, Duke SO, 1992. Production of hydroxybenzoic acids by *Bradyrhizobium japonicum* strains treatment with glyphosate. Journal of Agricultural and Food Chemistry 40, 289–293.

Morales CL, Traveset A, 2008. Interspecific pollen transfer: magnitude, prevalence and consequences for plant fitness. Critical Reviews in Plant Science 27, 221–238.

Morjan WE, Pedigo LP, 2002. Suitability of transgenic glyphosate-resistant soybeans to green cloverworm (Lepidoptera: Nuctuidae). Journal of Economic Entomology 95, 1275–1280.

Morjan WE, Pedigo LP, Lewis LC, 2002. Fungicidal effects of glyphosate and glyphosate formulations on four species of entomopathogenic fungi. Environmental Entomology 31, 1206–1212.

Morris SH, 2007. EU biotech crop regulations and environmental risk: a case of the emperor's new clothes? Trends in Biotechnology 25, 2–6.

Motavalli PP, Kremer RJ, Fang M, Means NE, 2004. Impact of genetically modified crops and their management on soil microbially mediated plant nutrient transformations. Journal of Environmental Quality 33, 816–824.

Mueller DS, Nelson RL, Hartman GL, Pedersen WL, 2003. Response of commercially developed soybean cultivars and the ancestral soybean lines to *Fusarium solani* f.sp. *glycines*. Plant Disease 87, 827–831.

Mulugeta D, Boerboom CM, 2000. Critical time of weed removal in glyphosate-resistant *Glycine max*. Weed Science 48, 35–42.

Nakayama Y, Yamaguchi H, 2002. Natural hybridization in wild soybean (*Glycine max* ssp. *soja*) by pollen flow from cultivated soybean (*Glycine max* ssp. *max*) in a designed population. Weed Biology and Management 2, 25–30.

Nazarko OM, Van Acker RC, Entz MH, 2005. Strategies and tactics for herbicide use reduction in field crops in Canada: a review. Canadian Journal of Plant Science 85, 457–479.

Nelson KA, Renner KA, 2001. Soybean growth and development as affected by glyphosate and postemergence herbicide tank mixtures. Agronomy Journal 93, 428–434.

Nelson GC, Bullock DS, 2003. Simulating a relative environmental effect of glyphosate-resistant soybeans. Ecological Economics 45, 189–202.

Neve P, 2008. Simulation modeling to understand the evolution and management of glyphosate resistant weeds. Pest Management Science 64, 392–401.

Neve P, Sadler J, Powles SB, 2004. Multiple herbicide resistance in a glyphosate-resistant rigid ryegrass (*Lolium rigidum*) population. Weed Science 52, 920–928.

Neve P, Diggle AJ, Smith FP, Powles SB, 2003a. Simulating evolution of glyphosate resistance in *Lolium rigidum*. I. Population biology of a rare trait. Weed Research 43, 404–417.

Neve P, Diggle AJ, Smith FP, Powles SB, 2003b. Simulating evolution of glyphosate resistance in *Lolium rigidum*. II. Past, present and future glyphosate use in Australian cropping. Weed Research 43, 418–427.

Nienstedt KM, Brock TCM, van Wensem J, Montforts M, Hart A, Aagaard A, Alix A, Boesten J, Bopp SK, Brown C, Capri E, Forbes V, Köpp H, Liess M, Luttik R, Maltby L, Sousa JP, Streissl F, Hardy AR, 2012. Development of a framework based on an ecosystem services approach for deriving specific protection goals for environmental risk assessment of pesticides. Science of the Total Environment 415, 31–38.

Njiti VN, Schroeder D, Lightfoot DA, 2003. Roundup Ready soybean: glyphosate effects on *Fusarium solani* root colonization and sudden death syndrome. Agronomy Journal 95, 140–1145.

Nordgård L, Nguyen T, Midtvedt T, Benno Y, Traavik T, Nielsen KM, 2007. Lack of detectable uptake of DNA by bacterial gut isolates grown *in vitro* and by *Acinetobacter baylyi* colonizing rodents *in situ*. Environmental Biosafety Research 6, 149–160.

Norris RF, Kogan M, 2000. Interactions between weeds, arthropod pests, and their natural enemies in managed ecosystems. Weed Science 48, 94–158.

Norsworthy JK, Burgos NR, Oliver LR, 2001. Differences in weed tolerance to glyphosate involve different mechanisms. Weed Technology 15, 725–731.

Norsworthy JK, Ward S, Shaw D, Llewellyn R, Nichols R, Webster T, Bradley K, Frisvold G, Powles S, Burgos N, Witt W, Barrett M, 2012. Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Science, DOI:10.1614/WS-D-11–00155.1 (in press).

NRC, 2010. The impact of genetically engineered crops on farm sustainability in the United States. Committee on the Impact of Biotechnology on Farm-Level Economics and Sustainability. National Research Council, Washington DC.

Nurse RA, Hamill AS, Swanton CJ, Tardif FJ, Deen W, Sikkema PH, 2007. Is the application of a residual herbicide required prior to glyphosate application in no-till glyphosate-tolerant soybean (*Glycine max*)? Crop Protection 26, 484–489.

OECD, 2000. Consensus document on the biology of *Glycine max* (L.) *merr.* (soybean). Series on Harmonization of Regulatory Oversight in Biotechnology ENV/JM/MONO(2000)9, No. 15, 1–20, http://www.olis.oecd.org/olis/2000doc.nsf/LinkTo/NT00002C3A/$FILE/00085953.PDF.

OECD, 2001. Consensus document on compositional considerations for new varieties of soybean: key food and feed nutrients and anti-nutrients. Series on the Safety of Novel Foods and Feeds ENV/JM/MONO(2001)15, No. 2, 1–30, http://www.oecd.org/dataoecd/15/60/46815135.pdf.

Oerke EC, 2006. Crop losses to pests. The Journal of Agricultural Science 144, 31–43.

Ortiz-Perez E, Horner HT, Hanlin SJ, Palmer RG, 2006. Insect-mediated seed-set evaluation of 21 soybean lines segregating for male sterility at 10 different loci. Euphytica 152, 351–360.

Ortiz-Perez E, Mian RMA, Cooper RL, Mendiola T, Tew J, Horner HT, Hanlin SJ, Palmer RG, 2008. Seed-set evaluation of four male-sterile, female-fertile soybean lines using alfalfa leafcutting bees and honey bees as pollinators. Journal of Agricultural Science 146, 461–469.

Owen MDK, 2000. Current use of transgenic herbicide-resistant soybean and corn in the USA. Crop Protection 19, 765–771.

Owen MDK, 2005. Maize and soybeans – Controllable volunteerism without ferality? In: Gressel J (Ed), Crop Ferality and Volunteerism, OECD + Taylor & Francis, pp 149–165.

Owen MDK, 2008. Weed species shifts in glyphosate-resistant crops. Pest Management Science 64, 377–387.

Owen MDK, 2011. Weed resistance development and management in herbicide-tolerant crops: experience from the USA. Journal of Consumer Protection and Food Safety 6, 85–89.

Owen MDK, Zelaya IA, 2005. Herbicide-resistant crops and weed resistance to herbicides. Pest Management Science 61, 301–311.

Owen MDK, Pedersen P, De Bruin JL, Stuart K, Lux J, Franzenburg D, Grossnickle D, 2010. Comparisons of genetically modified and non-genetically modified soybean cultivars and weed management systems. Crop Science 50, 2597–2604.

Owen MDK, Young BG, Shaw DR, Wilson RG, Jordan DL, Dixon PM, Weller SC, 2011. Benchmark study on glyphosate-resistant crop systems in the United States. Part 2: Perspectives. Pest Management Science 67, 747–757.

Padgette SR, Taylor NB, Nida DL. Bailey MR. MacDonald J, Holden LR, Fuchs RL, 1996. The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans. Journal of Nutrition 126, 702–716.

Pálinkás Z, Zalai M, Szénási A, Dorner Z, Szekeres D, 2012. Botanical and arthropod diversity in GM HT maize treated with glyphosate or conventional herbicides. IOBC/wprs Bulletin 73, 69–73.

Parker RG, York AC, Jordan DL, 2006. Weed control in glyphosate-resistant corn as affected by preemergence herbicide and timing of postemergence herbicide application. Weed Technology 20, 564–570.

Payne SA, Oliver LR, 2000. Weed control programs in drilled glyphosate-resistant soybean. Weed Technology 14, 413–422.

Perry JN, Rothery P, Clark SJ, Heard MS, Hawes C, 2003. Design, analysis and power of the Farm-Scale Evaluations of genetically modified herbicide tolerant crops. Journal of Applied Ecology 40, 17–31.

Peterson RKD, Hulting AG, 2004. A comparative ecological risk assessment for herbicides used on spring wheat: the effect of glyphosate when used within a glyphosate-tolerant wheat system. Weed Science 52, 834–844.

Petit S, Boursault A, Le Guilloux M, Munier-Jolain N, Reboud X, 2011. Weeds in agricultural landscapes: a review. Agronomy for Sustainable Development 31, 309–317.

Philippot L, Kuffner M, Chèneby D, Depret G, Laguerre G, Martin-Laurent F, 2006. Genetic structure and activity of the nitrate-reducers community in the rhizosphere of different cultivars of maize. Plant Soil 287, 177–186.

Pidgeon JD, May MJ, Perry JN, Poppy GM, 2007. Mitigation of indirect environmental effects of GM crops. Proceedings of the Royal Society B: Biological Sciences 274, 1475–1479.

Pline-Srnic W, 2005. Technical performance of some commercial glyphosate-resistant crops. Pest Management Science 61, 225–234.

Polverari A, Buonaurio R, Guiderdone S, Pezatti M, Marte M, 2000. Ultrastructural observations and DNA degradation analysis of pepper leaves undergoing a hypersensitive reaction to *Xanthomonas campestris* p.v. *vesicatoria*. European Journal of Plant Pathology 106, 423–431.

Powell JR, Swanton CJ, 2008. A critique of studies evaluating glyphosate effects on diseases associated with *Fusarium* spp. Weed Research 48, 307–318.

Powell JR, Gulden RH, Hart MM, Campbell RG, Levy-Booth DJ, Dunfield KE, Pauls KP, Swanton CJ, Trevors JT, Klironomos JN, 2007. Mycorrhizal and rhizobial colonization of genetically modified and conventional soybeans. Applied and Environmental Microbiology 73, 4365–4367.

Powell JR, Campbell RG, Dunfield KE, Gulden RH, Hart MM, Levy-Booth DJ, Klironomos JN, Pauls KP, Swanton CJ, Trevors JT, Antunes PM, 2009a. Effect of glyphosate on the tripartite symbiosis formed by *Glomus intraradices*, *Bradyrhizobium japonicum*, and genetically modified soybean. Applied Soil Ecology 41, 128–136.

Powell JR, Levy-Booth DJ, Gulden RH, Asbil WL, Campbell RG, Dunfield KE, Hamill AS, Hart MM, Lerat S, Nurse RE, Pauls KP, Sikkema PH, Swanton CJ, Trevors JT, Klironomos JN, 2009b. Effects of genetically modified, herbicide-tolerant crops and their management on soil food web properties and crop litter decomposition. Journal of Applied Ecology 46, 388–396.

Powles SB, 2008. Evolved glyphosate-resistant weeds around the world: lessons to be learnt. Pest Management Science 64, 360–365.

Powles SB, 2010. Gene amplification delivers glyphosate-resistant weed evolution. Proceedings of the National Academy of Sciences of the United States of America 107, 955–956.

Powles SB, Yu Q, 2010. Evaluation in action: plants resistant to herbicides. Annual Review of Plant Biology 61, 317–347.

Powles SB, Lorraine-Colwill DF, Dellow JJ, Preston C, 1998. Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidum)* in Australia. Weed Science 46, 604–607.

Pratley J, Urwin N, Stanton R, Baines P, Broster J, Cullis K, Schafer D, Bohn J, Krueger R, 1999. Resistance to glyphosate in *Lolium rigidum*, I: bioevaluation. Weed Science 47, 405–411.

Preston C, 2010. Glyphosate-resistant rigid ryegrass in Australia. In: Nandula VK (Ed), *Glyphosate Resistance in Crops and Weeds: History, Development, and Management*, John Wiley & Sons, Inc., New York, pp 233–247.

Preston C, Wakelin AM, Dolman FC, Bostamam Y, Boutsalis P, 2009. A decade of glyphosate-resistant *Lolium* around the world: mechanisms, genes, fitness and agronomic management. Weed Science 57, 435–441.

Prince JM, Shaw DR, Givens WA, Owen MDK, Weller SC, Young BG, Wilson RL, Jordan DL, 2011a. Benchmark study: III. Survey on changing herbicide use patterns in glyphosate-resistant cropping systems. Weed Technology, DOI:10.1614/WT-D-11-00093.1 (in press).

Prince JM, Shaw DR, Givens WA, Owen MDK, Weller SC, Young BG, Wilson RG, Jordan DL, 2011b. Benchmark study: IV. Survey of grower practices for managing glyphosate-resistant weed populations. Weed Technology, DOI:10.1614/WT-D-11–00094.1 (in press).

Puricelli E, Tuesca D, 2005. Weed density and diversity under glyphosate-resistant crop sequences. Crop Protection 24, 533–542.

Pywell RF, James KL, Herbert I, Meek WR, Carvell C, Bell D, Sparks TH, 2005a. Determinants of overwintering habitat quality for beetles and spiders on arable farmland. Biological Conservation 123, 79–90.

Pywell RF, Warman EA, Carvell C, Sparks TH, Dicks LV, Bennett D,Wright A, Critchley CNR, Sherwood A, 2005b. Providing foraging resources for bumblebees in intensively farmed landscapes. Biological Conservation 121, 479–494.

Qi A, Perry JN, Pidgeon JD, Haylock LA, Brooks DR, 2008. Cost-efficacy in measuring farmland biodiversity – lessons from the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. Annals of Applied Biology 152, 93–101.

Ramessar K, Peremarti A, Gomez Galera S, Naqvi S, Moralejo M, Muñoz P, Capell T, Christou P, 2007. Biosafety and risk assessment framework for selectable marker genes in transgenic crop plants. A case of the science not supporting the politics. Transgenic Research 16, 261–280.

Rapparini G, Geminiani E, Campagna G, 2011. Per il diserbo della soia prevale il post-emergenza. L'Informatore Agrario 12, 79–86.

Ray JD, Kilen TC, Abel AC, Paris RL, 2003. Soybean natural crosspollination rates under field conditions. Environmental Biosafety Research 2, 133–138.

Raybould A, Tuttle A, Shore S, Stone T, 2010. Environmental risk assessment for transgenic crops producing output trait enzymes. Transgenic Research 19, 595–609.

Reddy KN, 2000. Weed control in soybean (*Glycine max*) with cloransulam and diclosulam. Weed Technology 14, 293–297.

Reddy KN, 2001. Glyphosate-resistant soybean as a weed management tool: opportunities and challenges. Weed Biology and Management 1, 193–202.

Reddy KN, Whiting K, 2000.Weed control and economic comparisons of glyphosate-resistant, sulfonylurea-tolerant, and conventional soybean (*Glycine max*) systems. Weed Technology 14, 204–211.

Reddy KN, Zablotowicz RM, 2003. Glyphosate-resistant soybean response to various salts of glyphosate and glyphosate accumulation in soybean nodules. Weed Science 51, 496–502.

Reddy KN, Norsworthy JK, 2010. Glyphosate-resistant crop production systems: impact on weed species shifts. In: Nandula VK (Ed), *Glyphosate Resistance in Crops and Weeds: History, Development, and Management*, John Wiley & Sons, Inc., New York, pp 165–193.

Reddy KN, Hoagland RE, Zablotowicz RM, 2000. Effect of glyphosate on growth, chlorophyll, and nodulation in glyphosate-resistant and susceptible soybean (*Glycine max*) varieties. Journal of New Seeds 2, 37–52.

Reus J, Leendertse P, Bockstaller C, Fomsgaard I, Gutsche V, Lewis K, Nilsson C, Pussemier L, Trevisan M, van der Werf H, Alfarroba F, Blümel S, Isart J, McGrath D, Seppälä T, 2002.

Comparison and evaluation of eight pesticide environmental risk indicators developed in Europe and recommendations for future use. Agriculture, Ecosystems & Environment 90, 177–187.

Reyes SG, 2005. Wet season population abundance of *Micraspis discolor* (Fabr.) (Coleoptera: Coccinellidae) and *Trichomma cnaphalocrosis* Uchida (Hymenoptera: Ichnuemonidae) on three transgenic corn hybrids in two sites in the Philippines. Asian Life Sciences 14, 217–224.

Rizzi A, Pontiroli A, Brusetti L, Borin S, Sorlini C, Abruzzese A, Sacchi GA, Vogel TM, Simonet P, Bazzicalupo M, Nielsen KM, Monier J-M, Daffonchio D, 2008. Strategy for *in situ* detection of natural transformation-based horizontal gene transfer events. Applied Environmental Microbiology 74, 1250–1254.

Rizzi A, Raddadi N, Sorlini C, Nordgård K, Nielsen KM, Daffonchio D, 2012. The stability and degradation of dietary DNA in the gastrointestinal tract of mammals – implications for horizontal gene transfer and the biosafety of GMOs. Critical Reviews in Food Science and Nutrition 52, 142–161.

Robinson RA, Sutherland WJ, 2002. Post-war changes in arable farming and biodiversity in Great Britain. Journal of Applied Ecology 39, 157–176.

Rodriguez E, Fernández-Anero FJ, Ruiz P, Campos M, 2006. Soil arthropod abundance under conventional and no tillage in a Mediterranean climate. Soil & Tillage Research 85, 229–233.

Ronald P, 2011. Plant genetics, sustainable agriculture and global food security. Genetics 188, 11–20.

Rosca II, 2004. Impact of genetically modified herbicide resistant maize on the arthropod fauna. IOBC/wprs Bulletin 27, 143–146.

Roschewitz I, Gabriel D, Tscharntke T, Thies C, 2005. The effect of landscape complexity on arable weed species diversity in organic and conventional farming. Journal of Applied Ecology 42, 873–882.

Salas RA, Dayan FE, Pan Z, Watson SB, Dickson JW, Scott RC, Burgos NR, 2012. *EPSPS* gene amplification in glyphosate-resistant Italian ryegrass (*Lolium perenne* ssp *multiflorum*) from Arkansas, USA. Pest Management Science, DOI:10.1002/ps.3342 (in press).

Salvucci RD, Aulicino M, Hungria M, Balatti PA, 2012. Nodulation capacity of Argentinean (*Glycine max* L. Merr) cultivars inoculated with commercial strains of *Bradyrhizobium japonicum*. American Journal of Plant Science 3, 130–140.

Sammons RD, Heering DC, Dinicola N, Glick H, Elmore GA, 2007. Sustainability and stewardship of glyphosate and glufosinate-resistant crops. Weed Technology 21, 347–354.

Sanden M, Johannessen LE, Berdal KG, Sissener N, Hemre G-I, 2011. Uptake and clearance of dietary DNA in the intestine of Atlantic salmon (*Salmo salar* L.) fed conventional or genetically modified soybeans. Aquaculture Nutrition 17, e750-e759.

Sandermann H, 2006. Plant biotechnology: ecological case studies on herbicide resistance. Trends in Plant Science 11, 324–328.

Sanogo S, Yang XB, Scherm H, 2000. Effects of herbicides on *Fusarium solani* f.sp. *glycines* and development of sudden death syndrome in glyphosate-tolerant soybean. The American Phytopathological society 90, 57–66.

Sanogo S, Yang XB, Lundeen P, 2001. Field response of glyphosate-tolerant soybean to herbicides and sudden death syndrome. Plant Disease 85, 773–779.

Sanvido O, Widmer F, Winzeler M, Bigler F, 2005. A conceptual framework for the design of environmental post-market monitoring of genetically modified plants. Environmental Biosafety Research 4, 13–27.

Sanvido O, Romeis J, Bigler F, 2007. Ecological impacts of genetically modified crops: ten years of field research and commercial cultivation. Advances in Biochemical Engineering / Biotechnology 107, 235–278.

Sanvido O, Romeis J, Bigler F, 2009. An approach for post-market monitoring of potential environmental effects of Bt-maize expressing Cry1Ab on natural enemies. Journal of Applied Entomology 133, 236–248.

Sanvido O, Romeis J, Bigler F, 2011a: Environmental change challenges decision-making during post-market environmental monitoring of transgenic crops. Transgenic Research 20, 1191–2101.

Sanvido O, De Schrijver A, Devos Y, Bartsch D, 2011b. Post market environmental monitoring of genetically modified herbicide tolerant crops. Journal für Kulturpflanzen 63, 211–216.

Sanvido O, Romeis J, Gathmann A, Gielkens M, Raybould A, Bigler F, 2012. Evaluating environmental risks of genetically modified crops – ecological harm criteria for regulatory decision-making. Environmental Science & Policy 15, 82–91.

Sartorato I, Berti A, Zanin G, Dunan CM, 2011. Modeling of glyphosate application timing in glyphosate-resistant soybean. Weed Science 59, 390–397.

Savin C, Purcell LC, Daigh A, Manfredini A, 2009. Response of mycorrhizal infection to glyphosate applications and P fertilization in glyphosate-tolerant soybean, maize, and cotton. Journal of Plant Nutrition 32, 1702–1717.

Schier A, 2006. Field study on the occurrence of ground beetles and spiders in genetically modified, herbicide tolerant corn in conventional and conservation tillage systems. Journal of Plant Diseases and Protection XX, 101–113.

Schmidt K, Wilhelm R, Schmidtke J, Beissner L, Mönkemeyer W, Böttinger P, Sweet J, Schiemann J, 2008. Farm questionnaires for monitoring genetically modified crops: a case study using GM maize. Environmental Biosafety Research 7, 163–179.

Schmidtke J, Schmidt K, 2007. Use of existing network for the general surveillance of GMP? Proposal of a reporting system and central reporting office. Journal of Consumer Protection and Food Safety 2, 79–84.

Screpanti C, Accinelli C, Vicari A, Catizone P, 2005. Glyphosate and glufosinate-ammonium runoff from a corn-growing area in Italy. Agronomy for Sustainable Development 25, 407–412.

Scursoni JA, Satorre EH, 2010. Glyphosate management strategies, weed diversity and soybean yield in Argentina. Crop Protection 29, 957–962.

Scursoni J, Forcella F, Gunsolus J, Owen M, Oliver R, Smeda R, Vidrine R, 2006. Weed diversity and soybean yield with glyphosate management along a north–south transect in the United States. Weed Science 54, 713–719.

Scursoni JA, Forcella F, Gunsolus J, 2007. Weed escapes and delayed weed emergence in glyphosate-resistant soybean. Crop Protection 26, 212–218.

Service RF, 2007. A growing threat down on the farm. Science 316, 1114–1117.

Shaner DL, 2000. The impact of glyphosate-tolerant crops on the use of other herbicides and on resistance management. Pest Management Science 56, 320–326.

Shaner DL, 2010. Testing methods for glyphosate resistance. In: Nandula VK (Ed), *Glyphosate Resistance in Crops and Weeds: History, Development, and Management*, John Wiley & Sons, Inc., New York, pp 93–118.

Shaner DL, Lindenmeyer RB, Ostlie MH, 2012. What have the mechanisms of resistance to glyphosate taught us? Pest Management Science 68, 3–9.

Shaw DR, Arnold JC, 2002. Weed control from herbicide combinations with glyphosate. Weed Technology 16, 1–6.

Shaw DR, Givens WA, Farno LA, Gerard PD, Jordan D, Johnson WG, Weller SC, Young BG, Wilson RG, Owen MDK, 2009. Using a grower survey to assess the benefits and challenges of glyphosate-resistant cropping systems for weed management in US corn, cotton, and soybean. Weed Technology 23, 134–149.

Shaw DR, Owen MDK, Dixon PM, Weller SC, Young BG, Wilson RG, Jordan DL, 2011. Benchmark study on glyphosate-resistant cropping systems in the United States. Part 1: Introduction to 2006–2008. Pest Management Science 67, 747–757.

Shipitalo MJ, Malone RW, Owens LB, 2008. Impact of glyphosate-tolerant soybean and glufosinate-tolerant corn production on herbicide losses in surface runoff. Journal of Environmental Quality 37, 401–408.

Simard MJ, Rouane S, Leroux GD, 2011. Herbicide rate, glyphosate/glufosinate sequences and corn/soybean rotation effects on weed seed banks. Weed Science 59, 398–403.

Sissener NH, Sanden M, Bakke AM, Krogdahl Å, Hemre G-I, 2009a. A long term trial with Atlantic salmon (*Salmo salar* L.) fed genetically modified soy; focusing general health and performance before, during and after the parr-smolt transformation. Aquaculture 294, 108–117.

Sissener NH, Bakke AM, Gu J, Penn MH, Eie E, Krogdahl Å, Sanden M, Hemre G-I, 2009b. An assessment of organ and intestinal histomorphology and cellular stress response in Atlantic salmon (*Salmo salar* L.) fed genetically modified Roundup Ready® soy. Aquaculture 298, 101–110.

Sissener NH, Martin SAM, Cash P, Hevrøy EM, Sanden M, Hemre G-I, 2010. Proteomic profiling of liver from Atlantic salmon (*Salmo salar*) fed genetically modified soy compared to the near-isogenic non-GM line. Marine Biotechnology 12, 273–281.

Smit E, Bakker PAHM, Bergmans H, Bloem J, Griffiths BS, Rutgers M, Sanvido O, Singh BK, van Veen H, Wilhelm R, Glandorf DCM, 2012. General surveillance of the soil ecosystem: An approach to monitoring unexpected adverse effects of GMO's. Ecological Indicators 14, 107–113.

Smith J, Potts SG, Woodcock BA, Eggleton P, 2008a. Can arable field margins be managed to enhance their biodiversity, conservation and functional value for soil macrofauna? Journal of Applied Ecology 45, 269–278.

Smith V, Bohan DA, Clark SJ, Haughton AJ, Bell JR, Heard MS, 2008b. Weed and invertebrate community composition in arable farmland. Arthropod-Plant Interactions 2, 21–30.

Solomon KR, Thompson DG, 2003. Ecological risk assessment for aquatic organisms from over-water uses of glyphosate. Journal of Toxicology and Environmental Health, Part B: Critical Reviews 6, 289–324.

Sosnoskie LM, Herms CP, Cardina J, 2006. Weed seedbank community composition in a 35-yr-old tillage and rotation experiment. Weed Science 54, 263–273.

Sosnoskie LM, Herms CP, Cardina J, Webster TM, 2009. Seedbank and emerged weed communities following adoption of glyphosate-resistant crops in a long-term tillage and rotation study. Weed Science 57, 261–270.

Sotherton, 1991. Conservation headlands, a practical combination of intensive cereal farming and conservation. In: Firbank LG, Carter N, Darbyshire JF, Potts GR (Eds), *The Ecology of Temperate Cereal Fields. Blackwell Scientific Publications*, Oxford, pp 373–397.

Soukup J, Jursík M, Nováková K, Laksarová M, Holec J, 2008. Differences in sensitivity to glyphosate among weed species – implication for weed control in HT maize. Journal of Plant Diseases and Protection XXI, 51–56.

Squire GR, Rodger S, Wright G, 2000. Community-scale seedbank response to less-intense rotation and reduced herbicide input at three sites. Annals of Applied Biology 136, 47–57.

Squire GR, Brooks DR, Bohan DA, Champion GT, Daniels RE, Haughton AJ, Hawes C, Heard MS, Hill MO, May MJ, Osborne JL, Perry JN, Roy DB, Woiwod IP, Firbank LG, 2003. On the rationale and interpretation of the farm-scale evaluations of genetically-modified herbicide-tolerant crops. Philosophical Transactions of the Royal Society B: Biological Sciences 358, 1779–1800.

Squire GR, Hawes C, Begg GS, Young MW, 2009. Cumulative impact of GM herbicide-tolerant cropping on arable plants assessed through species-based and functional taxonomies. Environmental Science and Pollution Research 16, 85–94.

Stadnik J, Karwowska M, Dolatowski ZJ, Swiatkiewicz S, Kwiatek K, 2011a. Effect of genetically modified insect resistant corn (MON 810) and glyphosate tolerant soybean meal (Roundup Ready) on physic-chemical properties of broilers breast and thigh muscles. Bulletin of the Veterinary Institute in Pulawy 55, 541–546.

Stadnik J, Karwowska M, Dolatowski ZJ, Swiatkiewicz M, Kwiatek K, 2011b. Effect of genetically modified feeds on physico-chemical properties of pork. Annals of Animal Science 11, 597–606.

Stewart CL, Nurse RE, Hamill AS, Sikkema PH, 2010. Environment and soil conditions influence pre- and postemergence herbicide efficacy in soybean. Weed Technology 24, 234–243.

Stewart CL, Nurse RE, Van Eerd LL, Vyn RJ, Sikkema PH, 2011. Weed control, environmental impact, and economics of weed management strategies in glyphosate-resistant soybean. Weed Technology 25, 535–541.

Stoate C, Boatman ND, Borralho RJ, Carvalho CR, De Snoo GR, Eden P, 2001. Ecological impacts of arable intensification in Europe. Journal of Environmental Management 63, 337–365.

Storkey J, 2006. A functional group approach to the management of UK arable weeds to support biological diversity. Weed Research 46, 513–522.

Storkey J, Cussans JW, 2007. Reconciling the conservation of in-field biodiversity with crop production using a simulation model of weed growth and competition. Agriculture, Ecosystems & Environment 122, 173–182.

Storkey J, Westbury DB, 2007. Managing arable weeds for biodiversity. Pest Management Science 63, 517–523.

Storkey J, Bohan DA, Haughton AJ, Champion GT, Perry JN, Poppy GM, Woiwod IP, 2008. Providing the evidence base for environmental risk assessments of novel farm management practices. Environmental Science & Policy 11, 579–587.

Storkey J, Meyer S, Still KS, Leuschner C, 2012. The impact of agricultural intensification and land-use change on the European arable flora. Proceedings of the Royal Society B: Biological Sciences 279, 1421–1429.

Stotzky G, 2004. Persistence and biological activity in soil of the insecticidal proteins from *Bacillus thuringiensis*, especially from transgenic plants. Plant and Soil 266, 77–89.

Strandberg B, Pedersen MB, 2002 Biodiversity in glyphosate tolerant fodder beet fields–timing of herbicide application. NERI Technical Report no. 410. Silkeborg, Denmark: National Environmental Research Institute, http://www2.dmu.dk/1_viden/2_Publikationer/3_fagrapporter/rapporter/FR410.pdf.

Streloke M, 2011. Risk assessment and management of herbicides: obligations of the new EU regulations. Journal of Consumer Protection and Food Safety 6, 55–59.

Struger J, Thompson D, Staznik B, Martin P, McDaniel T, Marvin C, 2008. Occurrence of glyphosate in surface waters of Southern Ontario. Bulletin of Environmental Contamination and Toxicology 80, 378–384.

Suharman I, Satoh S, Haga Y, Takeuchi T, Endo M, Hirono I, Aoki T, 2009. Utilization of genetically modified soybean meal in Nile tilapia Oreochromis niloticus diets. Fisheries Science 75, 967–973.

Suharman I, Satoh S, Haga Y, Takeuchi T, Hirono I, Aoki T, 2010. Suitability of genetically modified soybean meal in a dietary ingredient for common carp *Cyprinus carpio*. Fisheries Science 76, 111–117.

Sutherland WJ, Adams WM, Aronson RB, Aveling R, Blackburn TM, Broad S, Ceballos G, Côté IM, Cowling RM, Da Fonseca AB, Dinerstein E, Ferraro J, Fleishman E, Gascon C, Hunter Jr. M, Hutton J, Kareiva P, Kuria A, MacDonald DW, MacKinnon K, Madgwick FJ, Mascia MB, McNeely J, Milner-Gulland EJ, Moon S, Morley CG, Nelson S, Osborn D, Pai M, Parsons ECM, Peck LS, Possingham H, Prior SV, Pullin AS, Rands MRW, Ranganathan J, Redford KH, Rodriguez JP, Seymour F, Sobel J, Sodhi NS, Stott A, Vance-Borland K, Watkinson AR, 2009. One hundred questions of importance to the conservation of global biological diversity. Conservation Biology 23, 557–567.

Swanton CJ, Shrestha A, Chandler K, Deen W, 2000. An economic assessment of weed control strategies in no-till glyphosate-resistant soybean (*Glycine max*). Weed Technology 14, 755–763.

Sweet J, Simpson E, Law J, Lutman P, Berry K, Payne R, Champion G, May M, Walker K, Wightman P, Lainsbury M, 2004. Botanical and Rotational Implications of Genetically Modified Herbicide Tolerance (BRIGHT) HGCA Project Report 353, 265.

Swiatkiewicz, S., Swiatkiewicz M, Koreleski J, Kwiatek K, 2010a. Nutritional efficiency of genetically modified insect resistant corn (MON 810) and glyphosate-tolerant soybean meal (Roundup Ready) for broilers. Bulletin of the Veterinary Institute in Pulawy 54, 43–48.

Swiatkiewicz S, Koreleski J, Arczewska J, Twardowska M, Kwiatek K, Tomczyk G, Kozaczynski W, Mazur M, Bednarek D, 2010b. Safety of transgenic feed materials in poultry nutrition – results of Polish study. Zycie Weterynaryjne 85, 161–165.

Swiatkiewicz M, Hanczakowska ME, Twardowska Mazur MM, Kwiatek K, Kozaczynski W, Swiatkiewicz S, Sieradzki Z, 2011. Effect of genetically modified feeds on fattening results and transfer of transgenic DNA to swine. Bulletin of the Veterinary Institute in Pulawy 55, 121–125.

Szekeres D, Kádár F, Dorner Z, 2008. Ground beetle (Coleoptera: Carabidae) in transgenic herbicide tolerant maize hybrids: impact of the transgenic crops or the weed control practice? IOBC/wprs Bulletin 33, 105–110.

Taylor NB, Fuchs RL, MacDonald J, Shariff AR, Padgette SR, 1999. Compositional analysis of glyphosate-tolerant soybeans treated with glyphosate. Journal of Agricultural and Food Chemistry 47, 4469–4473.

Taylor RL, Maxwell BD, Boik RJ, 2006. Indirect effects of herbicides on bird food resources and beneficial arthropods. Agriculture, Ecosystems & Environment 116, 157–164.

Teshima R, Akiyama H, Okunuki H, Sakushima J. Goda Y, Onodera H, Sawada J, Toyoda M, 2000. Effect of gm and non-gm soybeans on the immune system of BN rats and B10A mice. Journal of the Food Hygienic Society of Japan 41, 188–193.

Thieme T, 2010. Impact of Roundup Ready® maize production systems on NTO's 'North Europe', http://www.slideshare.net/smamu/t-thieme.

Thomas CFG, Marshall EJP, 1999. Arthropod abundance and diversity in differently vegetated margins in arable fields. Agriculture, Ecosystems & Environment 72, 131–144.

Thomas CFG, Parkinson L, Griffiths GJK, Fernandez A, Marshall EJP, 2001. Aggregation and temporal stability of carabid beetle distributions in field and hedgerow habitats. Journal of Applied Ecology 38, 100–116.

Tingle CH, Chandler JM, 2004. The effect of herbicides and crop rotation on weed control in glyphosate-resistant crops. Weed Technology 18, 940–946.

Tooley J, Brust G, 2002. Weed seed predation by carabid beetles. In: Holland JM (Ed), *The Agroecology of Carabid Beetles*, The Game Conservancy Trust, Fordingbridge, UK, pp 215–228.

Townsend JP, Bøhn T, Nielsen KM, 2012. Assessing the probability of detection of horizontal gene transfer events in bacterial populations. Frontiers in Antimicrobials, Resistance and Chemotherapy, DOI:10.3389/fmicb.2012.00027 (in press).

Tuesca D, Puricelli E, Papa JC, 2001. A long term study of weed flora shifts in different tillage systems. Weed Research 41, 369–382.

Ulber L, Nordmeyer H, Zwerger P, 2012. Resistance risk assessment within herbicide authorisation – a call for sensitivity data. Pest Management Science, DOI:10.1002/ps.3322 (in press).

USDA-ISO, 2006. U.S.A. Department of Agriculture, Agricultural Research Service. USDA Iowa State University Database on the isoflavone content of foods, Release 1.3, Nutrient Data Laboratory Website http://www.ars.usda.gov/ba/bhnrc/ndl.

Van Acker RC, Swanton CJ, Wiese SF, 1993. The critical period of weed control in soybean (*Glycine max*). Weed Science 41, 194–200.

van den Eede G, Aarts H, Bukh HJ, Corthier G, Flint HJ, Hammes W, Jacobsen B, Midtvedt T, van der Vossen J, von Wright A, Wackernagel W, Wilcks A, 2004. The relevance of gene transfer to the safety of food and feed derived from genetically modified (GM) plants. Food and Chemical Toxicology 42, 1127–1156.

van der Werf HMC, 1996. Assessing the impact of pesticides on the environment. Agriculture, Ecosystems & Environment 60, 81–96.

Vangessel MJ, Ayeni AO, Majek BA, 2000. Optimum glyphosate timing with and without residual herbicides in glyphosate-resistant soybean (*Glycine max*) under full-season conventional management. Weed Technology 14, 140–149.

Vasileiadis VP, Froud-Williams RJ, Eleftherohorinos IG, 2007. Vertical distribution, size and composition of the weed seedbank under various tillage and herbicide treatments in a sequence of industrial crops. Weed Research 47, 222–230.

Vencill W, Nichols RL, Webster, Soteres J, Mallory-Smith C, Burgos NR, Johnson WG, McClelland MR, 2012. Herbicide resistance: Toward an understanding of resistance development and the impact of herbicide-resistant crops. Weed Science, DOI:10.1614/WS-D-11–00206.1 (in press).

Vereecken H, 2005. Mobility and leaching of glyphosate: a review. Pest Management Science 61, 1139–1151.

Verschwele A, 2011. Is there a weed shift in Roundup Ready maize? Journal für Kulturpflanzen 63, 203–210.

Verschwele A, Mülleder N, 2008. Investigations on weed infestation in the multi-year cultivation of glyphosate-resistant maize. Journal of Plant Diseases and Protection XXI, 57–62.

Vickery JA, Feber RE, Fuller RJ, 2009. Arable field margins managed for biodiversity conservation: a review of food resource provision for farmland birds. Agriculture, Ecosystems & Environment 133, 1–13.

Vidal RA, Trezzi MM, De Prado R, Ruiz-Santaella JP, Vila-Aiub M, 2007. Glyphosate resistant biotypes of wild poinsettia (*Euphorbia heterophylla* L.) and its risk analysis on glyphosate-tolerant soybeans. Journal of Food, Agriculture & Environment 5, 265–269.

Vila-Aiub MM, Balbi MC, Distéfano AJ, Fernández L, Hopp E, Yu Q, Powles SB, 2012. Glyphosate resistance in perennial *Sorghum halepense* (Johnsongrass), endowed by reduced glyphosate translocation and leaf uptake. Pest Management Science 68, 430–436.

Vitta JI, Tuesca D, Puricelli E, 2004. Widespread use of glyphosate tolerant soybean and weed community richness in Argentina. Agriculture, Ecosystems & Environment 103, 621–624.

Wait JD, Johnson WG, Massey RE, 1999. Weed management with reduced rates of glyphosate in no-till, narrow-row, glyphosate-resistant soybean (*Glycine max*). Weed Technology 13, 478–483.

Walker KJ, Critchley CNR, Sherwood AJ, Large R, Nuttall P, Hulmes S, Rose R, Mountford JO, 2007. The conservation of arable plants on cereal field margins: an assessment of new agri-environment scheme options in England, UK. Biological Conservation 136, 260–270.

Waltz E, 2010. Glyphosate resistance threatens Roundup hegemony. Nature Biotechnology 28, 537–538.

Wardle DA, Parkinson D, 1990. Influence of the herbicide glyphosate on soil microbial community structure. Plant and Soil 122, 29–37.

Wardle DA, Parkinson D, 1992. Influence of the herbicides 2,4-D and glyphosate on soil microbial biomass and activity: A field experiment. Soil Biology & Biochemistry 24, 185–186.

Watkinson AR, Freckleton RP, Robinson RA, Sutherland WJ, 2000. Predictions of biodiversity response to genetically modified herbicide-tolerant crops. Science 289, 1554–1557.

Wauchope RD, Estes TL, Allen R, Baker JL, Hornsby AG, Jones RL, Richards RP, Gustafson DI, 2002. Predicted impact of transgenic, herbicide-tolerant corn on drinking water quality in vulnerable watersheds of the mid-western USA. Pest Management Science 58, 146–160.

Weaver MA, Krutz LJ, Zablotowicz RM, Reddy KN, 2007. Effects of glyphosate on soil microbial communities and its mineralization in a Mississippi soil. Pest Management Science 63, 388–393.

Weber CR, Hanson WD, 1961. Natural hybridization with and without ionizing radiation in soybeans. Crop Science 1, 389–392.

Webster EA, Tilston EL, Chudek JA, Hopkins DW, 2008. Decomposition in soil and chemical characteristics of pollen. European Journal of Soil Science 59, 551–558.

Webster TM, Sosnoskie LM, 2010. Loss of glyphosate efficacy: a changing weed spectrum in Georgia cotton. Weed Science 58, 73–79.

Werth JA, Preston C, Taylor IN, Charles GW, Roberts GN, Baker J, 2008. Managing the risk of glyphosate resistance in Australian glyphosate-resistant cotton production systems. Pest Management Science 64, 417–421.

Werth J, Walker S, Boucher L, Robinson G, 2010. Applying the double knock technique to control *Conyza bonariensis*. Weed Biology and Management 10, 1–8.

Westerman PR, Liebman M, Menalled F, Heggenstaller AH, Hartzler RG, Dixon PM, 2005. Are many little hammers effective? Velvetleaf (*Abutilon theophrasti*) population dynamics in two and four year crop rotation systems. Weed Science 53, 382–392.

Westra P, Wilson RG, Miller SD, Stahlman PW, Wicks GW, Chapman PL, Withrow J, Legg D, Alford C, Gaines TA, 2008. Weed population dynamics after six years under glyphosate and conventional herbicide-based weed control. Crop Science 48, 1170–1177.

Whittingham MJ, 2011. The future of agri-environment schemes: biodiversity gains and ecosystem delivery? Journal of Applied Ecology 48, 509–513.

Widmer F, Seidler RJ, Donegan KK, Reed GL, 1997. Quantification of transgenic plant marker gene persistence in the field. Molecular Ecology 6, 1–7.

Wiesbrook ML, Johnson WG, Hart SE, Bradley PR, Wax LM, 2001. Comparison of weed management systems in narrow-row, glyphosate-resistant and glufosinate-resistant soybean (*Glycine max)*. Weed Technology 15, 122–128.

Wilhelm R, Sanvido O, Castanera P, Schmidt K, Schiemann J, 2010. Monitoring the commercial cultivation of *Bt* maize in Europe – conclusions and recommendations for future monitoring practice. Environmental Biosafety Research 8, 219–225.

Wilkinson MJ, Sweet J, Poppy GM, 2003. Risk assessment of GM plants: avoiding gridlock? Trends in Plant Sciences 8, 208–212.

Wilson PJ, Aebischer NJ, 1995. The distribution of dicotyledonous arable weeds in relation to distance from the field edge. Journal of Applied Ecology 32, 295–310.

Wilson RG, Miller SD, Westra P, Kniss AR, Stahlman PW, Wicks GW, Kachman SD, 2007. Glyphosate-induced weed shifts in glyphosate-resistant corn or a rotation of glyphosate-resistant corn, sugarbeet, and spring wheat. Weed Technology 21, 900–909.

Wilson RG, Young BG, Matthews JL, Weller SC, Johnson WG, Jordan DL, Owen MDW, Dixon PM, Shaw DR, 2011. Benchmark study on glyphosate-resistant cropping systems in the United States. Part 4: Weed management practices and effects on weed populations and soil seedbanks. Pest Management Science 67, 771–780.

Witmer JE, Hough-Goldstein JA, Pesek JD, 2003. Ground-dwelling and foliar arthropods in four cropping systems. Environmental Entomology 32, 366–376.

Yang X, Harrison K, Riedel RM, 2002. Soybean (*Glycine max*) response to glyphosate and soybean cyst nematode (*Hererodera glycines*). Weed Technology 16, 392–399.

Yoshimura Y, 2011. Wind tunnel and field assessment of pollen dispersal in soybean [*Glycine max* (L.) Merr.]. Journal of Plant Research 124, 109–114.

Yoshimura Y, Matsuo K, Yasuda K, 2006. Gene flow from GM glyphosate-tolerant to conventional soybeans under field conditions in Japan. Environmental Biosafety Research 5, 169–173.

Yoshimura Y, Mizuguti A, Matsuo K, 2011. Analysis of the seed dispersal patterns of wild soybean as a reference for vegetation management around genetically modified soybean fields. Weed Biology and Management 11, 210–216.

Young BG, 2006. Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. Weed Technology 20, 301–307.

Young BG, Young JM, Gonzini LC, Hart SE, Wax LM, Kapusta G 2001. Weed management in narrow- and wide-row glyphosate-resistant soybean (*Glycine max*). Weed Technology 15, 112–121.

Yu Q, Cairns A, Powles S, 2007. Glyphosate, paraquat and ACCase multiple herbicide resistance evolved in a *Lolium rigidum* biotype. Planta 225, 499–513.

Zabaloy MC, Gómez E, Garland JL, Gómez MA, 2012. Assessment of microbial community function and structure in soil microcosms exposed to glyphosate. Applied Soil Ecology, DOI:10.1016/j.apsoil.2011.12.004 (in press).

Zablotowicz RM, Reddy KN, 2004. Impact of glyphosate on the *Bradyrhizobium japonicum* symbiosis with glyphosate-resistant transgenic soybean: A mini-review. Journal of Environmental Quality 33, 825–831.

Zablotowicz RM, Reddy KN, 2007. Nitrogenase activity, nitrogen content, and yield responses to glyphosate in glyphosate-resistant soybean. Crop Protection 26, 370–376.

Zhou J, Harrigan GG, Berman KH, Webb EG, Klusmeyer TH, Nemeth MA, 2011. Stability in the composition equivalence of grain from insect-protected maize and seed from glyphosate-tolerant soybean to conventional counterparts over multiple seasons, locations, and breeding germplasms. Journal of Agricultural and Food Chemistry 59, 8822–8828.

Zhu Y, Li D, Wang F, Yin J, Jin H, 2004. Nutritional assessment and fate of DANN of soybean meal from Roundup Ready or conventional soybeans using rats. Archives of Animal Nutrition 58, 295–310.

Zobiole LHS, Oliveira Jr RS, Kremer RJ, Constantin J, Yamada T, Castro C, Oliveira FA, Oliveira Jr A, 2010a. Effects of glyphosate on symbiotic N_2 fixation and nickel concentration in glyphosate-resistant soybeans. Applied Soil Ecology 44, 176–180.

Zobiole LHS, de Oliveira Jr RS, Huber DM, Constantin J, de Castro C, de Oliveira FA, de Oliveira Jr A, 2010b. Glyphosate reduces shoot concentrations of mineral nutrients in glyphosate-resistant soybeans. Plant Soil 328, 57–69.

Zobiole LHS, Kremer RJ, Oliveira Jr RS, Constantin J, 2011. Glyphosate affects micro-organisms in rhizospheres of glyphosate-resistant soybean. Journal of Applied Microbiology 110, 118–127.