SCIENTIFIC OPINION

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[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 placing on the market of genetically modified maize MON 88017 for cultivation. The EFSA GMO Panel considers that maize MON 88017 is unlikely to have any adverse effect on the environment, except for the possible resistance evolution to the Cry3Bb1 protein in coleopteran target pests; resistance evolution may lead to altered pest control practices that may cause adverse environmental effects. The cultivation management of maize MON 88017 could result in environmental harm. The EFSA GMO Panel therefore recommends managing the use of glyphosate on maize MON 88017 within diversified cropping regimes that have similar or reduced environmental impacts compared with conventional maize cultivation. The EFSA GMO Panel recommends the deployment of insect resistance management strategies and case-specific monitoring to address (1) the possible resistance evolution to the Cry3Bb1 protein in coleopteran target pests, (2) changes in botanical diversity within fields due to novel herbicide regimes, and (3) resistance evolution to glyphosate in weeds due to novel herbicide regimes. The EFSA GMO Panel agrees with the general surveillance plan of the applicant, but requests that the proposals made to strengthen general surveillance are implemented. Whilst the scope of this application only covers the cultivation of maize MON 88017, this Scientific Opinion also updates the previous EFSA GMO Panel safety evaluation of the food and feed uses, import and processing of maize MON 88017 and derived products. The EFSA GMO Panel concludes that the information available for maize MON 88017 addresses the scientific comments raised by Member States and that maize MON 88017, as described in this application, is as safe as its conventional counterpart and commercial maize varieties with respect to potential adverse effects on human and animal health. If subjected to appropriate management measures, the cultivation management of maize MON 88017 is unlikely to raise safety concerns for the environment.

[1] On request from the Competent Authority of the Czech Republic for an application (EFSA-GMO-CZ-2008-54) submitted by Monsanto, Question No EFSA-Q-2008-312, adopted on 19 October 2011.

[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. 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 expert Ilona Krypsin-Sorensen, as well as the EFSA staff member Yann Devos for the preparatory work on this Scientific Opinion, and the EFSA staff members Anna Christodoulidou (FF), Yann Devos (ENV) and Zoltán Divéki (MC) for the support provided to the development of this EFSA scientific output.

Suggested citation: EFSA Panel on Genetically Modified Organisms (GMO); 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. EFSA Journal 2011;9(11):2428. [152 pp.] doi:10.2903/j.efsa.2011.2428. Available online: www.efsa.europa.eu/efsajournal

KEY WORDS

GMO, maize (*Zea mays*), MON 88017, insect resistance, herbicide tolerance, *cry3Bb1*, CP4 *epsps*, risk assessment, food and feed safety, environment, environmental safety, food and feed uses, import and processing, cultivation, Regulation (EC) No 1829/2003

SUMMARY

Following the submission of an application (Reference EFSA-GMO-CZ-2008-54) 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 insect resistant and herbicide tolerant genetically modified (GM) maize MON 88017 (Unique identifier MON-88Ø17-3) for cultivation. Whilst the scope of this application only covers the cultivation of maize MON 88017, this Scientific Opinion also updates the previous EFSA GMO Panel safety evaluation of the food and feed uses, import and processing of maize MON 88017 and derived products.

In delivering its Scientific Opinion, the EFSA GMO Panel considered the application EFSA-GMO-CZ-2008-54, additional information supplied by the applicant, scientific comments submitted by Member States, the environmental risk assessment report of the Belgian Competent Authority (BE CA), and relevant scientific publications.

Maize MON 88017 expresses (1) a Cry3Bb1 insecticidal protein, derived from *Bacillus thuringiensis* subsp. *kumamotoensis*, which confers protection against coleopteran target pests belonging to the genus *Diabrotica* such as Western corn rootworm (*Diabrotica virgifera virgifera*), and (2) the enzyme CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS), which is derived from *Rhizobium radiobacter* (formerly named as *Agrobacterium tumefaciens*) strain CP4, and renders maize MON 88017 tolerant to the herbicidal active substance glyphosate.

The EFSA GMO Panel evaluated maize MON 88017 with reference to its intended uses and appropriate principles described in its guidelines for 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 for 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 target proteins. An evaluation of the comparative analyses of composition, agronomic and phenotypic characteristics was undertaken, and the safety of the new proteins, both individually and in combination, 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 establish that maize MON 88017 contains one copy of an intact CP4 *epsps* expression cassette and a *cry3Bb1* cassette in a single locus. No other parts of the plasmid used for transformation are present in the transformed plant. Updated bioinformatic analysis of the open reading frames at the junctions between the inserted DNA and maize genomic DNA did not raise safety concerns. The stability of the inserted DNA and the insect resistance and herbicide tolerance traits was confirmed over several generations. Updated analyses of the levels of newly expressed proteins in various plant parts collected from field trials performed in Europe did not raise safety concerns.

The EFSA GMO Panel compared the composition, agronomic and phenotypic characteristics of maize MON 88017 with its conventional counterpart, assessed all statistical differences identified, and came to the conclusion that maize MON 88017 is compositionally not different from its conventional counterpart, except for the newly expressed Cry3Bb1 and CP4 EPSPS proteins. With the exception of the presence of the newly expressed Cry3Bb1 and CP4 EPSPS proteins, maize MON 88017 is also compositionally and agronomically equivalent to commercial maize varieties. The risk assessment of the newly expressed proteins and the whole crop included an analysis of data from analytical and bioinformatic studies, as well as *in vitro* and *in vivo* studies. The EFSA GMO Panel concludes that maize MON 88017 is as safe as its conventional counterpart and that the overall allergenicity of the whole plant is not changed.

Since the scope of the current application covers cultivation, the environmental risk assessment considered the environmental impact of full-scale commercialisation of maize MON 88017.

The BE CA (including its Biosafety Advisory Council) provided to EFSA its report on the environmental risk assessment of maize MON 88017 (dated 28 September 2010) on 6 October 2010 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 BE 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 maize MON 88017 has no altered agronomic and phenotypic characteristics, except for the specific target pest resistance and herbicide tolerance. The likelihood of unintended environmental effects due to the establishment, survival and spread of maize MON 88017 is considered to be extremely low, and will be no different from that of conventional maize 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 *cry3Bb1* and CP4 *epsps* genes from maize MON 88017 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.

The possible resistance evolution to the Cry3Bb1 protein in coleopteran target pests is identified by the EFSA GMO Panel as a concern associated with the cultivation of maize MON 88017, as resistance evolution may lead to altered pest control practices that may cause adverse environmental effects.

Based on the evidence provided by the applicant and relevant scientific literature on maize MON 88017, the EFSA GMO Panel concludes that there are no indications of adverse effects on non-target organisms due to unintended changes in maize MON 88017, and therefore considers *trait*-specific information appropriate to assess whether maize MON 88017 poses a risk to non-target organisms.

The evidence provided by the applicant indicates that the protein sequences of the Cry3Bb1 protein variants of maize MON 88017, MON 863 and MON 853 are similar, and that the biological activity of these Cry3Bb1 protein variants is equivalent. Therefore, the EFSA GMO Panel considers that information generated to evaluate potential adverse effects on non-target organisms due to the expression of the Cry3Bb1 protein in maize MON 863 or MON 853 can be used to inform the environmental risk assessment of maize MON 88017.

The EFSA GMO Panel concludes that potential adverse effects of maize MON 88017 due to the expression of the Cry3Bb1 protein to non-target terrestrial (plant- and ground-dwelling), soil and aquatic arthropods are expected to be negligible in the context of its proposed uses. Rearrangements of species assemblages at different trophic levels in crop stands are commonly associated with any pest management practice, but the EFSA GMO Panel considers that maize MON 88017 will not cause reductions to natural enemies that are significantly greater from those caused by conventional cultivation where insecticides are used to control corn rootworms. Based on the evidence supplied by the applicant, the EFSA GMO Panel has no reason to believe that maize MON 88017 will adversely affect honeybees. Few studies have assessed the impact of the Cry3Bb1 protein on non-target aquatic arthropods and the fate of the Cry3Bb1 protein in senescent and decaying maize detritus in aquatic environments, but available data indicate it is unlikely that the Cry3Bb1 protein in maize MON 88017 products would cause adverse effects on non-target aquatic arthropods in the context of its proposed uses. In addition, there is no evidence to indicate that maize MON 88017 is likely to cause adverse effects on non-target organisms that are not arthropods in the context of its proposed uses.

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.

On the basis of the data provided by the applicant and those obtained from a literature survey, the likelihood of adverse effects to non-target organisms is foreseen to be very low, and limited to non-target chrysomelid larvae. However, the risk of maize MON 88017 to valued (non-pest) chrysomelid

species in the field is likely to be minimal due to their low occurrence and abundance in maize fields and due to the low likelihood of encountering harmful amounts of pollen from maize MON 88017 in and around maize fields. Moreover, the activity of the Cry3Bb1 protein on adult non-target chrysomelid species is expected to be limited.

The EFSA GMO Panel considers that Cry3Bb1 protein concentrations in decaying plant residues from maize MON 88017 decrease rapidly in soil, indicating that non-target soil organisms are exposed to relatively low Cry3Bb1 protein concentrations within a few months after harvest. There is no evidence for accumulation of the Cry3Bb1 protein on agricultural fields cultivated repeatedly with maize MON 88017 or comparable maize events (e.g., MON 863), despite its potential to bind to surface-active particles. Effects of crops on soil microbial communities, which are especially expected in the rhizosphere or on decaying plant material, depend more on their species, variety or age than whether they are genetically modified. Rearrangements in structural diversity and population abundance of non-target soil organisms occur frequently in the agricultural environment. They are typically associated with several sources of variation, caused by natural variability (e.g., soil heterogeneity, weather conditions) and agricultural practices (e.g., soil tillage, crop rotation, irrigation measures) and are thus not necessarily an indication of environmental harm. The EFSA GMO Panel concludes that potential effects on soil microorganisms and microbial communities, as well as the ecological functions they provide, due to the cultivation of maize MON 88017, if they occur, will be transient and minor, and are likely to be smaller or within the range currently caused by other agronomic and environmental factors.

There are no indications that the expression and biological activity of the Cry3Bb1 and CP4 EPSPS proteins expressed in maize MON 88017 are affected by potential interactions between these two proteins. However, the EFSA GMO Panel took account of crop management in the environmental risk assessment, as interactions between biota may occur under different weed and pest management regimes, irrespective of interactions between the newly expressed proteins.

The EFSA GMO Panel is of the opinion that potential adverse environmental effects of the cultivation of maize MON 88017 are associated with the use of the complementary glyphosate-based herbicide regimes. These potential adverse environmental effects comprise (1) a reduction in farmland biodiversity, (2) changes in botanical diversity due to weed shifts, with the selection of weed communities mostly composed of tolerant species, and (3) the selection of glyphosate resistant weeds. The potential harmful effects could occur at the level of arable weeds, farmland biodiversity, food webs and the ecological functions they provide. The magnitude of these potential adverse environmental effects will depend upon a series of factors, including the specific herbicide and cultivation management applied at the farm level, the crop rotation and the characteristics of receiving environments.

The EFSA GMO Panel considers that the use of glyphosate-based herbicides at recommended field application rates of glyphosate on maize MON 88017 is unlikely to cause adverse effects to soil microbial communities or beneficial functions mediated by them.

The conclusions of the EFSA GMO Panel on the environmental safety of maize MON 88017 are consistent with those of the BE CA. The BE CA concluded that "*based on the information in the application, the additional information received by the applicant, the information found in peer-reviewed studies and the scientific comments raised by the member states, no risks concerning the environment and human and animal health were identified as a result of cultivation of MON 88017, except for potential indirect adverse effects related to the use of glyphosate over the top of the crop*" (see overall conclusions of the environmental risk assessment report of the BE CA). In its evaluation, the BE CA identified potential adverse effects of the herbicide used on maize MON 88017 on the environment, and they considered that "*the use of glyphosate 'over the top of the crop' must not interfere with biological functions of non-target organisms (such as biological control and decomposition)*" (section 2.8 of the environmental risk assessment report of the BE CA). The BE CA

did not consider the evaluation of the potential weed resistance evolution was within their remit, as it should be considered under Regulation (EC) No 1107/2009.

The EFSA GMO Panel considers that the applicant provided conservative predictions on the duration of susceptibility of Western corn rootworm to the Cry3Bb1 protein with a 20 % refuge, though recognises that all modelling exercises are subject to scientific uncertainty, and that caution is recommended when predicting future responses of Western corn rootworm in the EU based on experiences elsewhere, as resistance evolution in target insect pests is dependent upon many factors. Moreover, scientific uncertainty related to the appropriateness of the 'high dose/refuge strategy' in delaying resistance evolution in Western corn rootworm remains. Therefore, the EFSA GMO Panel, while agreeing with the 'high dose/refuge strategy', recommends further research is conducted by the applicant to confirm that the underlying assumptions of this strategy are met for the Western corn rootworm, along with the periodic re-evaluation of the adequacy and efficacy of this insect resistance management strategy.

Since the life cycle of Western com rootworm extends over two consecutive maize growing seasons in the EU, the EFSA GMO Panel considers that areas designed to deliver susceptible Western corn rootworm adults are suitable as refuge only if they have been cropped with non-Cry3Bb1-expressing maize for at least two successive years.

The EFSA GMO Panel advocates the deployment of diversified resistance management strategies, along with more integrated methods to control pests targeted by Bt-crops.

The EFSA GMO Panel conclusions on the potential for target insect resistance evolution and its recommendation to periodically re-evaluate the adequacy of the insect resistance management strategy are consistent with those of the BE CA. "*Given the current knowledge gaps*", the BE CA supported "*the proposed refuge strategy as described in the IRM* [insect resistance management] *plan*", but was of the opinion that "*the IRM plan needs further development and continuous updating taking into account the results of ongoing scientific research*" (see overall conclusions of the environmental risk assessment report of the BE CA).

The EFSA GMO Panel anticipates that the repeated use of glyphosate at recommended application rates on continuous maize MON 88017 and/or other glyphosate tolerant crops grown in rotation may result in reductions in botanical diversity and/or weed density in maize fields to a level that might adversely affect food chains and webs, but not necessarily biological control functions, at the field and landscape level. Such a reduction in 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 on maize MON 88017 is used within diversified cropping regimes that have similar or reduced adverse effects on farmland biodiversity compared with conventional maize cultivation. Possible mitigation measures include protecting adjacent habitats from herbicide drift, (re)introduction and better management of field margins or other 'out of crop' measures, less intense in-crop weed management, and especially rotating crops.

The cultivation of maize MON 88017 in monoculture or in rotation with other glyphosate tolerant crops, in conjunction with the repeated and/or exclusive application of glyphosate-based herbicides will cause changes in weed flora, and will favour the evolution and spread of glyphosate resistant weeds due to the selection pressure exerted by glyphosate. This, in turn, may affect food webs, and the functional value of weed vegetation for organisms of higher trophic levels (reduced functional biodiversity). Under such situations, the EFSA GMO Panel recommends that risk mitigation measures are put in place to delay resistance evolution. 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 post-emergence 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.

With regard to weed resistance management, the BE CA noted that "*a glyphosate resistance management plan was set up* [by the applicant] *in the framework of Directive 91/414/EEC* [which was repealed by Regulation (EC) No 1107/2009 on 14 June 2011] *to address the potential development of resistant weeds*", and "*therefore not reconsidered in his* [its] *evaluation*" (section on monitoring of the environmental risk assessment report of the BE CA).

The EFSA GMO Panel gave its opinion and made 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) the possible resistance evolution to the Cry3Bb1 protein in coleopteran target pests, (2) changes in botanical diversity within fields due to novel herbicide regimes, and (3) resistance evolution to glyphosate in weeds due to novel herbicide regimes. 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 of the applicant (1) to establish farmer questionnaires as a reporting format of any on-farm observations of effects associated with the cultivation of maize MON 88017, (2) to use existing monitoring networks which observe changes in biota and production practices from farm up to regional level to obtain data on environmental impacts in the landscape where maize MON 88017 is cultivated, (3) to review all new scientific, technical and other information pertaining to maize MON 88017, and (4) to develop stewardship programs for the introduction, marketing, management and stewardship of maize MON 88017, but requests that its proposals and those made by the BE CA to strengthen general surveillance are implemented. The EFSA GMO Panel agrees with the reporting intervals and modalities proposed by the applicant.

The evaluation of the BE CA "*was restricted to the scientific quality of the monitoring plans proposed* [by the applicant], *including the IRM* [insect resistance management] *plan and the general surveillance plan*". "*As no risks concerning the environment and human and animal health were identified as a result of cultivation of MON 88017, except for potential indirect adverse effects related to the use of glyphosate over the top of the crop*", the BE CA supported the applicant's view that "*case-specific monitoring is not considered necessary during the cultivation of MON 88017. However, to delay resistance evolution, an insect resistance management plan was provided by the applicant, comprising case-specific monitoring of the baseline susceptibility of Western corn rootworm populations to the Cry3Bb1 protein*". The BE CA considered that "*the importance of the refuge in delaying resistance against MON 88017 should be further investigated*" (section on monitoring of the environmental risk assessment report of the BE CA). "*Given the current knowledge gaps*", the BE CA argued that "*the IRM plan needs further development and continuous updating taken into account the results of ongoing scientific research*" (see overall conclusions of the environmental risk assessment report of the BE CA).

The BE CA considered that "*the applicant should have linked the ERA issue* [potential indirect adverse effects related to the use of glyphosate over the top of the crop] *better to monitoring*", and therefore requested that "*the potential consequences for biological functions of non-target organisms due to the use of glyphosate are better considered in the post-market monitoring plan and that the proposals made in its report are implemented*" (section on overall conclusion of the environmental risk assessment report of the BE CA). While the BE CA stated that "*the risk assessment should have taken the unpredictability of farm management and its consequences for biological functions better into account, e.g. by relating this to monitoring*" (section 2.8 of the environmental risk assessment

report of the BE CA), it did not require case-specific monitoring of impacts of the specific cultivation, management and harvesting techniques associated with the cultivation of maize MON 88017.

The BE CA stated that "*farmer questionnaires are a good tool to detect changes in biological functions, but that the questionnaire should be adapted to cover this issue*" (section 2.8 of the environmental risk assessment report of the BE CA). The BE CA was also of the opinion that the "*current general surveillance plan needs to be adapted to allow identification of unanticipated adverse effects on non-target organisms (see 2.8 and Annex III), and of management regimes that do not have an environmental performance at least as good as current regimes*" (section on monitoring of the environmental risk assessment report of the BE CA).

In conclusion, the EFSA GMO Panel considers that the information available for maize MON 88017 addresses the scientific comments raised by Member States and that maize MON 88017, as described in this application, is as safe as its conventional counterpart and commercial maize varieties with respect to potential adverse effects on human and animal health. The EFSA GMO Panel also concludes that maize MON 88017 is unlikely to have any adverse effect on the environment, except for the possible resistance evolution to the Cry3Bb1 protein in coleopteran target pests; resistance evolution may lead to altered pest control practices that may cause adverse environmental effects. The cultivation management of maize MON 88017 could result in environmental harm. The EFSA GMO Panel therefore recommends managing the use of glyphosate on maize MON 88017 within diversified cropping regimes that have similar or reduced environmental impacts compared with conventional maize cultivation. The EFSA GMO Panel recommends the deployment of insect resistance management strategies and case-specific monitoring to address (1) the possible resistance evolution to the Cry3Bb1 protein in coleopteran target pests, (2) changes in botanical diversity within fields due to novel herbicide regimes, and (3) resistance evolution to glyphosate in weeds due to novel herbicide regimes. If subjected to appropriate management measures, the cultivation management of maize MON 88017 is unlikely to raise safety concerns for the environment.

TABLE OF CONTENTS

BACKGROUND

On 21 April 2008, the European Food Safety Authority (EFSA) received from the Competent Authority of Czech Republic an application (Reference EFSA-GMO-CZ-2008-54) for authorisation of the insect resistant and herbicide tolerant genetically modified (GM) maize MON 88017 (Unique Identifier MON-88Ø17-3), submitted by Monsanto under Regulation (EC) No 1829/2003. The scope of this application covers cultivation of maize MON 88017. Whilst the scope of this application only covers the cultivation of maize MON 88017, this Scientific Opinion also updates the previous EFSA GMO Panel safety evaluation of the food and feed uses, import and processing of maize MON 88017 and derived products (EFSA, 2009a).

After receiving the application EFSA-GMO-CZ-2008-54 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 7 July 2008, EFSA received additional information requested under completeness check (requested on 9 June 2008). On 12 September 2008, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

On 26 August 2008, 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 Belgian Competent Authority (BE CA) to evaluate the initial environmental risk assessment of application EFSA-GMO-CZ-2008-54 for the placing on the market of maize MON 88017 for cultivation. This call was initiated by EFSA on 7 May 2008 and the BE CA gave its conformity on 14 August 2008.

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 (12 December 2009) within which to make their opinion known.

The BE CA asked the applicant for additional information on maize MON 88017 on 10 December 2008, 8 April 2009, 11 September 2009, 26 January 2010, and on 13 September 2010. The applicant provided the requested information on 23 February 2009, 15 June 2009, 17 November 2009, 12 May 2010, and on 17 September 2010, respectively.

The BE CA (including its Biosafety Advisory Council) provided to EFSA its report on the environmental risk assessment of maize MON 88017 (dated 28 September 2010) on 6 October 2010 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 maize MON 88017 for cultivation 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 BE CA; and relevant scientific publications.

The EFSA GMO Panel asked the applicant for additional information on maize MON 88017 on 20 October 2010, 8 March 2011, and on 20 April 2011. The applicant provided the requested information on 04 April 2011 and 23 May 2011, respectively. Additional information was also spontaneously provided by the applicant on 19 January 2010. After receipt and evaluation of the full data package, the EFSA GMO Panel finalised its risk assessment evaluation of maize MON 88017.

In giving its Scientific Opinion on maize MON 88017 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 BE 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 safety of the food and feed uses, import and processing of maize MON 88017 itself or as a component of stacked maize events has been evaluated previously by the EFSA GMO Panel under Regulation (EC) 1829/2003 (EFSA, 2009a,e, 2010a,b). The Commission Decisions 2009/814/EC and 2010/429/EC authorised the placing on the market of products containing, consisting of, or produced from maize MON 88017 and MON 88017 x MON 810 pursuant to Regulation (EC) No 1829/2003, respectively (EC, 2009, 2010).

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific risk assessment of maize MON 88017 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

Maize MON 88017 was developed to provide:

(1) protection against certain coleopteran target pests belonging to the genus *Diabrotica* such as the larvae of Western corn rootworm (*Diabrotica virgifera virgifera*), Northern corn rootworm (*Diabrotica barberi*), Southern corn rootworm (*Diabrotica undecimpunctata howardi*) and Mexican corn rootworm (*Diabrotica virgifera zea*) by the introduction of a part of a *B. thuringiensis* subsp. *kumamotoensis* gene, encoding the insecticidal Cry3Bb1 protein (Donovan et al., 1992; Galitsky et al., 2001; Vaughn et al., 2005; Gray et al., 2007). The mode of action of the Cry3Bb1 protein and other Cry proteins is to bind selectively to specific receptors on the epithelial surface of the midgut of larvae of susceptible insect species, leading to death of larvae through pore formation, cell burst and subsequently septicemia (Broderick et al., 2006, 2009; OECD, 2007; Bravo and Soberón, 2008; Kaiser-Alexnat, 2009; Raymond et al., 2009; Soberón et al., 2009; Sayed et al., 2010; Van Frankenhuyzen et al., 2010; Sanahuja et al., 2011).

At present, the Western corn rootworm is the only species from the corn rootworm complex present in the EU. It has been introduced to the EU from the USA, where it is endemic. It was first detected in Europe in 1992, and has since spread across the continent (Hummel, 2003; Miller et al., 2005; Kiss et al., 2005; Boriani et al., 2006; Ciosi et al., 2008; Gray et al., 2009; Meinke et al., 2009), resulting in well established pest populations in 15 European countries[4]. Western corn rootworm is univoltine species and females lay eggs from mid-summer till autumn, mainly in maize fields where they overwinter, and larvae hatch the following spring. Larvae have limited mobility in the soil and feed on maize roots in their vicinity, thereby decreasing nutrient and water uptake and plant stability. Newly hatched Western corn rootworm larvae begin feeding on fine root hairs and burrowing into root tips. As the larvae grow larger, they feed and tunnel into primary roots (Meinke et al., 2009). The bulk of plant damage is caused by second and third instars, but adults feeding on silk and grains can be particularly damaging in seed and sweet maize production (Tuska et al., 2002). Western corn rootworm is considered a serious threat to agriculture in the EU (FCEC, 2009; Dillen et al., 2010a,b; Wesseler and Fall, 2010), where this pest species is expected to expand further (Hemerik et al., 2004; Moeser and Vidal, 2005).

(2) tolerance to the herbicidal active substance glyphosate by the introduction of a gene coding for the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *R. radiobacter* (formerly named as *A. tumefaciens*) strain CP4 (CP4 EPSPS). Glyphosate is normally phytotoxic to a broad range of plants. Its mode of action occurs by binding to and inactivating the EPSPS protein, which is a 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 maize MON 88017, a gene has been introduced that codes for the expression of the CP4 EPSPS protein, which is insensitive towards inhibition by glyphosate. This protein is similar to the native EPSPS found in wild-type plants, but it is not inactivated by glyphosate thus allowing the crop to be protected from the recommended dosages of glyphosate (Green, 2009; Dill et al., 2010). The CP4 *epsps* gene naturally contains a single point mutation that switches the nucleotide guanine for cytosine, which in turn causes the amino acid alanine to be substituted for glycine (Heck et al., 2005).

Maize MON 88017 was assessed with reference to its intended uses and the appropriate principles described in the EFSA GMO Panel guidelines for the risk assessment of GM plants and derived food

[4] http://extension.entm.purdue.edu/wcr/; http://www.eppo.org/QUARANTINE/Diabrotica_virgifera/diabrotica_virgifera.htm#map-dia; http://w3.mkk.szie.hu/dep/nvtt/wcrnet/wcrnet-2.htm; http://www.iwgo.org/dist_map.htm

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-CZ-2008-54, and also (1) a review of all peer-reviewed scientific literature on maize MON 88017, (2) a report on areas and quantity of production and use in Europe of maize MON 88017 and information on known and estimated human and animal exposure, (3) updated molecular characterisation, including sequence data for the flanking regions, (4) updated information on allergenicity and toxicology, (5) updated information on environmental issues, (6) post-market (environmental) monitoring plan, and (7) the additional information submitted by the applicant in reply to questions from both the EFSA GMO Panel and BE 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 BE 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[5], and have been considered throughout this EFSA GMO Panel Scientific Opinion.

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

Whilst the scope of this application only covers the cultivation of maize MON 88017, this Scientific Opinion also updates the previous EFSA GMO Panel safety evaluation of the food and feed uses, import and processing of maize MON 88017 and derived products (EFSA, 2009a).

3.1.1. Tranformation process and vector constructs

Maize MON 88017 was developed to express a modified Cry3Bb1 protein, derived from *B. thuringiensis* subsp. *kumamotoensis*, providing protection against certain coleopteran target pests, and the CP4 EPSPS protein derived from *A. tumefaciens* (also known as *R. radiobacter*) strain CP4 which provides tolerance to glyphosate-based herbicides.

Maize MON 88017 was produced by *A. tumefaciens*-mediated transformation of immature embryos with the PV-ZMIR39 plasmid[6]. PV-ZMIR39 is part of a binary vector system. The T-DNA contains two expression cassettes, one for CP4 *epsps* and one for *cry3Bb1*. The CP4 *epsps* cassette contains CP4 *epsps* coding sequence joined to a chloroplast transit peptide and driven by the promoter (P-*ract*1) and the first intron (*ract*1 intron) of the rice *actin* 1 gene, and the nopaline synthase terminator sequences (*nos* 3'). The *cry3Bb1* cassette contains *cry3Bb1* coding sequence, joined to the sequence coding for 5' untranslated leader of the wheat chlorophyll a/b-binding protein, and the *ract1* intron, and driven by the enhanced 35S *Cauliflower mosaic virus* promoter (P-e35S) and the terminator of the heat shock protein 17.3 (*tahsp*17 3'). The maize MON 88017 *cry3Bb1* sequence was modified to encode six specific amino acid substitutions with respect to the Cry3Bb1 protein from the wild-type *B. thuringiensis* (subsp. *kumamotoensis*) strain EG4691.

3.1.2. Transgenic constructs in maize MON 88017

Molecular characterisation data established that maize MON 88017 contains one copy of the T-DNA and that vector backbone sequences are absent (EFSA, 2009a)[7]. Bioinformatic analyses indicated that the flanking regions of the insert in maize MON 88017 show a significant level of similarity to maize genomic DNA sequences and that the pre-insertion locus was preserved, except for the deletion of

[5] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2008-312
[6] Technical dossier / Sections C and D1
[7] Technical dossier / Section D2

26 bp and the addition of 20 bp (EFSA, 2009a). These analyses also indicated that the insert lies upstream of a transcribed region potentially coding for a protein with sequence similarity to putative purine permeases (EFSA, 2009a). This was confirmed by an updated bioinformatic analysis[8].

Updated bioinformatic analysis also revealed no biologically relevant similarity to allergens or toxins for any of the putative peptides that might be produced from open reading frames spanning the junction regions.

3.1.3. Information on the expression of the insert

Southern analysis of maize MON 88017 and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations (EFSA, 2009a)[9].

The levels of newly expressed proteins Cry3Bb1 and CP4 EPSPS in various parts of maize MON 88017 were analysed by enzyme-linked immunosorbent assay (ELISA). Samples for analysis were collected from field trials conducted in the USA in 2002 (3 locations), in Argentina in 2003/2004 (4 locations) and in Europe during 2006 (3 locations in Germany and 4 locations in Spain). The analysis of the samples collected from field trials performed in Argentina and USA was previously assessed by the EFSA GMO Panel (EFSA, 2009a). For the European field trials, over season leaf, over season root, over season whole plant, forage root, senescent root, pollen, silk, and grain tissues collected from each plot at all field sites were analysed. The plants were treated with glyphosate-based herbicides.

Results indicate that the levels of the Cry3Bb1 and CP4 EPSPS proteins show a decline in samples collected over the growing season, similar to that reported for maize MON 88017 grown in the USA in 2002. This is also in agreement with the published results of field trials conducted in Germany between 2005-2007 (Nguyen and Jehle, 2009). The ranges of Cry3Bb1 and CP4 EPSPS protein levels in various samples obtained from the European trials at the developmental stages where the expression was the highest are summarised in Table 1, below.

Table 1. Ranges in the levels of the Cry3Bb1 and CP4 EPSPS proteins in various plant parts of maize MON 88017 (µg/g dry weight)

Plant parts	Cry3Bb1	CP4 EPSPS
Leaf (OSL1; V2-4)	220-400	120-300
Root (OSR1; V2-4)	110-300	23-71
Whole plant (OSWP2 for Cry3Bb1; OSWP1 for CP4 EPSPS)	140-330	82-230
Silk	110-220	NA
Pollen	10-19	160-330
Grain	6-15	2.4-2.5

NA: not assayed

The results showed that the means and ranges of Cry3Bb1 and CP4 EPSPS proteins in maize MON 88017 grown in 2006 in Europe were generally lower than those observed in samples collected from maize MON 88017 grown in 2002 in the USA. Variations in protein levels such as those observed are not unexpected in different field trials, and do not pose a safety concern *per se*.

[8] Additional information received on 19/01/2010
[9] Technical dossier / Section D3

3.2. Conclusion

The molecular characterisation data establish that maize MON 88017 contains one copy of an intact CP4 *epsps* expression cassette and a *cry3Bb1* cassette in a single locus. No other parts of the plasmid used for transformation are present in the transformed plant. Bioinformatic analysis of the open reading frames at the junctions between the inserted DNA and maize genomic DNA did not reveal safety concerns. The stability of the inserted DNA and the herbicide tolerance and insect resistance traits was confirmed over several generations. The levels of the Cry3Bb1 and CP4 EPSPS proteins were analysed in European field trials and the results do not raise a safety concern.

4. Comparative analysis

4.1. Evaluation of relevant scientific data

Whilst the scope of this application only covers the cultivation of maize MON 88017, this Scientific Opinion also updates the previous EFSA GMO Panel safety evaluation of the food and feed uses, import and processing of maize MON 88017 and derived products (EFSA, 2009a).

4.1.1. Choice of comparator and production of material for the compositional assessment[10]

Materials for the comparative assessment were produced during field trials in the USA and Argentina, with each trial being carried out during a single season (see also McCann et al., 2007). Supplementary compositional data were obtained from field trials that had been carried out in Europe. Maize MON 88017 was compared with its conventional counterpart (maize LH59XLH198 in the USA field trial and with maize DKC61-24 in the Argentinean field trial, treated with other herbicides than glyphosate). In addition to maize MON 88017 and the conventional counterpart, four commercial maize reference varieties were planted at each test site. Grain and forage from plants were harvested from field trials performed at three replicated locations during a single season in the USA (2002) and four replicated locations in Argentina during the 2003-2004 season, and used in the compositional analysis. In the EU field trial carried out during the 2006 season, maize MON 88017 was compared with conventional counterparts consisting of the varieties designated as maize DKC3945 and DKC5143 in three Northern (Germany) and four Southern (Spain) EU test sites, respectively. In each European test site, three commercial maize reference varieties were planted alongside the GM maize and its conventional counterpart. In these USA, Argentinean, and EU field trials, maize MON 88017 plants were treated with glyphosate. In the frame of application EFSA-GMO-CZ-2005-27 (see EFSA, 2009a), the applicant also provided data on the composition of forage and grain of maize MON 88017 not treated with glyphosate in comparison with conventional counterpart grown at three locations in Germany and at three locations in Spain during the 2007 growing season[11].

4.1.2. Compositional analysis

The data on composition of material from field trials in the USA (2002) and Argentina (2003-2004), were analysed statistically for each individual location and for all locations combined. These data have previously been assessed in the frame of application EFSA-GMO-CZ-2005-27 (EFSA, 2009a). The choice of compounds analysed was in accordance with the recommendations of the OECD Consensus Document on key compositional parameters to be measured in new varieties of maize (OECD, 2002).

The data from proximate and mineral analyses (fat, protein, total carbohydrate, acid detergent fibre (ADF), neutral detergent fibre (NDF), ash, moisture, phosphorus, and calcium) of forage from maize MON 88017 (treated with glyphosate) were compared with compositional data for forage from the conventional counterpart, the commercial maize reference varieties included into the same field trials, and to typical ranges of the analysed constituents in commercial maize varieties reported in the scientific literature and in previous field trials conducted by the applicant. No statistically significant differences between maize MON 88017 and the conventional counterpart were observed in forage from field trials in the USA. In samples of forage from field trials in Argentina, significant differences

[10] Technical dossier / Sections D7.1, D7.2 and D7.3
[11] Additional information received on 06/10/2008

were observed in phosphorus and total fat. However, these differences were detected only in some but not in all locations.

The grains of maize MON 88017 and its conventional counterpart were analysed for the same proximate parameters as forage, and for total dietary fibre (TDF), amino acids (18 amino acids including aromatic amino acids), fatty acids, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), vitamins (vitamin B1, vitamin B2, vitamin B6, folic acid, vitamin E and niacin), and some other secondary metabolites (phytic acid, raffinose, furfural, p-coumaric acid and ferulic acid).

In summary, the compositional analysis of grains from maize MON 88017 occasionally revealed statistically significant differences in the levels of some compounds compared with the conventional counterpart. In most cases, differences were observed within a single site only and were not consistently observed both each year and at each location. The levels of the compounds that significantly differed from the corresponding levels in the conventional counterpart were within the ranges reported for conventional maize, including commercial maize reference varieties tested within the same field trials, scientific literature data for commercial maize varieties, and data on conventional maize from previous field trials. The isoleucine level in grains of maize MON 88017 and the lysine level in grains of the conventional counterpart were found to be above the highest values reported in the commercial maize varieties in field trials in Argentina in 2003-2004. However, they were still within the scientific literature and historical ranges for maize grains. The same was true for lower limits of the ranges of p-coumaric acid, vitamin B1, and zinc in grains of maize MON 88017 and zinc in its conventional counterpart, which fell outside the range of the commercial maize reference varieties tested in the same field trial, but not outside the values reported in scientific literature and/or historic ranges.

In grains from the field trials in the USA in 2002, the level of vitamin B1 was consistently significantly lower in grains of maize MON 88017 than in grains of the corresponding conventional counterpart. The reduced levels were observed at each location. However, in the field trial performed in Argentina in 2003-2004, vitamin B1 levels in grains of maize MON 88017 were significantly different from the ones in the conventional counterpart onlyin two sites out of four; i.e., they were higher in one location and lower in the other. Notably, all vitamin B1 levels in grains of maize MON 88017 and non-GM maize in materials from the three years fell within the range reported in the scientific literature and in the range of historical data obtained from previous field trials provided by the applicant. In addition, grains of maize MON 88017 showed statistically significantly higher content of linoleic acid and a lower content of oleic acid in each location and across locations during the field trial in Argentina. Similar differences were observed in the field trials in the USA in 2002, but not at each location and for linoleic acid also across all locations. Ranges for both fatty acids fell within ranges reported in the scientific literature and historical ranges. In addition, it is reported in literature that the fatty acid composition of maize grains can vary substantially between maize varieties (e.g., Dunlap et al., 1995).

The data on the composition of forage and grain samples from the European field trials carried out in 2006, which have been provided in the current application EFSA-GMO-CZ-2008-54, included the same compositional parameters as in the USA and Argentina, while grain samples had been analysed additionally for provitamin A. In grain from the field trials in Northern Europe (Germany) and Southern Europe in 2006, the vitamin B1 level was statistically significantly lower than the level in the conventional counterpart. Other statistically significant differences in the overall across-site analysis in Southern Europe included lower levels of NDF in forage, and higher potassium and provitamin A in grain. Because of contamination of grain from non-GM maize commercial varieties with transgenic material caused by cross-pollination in the Spanish field trial, these grain samples were replaced with others from six commercial varieties obtained from five different locations in Spain. The abovementioned parameters showing differences in the European field trial in 2006 were within the ranges of reference varieties and scientific literature/database values.

Additional compositional analysis of forage and grain from maize MON 88017 not treated with the target herbicide (glyphosate) and grown in Northern (Germany) and Southern (Spain) Europe in 2007 was provided by the applicant at the EFSA GMO Panel's request during the evaluation of the application EFSA-GMO-CZ-2005-27 (see EFSA, 2009a). The same parameters had been measured as for the 2006 field trials. Statistical analysis of the compositional field trial data from Germany showed significantly different levels between maize MON 88017 and its conventional counterpart for potassium and raffinose at all three field trial sites and for vitamin B1 at two sites. However, the values of these parameters fell within the tolerance intervals defined by the reference maize varieties. Similarly, the statistical analysis of the Spanish data showed significant differences between maize MON 88017 and its conventional counterpart for four analytes at both sites; i.e., for methionine, iron, beta carotene and vitamin B1. Levels of additional twelve analytes differed between maize MON 88017 and the conventional counterpart at one site. However, all these statistically significant differences fell within the tolerance intervals defined by the reference varieties.

The composition of maize MON 88017 is also the subject of two reports published in scientific literature. Both reports focus on selected compositional parameters, namely lignin patterns and fatty acid profiles of root and leaf tissues of maize MON 88017 and conventional counterpart grown at a single field trial site in Germany in 2005. While several differences were found between maize MON 88017 and its conventional counterpart, the authors concluded that these fell within the range of conventional maize varieties (Poerschmann et al., 2008, 2009). The EFSA GMO Panel finds these studies of limited value for the comparative assessment given their small scale.

The EFSA GMO Panel considered the observed compositional differences between maize MON 88017 and its conventional counterpart in the light of the field trial design, the biological variation and the levels of the compounds in commercial maize reference varieties, and concludes that maize MON 88017 is compositionally equivalent to its conventional counterpart and conventional maize, except for the introduced traits and statistically significantly lower vitamin B1 content measured in Spanish and USA field trials. However, the vitamin B1 content of grain obtained from these trials fell within the background ranges.

The EFSA GMO Panel considers that the set of compositional data supplied by the applicant is in compliance with the principles described in its Guidance Document for the risk assessment of GM plants and derived food and feed (EFSA, 2006a, 2011b).

4.1.3. Agronomic traits and GM phenotype[12]

During field trials over several seasons and at different locations, phenotypic and agronomic data related to dormancy and germination, emergence and vegetative growth, reproductive growth, seed retention, and stress (i.e., disease, biotic and abiotic stress responses) were collected. Both in field trials in Spain and Germany, the seeding vigour, early stand count, number of days after planting to 50 % pollen shed and 50 % silking, stay green, ear height (Spanish sites only), ear length (German sites only), plant height, number of dropped ears, number of stalk and root lodged plants, final stand count, grain moisture and yield were measured.

Statistically significant differences between maize MON 88017 and the corresponding conventional counterpart were observed for seedling vigour over two seasons, with a higher average for maize MON 88017 in a single season and a lower in the other season. Two significant differences were found between maize MON 88017 and the conventional counterpart in the German field trial. Maize MON 88017 plants were shorter than the conventional counterpart (254.2 vs. 262.9 cm) and had lower yield (10.3 vs.11.4 tonnes/ha) compared with the conventional counterpart. However, these differences were not observed at all locations. The EFSA GMO Panel also noticed that the other biological characteristics examined were not consistently changed across all locations. In the Spanish field trial, no differences were detected for any of the assessed phenotypic characteristics. The EFSA GMO Panel is of the opinion that the observed differences are not biologically relevant.

[12] Technical dossier / Section D7.4

4.2. Conclusion

Based on the compositional, agronomic and phenotypic analysis of maize MON 88017, the conventional counterpart and commercial maize reference varieties, the EFSA GMO Panel concludes that maize MON 88017, as assessed in this application, is compositionally, agronomically and phenotypically not different from its conventional counterpart, and is equivalent to reference maize varieties, except for the Cry3Bb1 and CP4 EPSPS proteins.

5. Food/Feed safety assessment

5.1. Evaluation of relevant scientific data

Whilst the scope of this application only covers the cultivation of maize MON 88017, this Scientific Opinion also updates the previous EFSA GMO Panel safety evaluation of the food and feed uses, import and processing of maize MON 88017 and derived products (EFSA, 2009a).

5.1.1. Product description and intended use[13]

The genetic modification in maize MON 88017 is intended to improve agronomic performance only and is not intended to influence the nutritional properties, processing characteristics or overall use of maize MON 88017 as a crop (see section 1, above).

5.1.2. Effect of processing[14]

Since maize MON 88017 is compositionally equivalent to conventional maize (section 4.1.2, above), except for the newly expressed proteins (section 3.1.3, above), the characteristics of processed products derived from maize MON 88017 are not expected to be different from conventional maize varieties.

5.1.3. Toxicology[15]

5.1.3.1. Proteins used for safety assessment

Given the low levels of the Cry3Bb1 and CP4 EPSPS proteins in maize MON 88017 and challenges pertaining to the isolation of a sufficient quantity of purified proteins from this maize for safety testing, the applicant used proteins produced in recombinant *Escherichia coli* strains.

The equivalence of the Cry3Bb1 protein produced in maize MON 88017 grains to the one produced in *E. coli* was demonstrated by SDS PAGE, western analysis, MALDI-TOF mass spectrometry, protein glycosylation analysis and insect bioactivity assay.

The equivalence of the CP4 EPSPS protein in maize MON 88017 grains to the one produced in *E. coli* was proved by SDS PAGE followed by western analysis, protein glycosylation analysis, and enzymatic activity assay. The identity of the 45-kDa protein identified in plants as CP4 EPSPS was further corroborated with the aid of MALDI-TOF mass spectrometry and N-terminal sequence analysis.

The EFSA GMO Panel therefore accepts the *E. coli*-derived Cry3Bb1 and CP4 EPSPS proteins as appropriate substitute test materials for the plant Cry3Bb1 and CP4 EPSPS proteins in the safety studies.

5.1.3.2. Toxicological assessment of expressed novel proteins in maize MON 88017

The Cry3Bb1 MON 863 protein variant, which differs only by one amino acid from the Cry3Bb1 MON 88017 protein variant, has been evaluated previously by the EFSA GMO Panel (EFSA,

[13] Technical dossier / Section D7.5
[14] Technical dossier / Section D7.6
[15] Technical dossier / Section D7.8

2004a,b) and has been regarded as safe. Because of the single amino acid substitution in the Cry3Bb1 MON 88017 protein variant, a risk assessment of this protein has been undertaken.

EPSPS enzymes occur in plants, fungi and microorganisms and are thus consumed as part of the normal diet by humans and animals. No adverse effects associated with the intake of these proteins have been identified. Genetically modified crops containing the EPSPS protein from *A. tumefaciens* strain CP4 (CP4 EPSPS) are regarded as being as safe as the respective conventional crops for human and/or animal consumption (ACNFP, 1994; SCP, 1998a,b; EFSA, 2003).

Bioinformatic analysis[16]

Bioinformatic analyses of the amino acid sequences of the Cry3Bb1 and CP4 EPSPS proteins expressed in maize MON 88017 have been carried out using the TOXIN_2009 database and FASTA. These analyses revealed no relevant homology between the test proteins and known toxic proteins.

In vitro digestibility

The stability of the Cry3Bb1 and CP4 EPSPS proteins isolated from recombinant *E. coli* were tested *in vitro* with simulated gastric fluid (SGF). No intact Cry3Bb1 protein (ca. 75 kDa) was detected after incubation in SGF for 15 seconds, whereas lower-weight bands occurred transiently but disappeared after four minutes when the samples were analysed using SDS PAGE and protein staining. Using western analysis, no intact protein was detected after incubation in SGF for 15 seconds.

No degradation products of the CP4 EPSPS protein were observed after 15 seconds of incubation in SGF, as demonstrated by SDS PAGE and protein staining and confirmed by western analysis. In addition, the enzymatic activity of the CP4 EPSPS protein was almost lost at the first time point of sampling; i.e., after two minutes of incubation. Confirmation of rapid degradation of the CP4 EPSPS protein in SGF was also observed in a recently published scientific paper (Shim et al., 2010).

The *in vitro* digestion experiments demonstrated that the Cry3Bb1 and CP4 EPSPS proteins are rapidly degraded under simulated gastric conditions. In addition, the degradation of the Cry3Bb1 protein was also tested using *in vitro* simulated intestinal fluid (SIF). Western analysis showed that this protein was degraded within one minute in SIF. Protease resistant fragments were observed until 24 hours. More than 94 % of the enzymatic activity of the CP4 EPSPS protein incubated in SIF disappeared within 4.5 hours in SIF.

Acute oral toxicity

In single dose toxicity studies with mice, no adverse effects were observed after administration of the Cry3Bb1 and CP4 EPSPS proteins at doses of 1,930 mg/kg body weight and 572 mg/kg body weight, respectively.

The EFSA GMO Panel is of the opinion that the single dose acute oral toxicity study does not add relevant information for the safety assessment of these proteins.

5.1.3.3. Toxicological assessment of new constituents other than proteins

No new metabolites other than the Cry3Bb1 and CP4 EPSPS proteins are produced in maize MON 88017, and no biologically relevant changes in the composition were detected in the comparative compositional analyses.

[16] Additional information received on 19/01/2010

5.1.3.4. Toxicological assessment of the whole GM food/feed[17]

The applicant provided a subchronic (13-week) feeding study with rats using grains of maize MON 88017 as a component of the diet. Groups of 20 male and 20 female rats (Crl:CD(SD) IGS BR) were fed diets containing 11 % or 33 % (w/w) grains from maize MON 88017 treated with glyphosate or 33 % (w/w) grains from the conventional counterpart (maize LH59xLH198). Additional data on the background variation of the measured parameters were obtained from a parallel study with six dietary groups of rats fed diets each of which contained 33 % grains from a conventional reference maize variety (Healy et al., 2008).

Animals were examined twice daily for clinical appearance. Individual body weights and food consumption were recorded weekly. At the end of the experiment, clinical pathological evaluation was performed, including haematology, serum chemistry and urine analysis. In addition, a complete necropsy was carried out including organ weight determinations, macroscopic examinations and histopathology.

There were no statistically significant differences in the mean body weight between the groups and no relevant differences in food consumption.

For haematology parameters, a statistically significantly higher mean absolute neutrophil count was observed only in females fed 33 % maize MON 88017 compared with the conventional counterpart group. There was no difference in the relative neutrophil count between rats fed 33 % maize MON 88017 and the conventional counterpart group. Since the higher mean absolute neutrophil count was within the range of normal variation and there were no differences in related parameters, the observed difference was considered as an incidental finding. There were no findings in serum chemistry parameters, urine analysis, organ weight determinations and microscopic examinations related to feeding rats with diets containing maize MON 88017.

The EFSA GMO Panel concludes that the 13-week feeding study in rats gave no indication of adverse effects.

5.1.4. Allergenicity[18]

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, 2010g, 2011b).

5.1.4.1. Assessment of allergenicity of the newly expressed proteins

Bioinformatics-supported comparisons of the amino acid sequences of the plant-expressed Cry3Bb1 and CP4 EPSPS proteins with sequences of known allergens were performed. Searches using the FASTA algorithm and the allergen database AD_2009 indicated no similarity of the Cry3Bb1 and CP4 EPSPS proteins with known allergenic proteins. In addition, when the criterion of an identical 8-aa contiguous amino acid stretch was applied, the Cry3Bb1 and CP4 EPSPS sequences yielded no positive outcomes.

The studies on degradation of the Cry3Bb1 and CP4 EPSPS proteins in simulated mammalian gastric fluid, which are also relevant for the assessment of potential allergenicity, have been described in section 5.1.3.2 (above). The studies showed that most of the test proteins were degraded by pepsin within seconds.

[17] Technical dossier / Section D7.8.4
[18] Technical dossier / Section D7.9

Based on the available information, the EFSA GMO Panel considers that the newly expressed Cry3Bb1 and CP4 EPSPS proteins in maize MON 88017 are unlikely to be allergenic. This is in line with previous Scientific Opinions on events expressing the Cry3Bb1 (EFSA, 2004a,b) and EPSPS proteins (ACNFP, 1994; SCP, 1998a,b; EFSA, 2003, 2007).

5.1.4.2. Assessment of allergenicity of the whole GM plant

The issue of a potential increased allergenicity of maize MON 88017 does not appear relevant to the EFSA GMO Panel since maize is not considered a common allergenic food. However, rare cases of occupational allergy to maize dust have been reported in the scientific literature (Jeebhay and Quirce, 2007; Bardana, 2008). The EFSA GMO Panel is also aware that few cases of food allergy to maize have been specifically observed in some geographically restricted areas where maize is a common food and that, in the few cases reported, the major maize allergens have been identified.

In the context of the present application, there is no reason to expect an altered pattern of expression of endogenous proteins/potential allergens in the GM maize and thereby significantly change of the overall allergenicity of the whole plant. In addition, given all the available information, the EFSA GMO Panel sees no reason to expect that the use of maize MON 88017 would significantly increase the intake and exposure to maize.

5.1.5. Nutritional assessment of GM food/feed[19]

The applicant has provided a 42-day feeding study with broiler chicken to analyse the nutritional value of grain from the maize MON 88017 treated with glyphosate, the near-isogenic counterpart (LH59xLH198) and five commercial maize varieties. 100 birds per treatment were fed diets containing approximately 55 % (w/w) of maize grains during the first half and 60 % during the second half of the experiment. Weight gain, feed consumption and carcass parameters (weight, weight of carcass parts and compositional analysis of breast and thigh meat) were measured. Out of 56 statistical comparisons performed between the test and the control animals, there were statistically significant differences in feed intake of males, average thigh weight of males and percent drum weight per chill weight of the carcass of males. Although these parameters differed statistically between chickens fed maize MON 88017 and the conventional counterpart, the parameters were in the biological range of the commercial maize varieties.

The outcomes of the broiler feeding study support the conclusion on the compositional analysis summarised above (section 4.1.2, above), stating that grains of maize MON 88017 are compositionally not different to grains of the conventional counterpart and are equivalent to reference maize lines.

5.1.6. Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that maize MON 88017 is less safe or nutritious than its conventional counterpart and other conventional maize varieties. Therefore, and in line with its Guidance Document for the risk assessment of GM plants and derived food and feed (EFSA, 2006a), the EFSA GMO Panel considers that post-market monitoring of the GM food/feed is not necessary.

5.2. Conclusion

The Cry3Bb1 and CP4 EPSPS proteins expressed in maize MON 88017 are degraded in simulated digestive and intestinal fluids, and bioinformatics-supported analyes revealed no homology to known toxic or allergenic proteins. No toxicity of the Cry3Bb1 and CP4 EPSPS proteins was observed in an acute toxicity study with mice where the proteins were administered orally at high doses. A subchronic (90-day) feeding study with rats fed diets containing grains from maize MON 88017 raised no concerns. In addition, a 42-day feeding study with broiler chickens provided evidence that maize

[19] Technical dossier / Section D7.10

MON 88017 is as nutritious as its conventional counterpart and other maize varieties included in the study.

In conclusion, the EFSA GMO Panel considers that maize MON 88017 is as safe and as nutritious as its conventional counterpart and commercial maize varieties, and concludes that this maize and derived products are unlikely to have any adverse effects on human and animal health, in the context of its intended use.

6. Environmental risk assessment and risk management strategies

6.1. Evaluation of relevant scientific data

The scope of application EFSA-GMO-CZ-2008-54 is for cultivation of maize MON 88017. Therefore, the environmental risk assessment is concerned with potential direct and indirect environmental effects of the cultivation and the spread of maize MON 88017 into non-cultivated environments. Since this EFSA GMO Panel Scientific Opinion also updates its previous safety evaluation of the food and feed uses, import and processing of maize MON 88017 and derived products (EFSA, 2009a), indirect exposure through manure and faeces from animals fed maize MON 88017 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 BE CA provided to EFSA its report on the environmental risk assessment of maize MON 88017 (dated 28 September 2010) on 6 October 2010 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 BE 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

6.2.1. Changes in plant fitness due to the genetic modification[20]

A series of field trials with maize MON 88017 was conducted by the applicant across 18 USA corn belt locations over two years (8 locations in 2001[21] and 10 locations in 2002[22]) and across eight representative EU maize growing locations in 2006 (4 locations in Germany and 4 locations in Spain)[23] to compare the agronomic performance and field characteristics of maize MON 88017 with its comparators.

Information on phenotypic and agronomic characteristics of maize MON 88017 and its comparators was generated to compare their growth habit, vegetative vigour and reproduction characters. Several endpoints related to growth habit, vegetative growth, reproduction, and yield and grain characteristics were measured.

A randomised split-plot design with four replications was used in the field study conducted in 2001 and a randomised block design with three replications in the field study of 2002 and 2006. In the field trials performed in the USA, maize MON 88017 and its comparators received the same conventional herbicide treatments, whereas glyphosate-based herbicides were applied on maize MON 88017 in the

[20] Technical dossier / Sections B2, B3, B4, D4, D9.1 and D9.2
[21] Technical dossier / Section D4 / Pages 72-87 / Annex: Rosenbaum et al. (2003)
[22] Technical dossier / Section D4 / Pages 72-87 / Annex: Pester and Woodrun (2003)
[23] Technical dossier / Section D4 / Pages 72-87 / Annex: Martin and De Billot (2008)

EU field trials[24]. In the USA field trials, negative segregants and no conventional maize lines were used as comparators. Since potential unintended changes in the GM plant due to the genetic modification (i.e., transformation process) cannot be completely discounted using only a negative segregant as a comparator (EFSA, 2011a), the EFSA GMO Panel considered that the USA field trials were less appropriate to study phenotypic and agronomic characteristics of maize MON 88017.

The breeding tree provided by the applicant[25] confirmed that the near-isogenic line used in the agronomic and phenotypic field trials in the EU had a comparable genetic background with maize MON 88017.

The EU agronomic and phenotypic field trial data did not show major changes in plant characteristics that indicate altered fitness, persistence and invasiveness of maize MON 88017 plants, though there is a potential for enhanced biomass production under infestation of target pests or when glyphosate-based herbicides are applied. A number of endpoints (i.e., plant height, yield) showed statistically significant differences in the across-location comparisons between maize MON 88017 and its near-isogenic line in the EU. These differences were not consistently observed in each location, and were not considered biologically meaningful with respect to persistence and invasiveness potential. Hence, the range of values for agronomic and phenotypic characteristics was shown to fall within the range of values observed for traditional maize hybrids. 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 maize MON 88017 as compared with its conventional counterpart.

Laboratory experiments, analysing seed dormancy[26] and pollen morphology and viability[27], revealed no relevant differences in seed germination, pollen morphology and pollen viability characteristics between maize MON 88017 and its conventional counterpart.

It is considered very unlikely that the establishment, spread and survival of maize MON 88017 would be increased due to the insect resistance and herbicide tolerance traits. These traits can only be regarded as providing a potential selective advantage to maize MON 88017 under infestation of target pests and/or when glyphosate-based herbicides are applied. Moreover, it is considered very unlikely that maize MON 88017 plants or their progeny will differ from conventional maize 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, below). Maize is highly domesticated and generally unable to survive in the environment without management intervention (Baker, 1974; Bagavathiannan and Van Acker, 2008). The survival of maize is limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens, herbivores and cold climatic conditions (van de Wiel et al., 2011). Maize plants are only winter hardy in European regions with mild winters, and in those situations maize kernels remaining in the field after harvest can germinate, grow, flower, and locally cross-pollinate neighbouring maize plants. The occurrence of maize volunteers was reported in Spain and other European regions (Gruber et al., 2008; Palaudelmàs et al., 2009), but these plants grow weakly and tend to flower asynchronously with the cultivated maize crops in which they occur (Palaudelmàs et al., 2009). While maize MON 88017 volunteers occurring in cultivated areas will be tolerant to glyphosate, they are normally controlled by current agricultural practices, including the use of selective herbicides and/or cultivation techniques (Beckie et al., 2006; Deen et al., 2006)[28]. If maize MON 88017 is rotated with broad leaf crops (such as soybean, oilseed rape, sugar beet, sunflower), potential volunteers can easily be controlled with selective graminicide herbicides. For the rotation of glyphosate tolerant maize and soybean, the control of maize volunteers in soybean has been achieved by the use of herbicide regimes involving a graminicide herbicide(s) and glyphosate-based herbicides in the USA (Deen et al., 2006). The EFSA GMO Panel

[24] Technical dossier / Section D4 / Pages 72-87 / Annex: AF/10289/ME (production plan 06-10-50-01)
[25] Additional information received on 23/02/2009 / Request 4, Pages 30-31
[26] Technical dossier / Section D4 / Pages 87-88 / Annex: Rosenbaum and Horak (2003)
[27] Technical dossier / Section D4 / Pages 88-89 // Additional information received on 15/06/2009 / Request 4 / Page 11 / Annex: Rosenbaum and Pester (2004)
[28] Additional information received on 04/04/2011 / Request 2.4, Page 28

notes that mechanical weed control such as hoeing is the only solution for weed control if maize MON 88017 is rotated with another maize crop (either conventional or tolerant to glyphosate), as effective herbicides cannot be applied without killing the rotational maize crop itself (Davis et al., 2008). Maize MON 88017 volunteers are likely to be controlled by the herbicide programmes applied in glufosinate-ammonium tolerant crops (Feng et al., 2010; Green and Castle, 2010; Green and Duke, 2011).

Note that the possible impact of maize MON 88017 volunteers on the efficiency of the insect resistance management plan is considered in section 6.3.1.3, below.

Despite cultivation for centuries, maize 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 maize MON 88017 and any change in survival (including over-wintering), persistence and invasiveness capacity. Because the general characteristics of maize MON 88017 are unchanged, insect resistance and herbicide tolerance are not likely to provide a selective advantage outside of cultivation in Europe.

Since maize MON 88017 has no altered agronomic and phenotypic characteristics, except for the specific target pest resistance and herbicide tolerance, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects due to the establishment and survival of maize MON 88017 will be no different to that of conventional maize varieties.

The conclusion of the EFSA GMO Panel is consistent with that of the BE CA. The BE CA concluded that "*given (a) the biology of maize (see 1.1), (b) the information that maize line MON 88017 does not exhibit characteristics that would cause it to be more weedy than other maize hybrids (see 1.3.4) and (c) that the traits conferred to MON 88017 are not expected to change the persistence and invasiveness of maize, as maize is incapable of surviving without human assistance under European conditions, it can be concluded that the likelihood of MON 88017 to become more persistent or invasive is negligible*". For the evaluation of the agronomic and phenotypic field trials, the BE CA also focused "*on the study conducted in the EU, as the set-up of both USA field trials was considered less appropriate to study phenotypic and agronomic characteristics of MON 88017 (negative segregants were used as control and no conventional maize lines were used as comparator)*" (sections 1.3.4, 2.1 and 2.2 of the environmental risk assessment report of the BE CA).

6.2.2. Gene transfer

The EFSA GMO Panel evaluated the potential for horizontal and vertical gene flow of maize MON 88017, 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.

6.2.2.1. Plant to bacteria gene transfer and its consequences[29]

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 of plants, which may also include DNA derived from 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 bacteria.

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, 2009b and references therein; Brigulla and Wackernagel, 2010; Ma et al., 2011). The main barriers for horizontal gene transfer from plants to

[29] Technical dossier / Section D6

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 maize MON 88017, 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 gene 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, 2009b). The scope of this application is for cultivation. Therefore, the main exposure to DNA would occur in agricultural soils. Since this EFSA GMO Panel Scientific Opinion also updates its previous safety evaluation of the food and feed uses, import and processing of maize MON 88017 and derived products (EFSA, 2009a), exposure to DNA in gastrointestinal systems is also considered.

It is expected that bacteria in the digestive tract of humans, domesticated animals, and other animals feeding on maize MON 88017 will be exposed to low levels of fragmented products of the ingested DNA, including the recombinant genes (section 5.1.1, above). Genomic DNA is a component of many food and feed products derived from maize, but becomes substantially degraded during food/feed processing, and in the process of digestion in the human or animal gastrointestinal tracts (Jonas et al., 2001; van den Eede et al., 2004; Ramessar et al., 2007). The DNA is increasingly degraded in the digestive tract, so no full-length genes from plants have been detected in the large intestine or in faeces (EFSA, 2009b and references therein). In *in vivo* experiments with broilers fed Bt-maize, the *cry1Ab* gene was degraded to fragments smaller than 500 bp along the digestive tract (Rossi et al., 2005). Similarly, Chambers et al. (2002) fed chickens with GM maize to explore the *in vivo* fate of the bacterial ampicillin resistance gene bla_{TEM} in bacteria and GM maize. The gene was found in the stomach contents, but not in the lower intestine of animals fed GM maize. In case of Roundup Ready maize (event 39T67), the presence of *epsps* genes due to feeding on the GM plant material was reported in soil micro-arthropods, nematodes, macro-arthropods and earthworms within a field where the crop was grown (Gulden et al., 2008; Hart et al., 2009b).

Soil bacteria may also be exposed to extracellular DNA released from plant cells into the soil environment throughout and after the growing season (reviewed by Levy-Booth et al., 2007). During active plant growth, free plant DNA may originate from sloughed off root cap cells (Hawes et al., 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 (de Vries et al., 2003; Webster et al., 2008) and DNA release from decomposing plant residue remaining in agricultural areas after harvest, and which is 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 only a transient component of the total DNA pool in soil (Levy-Booth et al., 2007; Nielsen et al., 2007; Gulden et al., 2008). 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 is degraded shortly after harvest. It can therefore be concluded that the concentration of extracellular DNA fragments (including the *cry3Bb1* and CP4 *epsps* genes of maize MON 88017) in gastrointestinal tracts, soil or other environments is relatively low.

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, 2011; EFSA, 2009b). 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, 2009b; Rizzi et al., 2011). 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., 2011). 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 gene context (i.e., the surrounding/neighbouring sequences) of the recombinant gene(s) in the plant (EFSA, 2009b).

Homologous recombination efficiently facilitates integration of non-mobile, chromosomal DNA fragments into bacterial genomes (see EFSA, 2009b 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 *cry3Bb1* and CP4 *epsps* genes in maize MON 88017 are derived from bacterial genes, and theoretically they could provide sufficient DNA similarity for homologous recombination to take place in bacteria already possessing similar genes.

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 *cry3Bb1* and CP4 *epsps* genes in maize MON 88017 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 *cry3Bb1* cassette from maize MON 88017 is transferred to bacterial cells, the expression of the *cry3Bb1* gene cannot be excluded due to the presence of the P-e35S promoter (section 3.1.1, above), which has been shown to be functional in some bacteria (Assaad and Signer, 1990; Lewin et al., 1998; Jacob et al., 2002). Therefore, despite its high unlikelihood, the EFSA GMO Panel takes into account that expression might occur.

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 due to the lack of selective advantage for gene transfer recipients in case they would be able to express their acquired recombinant genes. The hypothesised low level exposure of environmental bacterial communities to the maize MON 88017 *cry3Bb1* and CP4 *epsps* genes 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. In the unlikely event that the above mentioned genes and regulatory elements are taken up by bacteria, no selective advantage is anticipated, because *cry* and *epsps* genes are already occurring in various bacterial species in the environment.

The unlikelihood of double homologous recombination, the wide environmental presence of genetically diverse natural variants of the recombinant DNA coding sequences, and the absence of an identified plausible selective advantage that would be provided to hypothesised transformed bacteria,

suggest it is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the human and/or animal digestive tract or in the environment (EFSA, 2009b). Hence, in the rare but theoretically possible case of transfer of the *cry3Bb1* and CP4 *epsps* genes from maize MON 88017 to 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.

In its evaluation, the EFSA GMO Panel did not identify properties with the inserted DNA in maize MON 88017 that would change its likelihood of horizontal transfer compared with other plant genes. A selective advantage of hypothesised rare horizontal transfer of the recombinant genes (*cry3Bb1*, CP4 *epsps*) to environmental bacteria has not been identified. Therefore, the EFSA GMO Panel concludes that the recombinant DNA in maize MON 88017 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 BE CA. The BE CA concluded that "*the possibility of horizontal gene transfer between the GM plant and micro-organisms is considered as a rare event under natural conditions (EFSA, 2006b; Nielsen & Townsend, 2004; Keese, 2008). In the very unlikely case of transfer, maintenance and functional expression of the cp4 epsps or cry3Bb1 gene in micro-organisms of the receiving environment, no impact on the ecology of micro-organism communities and no adverse effect on human/animal health or to the environment are expected*" (section 2.3 of the environmental risk assessment report of the BE CA).

6.2.2.2. Plant to plant gene transfer and its consequences[30]

Maize is a cross-pollinating plant, relying on wind for the dispersal of its pollen. While maize pollen can be collected by honeybees and other insects, these pollinating insects play a minor role in the cross-pollination of maize plants (Eastham and Sweet, 2002; Malone and Burgess, 2009).

Compared with other wind-pollinated species, pollen grains of maize are relatively large (an average diameter of 90 μm) and heavy (0.25 μg) (Raynor et al., 1972; Di-Giovanni et al., 1995). Due to their characteristics, maize pollen grains settle to the ground rapidly (Aylor et al., 2003) and have usually a short flight range (Jarosz et al., 2005). Approximately 95-99 % of the released pollen is deposited within about 50 m from the source. However, vertical wind movements or gusts during pollen shedding can lift pollen up high in the atmosphere and distribute it over significant distances up to kilometres (Jarosz et al., 2005; Astini et al., 2009; Vogler et al., 2009; Hofmann et al., 2010). Concentrations of viable pollen considerably decrease with height (Aylor et al., 2006) and distance (Jarosz et al., 2005) from the source. Very low levels of cross-pollination can occur over distances up to kilometres under suitable climatic conditions (Bannert and Stamp, 2007; Delage et al., 2007; Langhof et al., 2010; Kawashima et al., 2011), but most cross-pollination events occur within 40 m of the pollen source (reviewed by Eastham and Sweet, 2002; Devos et al., 2005, 2009b; van de Wiel and Lotz, 2006; Hüsken et al., 2007; Langhof and Rühl, 2008; Sanvido et al., 2008; Ricroch et al., 2009; van de Wiel et al., 2009; Czarnak-Klos and Rodríguez-Cerezo, 2010; Riesgo et al., 2010).

Maize pollen is susceptible to desiccation, and water loss in pollen grains during dispersal reduce their ability to germinate on the stigma (Aylor, 2004). In addition, the water content of maize pollen affects its flight dynamics (Aylor, 2002, 2003; Aylor et al., 2003). During drying, the shape of maize pollen changes from a prolate spheroid to a crinkled, prismatic solid, and its density increases by approximately 16 %, and its settling speed decreases by approximately 34 %. These physical changes impact potential transport distances of pollen. In general, the lightest pollen will travel the longest distances, but it will be the least viable (Aylor, 2002).

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

[30] Technical dossier / Section D9.3

consequences of transgene flow on ecosystems by considering the spread and fitness of hybrid and backcross progeny, as well as exposure to non-target organisms (section 6.2.4, below).

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 maize can hybridise and form backcross progeny (Eastham and Sweet, 2002; OECD, 2003). The only recipient plants that can be cross-fertilised by maize are other cultivated maize varieties and types (Devos et al., 2005, 2009b; van de Wiel and Lotz, 2006; Hüsken et al., 2007; Sanvido et al., 2008; Bitocchi et al., 2009; Ricroch et al., 2009; Czarnak-Klos and Rodríguez-Cerezo, 2010). Since the molecular analysis and food/feed safety evaluation did not raise safety concerns (sections 3 to 5, above; EFSA, 2009a), the EFSA GMO Panel does not consider cross-pollination in maize an environmental risk, but an agricultural management and coexistence issue that is not within its remit.

Seed-mediated establishment of maize and its survival outside cultivation is rare in spite of extensive cultivation in many countries and accidental seed dispersal. Maize plants have lost their ability to release seeds from the cob, so most seed dispersal is due to harvesting and post-harvest activities of farmers. The occurrence of some GM maize plants outside cropped area has been reported in Korea and is attributed to seed spillage during import, transportation, storage, handling and processing (Kim et al., 2006; Lee et al., 2009; Park et al., 2010). However, survival of maize 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 maize MON 88017, it is considered very unlikely that it or its progeny will differ from conventional maize varieties in their ability to establish feral populations under European environmental conditions. The insect resistance and herbicide tolerance traits are 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 maize varieties (Raybould et al., 2011b), maize MON 88017 plants are not likely to establish feral populations under European environmental conditions. The contribution of occasional feral GM maize plants to pollen flow into agricultural fields will be extremely small, compared with that from the crop. Moreover, field observations performed on maize volunteers after GM maize cultivation in Spain revealed that maize volunteers had a low vigour, rarely had cobs and produced pollen that cross-pollinated adjacent plants only at low levels (Palaudelmàs et al., 2009; section 6.2.1, above). In comparison with GM maize volunteers, the vigour of occasional feral GM maize plants will be reduced due to the less suitable habitat than agricultural fields.

In conclusion, since maize MON 88017 has no altered agronomic and phenotypic characteristics, except for the specific target pest resistance and herbicide tolerance (section 6.2.1, above), the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from maize MON 88017 is considered to be extremely low.

The conclusion of the EFSA GMO Panel is consistent with that of the BE CA on maize MON 88017. The BE CA concluded that "*as there are no wild relatives of maize in the EU, vertical gene flow through cross-pollination from GM maize fields is restricted to plants of the same species. Gene flow might also result from the adventitious presence of GM maize kernels in conventional maize seeds or from seed spillage during transport. Gene transfer might thus result in the occurrence of GM maize volunteers. As the control of these volunteers will be the same as for non-GM maize volunteers, the occurrence of GM maize volunteers will not raise any novel environmental concerns compared to non-GM volunteers*" (section 2.3 of the environmental risk assessment report of the BE CA).

6.2.3. Interactions of the GM plant with target organisms[31]

The potential of maize MON 88017 to cause adverse effects through direct or indirect interactions between the GM plant and target organisms was evaluated by EFSA GMO Panel, and this evaluation is described below.

6.2.3.1. Adverse effects due to resistance evolution to the Cry3Bb1 protein in coleopteran target pests

Maize MON 88017 expresses the *cry3Bb1* gene, which provides protection against certain coleopteran target pests belonging to the genus *Diabrotica*. The applicant tested the biological activity of the Cry3Bb1 protein on a number of insect species via laboratory bioassays[32]. The toxicity of the Cry3Bb1 protein was shown to be restricted to certain coleopteran pests, namely those from the Chrysomelidae family (leaf beetles). Of the tested pest insect species, only the larvae of Western corn rootworm (Chrysomelidae: *D. v. virgifera*) and the Colorado potato beetle (Chrysomelidae: *L. decemlineata*) were shown to be sensitive to the Cry3Bb1 protein (see also Donovan et al., 1992; Meissle and Romeis, 2009a)[33]. Other non-pest Chrysomelidae are also sensitive to the Cry3Bb1 protein, as described in section 6.2.4, below.

Because resistance to chemical insecticides is known to evolve in insect pests, including Western corn rootworm (Onstad, 2008; Miller et al., 2009; Whalon et al., 2011), the potential evolution of insect resistance to Cry proteins constitutively expressed in Bt-crops is considered a relevant environmental and agronomic concern by the scientific community (e.g., Tabashnik et al., 2008a,b, 2009; BEETLE report, 2009). Resistance evolution to the Cry3Bb1 protein is not considered a direct environmental harm, but the consequences of the establishment of Cry3Bb1-resistant Western corn rootworm populations could be that farmers have to use other pest control tools (e.g., chemical insecticides) with a higher environmental load, to displace biocontrol programs at a larger scale, or have to alter their cultivation/farming system (i.e., rotate maize with other crops) (Andow, 2008).

Instances of field resistance to Bt-maize expressing Cry1 type proteins have been reported for two lepidopteran target pests that are not present in the European fauna (reviewed by Tabashnik et al., 2009; Huang et al., 2011): *Busseola fusca* (Van Rensburg, 2007; Kruger et al., 2009, 2011b) and *Spodoptera frugiperda* (Matten et al., 2008; Moar et al., 2008; Tabashnik, 2008; Tabashnik et al., 2008a; Storer et al., 2010). Field resistance is defined as a genetically based decrease in susceptibility of a population to a toxin caused by exposure of the population to the toxin in the field (Tabashnik, 1994; Andow, 2008). The first instance of field resistance to Bt-maize expressing Cry1 proteins has been reported in a population of the African stem borer (*Busseola fusca*) in South Africa, where some larvae were able to survive on Cry1Ab-expressing maize (Van Rensburg, 2007; Kruger et al., 2009, 2011b). The second instance concerns fall armyworm, *Spodoptera frugiperda*. Larvae surviving on Cry1F-expressing maize in some fields in the USA (Puerto Rico) were collected and exposed to high concentrations of the Cry1F protein in laboratory bioassays, where no mortality was observed (Matten et al., 2008; Moar et al., 2008; Tabashnik, 2008; Tabashnik et al., 2008a). Storer et al. (2010) confirmed via laboratory bioassays that *S. frugiperda* collected from the affected area exhibited lower sensitivity to the Cry1F protein compared with typical colonies from other regions, and that the resistance was shown to be autosomally inherited and highly recessive.

In case of Western corn rootworm, the possible evolution of resistance to the Cry3Bb1 protein has been demonstrated for maize MON 863 under artificial selection experiments under greenhouse conditions (Meihls et al., 2008) and recently confirmed under field conditions in some populations in Iowa, USA (Gassmann et al., 2011).

- Meihls et al. (2008) exposed rootworm colonies to Bt-maize in the greenhouse under four selection regimes (1) continuous exposure (larvae were reared on Bt-maize throughout the larval

[31] Technical dossier / Section D9.4

[32] Technical dossier / Section D7.8 / Pages 136-137 / Annex: Head et al. (2001)

[33] Technical dossier / Section D7.8 / Pages 136-137 // Additional information received on 23/02/2009 / Request 1 / Pages 2-3 / Annexes: Astwood et al. (2001) & Duan et al. (2003)

development period), (2) neonate exposure (larvae were placed on Bt-maize as neonates, then shifted to non-Bt-maize to complete development), (3) late exposure (larvae ate non-Bt-maize as neonates and completed development on Bt-maize), and (4) no exposure (larvae were reared on non-Bt-maize). After three and six generations of greenhouse selection, the colony that was continuously exposed to Bt-maize was highly resistant; larval survival on Bt-maize was equivalent to survival on the non-Bt-maize counterpart. After three generations of selection, the LC_{50} of the continuous exposure colony was approximately 22-fold greater than that of the unexposed control colony. After six generations of selection, percent survival on Bt-maize relative to its non-Bt-maize counterpart was 11.7-fold greater in the field for the continuous exposure colony than for the control colony (Meihls et al., 2008).

- Gassmann et al. (2011) reported that the survival of Western corn rootworm on Cry3Bb1-expressing maize in laboratory bioassays was significantly higher for individuals from problem fields where farmers reported severe root injury to Cry3Bb1-expressing maize than from control fields where such injury was not reported. In all problem fields studied, Cry3Bb1-expressing maize had been grown for at least three consecutive years (Gassmann et al., 2011), corresponding to three generations of selection (Gray et al., 2009).

These results demonstrate that Western corn rootworm will evolve resistance to Cry3Bb1-expressing maize rapidly under conditions of continuous exposure (Meihls et al., 2008; Tabashnik, 2008; EPA, 2010; Gassmann et al., 2011; Oswald et al., 2011; see also Lefko et al., 2008 and Nowatzki et al., 2008 for the Cry34Ab1/Cry35Ab1-expressing maize event DAS-59122-7, and Meihls et al., 2011 for the mCry3A-expressing maize event MIR604).

The possible resistance evolution to the Cry3Bb1 protein in coleopteran target pests is identified by the EFSA GMO Panel as a concern associated with the cultivation of maize MON 88017, as resistance evolution may lead to altered pest control practices that may cause adverse environmental effects.

The conclusion of the EFSA GMO Panel is consistent with that of the BE CA (section on monitoring of the environmental risk assessment report of the BE CA).

6.2.3.2. Adverse effects on target organisms due to the expression of the CP4 EPSPS protein

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 BE CA and Member States, because the protein does not interact with any specific target organisms. The CP4 EPSPS protein renders maize MON 88017 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, below.

6.2.4. Interactions of the GM plant with non-target organisms[34]

The potential of maize MON 88017 to have direct or indirect adverse effects on non-target organisms and the ecological functions they provide, 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).

[34] Technical dossier / Section D9.5

For the assessment of potential adverse effects on non-target organisms due to the transgene product(s), a tiered approach is followed that relies mostly on *trait*-specific information. Based on the familiarity with the transgene product (e.g., knowledge on its mode of action and spectrum of activity) environmental consequences on non-target organisms can be anticipated. Therefore, a specific hypothesis-driven assessment can be followed, where information collected in lower tiers directs the extent and nature of the experimentation conducted in higher tiers (Romeis et al., 2008a).

Because of the unpredictable nature of some unintended changes in the GM plant, a weight-of-evidence approach is followed that relies on *in planta* [*event*-specific] data for the assessment of their potential adverse effects on non-target organisms. This assessment concentrates on the detection of unintended changes in the GM plant itself at molecular, compositional and agronomic/phenotypic level. Since for instance compositional analyses do not necessarily target specific metabolites known to be involved in plant-non-target organism interactions, *in planta* [event-specific] data are required on effects on non-target organisms that fulfil important ecological functions/services to the plant ecosystem under consideration (EFSA, 2010d,e). The evaluation of potential adverse effects on non-target organisms due to intended and unintended changes in maize MON 88017 are described below.

6.2.4.1. Adverse effects on non-target organisms due to unintended changes in maize MON 88017

The molecular characterisation of the DNA insert and flanking regions of maize MON 88017 did not indicate unintended changes due to the insertion (section 3, above). Moreover, no biologically relevant differences in the composition of key analytes or agronomic and phenotypic characteristics were identified between maize MON 88017 and its near-isogenic line (McCann et al., 2007; Poerschmann et al., 2009; EFSA, 2009a). Therefore, the EFSA GMO Panel concludes that there are no indications of unintended changes in maize MON 88017 at the molecular, compositional and agronomic/phenotypic level.

In the course of its evaluation, the EFSA GMO Panel noted that most of the data on plant-non-target organism interactions, provided by the applicant, were not *event*-specific, as the studies were done, either with maize MON 863 or MON 853, instead of maize MON 88017.

In order to reliably conclude on potential adverse effects on non-target organisms due to unintended changes in maize MON 88017, the EFSA GMO Panel requested the applicant to review all *event*-specific studies on main functional groups of non-target organisms. Following this request, the applicant performed two studies with honeybees (*Apis mellifera*) that were exposed to pollen from maize MON 88017[35]. A first study was conducted with honeybee larvae to assess potential dietary and development effects of maize MON 88017 pollen. There were no differences in development or survival of larvae between the two maize pollen-based treatments, nor were any adverse effects on the development or survival of any of the larvae observed. A second study was conducted with honeybee adults to assess potential dietary effects of maize MON 88017 pollen over a 14-day feeding period. Adults were allowed to feed *ad libitum* pollen from maize MON 88017 in a honey-based diet for the duration of 14 days. Honeybees were observed for mortality and behavioural abnormalities. There were no differences in survival of adults between the two maize pollen-based treatments, nor were any behavioural abnormalities observed.

The applicant also provided a literature overview of recent laboratory, greenhouse and field studies on main functional groups of non-target organisms conducted with maize MON 88017[36]. None of these studies provided indications of adverse effects on non-target organisms due to unintended changes in maize MON 88017.

Based on the evidence provided by the applicant and relevant scientific literature on maize MON 88017, the EFSA GMO Panel concludes that there are no indications of adverse effects on non-

[35] Additional information received on 04/04/2011 / Request 1.3 / Pages 7-12 // Additional information received on 23/05/2011 / Request 1 / Page 2 / Annexes: Richards (2011a,b)

[36] Additional information received on 04/04/2011 / Request 1.3 / Pages 7-12 / Appendix 2

target organisms due to unintended changes in maize MON 88017. Since there are no indications of unintended changes, the EFSA GMO Panel considers *trait*-specific information appropriate to assess whether maize MON 88017 poses a risk to non-target organisms. The assessment of potential adverse effects on non-target organisms due to the expression of the Cry3Bb1 and CP4 EPSPS proteins is described in the below sections 6.2.4.2 and 6.2.4.3, respectively.

The conclusion of the EFSA GMO Panel is consistent with the evaluation carried out by the BE CA on maize MON 88017. The BE CA considered that "*impacts on non-target organisms due to unintended changes of composition or morphology of the GM maize were not expected to occur, as no compositional and phenotypic differences have been found between the GM maize and its non-GM comparator*" (section 2.5 of the environmental risk assessment report of the BE CA).

6.2.4.2. Adverse effects on non-target organisms due to the expression of the Cry3Bb1 protein

Biological equivalence of maize MON 863, MON 853 and MON 88017 Cry3Bb1 protein variants

In its evaluation of the environmental risk assessment of maize MON 88017, the EFSA GMO Panel considered, when appropriate, information available from other GM maize events expressing the Cry3Bb1 protein, in particular maize event MON 863 and product candidate line MON 853. Studies, presented in the application, to determine the target specificity of the Cry3Bb1 protein and its potential impact on non-target organisms, have been conducted mostly in the context of the evaluation of maize MON 863, either using the Cry3Bb1 MON 863 or MON 853 variants. Therefore, the EFSA GMO Panel indicates in its Scientific Opinion where information derived from maize MON 863 or MON 853 is used for the evaluation of potential environmental impacts of maize MON 88017.

The amino acid sequence of the Cry3Bb1 proteins present in maize MON 863 and MON 853 share a high identity with that of maize MON 88017; they share an amino acid identity of > 99.8 % with MON 88017 and differ by one of 653 amino acids from that of maize MON 88017[37]. The Cry3Bb1 protein variants in maize MON 88017 and MON 863 differ from one another by one of 653 amino acids at position 166 in maize MON 863, where glycine is present instead of aspartic acid. The Cry3Bb1 protein variant produced in maize MON 88017 differs in its amino acid sequence by six amino acids from the wild-type Cry3Bb1 protein. Structural data for the wild-type Cry3Bb1 protein and the Cry3Bb1 protein variants derived from maize MON 863 and MON 853 or produced by *B. thurigiensis* fermentation were provided by the applicant and were compared with each other. The structural data of the analysed Cry3Bb1 protein variants indicated that the amino acid substitutions do not alter their overall 3-dimensional structure[38]. However, a similar analysis was not conducted for the Cry3Bb1 MON 88017, MON 863 and MON 853 protein variants. Therefore, the BE CA noted that "*experiments describing a side-by-side comparison of the different protein sources, indicating that the amino acid substitution is not expected to have an impact on protein structure and eventually biological activity, are (partly) lacking*" (section 2.4 of the environmental risk assessment report of the BE CA).

The applicant demonstrated that the Cry3Bb1 MON 853 protein variant is biologically and hence functionally equivalent to the MON 863 Cry3Bb1 variant[39]. Moreover, no statistical differences in biological activity between the two Cry3Bb1 protein variants of maize MON 88017 and MON 863 against two susceptible coleopteran species, Western corn rootworm and Colorado potato beetle (*Leptinotarsa decemlineata*), were observed in laboratory bioassays, indicating the functional equivalence of both variants[40]. An analysis of several bioassays on the Colorado potato beetle with the *E. coli*-produced Cry3Bb1 MON 88017 and MON 863 protein variants showed overlap in LC_{50} values between both variants, confirming biological equivalence[41].

[37] Technical dossier / Section D3 / Page 25
[38] Technical dossier / Section D8 / Pages 189-190 / Annex: Astwood et al. (2001)
[39] Technical dossier, Section D8 / Pages 189-190 / Annex: Astwood et al. (2001)
[40] Technical dossier, Section D8 / Pages 189-190 / Annex: Duan et al. (2003)
[41] Additional information received on 23/02/2009 / Request 1 / Pages 2-3

The equivalence of the Cry3Bb1 protein produced by *E. coli* and maize MON 88017 was shown and previously evaluated by the EFSA GMO Panel (see EFSA, 2009a)[42].

The evidence provided by the applicant indicates that the protein sequences of the Cry3Bb1 protein variants of maize MON 88017, MON 863 and MON 853 are similar, and that the biological activity of these Cry3Bb1 protein variants is equivalent. Therefore, the EFSA GMO Panel considers that information generated to evaluate potential adverse effects on non-target organisms due to the expression of the Cry3Bb1 protein in maize MON 863 or MON 853 can be used to inform the environmental risk assessment of maize MON 88017. The average and range in levels of the Cry3Bb1 protein in various parts from maize MON 88017 and MON 863, used to extrapolate *trait*-specific information for maize MON 863 to maize MON 88017, are summarised in Table 2, below.

Table 2. Means and ranges in the levels of the Cry3Bb1 protein in various plant parts of maize MON 88017 and MON 863 (µg/g fresh weight)

Plant parts	MON 88017 2006 EU trials [43]	MON 88017 2002 USA trials [44]	MON 863 1999 USA trials [45]
Leaf stage (OSL1; V2-4)	53 (37-67)	60 (22-84)	81 (65-93)
Leaf stage (OSL2; V6-8)	59 (51-70)	59 (49-73)	NA
Leaf stage (OSL3; V10-12)	49 (38-78)	55 (43-74)	NA
Leaf stage (OSL4)	48 (25-70)	49 (39-61)	NA
Root (OSR1; V2-4)	20 (12-34)	34 (21-44)	NA
Root (OSR2; V6-8)	24 (13-37)	30 (22-48)	NA
Root (OSR3; V10-12)	14 (10-18)	25 (18-44)	41 (25-56)
Root (OSR4; pre-VT)	15 (12-21)	23 (14-30)	NA
Grain	8 (5-13)	9 (6-13)	70 (49-86)
Silk	16 (11-24)	31 (26-38)	10 (no range)
Pollen	9 (5-12)	13 (10-17)	62 (30-93)

NA: not assayed

The EFSA GMO Panel conclusions on the biological equivalence of the MON 863, MON 853 and MON 88017 Cry3Bb1 protein variants are consistent with those of the BE CA. "*Given that the variations in protein sequences between the Cry3Bb1 variants are small and that the biological activity of MON 863 and MON 88017 Cry3Bb1 is equivalent*", the BE CA concluded that "*studies conducted with maize MON 853 or MON 863 can be used to assess the safety of maize MON 88017*", but that "*the biological equivalence between the MON 853 and the MON 88017 Cry3Bb1 protein variant could have been better demonstrated*" (section 2.4 of the environmental risk assessment report of the BE CA).

Effects on non-target terrestrial (plant- and ground-dwelling) arthropods

Non-target terrestrial (plant- and ground-dwelling) arthropods: It has been described that up to a 1,000 non-target arthropod species can occur in maize fields in the EU (Knecht et al., 2010). Therefore, several non-target arthropods are likely to be exposed to Bt-maize plants and the Cry protein(s) they express when cultivated. These non-target arthropods can be exposed to Cry proteins when feeding on plant material (including pollen) or honeydew excreted from sap-sucking species,

[42] Technical dossier / Section D7.8.1 / Pages 133-134 / Annex: Bonner et al. (2003a)
[43] Technical dossier / Section D3 / Pages 57-70 / Annex: Niemeyer and Silvanovich (2007)
[44] Technical dossier / Section D3 / Pages 57-70 / Annex: Bhakta et al. (2003)
[45] Technical dossier / Section D3 / Pages 57-70 / Annex: Dudin (2001)

and/or when feeding on prey/host organisms which have previously been feeding on Bt-maize (Andow et al., 2006; Romeis et al., 2006, 2008a,b; Lundgren, 2009). These species however are only at risk if the Cry proteins show toxicity at a realistic level of exposure (e.g., Head et al., 2001; Dutton et al., 2002; Harwood et al., 2005; Vojtech et al., 2005; Obrist et al., 2005, 2006a,b,c; Torres et al., 2006; Raybould, 2007; Torres and Ruberson, 2008; Meissle and Romeis, 2009a,b; Romeis and Meissle, 2011). Because not all of the exposed species can be tested from a practical viewpoint, the toxicity of Cry proteins is tested generally on a representative subset of species using a tiered approach (Garcia-Alonso et al., 2006; Rose, 2007; Romeis et al., 2006, 2008a). In case of maize MON 88017, the applicant clarified that a representative subset of non-target arthropod species was selected for testing purposes based on the ecological relevance of the species, the likely exposure of the species to maize MON 88017 under field conditions, species susceptibility to the Cry3Bb1 protein, and testability[46].

The applicant conducted and reviewed a series of lower-tier studies (dietary bioassays) on several non-target arthropod species representative of different functional groups, including natural enemies (predators and parasitoids), pollinators, herbivores and decomposers[47]. In general, most of the lower-tier studies conducted or reviewed by the applicant adhere to the general principles of good laboratory study design (see Rose, 2007; Romeis et al., 2011 for recommendations for the design of laboratory studies on non-target arthropods), and therefore can be used to inform the risk assessment. The BE CA considered that "*the dietary toxicity tests conducted by the applicant on non-target insects and herbivores (putative targets) were overall well-conducted, except for the Chrysoperla carnea larvae*"[48]. For this study, the BE CA did not consider that "*the test species was sufficiently exposed to the Cry3Bb1 toxin*". "*Given that, due to the mode of feeding, lacewing larvae will not be exposed much (if at all) to Cry3Bb1 in the field, this study was considered of less relevance*" by the BE CA (section 2.5.1 of the environmental risk assessment report of the BE CA and its annexes). Recent findings reported by Li et al. (2008, 2010) demonstrated that adult *C. carnea* is not affected by Cry3Bb1-containing pollen and is not sensitive to the Cry3Bb1 protein at concentrations exceeding the levels in pollen. It can therefore be concluded that pollen of maize MON 88017 will pose a negligible risk to adult *C. carnea*.

The applicant's lower-tier study conducted with the minute pirate bug *Orius insidiosus* (Duan et al., 2008b) reported that 3-5 day old nymphs (probably late second instars considering a temperature of 25°C) of *O. insidiosus* reared on honeybee collected pollen (mixed to a 40:60 ratio with water/buffer or dissolved Cry3Bb1 protein) developed to the adult stage in about 5-7 days (with an overall average of approximately six days), with a survival rate close to 90 %. These rapid development and high survival rates (see also Duan et al., 2007) are not in line with previous studies on the biology of this predatory bug (Isenhour and Yeargan, 1981; Kiman and Yeargan, 1985; Pilcher et al., 1997; Bonte and De Clercq, 2008, 2011; Lundgren, 2009). Evidence shows that the developmental success of *O. insidiosus* is consistently lower when fed pollen compared with lepidopteran eggs. When fed pollen only, survival rates of *O. insidiosus* vary with pollen species and quality (Kiman and Yeargan, 1985; Pilcher et al., 1997; Lundgren, 2009). Therefore, both the BE CA and the EFSA GMO Panel considered the rapid development and high survival rates on pollen reported in Duan et al. (2008b) unlikely, even if the *Orius* bugs received lepidopteran eggs during the 3-5 days prior to the experiment with the pollen: water mix.

The lower-tier studies conducted or reviewed by the applicant indicate the lack of toxicity to several species representative of different functional groups such as: ladybird beetle species, carabid beetle species, larvae and adults of the honeybee *A. mellifera*, the parasitic wasp *Nasonia vitripennis*, the green lacewing *C. carnea*, and the predatory spider *Theridion impressum* of the purified Cry3Bb1 protein (Bt-plant- or *E. coli*-produced) and/or Cry3Bb1-containing food (be it pollen of Cry3Bb1-expressing maize or prey fed Cry3Bb1-expressing maize material), confirming that the specificity of

[46] Additional information received on 23/02/2009 / Request 2 / Pages 4-8 // Additional information received on 15/06/2010 / Request 2 / Pages 2-3 // Additional information received on 04/04/2011 / Request 1.1 / Pages 4-6

[47] Technical dossier / Section D9.5 / Pages 186-208 // Additional information received on 04/04/2011 / Request 1.3 / Pages 7-12 / Appendix 2

[48] Technical dossier / Section D9.4 / Pages 184-201 / Annex: Palmer and Krueger (1999b)

the insecticidal activity of the Cry3Bb1 protein is limited to arthropod species of the Chrysomelidae family (see Table 3, below).

While most lower-tier studies, assessing the potential impact of Cry3Bb1-expressing maize on non-target arthropods (see Table 3 for an overview), did not reveal adverse effects to non-target terrestrial arthropods including Coleoptera (reviewed by OECD, 2007; Meissle and Romeis, 2008; EPA, 2010), adverse effects on the predatory ladybird beetle *Adalia bipunctata* and predatory rove beetle *Atheta coriaria* were reported by Schmidt et al. (2009) and Büchs et al. (2008), respectively.

- Schmidt et al. (2009) reported marginal mortality in larvae of the predatory coccinellid *A. bipunctata* when exposed to certain concentrations of microbially produced trypsin-activated Cry3Bb1 protein in its diet. In their laboratory study, Schmidt et al. (2009) fed different larval stages (L1-L4) of *A. bipunctata* with eggs of the flour moth *Ephestia kuehniella* that had been sprayed with solutions of microbially produced trypsin-activated Cry1Ab or Cry3Bb proteins. The authors suggested that the increased mortality of larvae was caused directly by the activated Cry proteins and raised questions about the postulated specificity and mode of action of Cry proteins in *A. bipunctata*. Since methodological flaws and inconsistencies in the Schmidt et al. (2009) study were brought to light by the scientific community (Meissle and Romeis, 2008; Rauschen, 2010; Ricroch et al., 2010; ZKBS, 2011), the EFSA GMO Panel supports the BE CA position not to take this study "*into consideration in the evaluation of the risk assessment*". While the authors tested qualitatively the presence of Cry proteins in the spray solution by immuno-strip assays, they did not quantify the actual intake of Cry proteins by the larvae. Given that young ladybird larvae do not consume entire prey items but instead puncture the prey and suck out the contents, it is questionable whether the test insects actually ingested Cry proteins (Álvarez-Alfageme et al., 2011). It was also noted that neither a dose-response relationship, nor sublethal effects (on developmental time and adult body weight) on surviving specimen were observed; both these features represent a typical response of sensitivity to Cry proteins. Furthermore, the control mortality in first instars was very high (21 %), which may underline the unsuitability of the bioassay design (Álvarez-Alfageme et al., 2011). Therefore, the EFSA GMO Panel is of the opinion that the Schmidt et al. (2009) study is not sufficient to identify a hazard, or to question the target specificity of the Cry3Bb1 protein. Recent laboratory bioassays with the Cry3Bb1 protein – provided either through spider mites that had consumed maize MON 88017, or as purified protein – revealed no adverse effects on different measurement endpoints of *A. bipunctata* larvae (Álvarez-Alfageme et al., 2011), confirming that the adverse effects reported by Schmidt et al. (2009) were likely artefacts of poor study design (Meissle and Romeis, 2008; Rauschen, 2010; Ricroch et al., 2010).

  Whilst the exposure route used by Schmidt et al. (2009) may constitute a useful model for lower-tier studies with coccinellids, any exposure through egg feeding in the field is very unlikely. The EFSA GMO Panel considers that the level of exposure of coccinellid larvae to the Cry3Bb1 protein will be low under natural conditions. Cry proteins are normally absent in aphids that feed on Bt-maize, which is the main food source of coccinellid larvae. Evidence indicates that aphids feeding on Bt-maize plants do not ingest considerable amounts of Cry proteins, if any (Head et al., 2001; Raps et al., 2001; Dutton et al., 2002; Lundgren and Wiedenmann, 2005; Obrist et al., 2006b; Ramirez-Romero et al., 2008; Meissle and Romeis, 2009a; Romeis and Meissle, 2011). In addition, the content of the Cry3Bb1 protein in pollen from maize MON 88017, which is likely to be the most common source for possible toxin ingestion for coccinellids, is low, ranging between 5-12 μg/g fresh weight (see Table 2).

- Büchs et al. (2008) indicated in a non-peer-reviewed proceedings abstract that the uptake of prey (saprophagous dipterans) fed plant tissues from maize MON 88017 can prolong the development time of *A. coriaria* larvae, and can reduce female fertility and offspring production in comparison with those reared on non-Bt-maize litter[49]. The Cry3Bb1 protein content was 42.4 ng/g fresh

[49] http://www.gmo-safety.eu/database/1028.research-impact-maize-cry-3bb1-non-target-organisms-living-soil.html

weight in dipteran larvae and 78.8 ng/g fresh weight in pupae when fed pollen from maize MON 88017 for a period of six days, and 263.9 ng/g fresh weight in larvae and 39.5 ng/g fresh weight in pupae when fed roots from maize MON 88017, indicating that *A. coriaria* larvae can be exposed to low concentrations of the Cry3Bb1 protein through their prey. However, it is very unlikely that the observed sublethal effects to this rove beetle species can be attributed to the toxicity of the Cry3Bb1 protein. Based on the feeding habit of individual *A. coriaria* beetles and effects reported, this species would have to be much more sensitive to the Cry3Bb1 protein than *Diabrotica* spp., which is considered unlikely. Moreover, unpublished data on the potential impact of the pure Cry3Bb1 protein on *A. coriaria* beetles did not reveal adverse effects under lower-tier conditions (Fernandez S, personal communication), and no significant changes in the abundance of rove beetles were observed in a 2-year higher-tier study with maize MON 88017 in the Czech Republic (Svobodová et al., 2012), though the number of rove beetles captured was limited.

Evidence from other coleopteran-active Cry proteins does not indicate adverse effects to rove beetles, suggesting that they are not sensitive to the tested coleopteran-active Cry proteins. Porcar et al. (2010) reported that, based on a 15-day laboratory bioassay covering 70 % of the life-span of adults, the adult mortality of *A. coriaria* when fed a diet containing the coleopteran-active Cry3Aa protein, did not differ statistically from that of the control group. A field study in Hungary with Bt-maize that expresses the coleopteran-active Cry34/35Ab1 proteins showed that the overall assemblage of rove beetles was not significantly affected by the Cry34/35Ab1 proteins through their diet (Balog et al., 2011). These studies confirm that the adverse effects reported by Büchs et al. (2008) were likely due to varietal or prey quality effects, rather than the toxicity of the Cry3Bb1 protein.

The applicant also reported on a series of higher-tier studies in which the potential impact of Cry3Bb1-expressing maize on several non-target terrestrial arthropod species in the EU and USA was assessed[50]. No negative impact of Cry3Bb1-expressing maize was observed on field densities of abundantly occurring acarids; chilopods; coleopteran species, including carabids, chrysomelids, coccinellids, elaterids, lathridiids, nitidulids and staphylinids; dipteran species, including syrphids; the hemipteran species *O. insidiosus*; the homopteran species *Rhopalosiphum maidis*; the neuropteran species *C. carnea*; hymenopterans, including braconids and formicids; orthopterans, including gryllids; and spiders in the USA. In the EU, no negative impact of maize MON 88017 was revealed on the abundance of spiders (Svobodová et al., 2012), carabids (Priesnitz, 2010; Svobodová et al., 2012), chrysomelids (Rauschen et al., 2010a), coccinellids (Rauschen et al., 2010a), staphylinids (Svobodová et al., 2012), and the hemipteran species *Trigonotylus caelestialium* (Rauschen et al., 2009) and *Zyginidia scutellaris* (Rauschen et al., 2008, 2010b). The results of these higher-tier studies confirm the conclusions of lower-tier studies (see Table 3, below), indicating that the Cry3Bb1 protein has little or no activity on species other than chrysomelids.

At present, the EFSA GMO Panel is not aware of identified significant adverse effects of the Cry3Bb1 protein on non-target terrestrial arthropods. Lower- and higher-tier studies showed minimal to undetectable changes in non-target terrestrial arthropods (e.g., Marvier et al., 2007; Duan et al., 2008a; Meissle and Romeis, 2008; Wolfenbarger et al., 2008; Malone and Burgess, 2009; Naranjo, 2009). According to Lövei et al. (2009), the majority of lower-tier studies with Cry3A/Bb proteins reported fewer effects on natural enemies in either direction compared with other toxin classes considered in their review (see also Andow et al., 2009; Shelton et al., 2009a,b for further details). The EFSA GMO Panel therefore concludes that adverse effects of maize MON 88017 due to the expression of the Cry3Bb1 protein on non-target terrestrial (plant- and ground-dwelling) arthropods are expected to be negligible, except for chrysomelids (see below).

[50] Technical dossier / Section D9.5 / Pages 186-208 // Additional information received on 04/04/2011 / Request 1.3 / Pages 7-12 / Appendix 2

The conclusion of the EFSA GMO Panel is consistent with the evaluation carried out by the BE CA on maize MON 88017 who concluded that "*adverse effects on non-target insects is expected to be negligible*" (section 2.5.1 of the environmental risk assessment report of the BE CA).

Non-target chrysomelids: The activity of the Cry3Bb1 protein is likely to be broader than the target pest species or other putative chrysomelid targets such as the cereal leaf beetle, *Oulema melanopus*, and include other non-target Chrysomelidae. Chrysomelids are regularly found in maize fields and can be exposed to the Cry3Bb1 protein based on their herbivorous feeding habits, as shown for the genera *Chaetocnema*, *Longitarsus*, *Oulema* and *Phyllotreta* (Kiss et al., 2002, undated; Daly and Buntin 2005; Eckert et al., 2005; Harwood et al., 2005; Obrist et al., 2006b; Knecht et al., 2010; Rauschen et al., 2010a). In addition, a potential risk to valued (non-pest) chrysomelid species from maize MON 88017 is the ingestion of harmful amounts of pollen deposited on their host-plants in and around maize fields.

To be in a position to evaluate the potential impact of maize MON 88017 on non-target chrysomelids, the BE CA requested the applicant to address in its environmental risk assessment whether "*the cultivation of maize MON 88017 might impact on non-target Chrysomelids (including threatened and endangered chrysomelids, if relevant) occurring in and around maize fields in Europe*" (section 2.5.1 of the environmental risk assessment report of the BE CA and its annexes). The EFSA GMO Panel requested the applicant to provide an overview of chrysomelid species occurring in and around maize fields that could potentially be exposed to pollen from maize MON 88017 deposited on host-plants in and around maize fields during anthesis in all likely European receiving environments. Following these requests for clarification, the applicant provided (1) a list of non-target chrysomelids that are representative of the European fauna that could potentially be exposed to the Cry3Bb1 protein in and around maize MON 88017 fields[51], and (2) a theoretical exposure assessment to estimate whether pollen from maize MON 88017 deposited on the leaf surface of host-plants in and around maize fields during anthesis is likely to pose a risk to non-target chrysomelid species[52].

(1) Based on a literature review and targeted interrogations of a EU faunistic arthropod database (see Knecht et al., 2010), the applicant listed 30 different non-target chrysomelid species that may occur in and around maize fields, and indicated for each of these species whether maize is used as the main host-plant. The applicant argued that host-plants for most of the listed chrysomelid species do not belong to the Gramineae family. Only two non-target chrysomelid species, *Oulema melanopus* and *Phyllotreta vittula*, which are considered pests, were found to use maize as host-plant. This conclusion is consistent with the findings reported by Rauschen et al. (2010a) for Germany and Kiss et al. (2002, undated) for Hungary. Rauschen et al. (2010a) showed that Chrysomelidae are one of the most abundant families of Coleoptera in maize fields in Germany, but that their occurrence is mainly restricted to the chrysomelid pests *Phyllotreta* spp. (see also Kiss et al., 2002, undated for Hungary) and *Oulema lichenis*. Because leaf beetles that use maize as host-plant can induce visible damage to maize plants, a reduction of their densities is not considered an environmental concern (Rauschen et al., 2010a). The other Chrysomelidae found in maize in Europe are low in abundance due to their preference for other habitats or host-plants (Knecht et al., 2010).

No non-target chrysomelid species of conservation concern, occurring in and around maize fields, were identified by the applicant[53]. Only one of the 38 coleopteran species considered at risk across the EU under Directive 92/43/EEC on conservation of natural habitats and of wild fauna and flora belongs to the family of Chrysomelidae (*Macroplea pubipennis*). However, *M. pubipennis* has not been observed in maize fields.

[51] Additional information received on 04/04/2011 / Request 1.4a / Pages 14-16

[52] Additional information received on 23/02/2009 / Request 2 / Pages 14-16 // Additional information received on 15/06/2010 / Request 2 / Pages 6-8 // Additional information received on 04/04/2011 / Request 4 / Pages 13-14 & Request 4b / Page 17

[53] Additional information received on 04/04/2011 / Request 1.4a / Pages 14-16 / Annex: Dewar (2010)

(2) To give an indication of the potential risk of Cry3Bb1-containing pollen to non-target chrysomelid larvae, the applicant put toxicity endpoints in relation to exposure estimations, which resulted in the calculation of margins of safety as indicator of risk (also termed TER = toxicity-to-exposure ratios) for the Colorado potato beetle, which is known to be very sensitive to the Cry3Bb1 protein. These margins were calculated as the quotient of the 7-day LD_{50} values for larvae of the Colorado potato beetle, divided by maximum estimated exposure concentrations (EEC) for the Cry3Bb1 protein estimated within and at various distances (0, 1, 2 and 4-5 m) from the maize field. The maximum EEC for the Cry3Bb1 protein in a pollen-contaminated food substrate at a given distance from the edge of the maize field was calculated considering pollen deposition on the leaf surface of host-plants, number of pollen grains per gram fresh weight maize pollen, the maximum Cry3Bb1 protein concentration in pollen, and the fresh weight of the host-plants per cm^2. These margins were determined to be 6-fold within the maize field, 12-fold at the edge of the maize field, 18-fold at 1 m from the edge of the maize field, 49-fold at 2 m from the edge of the maize field, and 146-fold at 4 to 5 m from the edge of the maize field. The TER values indicate that the exposure to the Cry3Bb1 protein via pollen decreases with increasing distance from the maize MON 88017 field, and suggest that the maximum EEC of the Cry3Bb1 protein encountered through pollen in the field will be much lower than the Cry3Bb1 protein dose causing the death of 50 % of Colorado potato beetle larvae under laboratory conditions. However, to inform the environmental risk assessment, the calculated TER values should be compared with assessment factors (trigger values, levels of concern) defined by risk managers. If a trigger value for an organism is not met, then further steps in the risk assessment are generally required. If the TER value for an organism is below its respective trigger value, then the risk is deemed acceptable and no further studies are required. No TER trigger values for acute toxicity to non-target chrysomelids such as the Colorado potato beetle were defined by risk managers, or by the applicant. Therefore, the EFSA GMO Panel is not in a position to put the TER values calculated by the applicant into context.

Leopold et al.[54] calculated the amount of pollen grains from maize MON 88017 (each pollen grain containing 3.45×10^{-6} µg) that larvae of the Colorado potato beetle, the mustard beetle (*Phaedon cochleariae*) and the green dock leaf beetle (*Gastrophysa viridula*) have to ingest to reach their respective LD_{50} values (see also Felke, 2006; Leopold and Felke, 2008). L1 larvae of the Colorado potato beetle and the mustard beetle have to ingest 5,217 (3,644-6,618) and 85,797 (59,919-108,824) pollen grains from maize MON 88017, respectively, to reach their LD_{50} value. In case of the green dock leaf beetle, it was calculated that the LD_{50} value of L1 and L2 larvae would be reached after ingestion of 8,696 (6,073-11,029) and 74,493 (52,024-94,485) pollen grains from maize MON 88017, respectively.

For the non-target non-EU chrysomelid species, *Galerucella vittaticollis* (the strawberry leaf beetle), Shirai (2006) reported that larval survival and development were not adversely affected when exposed to pollen from maize MON 863 at doses of 500 and 2,000 grains per cm^2 for ten days after hatching. The pollen density of 2,000 grains per cm^2 represents a worst-case exposure scenario, as several parameters affect the likelihood of environmentally relevant exposure in the field (reviewed by Perry et al., 2010; Perry, 2011). First, the highest content of the Cry3Bb1 protein measured in pollen from maize MON 863 was shown to be up to seven times higher than that in maize MON 88017 pollen[55] (see Table 2). Second, in their study, Pleasants et al. (2001) reported that the maximum pollen density, observed on 0.2 % of milkweed (*Asclepias* spp.) host-plants occurring within maize fields, was 1,500-1,600 grains per cm^2, while most milkweed plants (approximately 90 %) contained less than 500 grains per cm^2. Average pollen densities on milkweed leaves decreased from 171 grains per cm^2 on plants occurring within the field, to 63 grains per cm^2 on plants located at the field margin (0 m), to 14-35 grains per cm^2 on plants sampled 1-2 m from the edge of the field, and finally to eight grains per cm^2 on plants sampled 4-5 m from the edge of the field (Pleasants et al., 2001). In the EU, pollen densities on goosefoot

[54] http://www.gmo-safety.eu/database/1026.research-side-effects-maize-cry3bb1-non-target-organisms.html

[55] Technical dossier / Section D3 / Pages 57-70 // Annexes: Bhakta et al. (2003) & Dudin (2001) & Niemeyer and Silvanovich (2007)

(*Chenopodium album*) and mustard plants (*Sinapis alba*) located in maize fields at the end of pollen shed ranged between 52-972 grains per cm^2 and 100-894 grains per cm^2, respectively (Gathmann et al., 2006). Third, rainfall events or heavy dew may wash pollen from the maize or host-plant leaves (Pleasants et al., 2001), or may result in lysis and bursting of the pollen grains (Li et al., 2010). Finally, the duration of pollen shed for cultivated maize can be variable, resulting in a different overlap with sensitive larvae occurring in or nearby maize fields (e.g., Oberhauser et al., 2001).

Even though the susceptibility of the larvae of most non-target chrysomelid species to the Cry3Bb1 protein is not known and data on some aspects of exposure, particularly plant-insect phenology, host-plant characteristics, pollen consumption and subsequent mortality in field conditions, are rare within Europe, it can be concluded that exposure to the Cry3Bb1 protein through pollen from maize MON 88017 is expected to be low. The theoretical exposure assessments (described above) indicate that the amount of pollen from maize MON 88017 found in and around maize fields is unlikely to adversely affect a significant proportion of non-target chrysomelid larvae.

The activity of the Cry3Bb1 protein on adult non-target chrysomelid species is expected to be limited (Rauschen et al., 2010a), as it was shown to be low in adult Western corn rootworm, whose first instar larvae are highly sensitive to the Cry3Bb1 protein (Al-Deeb et al., 2005; Nowatzki et al., 2006; Meissle et al., 2009, 2011a).

The EFSA GMO Panel concludes that the risk of maize MON 88017 to valued (non-pest) chrysomelid species in the field is likely to be minimal due to their low occurrence and abundance in maize fields and due to the low likelihood of encountering harmful amounts of pollen from maize MON 88017 in and around maize fields. Moreover, the activity of the Cry3Bb1 protein on adult non-target chrysomelid species is expected to be limited.

The conclusion of the EFSA GMO Panel is consistent with the evaluation carried out by the BE CA on maize MON 88017 who concluded that "*unacceptable adverse effects to non-target and endangered Chrysomelids from cultivation of maize MON 88017 are not expected*". "*Due to the low occurrence of non-pest chrysomelids in maize fields the risk to these non-target species in field is expected to be minimal, at least in Germany*" (section 2.5.1 of the environmental risk assessment report of the BE CA).

Effects on non-target soil arthropods

Collembolans and Acari are important in the breakdown and recycling of crop residues, and are key indicator species of soil functionality and quality. Since these micro-arthropods can be exposed to the Cry3Bb1 protein in the Bt-maize field environment, they and their ecological functions could be adversely affected by the cultivation of maize MON 88017.

In general, no negative effects of Cry proteins on collembolans and soil mites have been reported in the scientific literature (reviewed by Icoz and Stotzky, 2008). In lower-tier studies[56], the survival and reproduction of *Folsomia candida* fed leaf material of Cry3Bb1-expressing maize was not adversely affected by the Cry3Bb1 protein (EPA, 2010). The conclusion of the lower-tier study was supported by higher-tier studies on springtails performed with maize MON 88017 in the EU (Höneman et al., 2008) and maize MON 863 in the USA (Al-Deeb et al., 2003; Ahmad et al., 2005; Bitzer et al., 2005). No adverse effects of Cry3Bb1-expressing maize were reported on field densities of springtails and soil mites (Acari) in a nine months leaf litter-bag field study conducted in Switzerland with maize MON 88017, as compared with the non-Bt-treatment (Hönemann et al., 2008). Field trials conducted in the USA also showed that there were no significant differences in numbers of soil mites (Al-Deeb et al., 2003; Ahmad et al., 2005).

[56] Technical dossier / Section D9.4 / Pages 184-201 / Annex: Teixeira (1999)

The EFSA GMO Panel is of the opinion that there is no evidence to indicate that the cultivation of maize MON 88017 is likely to cause adverse effects on non-target soil arthropods such as springtails and soil mites due to the expression of the Cry3Bb1 protein.

The conclusion of the EFSA GMO Panel is consistent with the evaluation carried out by the BE CA on maize MON 88017. The BE CA concluded that "*adverse effects on non-target insects of the soil community are expected to be negligible*" (section 2.7.1 of the environmental risk assessment report of the BE CA).

Effects on non-target aquatic arthropods

Byproducts from GM plants (e.g., pollen, detritus) can be transported in water courses to downstream water bodies where non-target aquatic arthropods can be exposed to transgene product(s) through consumption (Axelsson et al., 2010, 2011). In case of Bt-maize, Rosi-Marshall et al. (2007) reported that byproducts of Bt-maize enters headwater streams in the USA and claimed on the basis of experimental data obtained under lower-tier conditions that this would reduce growth and increase mortality of some non-target aquatic arthropods, especially trichopteran species (see also Chambers et al., 2010; Tank et al., 2010). 50% of filtering trichopterans collected by Rosi-Marshall et al. (2007) from water streams during peak pollen shed had maize pollen grains in their guts and detritivorous trichopterans were located in accumulations of decomposing maize litter in the streams after harvest.

Few studies, assessing the impact of the Cry3Bb1 protein on non-target aquatic arthropods and the fate of the Cry3Bb1 protein in senescent and decaying maize detritus in aquatic environments, have been reported in the scientific literature so far (see Table 3), but data are available for the daphnid species *Daphnia magna* (APHIS, 2005; EPA, 2010), the dipteran species *Chironomus dilutus* (Prihoda and Coats, 2008a) and *Tipula (Nippotipula) abdominalis* (Jensen et al., 2010), the caddisflies *Lepidostoma* spp. and *Pycnopsyche scabripennis* (Jensen et al., 2010), and the isopod *Caecidotia communis* (Jensen et al., 2010). Based on exposure estimates, Carstens et al. (2011) identified shredders (Cummins et al., 1989) as the functional group most likely to be exposed to Cry proteins.

- No adverse toxic effects on *D. magna* were observed when fed high amounts of Cry3Bb1-expressing maize pollen mixed with water (APHIS, 2005; EPA, 2010). Questions have been raised about using maize pollen in aquatic invertebrate testing with *D. magna* because maize pollen is thought to be too large for ingestion by these filter feeders (EcoStrat, 2000; see also Bern, 1990) and, if ingested, to have a low food value for daphnids (Masclaux et al., 2011). However, there is some observational evidence that daphnids do ingest pollen (see Hadden, 1978 cited in Campbell, 1999). Daphnids fed maize pollen are actually yellow in colour, which can be indicative of ingestion of the test material, with no treatment mortality or behavioural change compared with untreated controls. Even though there is some observational evidence that daphnids do ingest pollen, there is no clear evidence that these filter feeders are capable of digesting pollen grains. The presence of a refractory wall reduces the digestibility of intact pollen grains by daphids, and hence the nutritional value of pollen for these filter feeders (Masclaux et al., 2011). Therefore, only a statement of no effect from exposure to pollen, and no statement on lack of toxicity can be made from the *D. magna* study provided by the applicant (EPA, 2010).

  Bøhn et al. (2008, 2010) revealed that *D. magna* fed a 100 % suspension of maize MON 810 flour under lower-tier conditions had a higher mortality and reduced fitness performance, as compared with the non-Bt-maize treatment, suggesting toxic effects of the Cry1Ab protein. However, it remains unclear whether the unusual delays in development of *D. magna* fed non-Bt-maize have been caused by nutritional deficiencies related to the maize-based diet or the presence of the Cry1Ab protein (EFSA, 2009d; Ricroch et al., 2010).

- Prihoda and Coats (2008a) observed a decrease in survival but no effect on the growth of the larvae of the *C. dilutus* when exposed to the Cry3Bb1 protein via root extracts of maize MON 863. The EFSA GMO Panel agrees with both the authors of the study and the BE CA that "*it remains unclear if the observed effects were due to the presence of Cry3Bb1 or other*

compounds in the root extracts, as no control treatments with increasing concentrations of non-Bt maize root extracts were included".

- No adverse effects on non-target aquatic shredding arthropods (two caddisflies (*Lepidostoma* spp. and *P. scabripennis*), a crane fly (*T. abdominalis*) and an isopod (*C. communis*)) were reported when fed senesced leaf tissues from Cry3Bb1-expressing maize (maize event MON 810 x MON 863) *ad libitum* for 30 days (Jensen et al., 2010; Lamp, 2010).

  Rosi-Marshall et al. (2007), who assessed the impact of an Cry1Ab-expressing but unspecified Bt-maize event, reported adverse effects on the trichopteran species (caddisflies) *Lepidostoma liba* and *Helicospyche borealis* when they were fed senesced Bt-maize leaves or Bt-maize pollen at a concentration of 2.75 gm^{-2} (a concentration that is two to three times higher than the maximum observed input rate of pollen in the field), respectively (but see ACRE, 2007b; Beachy *et al.*, 2008; Parrott, 2008; EFSA, 2009d). Under realistic conditions of exposure reflecting those reported in water courses, recent lower-tier bioassays with four different non-target aquatic shredding arthropod species (two caddisflies, a crane fly and an isopod) showed no effect on the larvae of caddisflies when fed senesced leaf tissues of Cry1Ab-expressing maize, whereas the negative effects observed on the crane fly and isopod were attributed to tissue-mediated differences among the isogenic line treatments (Jensen et al., 2010; Lamp, 2010). The authors attributed the lack of observable toxic effects in their study to the reduction of bioactivity of the Cry1Ab protein; maize tissues used were previously exposed for two weeks to environmental conditions. Moreover, Chambers et al. (2010) did not observe significant differences in total biomass of Trichoptera collected from streams close to GM and non-GM-maize in twelve streams adjacent to Bt-maize fields.

  Compared with the lepidopteran-active Cry1Ab protein, species in the order of Trichoptera are less likely to be affected by the coleopteran-active Cry3Bb1 protein because of their more distant phylogenetic relationship with the target Coleoptera.

Exposure of non-target organisms to Cry proteins in aquatic ecosystems is likely to be very low (Douville et al., 2005, 2007; Wolt and Peterson, 2010; Carstens et al., 2011). In decomposing maize MON 863 residues (i.e., leaf, stalk and root), a half-life of less than three days was found for the Cry3Bb1 protein, indicating that the duration of exposure of aquatic non-target arthropods to the Cry3Bb1 protein will be short (Prihoda and Coats, 2008a). Jensen et al. (2010) found no bioactivity of the Cry1Ab protein in senesced maize tissue after two weeks of exposure to terrestrial or aquatic environments in their lower-tier study with the European corn borer, suggesting rapid degradation of the protein (Griffiths et al., 2009). Even though the occurrence of maize detritus and detectable levels (0.56 ng/mL) of the Cry1Ab protein were reported in water bodies located at less than 500 m from maize fields up to six months after harvest in surveyed water streams in Indiana (USA), the Cry1Ab protein concentrations detected in water bodies were small compared with those measured in fresh maize plants (cf., the mean concentration (± SD) in stream water samples that were positive for the Cry1Ab protein was 14 ± 5 ng/L with a maximum concentration of 32 ng/L) (Tank et al., 2010). It was also shown that Cry1Ab-expressing maize tissue does not alter degradation rates, as compared with non-Bt-maize (Griffiths et al., 2009; Swan et al., 2009). Considering the probability of short-term exposure and acute effects to sensitive species, Wolt and Peterson (2010) indicated no concern in 99 % of cases, with limited opportunity for chronic effects, due to the rapid degradation of the Cry1Ab protein. Carstens et al. (2011) calculated that, even under worst-case conditions, the exposure of shredders to Bt-maize is low.

Using a simple standard pond scenario (1 ha pond, 2 m deep draining a 10 ha watershed planted with maize), the worst-case EEC was calculated to be 3.9 ng/mL Cry3Bb1 protein for maize MON 863 based on maize pollen loadings from airborne pollen deposition and agricultural run-off from maize plant tissue left in the field at the end of harvest (assuming that no degradation of the protein takes place) (EPA, 2010). Therefore, no substantial aquatic exposure to the Cry3Bb1 protein contained within maize plant tissue is expected (EPA, 2010; Carstens et al., 2011). In addition, whilst aquatic

coleopteran species are expected to occur in water bodies, even around agricultural areas, they are more likely to be abundant and stable in permanent water bodies (e.g., Schäfer et al., 2006).

After consideration of the published literature, the EFSA GMO Panel concludes it is unlikely that the Cry3Bb1 protein in maize MON 88017 products would cause adverse effects on non-target aquatic arthropods in the context of its proposed uses.

The conclusion of the EFSA GMO Panel is consistent with the evaluation carried out by the BE CA on maize MON 88017 who concluded that "*adverse effects on non-target insects is expected to be negligible*" (section 2.5.1 of the environmental risk assessment report of the BE CA).

Table 3. Overview of laboratory, greenhouse and field studies investigating the potential adverse effects of the Cry3Bb1 protein or of Cry3Bb1-expressing maize events (i.e., MON 88017, MON 863) on non-target arthropods

Order: Family	Species	Common name	Functional group	Habitat	Species of EU fauna	Type of study	Test material	Reference
Acari: Acaridae	*Rhizoglyphus robini*	Bulb mite	Herbivore	Terrestial	Yes	Tier 3	MON 863 root material	Carter et al. (2004)
Acari: Taxon	*Acari* spp.	Soil mites	Herbivore	Terrestial	Yes	Tier 3	MON 863	Al-Deeb et al. (2003); Ahmad et al. (2005)
							MON 88017	Höneman et al. (2008)
Aranea: Theridiidae	*Theridion impressum*	-	Predator	Terrestial	Yes	Tier 1b	MON 88017 pollen or prey fed MON 88017 material	Meissle and Romeis (2009b)
Aranea: Taxon	*Aranea* spp.	Spiders	Predator	Terrestrial	Yes	Tier 3	MON 88017	Svobodová et al. (2012)
							MON 863	Ahmad et al. (2005); Bhatti et al. (2005a,b)
Chilopoda: Taxon	*Centipedes*	Centipedes	Predator	Terrestrial	Yes	Tier 3	MON 863	Bhatti et al. (2005a)
Cladocera: Daphniidae	*Daphnia magna*	Water flea	Filter-feeder	Aquatic	Yes	Tier 1b	Cry3Bb1-expressing pollen	APHIS (2005); EPA (2010)
Collembola: Isotomidae	*Folsomia candida*	Springtail	Detrivore	Terrestrial	Yes	Tier 1b	MON 859 leaf material	[57]
							MON 863 leaf material	EPA (2010)
Collembola: Taxon	*Collembolans*	Springtails	Detrivore	Terrestrial	Yes	Tier 3	MON 88017	Höneman et al. (2008)
							MON 863	Al-Deeb et al. (2003); Ahmad et al. (2005); Bitzer et al. (2005)
Coleoptera: Bruchidae	*Callosobruchus maculatus*	Cowpea weevil	Herbivore	Terrestial	Yes	Tier 1a	Cry3Bb1 pure protein	[58]

[57] Technical dossier / Section D9.4 / Pages 184-201 / Annex: Teixeira (1999)
[58] Technical dossier / Section D9.4 / Pages 184-201 / Annex: Head et al. (2001)

Coleoptera: Carabidae	*Calathus fuscipes*	Ground beetle	Predator	Terrestial	Yes	Tier 1a	Cry3Bb1 pure protein	Priesnitz (2010)
						Tier 1b	Prey fed MON 88017 material	Büchs et al. (2008); Priesnitz (2010); [59]
						Tier 3	MON 88017	Priesnitz (2010)
	Calathus ambiguus	Ground beetle	Predator	Terrestial	Yes	Tier 1a	Cry3Bb1 pure protein	Priesnitz (2010)
						Tier 3	MON 88017	Priesnitz (2010)
	Harpalus caliginosus	Murky ground beetle	Predator, herbivore	Terrestial	Yes	Tier 1b	Cry3Bb1-expressing pollen	Mullin et al. (2005)
							MON 863 pollen	Ahmad et al. (2006a)
	Harpalus pensylvanicus	Ground beetle	Predator	Terrestial	Yes	Tier 1b	Cry3Bb1-expressing pollen	Mullin et al. (2005)
							MON 863 pollen	Ahmad et al. (2006a)
	Poecilus chalcites	Ground beetle	Predator	Terrestial	No	Tier 1a	Cry3Bb1 pure protein	Duan et al. (2006)
						Tier 1b	Cry3Bb1-expressing pollen	Mullin et al. (2005)
	Poecilus cupreus	Ground beetle	Predator	Terrestial	Yes	Tier 1b	Prey fed MON 88017 material	Büchs et al. (2008); [60]
	Pseudophonus rufipes	Ground beetle	Predator	Terrestial	Yes	Tier 1a	Cry3Bb1 pure protein	Priesnitz (2010)
						Tier 1b	Prey fed MON 88017 material	Priesnitz (2010)
						Tier 3	MON 88017	Priesnitz (2010)

[59] http://www.gmo-safety.eu/database/1028.research-impact-maize-cry-3bb1-non-target-organisms-living-soil.html
[60] http://www.gmo-safety.eu/database/1028.research-impact-maize-cry-3bb1-non-target-organisms-living-soil.html

Coleoptera: Carabidae	*Pterostichus melanarius*	Ground beetle	Predator	Terrestial	Yes	Tier 1a	Cry3Bb1 pure protein	Priesnitz (2010)
						Tier 1b	Cry3Bb1-expressing pollen	Mullin et al. (2005)
							Prey fed MON 88017 material	Priesnitz (2010)
						Tier 3	MON 88017	Priesnitz (2010)
	Carabids	Ground beetles	Predator	Terrestial	Yes	Tier 3	MON 88017	Priesnitz (2010)
								Svobodová et al. (2012)
							MON 863	Ahmad et al. (2005); Bhatti et al. (2005a)
Coleoptera: Chrysomelidae	*Chaetocnema pulicaria*	Corn flea beetle	Herbivore	Terrestrial	No	Tier 3	MON 863	Bhatti et al. (2005b)
	Chrysolina varians	-	Herbivore	Terrestial	Yes	Tier 1a	Cry3Bb1 pure protein	[61]
						Tier 1b	MON 88017 pollen	[62]
	Galerucella vittaticollis	Strawberry leaf beetle	Herbivore	Terrestial	Yes	Tier 1b	MON 863 pollen	Shirai (2006)
	Gastrophysa viridula	Green dock leaf beetle	Herbivore	Terrestial	Yes	Tier 1a	Cry3Bb1 pure protein	[63]
						Tier 1b	MON 88017 pollen	[64]
	Oulema lichenis	Cereal leaf beetle	Herbivore	Terrestial	Yes	Tier 3	MON 88017	Rauschen et al. (2010a)

61 http://www.gmo-safety.eu/database/1026.research-side-effects-maize-cry3bb1-non-target-organisms.html
62 http://www.gmo-safety.eu/database/1026.research-side-effects-maize-cry3bb1-non-target-organisms.html
63 http://www.gmo-safety.eu/database/1026.research-side-effects-maize-cry3bb1-non-target-organisms.html
64 http://www.gmo-safety.eu/database/1026.research-side-effects-maize-cry3bb1-non-target-organisms.html

Coleoptera: Chrysomelidae	*Phaedon cochleariae*	Mustard beetle	Herbivore	Terrestial	Yes	Tier 1a	Cry3Bb1 pure protein	[65]
						Tier 1b	MON 88017 pollen	[66]
	Phyllotreta spp.	Flea beetles	Herbivore	Terrestial	Yes	Tier 3	MON 88017	Rauschen et al. (2010a)
Coleoptera: Cicindelidae	*Cicindelids*	Tiger beetles	Herbivore	Terrestial	Yes	Tier 3	MON 863	Ahmad et al. (2005)
Coleoptera: Coccinellidae	*Adalia bipunctata*	2-spotted ladybird beetle	Predator	Terrestial	Yes	Tier 1a	Cry3Bb1 pure protein	Álvarez-Alfageme et al. (2011)
						Tier 1b	Prey fed MON 88017 material	Álvarez-Alfageme et al. (2011) [Schmidt et al. (2009)]
	Coccinella 7-punctata	7-spot ladybird	Predator	Terrestrial	Yes	Tier 3	MON 88017	Rauschen et al. (2010a)
	Coleomegilla maculata	Spotted ladybird beetle	Predator	Terrestial	No	Tier 1b	MON 863 pollen	Duan et al. (2002); Lundgren and Wiedenmann (2002); Ahmad et al. (2006a); Lövei et al. (2009); EPA (2010); [67]
							Prey fed MON 863 material	Lundgren and Wiedenmann (2005); Lövei et al. (2009)
						Tier 3	Cry3Bb1-expressing maize	McManus et al. (2005)
							MON 863	Al-Deeb and Wilde (2003); Lundgren et al. (2004, 2005); Bhatti et al. (2005b); Ahmad et al. (2006a)

65 http://www.gmo-safety.eu/database/1026.research-side-effects-maize-cry3bb1-non-target-organisms.html
66 http://www.gmo-safety.eu/database/1026.research-side-effects-maize-cry3bb1-non-target-organisms.html
67 Technical dossier / Section D9.4 / Pages 184-201 / Annexes: Duan et al. (2001a,b)

Coleoptera: Coccinellidae	*Epilachna vigintioctopunctata*	28-spotted ladybird beetle	Herbivore	Terrestial	No	Tier 1b	MON 863 pollen	Shirai (2006)
	Harmonia axyridis	Asian lady beetle	Predator	Terrestrial	Yes	Tier 3	MON 863	Bhatti et al. (2005b)
	Hippodamia convergens	Convergent ladybird beetle	Predator	Terrestial	Yes	Tier 1a	Cry3Bb1 pure protein	EPA (2010); [68]
						Tier 1b	MON 863 pollen	EPA (2010); [69]
						Tier 3	MON 863	Al-Deeb and Wilde (2003); Ahmad et al. (2006a)
	Propylea 14-punctata	14-spot ladybird	Predator	Terrestrial	Yes	Tier 3	MON 88017	Rauschen et al. (2010a)
	Scymnus spp.	Ladybeetles	Predator	Terrestrial	Yes	Tier 3	MON 863	Al-Deeb and Wilde (2003); Ahmad et al. (2006a)
	Stethorus punctillum	Ladybird beetle	Predator	Terrestial	Yes	Tier 1b	Prey fed MON 88017 material	Li and Romeis (2010)
Coleoptera: Curculionidae	*Sitophilus oryzae*	Weevil	Herbivore	Terrestial	Yes	Tier 1a	Cry3Bb1 pure protein	[70]
Coleoptera: Curculionidae	*Anthonomus grandis, Anthonomus eugenii*	Weevils	Herbivore	Terrestial	No	Tier 1a	Cry3Bb1 pure protein	[71]
Coleoptera: Elateridae	*Elaterids*	Wireworms	Herbivore	Terrestial	Yes	Tier 3	MON 863	Ahmad et al. (2005)
Coleoptera: Lathridiidae	*Cortinicara gibbosa*	Minute brown scavenger beetle	Saprovore	Terrestrial	Yes	Tier 3	MON 88017	Rauschen et al. (2010a)
Coleoptera: Nitidulidae	*Nitidulids*	Sap beetles	Herbivore	Terrestrial	Yes	Tier 3	MON 863	Bhatti et al. (2005a)

[68] Technical dossier / Section D9.4 / Pages 184-201 / Annexes: Palmer and Krueger (1999a) & Head et al. (2001)
[69] Technical dossier / Section D9.4 / Pages 184-201 / Annex: Bryan et al. (2001)
[70] Technical dossier / Section D9.4 / Pages 184-201 / Annex: Head et al. (2001)
[71] Technical dossier / Section D9.4 / Pages 184-201 / Annex: Head et al. (2001)

Coleoptera: Staphylinidae	*Atheta coriaria*	Rove beetle	Predator	Terrestrial	Yes	Tier 1a	Cry3Bb1 pure protein	Unpublished data (Fernandez S, personal communication)
						Tier 1b	Prey fed MON 88017 material	[72]; [Büchs et al. (2008)]
	Staphylinids	Rove beetles	Predator	Terrestrial	Yes	Tier 3	MON 88017	Hönemann et al. (2008)
								Svobodová et al. (2012)
							MON 863	Ahmad et al. (2005); Bhatti et al. (2005a)
Coleoptera: Tenebrionidae	*Tribolium castaneum*	Red flour beetle	Herbivore	Terrestial	Yes	Tier 1a	Cry3Bb1 pure protein	[73]
Diptera: Chironomidae	*Chironomus dilutus*	Midge	Detrivore	Aquatic	Yes	Tier 1b	MON 863 root extracts	Prihoda and Coats (2008a)
Diptera: Drosophilidae	*Drosophila melanogaster*	Common fruit fly	Detrivore	Terrestial	Yes	Tier 1b	MON 88017 leaf material	Knecht and Nentwig (2010)
Diptera: Phoridae	*Megaselia scalaris*	-	Detrivore	Terrestial	Yes	Tier 1b	MON 88017 leaf material	Knecht and Nentwig (2010)
Diptera: Sciaridae	*Lycoriella castanescens*	Dark-winged fungus gnat	Saprivore	Terrestial	Yes	Tier 1b	MON 88017 plant material	Büchs et al. (2008); [74]
Diptera: Syrphidae	*Syrphids*	Flower flies	Predator	Terrestrial	Yes	Tier 3	MON 863	Bhatti et al. (2005b)
Diptera: Tipulidae	*Tipula (Nippotipula) abdominalis*	Giant crane fly	Herbivore, shredder	Aquatic	No	Tier 1b	MON 810 x MON 863 leaf material	Jensen et al. (2010)
Diptera: Taxon	*Dipterans*	Flies (larvae)	Detrivore	Terrestial	Yes	Tier 3	MON 88017	Höneman et al. (2008)

[72] http://www.gmo-safety.eu/database/1028.research-impact-maize-cry-3bb1-non-target-organisms-living-soil.html
[73] Technical dossier / Section D9.4 / Pages 184-201 / Annex: Head et al. (2001)
[74] http://www.gmo-safety.eu/database/1028.research-impact-maize-cry-3bb1-non-target-organisms-living-soil.html

Hemiptera: Anthocoridae	*Orius insidiosus*	Minute pirate bug	Predator	Terrestial	Yes	Tier 1a	Cry3Bb1 pure protein	[75]; [Duan et al. (2008b)]
						Tier 3	MON 863	Al-Deeb and Wilde (2003); Bhatti et al. (2005b); Ahmad et al. (2006a)
							MON 88017	Svobodová et al. (2012)
Hemiptera: Aphididae	*Metopolophium dirhodum*	Rose-grain aphid	Herbivore (phloem sap feeder)	Terrestial	Yes	Tier 3	MON 88017	Meissle and Romeis (2009b); Romeis and Meissle (2011); Svobodová et al. (2012)
	Rhopalosiphum maidis	Corn leaf aphid	Herbivore (phloem sap feeder)	Terrestial	Yes	Tier 2	MON 863	Lundgren and Wiedenmann (2005)
						Tier 3	MON 863	Bhatti et al. (2005b)
	Rhopalosiphum padi	Bird cherry-oat aphid	Herbivore (phloem sap feeder)	Terrestial	Yes	Tier 2	MON 88017	Meissle and Romeis (2009b); Romeis and Meissle (2011)
						Tier 3	MON 88017	Meissle and Romeis (2009b); Romeis and Meissle (2011); Svobodová et al. (2012)
	Sitobion avenae	Grain aphid	Herbivore (phloem sap feeder)	Terrestial	Yes	Tier 3	MON 88017	Meissle and Romeis (2009b); Romeis and Meissle (2011)
Hemiptera: Miridae	*Trigonotylus caelestialium*	Rice leaf bug	Herbivore	Terrestial	Yes	Tier 3	MON 88017	Rauschen et al. (2009)
Hemiptera: Nabidae	*Nabis pseudoferus*	Damsel gug	Predator	Terrestial	Yes	Tier 3	MON 88017	[76]

[75] Technical dossier / Section D9.4 / Pages 184-201 / Annex: Teixeira (2005)

[76] http://www.gmo-safety.eu/database/1036.effects-cultivation-diabrotica-resistant-maize-ecosystem.html

Homoptera: Cicadellidae	*Zyginidia scutellaris* > *Psammotettix alienus* > *Javesella pellucid* > *Eupteryx atropunctata* > *Empoasca pteridis* > *Macrosteles laevis* > *Laodelphax striatella*	Plant and leafhoppers	Herbivore	Terrestial	Yes	Tier 3	MON 88017	Rauschen et al. (2008, 2010b)
Hymenoptera: Aphelinidae	*Nasonia vitripennis*	Wasp	Parasitoid	Terrestial	No	Tier 1a	Cry3Bb1 pure protein	EPA (2010); [77]
Hymenoptera: Apidae	*Apis mellifera*	Honeybee	Pollinator	Terrestial	Yes	Tier 1a	Cry3Bb1 pure protein	Duan et al. (2008a); EPA (2010); [78]
						Tier 1b	MON 88017 pollen	[79]
Hymenoptera: Braconidae	*Macrocentrus cingulum*	Braconid parasitoid	Parasitoid	Terrestrial	Yes	Tier 3	MON 863	Bhatti et al. (2005b)
Hymenoptera: Formicidae	*Formicids*	Ants	Detrivore	Terrestrial	Yes	Tier 3	MON 863	Ahmad et al. (2005); Bhatti et al. (2005a)
Hymenoptera: Taxon	*Hymenopterans*	Wasps	Parasitoid	Terrestrial		Tier 3	MON 863	Bhatti et al. (2005a,b)
Isopoda: -	*Caecidotea communis*	-	Herbivore, shredder	Aquatic	No	Tier 1b	MON 810 x MON 863 leaf material	Jensen et al. (2010)
Lepidoptera: Pyralidae	*Ostrinia nubilalis*	European corn borer	Herbivore	Terrestial	Yes	Tier 1a	Cry3Bb1 pure protein	[80]
Lepidoptera: Danaidae	*Danaus plexippus*	Monarch butterfly	Herbivore	Terrestial	No	Tier 1b	MON 863 pollen	Mattila et al. (2005); EPA (2010)
Lepidoptera: Noctuidae	*Helicoverpa zea*	Corn earworm	Herbivore	Terrestial	No	Tier 1a	Cry3Bb1 pure protein	[81]

[77] Technical dossier / Section D9.4 / Pages 184-201 / Annexes: Sindermann et al. (2002b) & Head et al. (2001)
[78] Technical dossier / Section D9.4 / Pages 184-201 / Annexes: Maggi (1999a,b, 2002) & Head et al. (2001)
[79] Additional information received on 04/04/2011 / Request 1.3 / Pages 7-12 // Additional information received on 23/05/2011 / Request 1 / Page 2 / Annexes: Richards (2011a,b)
[80] Technical dossier / Section D9.4 / Pages 184-201 / Annex: Head et al. (2001)
[81] Technical dossier / Section D9.4 / Pages 184-201 / Annex: Head et al. (2001)

Neuroptera: Chrysopidae	*Chrysoperla carnea*	Green lacewing	Predator	Terrestial	Yes	Tier 1a	Cry3Bb1 pure protein	Li et al. (2008); EPA (2010) [82]
						Tier 1b	MON 88017 pollen	Li et al. (2008, 2010)
						Tier 3	MON 863	Bhatti et al. (2005b)
Orthoptera: Gryllidae	*Gryllids*	Crickets	Herbivore	Terrestrial	Yes	Tier 3	MON 863	Ahmad et al. (2005); Bhatti et al. (2005a)
Thysanoptera: Thripidae	*Frankliniella occidentalis*	Thrip	Herbivore	Terrestrial	Yes	Tier 3	MON 88017	Svobodová et al. (2012)
Trichoptera: Limnephilidae	*Pycnopsyche scabripennis*	Caddisfly	Herbivore, shredder	Aquatic	No	Tier 1b	MON 810 x MON 863 leaf material	Jensen et al. (2010)
Trichoptera: Lepidostomatidae	*Lepidostoma* spp.	Caddisflies	Herbivore, shredder	Aquatic	Yes	Tier 1b	MON 810 x MON 863 leaf material	Jensen et al. (2010)

In its guidelines for the environmental risk assessment of GM plants (EFSA, 2010e) and for the assessment of potential impacts of GM plants on non-target organisms (EFSA, 2010d), the EFSA GMO Panel considered three main tiers, which comprise experimental studies under controlled conditions (e.g., laboratory studies under tier 1a and 1b and semi-field studies under tier 2) and field studies (tier 3). Tier 1a refers to *in vitro* studies carried out with purified metabolites, whereas tier 1b refers to *in planta* testing using bi- or multi-trophic experiments. Semi-field studies are outdoor experiments carried out with some containment that controls for variability, with manipulation treatments on relatively small experimental units (e.g., caged plants, screen houses).

[82] Technical dossier / Section D9.4 / Pages 184-201 / Annexes: Palmer and Krueger (1999b) & Head et al. (2001)

Effects on non-target organisms that are not arthropods

The potential of maize MON 88017 to have direct or indirect adverse effects on non-target organisms that are not arthropods, as well as the ecological functions they provide is described below, with a focus on nematodes, earthworms, enchytraeid worms, molluscs, mammals, birds and fish. Potential adverse effects on soil microorganisms are considered in section 6.2.6.2, below.

Nematodes are considered useful indicators of soil quality due to their great diversity and participation in many functions at different levels of food webs in soil and due to their presence in almost all soils with a high population density and a large number of species (Sochova et al., 2006).

Caenorhabditis elegans exposed to aqueous Cry3Bb1-containing solutions showed a dose-dependent inhibitory effect on the growth and reproduction, with EC_{50} values of 22.3 mg/L (0.29 µM) and 7.9 mg/L (0.10 µM), respectively, indicating susceptibility to the aqueous solution of the Cry3Bb1 protein (Höss et al., 2011). Higher-tier field studies conducted with Cry3Bb1-expressing maize in the EU (event MON 88017; Höss et al., 2011) and the USA (event MON 863; Al-Deeb et al., 2003) did not indicate significant differences in the abundance and diversity of nematodes in soil planted with Bt-maize and soil planted with its near-isogenic counterpart due to the low Cry3Bb1 protein concentrations in soil (section 6.2.6.1, below). Any effects on nematodes by Cry3Bb1-expressing maize and their products are likely to be minor compared with effects of agricultural practices, environmental stresses or differences between localities and maize varieties (e.g., Griffiths et al., 2005, 2006, 2007a,b).

Earthworms and enchytraeid worms play an important role in decomposing plant litter, and are responsible for numerous physical changes that affect the biological properties and processes in soil (e.g., structure, quality, functionality). They are considered important organisms in the regulation of nutrient cycling processes (Didden, 1993; Curry and Schmidt, 2006). Earthworms and enchytraeid worms can be exposed to Cry proteins, as Cry proteins can enter the soil by root exudates (Saxena et al., 2002, 2004), plant material (Webster et al., 2008), and by plant residues (Stotzky, 2004). If earthworm populations would be adversely affected by the cultivation of GM crops, this may have negative consequences on the ecological functions they provide.

A lower-tier study on the earthworm species *Eisenia fetida* exposed to pure Cry3Bb1 protein conducted by the applicant gave no indications of adverse impacts to this earthworm species following short-term exposure to high doses of the Cry3Bb1 protein[83]. Due to the very short exposure in duration, the BE CA considered that this study "*did not prove for the absence of adverse effects on Eisenia fetida, but rather gave indications of absence of adverse effects (see Annex I and II)*" (section 2.7.1 of the environmental risk assessment report of the BE CA). No adverse effects due to toxicity of the Cry3Bb1 protein have been detected in other lower-tier studies on the earthworm species *Lumbricus terrestris* (Ahmad et al., 2006b) and the enchytraeid worm species *Enchytraeus albidus* (Hönemann and Nentwig, 2009). Feeding *E. albidus* with diets containing leaf material from maize MON 88017 did not affect the survival and reproduction of adults (Hönemann and Nentwig, 2009).

In their higher-tier field study conducted in the USA, Zeilinger et al. (2010) did not observe significant differences in numbers and biomass of juvenile and adult earthworms (*Aporrectodea caliginosa*, *A. trapezoides*, *A. tuberculata* (collectively, the *A. caliginosa* species complex), and *L. terrestris*) between non-Bt-maize and Bt-maize (event MON 863) varieties during four years of cultivation. A nine months leaf litter-bag field study conducted in Switzerland with maize MON 88017 revealed no difference in decomposer communities (including Clitellata which represented 6 % of the total abundance of observed decomposers) when compared with the near-isogenic counterpart and other conventional maize varieties (Hönemann et al., 2008).

[83] Technical dossier / Section D9.4 / Pages 184-201 / Annex: Sindermann et al. (2002a)

So far, no higher-tier studies have been performed on enchytraeid worms to analyse the potential consequences of ingestion of Cry3Bb1-expressing maize plant material. Hönemann and Nentwig (2009) did not expect Cry3Bb1-expressing maize to endanger the survival or reproduction *E. albidus*, provided that organic matter of sufficient quality is available in the soil. Generally, enchytraeid worms do not feed on a single food source, but take up all degradable organic matter of adequate size in the field.

The Cry3Bb1 protein was detected in the gut and feaces of the molluscs, *Arion lusitanicus* and *Deroceras reticulatum*, after the slugs had fed on leaves from maize MON 88017, indicating possible exposure of slugs when feeding on Bt-maize. Following exposure, no differences in weight gain or loss of slugs were observed among the treatment groups (Zürbrugg and Nentwig, 2009). In a continuation of the study by Zürbrugg and Nentwig (2009) with experiments lasting for 16 weeks, no significant effects of maize MON 88017 were detected on the survival, weight change and oviposition of the slug *Arion vulgaris* (Hönemann and Nentwig, 2010).

The lack of toxicity of the Cry3Bb1 protein to birds[84] and mammals[85] was confirmed in toxicity studies, and the nutritional quality of maize MON 88017 tested with fish[86] in an nutritional equivalence study (APHIS, 2005) (sections 4 and 5, above; EFSA, 2009a for further details).

The EFSA GMO Panel is of the opinion that there is no evidence to indicate that the cultivation of maize MON 88017 is likely to cause adverse effects on non-target organisms that are not arthropods in the context of its proposed uses.

The conclusion of the EFSA GMO Panel is consistent with the evaluation carried out by the BE CA on maize MON 88017. The BE CA concluded that "*adverse effects on non-target organisms other than insects are expected to be negligible*" (section 2.5.1 of the environmental risk assessment report of the BE CA).

6.2.4.3. 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 maize MON 88017 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, 2009c for maize NK603; CERA, 2010). The CP4 EPSPS protein shares no significant homology with known toxic proteins (section 4, above; EFSA, 2009a)[87] 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 argued that the probability of direct adverse effects of maize MON 88017 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 maize MON 88017 and its conventional counterpart, and because the molecular characterisation of the DNA insert and flanking regions of maize MON 88017 did not raise safety concerns (section 4, above; EFSA, 2009a).

Lower-tier studies performed with the pure CP4 EPSPS protein confirmed the lack of acute toxicity of the CP4 EPSPS protein to the Colorado potato beetle[88], the European corn borer[89], the earthworm species *Eisenia fetida*[90] and mice (Harrison et al., 1996). The lack of toxicity of the CP4 EPSPS proteins to rats and broiler chickens fed diets containing maize MON 88017 was shown in toxicity studies and nutritional equivalence studies (section 4, above; EFSA, 2009a for further details). In its application, the applicant also referred to higher-tier studies conducted with maize NK603 in the EU

84 Technical dossier / Section D9.5 / Pages 186-208 / Annexes: MSL-16161 (1999) & MSL-19877 (2005)
85 Technical dossier / Section D9.5 / Pages 186-208 / Annexes: WIL-50283 (2005) & WIL-50284 (2005)
86 Technical dossier / Section D9.7 / Pages 211-212 / Annex: Li and Robinson (2004)
87 Technical dossier / Section D9.5 / Pages 190-191 / Annex: Astwood et al. (2001a)
88 Technical dossier / Section D9.5 / Pages 190-191 / Annex: Levine and Uffman (2007)
89 Technical dossier / Section D9.5 / Pages 190-191 / Annex: Uffman and Levine (2007)
90 Technical dossier / Section D9.5 / Pages 190-191 / Annex: Sindermann et al. (2004)

(Rosca, 2004; Rodriguez et al., 2006; Schier, 2006) and the Philipines (Reyes, 2005), or with soybean GTS 40-3-2 in the USA (Buckelew et al. 2000; Jasinski et al., 2003) to confirm that the exposure of non-target organisms to CP4 EPSPS-expressing crops poses no potential hazard, supporting the conclusions of lower-tier studies. The higher-tier study of Jasinski et al. (2003) suggested that the abundance of some beneficial organisms decreased in fields with glyphosate tolerant crops compared with fields planted with conventional crops. These reductions do not seem to be directly associated with the expression of CP4 EPSPS protein in herbicide tolerant crops, but are likely to be a consequence of changes in weed populations caused by different weed management regimes. The applicant also referred to field trials with soybean GTS 40-3-2 to show that no significant differences in the abundance of Collembolla were noted between glyphosate tolerant and conventional soybean under the same weed management regimes (Bitzer et al., 2002).

The non-target organism 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 notes that maize MON 88017 and other glyphosate tolerant crops have been extensively cultivated in the 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).

The conclusion of the EFSA GMO Panel on the absence of adverse effects of maize MON 88017 to non-target organisms due to the expression of the CP4 EPSPS protein is consistent with the evaluation carried out by the BE CA on maize MON 88017. The BE CA concluded that "*the expression of CP4 EPSPS is not expected to have adverse effects on non-target organisms*" (section 2.5.2 of the environmental risk assessment report of the BE CA).

Effects of the cultivation of maize MON 88017 and the use of glyphosate are considered in section 6.2.7, below.

6.2.4.4. Adverse effects on non-target organisms due to interactions between the Cry3Bb1 and CP4 EPSPS proteins

The activity of the Cry3Bb1 and CP4 EPSPS proteins expressed in maize MON 88017 is not likely to be affected by potential interactions among both proteins, as their mode of action differs (e.g., De Schrijver et al., 2007; Raybould et al., 2011a; Wolt, 2011). The data submitted by the applicant and the review of published literature did not indicate any interactions in the expression of the proteins or their biological activity compared with GM crops expressing similar single proteins. The EFSA GMO Panel therefore supports the conclusion of the BE CA who concluded that "*testing the combined effects of the two newly expressed proteins*" is "*not necessary*" (section 1.3.1 of the environmental risk assessment report of the BE CA).

6.2.4.5. Adverse effects on non-target organisms due to interactions between biota

The EFSA GMO Panel pinpoints the importance of accounting for farm management practices in any environmental risk assessment evaluation of each GM crop that combines insect resistance and herbicide tolerance traits, as interactions between biota may occur under different weed management regimes, irrespective of interactions between the newly expressed proteins (section 6.2.7, below).

6.2.5. Effects on human and animal health[91]

The molecular analysis and the food and feed safety assessment of maize MON 88017 did not raise safety concerns for human and animal health (sections 3 to 5, above; EFSA, 2009a). In its previous Scientific Opinion on maize MON 88017 (EFSA, 2009a), the EFSA GMO Panel concluded that

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

"*maize MON 88017 is as safe as conventional maize*", and that "*maize MON88017 and derived products are unlikely to have any adverse effect on human and animal health in the context of the intended uses*".

6.2.6. Interactions with biogeochemical processes and the abiotic environment[92]

The newly expressed proteins in maize MON 88017 can be introduced into the soil via physical damage to plant tissues, via decomposition of shed root cells during plant growth, via decomposing plant residues remaining in fields after harvest, which might be incorporated into the soil during tillage operations (Stotzky, 2004), and possibly via root exudates (e.g., Saxena et al., 2002, 2004; Icoz and Stotzky, 2007; Icoz et al., 2008), resulting in exposure of non-target soil organisms to the Cry3Bb1 protein. Whether nanograms of Cry3Bb1 proteins detected in the rhizosphere of Bt-maize are caused by root exudation or are derived from sloughed root cells and small root hairs as contaminants of soil samples remains debatable (Miethling-Graff et al., 2010). Indirect exposure through manure and faeces from animals fed maize MON 88017 was also considered, though most of the Cry3Bb1 protein would be degraded by enzymatic activity in the intestinal tract and subsequently by microbial processes in the manure.

6.2.6.1. Fate of the Cry3Bb1 protein in soil

Proteins can be a major source of energy, carbon and nitrogen for soil microorganisms. They are readily degradable by widely abundant extracellular microbial proteases (Jan et al., 2009) and there is no indication that Cry proteins would generally behave differently compared with other proteins (reviewed by Icoz and Stotzky, 2008). The fate and hence the persistence of Cry proteins in soil depends upon multiple factors, varying among environmental conditions (e.g., type of crop, soil characteristics, microbial activity, temperature) (reviewed by Icoz and Stotzky, 2008). Evidence retrieved from the scientific literature shows that the Cry3Bb1 protein is rapidly degraded in soil and has a short half-life (Ahmad et al., 2005; Icoz and Stotzky, 2007; Icoz et al., 2008; Miethling-Graff et al., 2010; Zurbrügg et al., 2010). Even though Cry proteins are degraded or inactivated in soil within weeks (e.g., Gruber et al., 2011a,b), a residual fraction can persist longer under certain conditions. Evidence has shown that, due to their chemical properties (e.g., surface charges), the Cry3Bb1 protein can be sorbed by organo-mineral surfaces, i.e., those provided by clay or humic particles, thereby reducing its availability for extracellar microbial proteases (Pagel-Wieder et al., 2006; Fiorito et al., 2008). This reduced availability slows down degradation rates of the Cry3Bb1 protein compared with purely water-dissolved protein, but does not completely preserve it from degradation. Prihoda and Coats (2008b) reported half-lives of the Cry3Bb1 protein in decomposing crop residues from maize MON 863 in microcosms of less than six days for leaf, root and stalk. After 25 days, less than 1 % of the Cry3Bb1 protein remained in the three analysed plant tissues (Prihoda and Coats, 2008b). In a litterbag study carried out during winter, the decomposition of the Cry3Bb1 protein in senescent leaves from maize MON 88017 was determined as 48 % after three weeks and 95 % after six weeks (Zürbrugg et al., 2010). Similarly, Miethling-Graff et al. (2010) reported almost 100 % decline in the Cry3Bb1 protein concentration in the roots of field-collected stubbles from maize MON 88017 within a seven months period. The Cry3Bb1 protein was found to be degraded more quickly than the Cry1Ab protein in soil under similar conditions (Baumgarte and Tebbe, 2005; Madliger et al., 2010, 2011; Miethling-Graff et al., 2010; Sander et al., 2010).

The potential accumulation of plant-produced Cry3Bb1 protein in soil following repeated and large-scale cultivation of Cry3Bb1-expressing maize has been studied (e.g., Ahmad et al., 2005; Icoz and Stotzky, 2007; Icoz et al., 2008; Miethling-Graff et al., 2010; Zurbrügg et al., 2010). Miethling-Graff et al. (2010) detected low concentrations of the Cry3Bb1 protein in soil, but did not observe an increase in detectable Cry3Bb1 protein during three years of continuous maize MON 88017 cultivation, indicating that there is no accumulation of this protein in soil on agricultural fields up to detectable amounts (ca. > 0.01 ng g^{-1} soil), despite its potential to bind to surface-active particles (Miethling-Graff et al., 2010).

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

The EFSA GMO Panel concludes that Cry3Bb1 protein concentrations in decaying plant residues from maize MON 88017 decrease rapidly. There is no evidence for accumulation of the Cry3Bb1 protein on agricultural fields cultivated repeatedly with maize MON 88017 or comparable maize events (e.g., maize MON 863), despite its potential to bind to surface-active particles. Therefore, non-target soil organisms will be exposed to relatively low Cry3Bb1 protein concentrations within a few months after harvest, even in cropping systems with repeated cultivation of maize MON 88017.

The conclusion of the EFSA GMO Panel is consistent with that of the BE CA who reported that "*laboratory and field studies showed that Cry3Bb1 does not persist in the soil environment and is degraded rapidly*" (section 2.7.1 of the environmental risk assessment report of the BE CA).

6.2.6.2. Adverse effects on soil microorganisms due to the expression of the Cry3Bb1 protein

A lower-tier study with the pure Cry3Bb1 protein did not reveal adverse effects on microorganisms or microbial-mediated carbon and nitrogen mineralisation processes in the soil[93]. Likewise, no deleterious effects on soil microbial biomass, activity or community structure were observed in higher-tier studies with maize MON 863 in the USA (Devare et al., 2004, 2007; Icoz et al., 2008; Lawhorn et al., 2009; Xue et al., 2011). The occasional differences observed in decomposition rates or numbers of some microbial groups were not consistent, as they were transient, and were driven primarily by environmental factors. In their 3-year field study in Germany, Miethling-Graff et al. (2010) did not detect any significant differences between the rhizosphere bacterial community structure of maize MON 88017 compared with the near-isogenic counterpart and two conventional maize varieties.

Field trials conducted in the USA with maize MON 863 showed no significant differences in numbers of protozoa and fungal propagules after four years of continuous cultivation (Icoz et al., 2008). Meissle et al. (2009) studied the interaction of the entomopathogenic fungus *Metarhizium anisopliae*, used as a microbial pest control agent, with maize MON 88017 and Western corn rootworm in a lower-tier study. While the development of larvae from Western corn rootworm was delayed when fed maize MON 88017, there was no difference in infection rates between maize MON 88017 and control maize, indicating that maize MON 88017 does not interfere with the biological control provided by *M. anisopliae*.

Cry proteins do not act as antimicrobials but rather as insecticides with a narrow host specificity. During the relatively long period of scientific exploration of Cry proteins and their potential biotechnological or agricultural applications, there has been no scientific report to the EFSA GMO Panel's knowledge that these proteins would exhibit persisting adverse effects on bacteria or other microorganisms or interfere with microbial activities (Icoz et al., 2008). Therefore, there is no indication of a hazard of maize MON 88017 or its Cry3Bb1 protein to soil microorganisms and the ecological functions they provide, including their contribution to biogeochemical processes. Where effects of GM crops on microbial communities have been reported, these effects were in general considered spatially and temporally limited, and small compared with those induced by differences in geography, temperature, seasonality, plant variety, soil type and changes in soil management (Sessitsch et al., 2004; Fang et al., 2005, 2007; Griffiths et al., 2005, 2006; Lilley et al., 2006; Filion, 2008; Icoz and Stotzky, 2008).

In case of Cry1Ab-expressing maize, various studies have reported decreases in the decomposition rate of Bt-maize (e.g., Saxena and Stotzky, 2001b; Flores et al., 2005; Poerschmann et al., 2005; Fang et al., 2007; Raubuch et al., 2007). These differences in decomposition rate have been shown to result from increased lignin contents in certain maize varieties, and not from an inhibition of soil microorganisms by the Cry1Ab protein (Griffiths et al., 2007b; Hönemann et al., 2008; Lehman et al., 2008a; Tarkalson et al., 2008). Altered lignin content in maize varieties is not an effect attributed to the insertion of the transgene, but from the genetic background of the maize varieties under consideration (Fernie et al., 2006; Griffiths et al., 2007; Lehman et al., 2008b, 2010; Zurbrügg et al., 2010; Yanni et al., 2011). The total lignin content in the roots and leaves of maize MON 88017 is

[93] Technical dossier / Section D9.8 / Pages 213-216 / Annex: Carson et al. (2005)

slightly higher (7 %) or similar, respectively, compared with the near-isogenic counterpart (Poerschmann et al., 2008; Zurbrügg et al., 2010). In their litter bag study, Zurbrügg et al. (2010) found that leaf litter from maize MON 88017 is readily degraded and that degradation did not differ from the near-isogenic counterpart, but varied among conventional maize varieties. Compositional plant properties including lignin contents among conventional maize varieties differed more than between maize MON 88017 and its near-isogenic counterpart, with maize MON 88017 falling within the variation found in conventional maize varieties (see also Lehman et al., 2008a, 2010 for Cry3Bb1-expressing maize events). The EFSA GMO Panel agrees with the BE CA that "*the small increase in lignin content in the roots*" is not expected to cause "*differences in carbon sequestration over the longer term*" (section 2.7.1 of the environmental risk assessment report of the BE CA).

The EFSA GMO Panel considers that Cry3Bb1 protein concentrations in decaying plant residues from maize MON 88017 decrease rapidly in soil, indicating that non-target soil organisms are exposed to relatively low Cry3Bb1 protein concentrations within a few months after harvest. There is no evidence for accumulation of the Cry3Bb1 protein on agricultural fields cultivated repeatedly with maize MON 88017 or comparable maize events (e.g., MON 863), despite its potential to bind to surface-active particles. Effects of crops on soil microbial communities, which are especially expected in the rhizosphere or on decaying plant material, depend more on their species, variety or age than whether they are genetically modified. Rearrangements in structural diversity and population abundance of non-target soil organisms occur frequently in the agricultural environment. They are typically associated with several sources of variation, caused by natural variability (e.g., soil heterogeneity, weather conditions) and agricultural practices (e.g., soil tillage, crop rotation, irrigation measures) and are thus not necessarily an indication of environmental harm. The EFSA GMO Panel concludes that potential effects on soil microorganisms and microbial communities, as well as the ecological functions they provide, due to the cultivation of maize MON 88017, if they occur, will be transient and minor, and are likely to be smaller or within the range currently caused by other agronomic and environmental factors.

The conclusion of the EFSA GMO Panel on the absence of direct effects of maize MON 88017 on biogeochemical processes and the abiotic environment due to the expression of the Cry3Bb1 protein is consistent with the evaluation carried out by the BE CA on maize MON 88017. The BE CA concluded that "*adverse effects on non-target microbes of the soil community are expected to be negligible*" (section 2.7.1 of the environmental risk assessment report of the BE CA).

6.2.6.3. Adverse effects on biogeochemical processes and the abiotic environment due to the expression of the CP4 EPSPS protein

No direct effects on biogeochemical processes and the abiotic environment of maize MON 88017 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 transformation, via lower-[94] and higher-tier studies performed with maize NK603 in France (Philippot et al., 2006), glyphosate tolerant maize in Canada (Hart et al., 2009), or with glyphosate tolerant maize and soybean in the USA (Liphadzi et al., 2005). Because the CP4 EPSPS protein of maize MON 88017 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, of which the wild-type variants are naturally occurring in the soil environment, is not expected to alter the natural interactions of maize plants with the abiotic environment. The EFSA GMO Panel is not aware of any reports of effects on biogeochemical processes and the abiotic environment due to this trait (Dunfield and Germida, 2004; Cerdeira and Duke, 2006; Powell et al., 2007; CERA, 2010).

The conclusion of the EFSA GMO Panel on the absence of potential adverse effects of maize MON 88017 on biogeochemical processes and the abiotic environment due to the expression of the

[94] Technical dossier / Section D9.8 / Pages 213-216 / Annex: Carson et al. (2004)

CP4 EPSPS protein is consistent with the evaluation carried out by the BE CA on maize MON 88017 (section 2.7.2 of the environmental risk assessment report of the BE CA).

6.2.7. Impacts of the specific cultivation, management and harvesting techniques[95]

Changes in pest management: Western corn rootworm larvae are currently controlled chemically through the use of maize seed coated with systemic insecticides and/or soil insecticides (applied at planting) (Levine and Oloumi-Sadeghi, 1991; Széll et al., 2005; Boriani et al., 2006; Ma et al., 2009; van Rozen and Ester, 2010; Meissle et al., 2011b). Foliar broad-spectrum insecticides are applied to suppress adult populations, especially in continuous maize, in order to decrease egg-laying by adult females and hence the number of overwintering eggs and hatching larvae in the subsequent year (Levine and Oloumi-Sadeghi, 1991; Boriani et al., 2006). Foliar insecticides are also applied to prevent silk clipping by adults in seed and sweet maize production, where high grain quality is essential for marketing (Levine and Oloumi-Sadeghi, 1991; Tuska et al., 2002; Boriani et al., 2006; van Rozen and Ester, 2010; Meissle et al., 2011b). Crop rotation is highly effective in controlling Western corn rootworm, as females lay their eggs mainly in maize fields and the larvae hatching in the following year do not survive well on other crop roots (Levine and Oloumi-Sadeghi, 1991; Kiss et al., 2005, Boriani et al., 2006; Meissle et al., 2011b). The evolution of rotation-resistant pest populations jeopardises the efficiency of crop rotations in controlling Western corn rootworm. In some regions of the USA, Western corn rootworm has adapted its egg-laying behaviour to lay eggs in crops other than maize, leading to damage in first-year maize in spite of crop rotation (Levine and Oloumi-Sadeghi, 1996; Onstad et al., 1999, 2001b; Levine et al., 2002; Mitchell et al., 2004; Gray et al., 2009; Miller et al., 2009). Such a rotation-resistant Western corn rootworm variant has not evolved in the EU yet (Kiss et al., 2005). In regions where Western corn rootworm populations have been detected, but are not yet established[96], mandatory eradication programs require the application of chemical insecticides and planting restrictions of maize in buffer zones surrounding new introduction points (FCEC, 2009; Carrasco et al., 2010). Pest management in maize MON 88017 will differ from that currently practiced in conventional maize cropping systems; less or no treatments with broad-spectrum insecticides are needed to control Western corn rootworm. Therefore, maize MON 88017 is expected to result in a reduced environmental load by chemical insecticides (Alston et al., 2002; Rice, 2004), and lead to fewer adverse side-effects on non-target arthropods in the maize ecosystem, when it replaces chemical insecticides (Marvier et al., 2007; EFSA, 2008b; Wolfenbarger et al., 2008; Naranjo, 2009). As indicated in section 6.2.3.1, the cultivation of maize MON 88017 could lead to the evolution of resistance in the target pest and so cultivation practices will need to be adapted accordingly.

Changes in weed management: The applicant concluded that "*the in-crop use of glyphosate should not be considered as a novel agronomic or management technique specific to MON 88017, 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) maize MON 88017 in cropping systems is a change in the cultivation and management of this maize compared with conventional maize. Currently, the control of weeds in maize is mostly achieved by using pre-emergence soil acting residual herbicides and/or post-emergence selective herbicides in Europe. Very little control using cultural or mechanical means alone, without the use of selective herbicides, is applied in current European maize cropping systems (Bastiaans et al., 2008), though some has been suggested particularly for continuous maize or organic systems (Dewar, 2009; Meissle et al., 2010).

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

[95] Technical dossier / Section D9.9

[96] http://extension.entm.purdue.edu/wcr/; http://www.eppo.org/QUARANTINE/Diabrotica_virgifera/diabrotica_virgifera.htm#map-dia; http://w3.mkk.szie.hu/dep/nvtt/wcrnet/wcrnet-2.htm; http://www.iwgo.org/dist_map.htm

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. Where 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). However, the environmental impact indices used for these calculations are generally based on residual, persistence and ecotoxicity characteristics, and do not relate to the efficacy and hence the biodiversity impact of herbicides (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-GMHT 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 (Chassy et al., 2003; ACRE, 2007a; Morris, 2007; Sanvido et al., 2007, 2011a,b; Ehlers, 2011). While 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. Herbicide regimes in maize cropping systems

Herbicide regimes in conventional maize in Europe

The sensitivity of maize to early weed competition is well-understood and the need for efficient weed control in the early maize growth stages often requires herbicide use with soil (residual) activity. Maize is very delicate in its early growth stages; it is very susceptible to competition for resources such as water, nutrients and light (Johnson et al., 2000; Lehoczky et al., 2004; Dewar, 2009; Teasdale and Cavigelli, 2010). Therefore, it is important to protect the early growth stages from weed interference until crop canopy development naturally limits the emergence of weeds (this usually takes place around the 8th leaf stage of maize). Three different herbicidal weed management strategies are possible in conventional maize for the control of annual and perennial grass and broadleaf weeds (Champion et al., 2003; Lehoczky et al., 2004; Beckie et al., 2006):

(1) application(s) pre-emergence of the crop;

(2) application(s) early post-emergence, ideally in the 2nd to the 4th leaf stage of maize;

(3) sequential applications, where a combination of herbicides with soil (residual) activity is applied pre-emergence followed by a mixture of post-emergence herbicides with foliar activity.

The choice of herbicides, applied alone or in tank mixtures, is driven by the need to cope with a wide spectrum of weeds present, which can vary greatly according to climate, soil type, season, field history, rotation, weed life cycles and cultivation practices (Dewar, 2010; Meissle et al., 2010).

Glyphosate is a broad-spectrum contact systemic herbicidal active substance used for the control of most annual and many perennial weeds (Duke and Powles, 2008b), but with little or no soil acting (residual) properties. It is absorbed by green leaves and stems from where it is translocated into plant tissues via the apoplast and the symplast. Glyphosate uptake by roots is minimal, so there is no long-term exposure of weeds to herbicidal activity. In the EU, glyphosate is currently used in conventional cropping by some farmers as a pre-sowing or pre-emergence herbicide. It is used pre-emergence of the crop for removing emerged weeds (Monsanto, 2010). 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 (Duke and Powles, 2008b; Dewar, 2009), or it is used through weed wipers when weeds (especially perennials) are taller than the crop[97].

Glyphosate-based herbicide regimes in genetically modified herbicide tolerant (GMHT) maize

Glyphosate-based herbicides will 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 weed emergence (Gianessi, 2005; Cerdeira and Duke, 2006). The efficacy of glyphosate at controlling weeds is less dependent on weed size, 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; Dewar, 2009, 2010). It is expected that the introduction of glyphosate in GMHT maize will replace or reduce the use of other herbicidal active substances used pre-emergence or early post-emergence of the crop (Dewar, 2010). However, the control of larger and perennial weeds will require higher application rates.

Several strategies have been proposed for controlling weeds in GMHT maize depending upon the spectrum and density of weeds present at or just after sowing (reviewed by Beckie et al., 2006; Devos et al., 2008; Dewar, 2009, 2010)[98].

(1) A single application (or sequential applications) of glyphosate-based herbicide alone, with no use of pre-emergence herbicides. However, note that field trials have shown that the use of glyphosate alone, applied post-emergence on one occasion at the recommended application rates can be inadequate to control all the weeds present throughout a full growing season (Gianessi et al., 2002; Gower et al., 2002, 2003; Grichar and Minton, 2006; Parker et al., 2006). In a trial of glyphosate tolerant maize (event NK603) in the Czech Republic, Soukup et al. (2008) only achieved acceptable herbicide efficacy for a single application of glyphosate when applied at dose rates above 1,440 g/ha active substance (ai). Additionally, the achievement of acceptable levels of herbicide efficacy depends upon the correct timing of glyphosate application (Johnson et al., 2000; Thomas et al., 2004, 2007; Beckie et al., 206). 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 also increase weed pressure in subsequent years (Myers et al., 2005). Recommended strategies to avoid weed re-infestation involve the use of two post-emergence applications of glyphosate (Gower et al., 2002, 2003; Gehring and Mülleder, 2004). In case of maize NK603, both Soukup et al. (2008) and Verschwele and Mülleder (2008) achieved optimal herbicide efficacy by providing a double application of glyphosate, each with a dose rate of 1,080 g/ha ai. In its label proposals, the applicant recommended the use of glyphosate at dose rates ranging between 1,440 and 2,160 g/ha ai in two applications[99]. However, the increased frequency of glyphosate use 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*.

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

98 Additional information received on 04/04/2011 / Request 2.1 / Page 19 / Appendix 3

99 Additional information received on 23/02/2009 / Request 3 / Pages 19-20 / Attachment III: Seguridad del Herbicida roundup Ready® y su empleo sobre variedades modificadas geneticamente para tolerancia a glifosato. Cuaderno Técnico n°6, Monsanto Agricultura Espana, SL, Av. De Burgos, 17. 28036 Madrid, pp. 59

(2) 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 (Gower et al., 2002, 2003; Champion et al., 2003; Cox et al., 2006). To limit such early-season competition and avoid maize yield losses, another strategy involves the use of other herbicides, especially residual herbicides applied pre-emergence (Grichar and Minton, 2006; Nurse et al., 2006). Glyphosate has little or no residual activity when applied to the soil surface where it strongly binds to soil particles. Moreover, glyphosate uptake by the plant roots is minimal. If glyphosate is applied pre-emergence, 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 (Thomas et al., 2004, 2007; Parker et al., 2006) 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. For this weed control strategy, Dewar (2010) recommended that the pre-emergence herbicide be applied at a reduced dose rate and that glyphosate be applied at a dose rate of 1,080 g/ha ai. In this situation, the application rates of glyphosate proposed by the applicant in its label proposals are within the range of 720 to 1,440 g/ha ai.

(3) A single application of a glyphosate-based herbicide in combination with other compatible post-emergence herbicides with residual activity. This strategy has been recommended by Gianessi (2008) and Soukup et al. (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 (Johnson et al., 2000; Thomas et al., 2007; Dill, 2005; Tharp et al., 2004; Grichar and Minton, 2006; Parker et al., 2006; Young, 2006; Zuver et al., 2006) and facilitate the control of weeds that are less susceptible to glyphosate (e.g., Norsworthy et al., 2001; Soukup et al., 2008). Based on field studies conducted at 35 sites throughout the north-central USA, Gower et al. (2003) concluded that the optimum timing for a single glyphosate application to avoid maize yield loss is when weeds are less than 10 cm in height, no later than 23 days after maize planting, and when maize growth was not more advanced than the 4th leaf stage. When mixed with another herbicide with residual activity, the applicant proposed application rates of glyphosate ranging from 720 to 1,080 g/ha ai. The use of glyphosate-based herbicides at a dose rate of 1,080 g/ha ai in conjunction with the soil-active herbicidal active substance acetochlor resulted in high herbicide efficacy in field trials in the Czech Republic (Soukup et al., 2008).

(4) Several applications of broad-spectrum herbicides, including glyphosate. In cases of high weed pressure, other chemical-containing weed control strategies suggested to include the sequential application of glyphosate in conjunction with residual herbicides applied pre-emergence or early post-emergence (Thomas et al., 2007; Dewar, 2010); or the use of other broad-spectrum herbicides (such as dicamba) in combination with glyphosate (Dewar, 2009, 2010; Green and Castle, 2010; Green, 2011; Green and Owen, 2011).

In conclusion, pre- or post-emergence residual herbicides could be used in combination with the post-emergence application of glyphosate around the 4th and 6th leaf stage, in order to give optimum control and yield protection during the most vulnerable maize growth stages (Soukup et al., 2008; Dewar, 2009).

Recommended herbicide regimes for maize MON 88017 by the applicant

In its application under Regulation (EC) No 1829/2003, the applicant provided provisional recommendations on the application rates of glyphosate on maize MON 88017. Reference was made to recommendations made to Spanish farmers for the use of Roundup Ready[100]. More generally, it is assumed that the applicant will recommend herbicide regimes where the maximum total application

[100] Additional information received on 23/02/2009 / Request 3 / Pages 19-20 / Attachment III: Seguridad del Herbicida roundup Ready® y su empleo sobre variedades modificadas geneticamente para tolerancia a glifosato. Cuaderno Técnico n°6, Monsanto Agricultura Espana, SL, Av. De Burgos, 17. 28036 Madrid, pp. 59

rate of glyphosate is 2,880 g/ha ai administered in up to two applications per crop. Application rates ranging from 1,440 to 2,880 g/ha ai are expected to provide effective control of difficult to control perennial weeds, whilst lower application rates (generally 540-1,440 g/ha ai) might be sufficient to give effective control of annual weeds in maize. These proposed application rates are currently being 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), but are indicative of the range of application rates, mixtures and systems that might be applied in the future[101]. Hence, the applicant suggested that an appropriate recommended rate of glyphosate should be determined by the weed species, density and growth stages, and by mixture patterns (according to local good agricultural practices).

Proportion of maize in crop rotations

The applicant noted that maize MON 88017 will be used by farmers as any other maize, appearing in rotational-planning adapted to each specific geographic region and subject to changes in rotation for economic and customer/stakeholder demand reasons[102]. Maize-based cropping systems, with different shares of maize in crop rotations, are dominant in European arable systems (FCEC, 2009; Meissle et al., 2010; Vasileiadis et al., 2011). In the northern EU region, maize is mostly cultivated as non-irrigated continuous silage maize or rotated with grasses. In the eastern EU region (e.g., Hungary, Romania), maize is mostly cultivated as grain maize in rotation programs after wheat such as soybean (or other plant from the leguminosae family)-wheat-maize-sunflower or wheat-wheat-maize-sunflower or wheat-wheat-maize-oilseed rape), or as non-irrigated continuous grain maize. In southwest EU (e.g., Portugal, Spain), grain maize is commonly irrigated and either grown continuously or rotated with other crops that need also irrigation. The usual rotation is with winter wheat, cotton or sugar beet, but other crops such as sunflower, alfalfa or tomatoes can appear in the scheme. In the southern region (e.g., Po Valley, Italy), grain maize irrigated and rotated (mainly with winter wheat or soybean) is the main system identified, while other important systems include silage maize rotated and irrigated, as well as continuous and irrigated grain maize (reviewed by Vasileiadis et al., 2011).

Conclusion

EU countries show considerable variation in herbicide use in maize depending on the crop type (grain, forage, sweet, etc.), the weed species (including crop volunteers) present, meteorological and agro-environmental conditions, farming systems (including weed resistance evolution management, rotation systems), economics, the growing season, and farmers' behaviour. Herbicide regimes are influenced by weed species and biology, since not all weeds are equally susceptible to glyphosate (e.g., Norsworthy et al., 2001; Soukup et al., 2008) and by factors influencing integrated pest and crop management. Hence, where weeds are required for soil erosion management, cover or protection, or as refuges for beneficial insects, then management will vary accordingly. If farmers consider weed management as interacting with Western corn rootworm management, then they will adopt practices accordingly, especially in relation to management of maize volunteers in other crops in a rotation. Variations between locally-adopted herbicide regimes and cultivation management (including conservation tillage) for GMHT maize would be expected, in response to these factors. Therefore, it is anticipated that glyphosate-based herbicide regimes will represent a mixture of the strategies outlined above, and involve different numbers of applications (single vs. sequential), doses, timing of application, and the use of other herbicides (including soil acting residuals) in association with glyphosate. Nonetheless, to ensure the safe use of the GMHT technology, it is important that individual farmers adopt integrated weed management strategies based on use of multiple options (cultural, mechanical and chemical). Oversimplification of weed management due to the introduction of glyphosate tolerant crops has occurred in the USA (Bonny, 2008, 2011).

[101] Additional information received on 04/04/2011 / Request 2.1 / Page 19 / Appendix 3
[102] Additional information received on 04/04/2011 / Request 2.4 / Pages 26-28

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

The EFSA GMO Panel considers that since farming systems are highly dynamic, the introduction of widespread broad-spectrum herbicide-based systems may lead to changes in management. For the reasons stated below, 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 other maize, 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 MON 88017 in the E.U. is considered no different from any other maize*".

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 ecosystem service 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 (Brookes et al., 2003; Dewar et al., 2003; May et al, 2005). In addition, the use of a broad-spectrum herbicide to control both monocotyledons and dicotyledons within the maize phase of a rotation may be compensated by a reduction in herbicide control of dicotyledonous weeds in other crops within the rotation, particularly if these are also cereals (Heard et al., 2005), although this effect cannot be generalised. Furthermore, the use of glyphosate allows greater adoption of no- or reduced-tillage systems (Locke et al., 2008; Givens et al., 2009b). These systems contribute to different extent 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[103]).

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 botanical diversity due to weed shifts, with the selection of weed communities mostly composed of tolerant species, (3) select for glyphosate resistant weeds, and (4) impact soil microbial communities (see also EFSA, 2009c). These potential adverse indirect environmental effects of the cultivation of maize MON 88017 are discussed below.

Impact on farmland biodiversity

A few studies have assessed the impact of glyphosate-based herbicide regimes used in GMHT maize cultivation in Europe (Soukup et al., 2008; Verschwele and Mülleder, 2008; Albajes et al., 2008, 2009, 2010, 2011; Szekeres et al., 2008; Thieme, 2010). In addition, 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. Additionally, there are some other studies of herbicide tolerant crops in European countries that have compared the environmental impact of conventional production systems with that of GMHT cropping systems (Madsen and Jensen, 1995; Bückmann et al., 2000; Coyette et al., 2002).

Indirect effects on farmland biodiversity associated with the use of glufosinate-ammonium- and glyphosate-based herbicides in GMHT cropping systems, including maize, were studied extensively in the FSEs (Firbank et al., 2003a). Results showed that herbicide regimes used with glufosinate-ammonium tolerant maize had less adverse impact on farmland biodiversity, compared with non-GM maize treated with conventional herbicides. In the maize growing season, the weed density in glufosinate-ammonium tolerant maize was approximately two to three fold higher throughout the season, and biomass was 1.85-fold higher than in conventionally-managed maize. Due to the greater weed control exerted by conventional herbicide regimes, as compared with those used with glufosinate-ammonium tolerant maize, biomass of dicotyledonous weeds and counts of their seed-rain were greater in GMHT maize (Heard et al., 2003a,b). There were few effects on major groups of invertebrates, though there were more soil-dwelling detritivores in glufosinate-ammonium tolerant maize, especially in August, and more herbivores and their parasitoids in June (Hawes et al., 2003). In July, the seed feeding carabid *Harpalus rufipes* was more frequent in glufosinate-ammonium tolerant maize fields (Brooks et al. 2003). Consumer-resource ratios were similar between herbicide regimes, except that there were more invertebrate predators per herbivore in glufosinate-ammonium tolerant maize. In glufosinate-ammonium tolerant maize, weed seed rain, important in the diets of 17 granivorous bird species, was higher than in conventionally-managed maize, though the difference was only significant for the following seven species: *Pedrix pedrix*, *Columba oenas*, *Columba palumbus*, *Carduelis chloris*, *Pyrrhula pyrrhula*, *Emberiza schoeniclus* and *Emberiza cirlus* (Gibbons et al., 2006). In subsequent conventional crops, the beneficial effect of herbicide regimes was detectable in the weed soil seedbank. Soil seedbanks following glufosinate-ammonium tolerant maize were 1.23-fold higher than following conventional maize for both the first and second years (Firbank et al., 2005a). While long-term effects on farmland biodiversity have been predicted at the landscape level due to the continuous cultivation of GMHT crops in association with the exclusive use of

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

glyphosate-based herbicides (Heard et al., 2005, 2006; Squire et al., 2009), such effects have not been confirmed by field data. In the second year of maize cultivation, there was no overall trend of herbicide regime ratios being greater or smaller when taken across taxa (Heard et al., 2006).

Caution is required when interpreting, extrapolating and scaling up the observations made under the conditions of the FSEs. First, the GMHT maize used in the FSEs was tolerant to the herbicidal active substance glufosinate-ammonium, whereas maize MON 88017 will be used in association with glyphosate. Glyphosate is a very effective broad-spectrum herbicidal active substance that provides more consistent control than glufosinate-ammonium in particular cases (see e.g., Leroux et al., 2006; Zuver et al., 2006). Glufosinate-ammonium behaves likes a contact herbicide, so unlike glyphosate, it must be applied to small weeds and is not as effective on perennials that require significant translocation for complete control (Green, 2011; Green and Owen, 2011). Second, herbicide regimes applied in non-GMHT maize included the herbicidal active substances atrazine, simazine and cyanazine in the FSEs (Champion et al., 2003). Considering that these herbicidal active substances have been withdrawn from the EU market, further analysis of the FSE data was deemed necessary. The reanalysis indicated that the replacement of triazine herbicides by less efficient conventional herbicides slightly reduced the net beneficial effect of herbicide regimes in GMHT maize, but did not eliminate it (Perry et al., 2004; Brooks et al., 2005). Third, the herbicide regimes used with glufosinate-ammonium tolerant maize in the FSEs might not fully reflect real agricultural practice, as the application of glufosinate-ammonium-based herbicides was limited to a single spray applied at dose rates lower than 0.800 kg/ha ai in most cases (Champion et al., 2003). In practice, however, it is reasonable to assume that other herbicide regimes than the one used in the FSEs will be implemented, resulting in a different impact on farmland biodiversity. Whilst the impact of glyphosate tolerant maize was not tested in the FSEs, it has been suggested that it might be similar to that which occurred in glyphosate tolerant sugar beet (Dewar, 2010). Reductions in the number of weeds in glyphosate-treated sugar beet, compared with conventionally-treated sugar beet, resulted in significant reductions in weed biomass, and in subsequent weed seed production later in the season and in the following crops (Dewar et al., 2005).

The above studies confirm 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 and on the herbicides used in both systems. The extent and direction of the effects of weed management on weeds and invertebrates is dependent on the relative efficacy of the existing conventional regimes and the forthcoming GMHT herbicide regimes. Extensive research has shown that impacts on biodiversity also depend greatly upon the management of crops, rotations, and upon 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 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. Predicting future changes in the timing of herbicide applications as had already occurred in the USA, where uptake of GMHT crops was driven by the perceived profitability of cropping, Heard et al. (2005) noted that alterations to the frequency of high-density weed patches in the landscape could have important implications, if the spatial distribution of weeds across the landscape affects interactions with higher trophic levels. Farmland birds that forage extensively on weed seeds in winter, aggregating in direct response to their abundance, may be particularly affected. Changes to rotations themselves are also likely, and may have considerable effects (both beneficial or adverse ones). Indeed, biodiversity differences between crops were shown to be comparable to those between treatments in separate studies (Firbank et al., 2003b; Lutman et al., 2008). All of the factors above will vary from region to region, from Member State to Member State, from season to season, and from biodiversity component to biodiversity

component. These factors depend not only on the nature of the particular receiving environment, but on weed pressure, soil type and climatic conditions. For these reasons, whilst meaningful conclusions can be drawn from general principles, the EFSA GMO Panel acknowledges that there are considerable challenges to making accurate predictions on the environmental consequences of the use of herbicides in 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. The complex nature of all these dynamic effects is of course be modulated further by market forces and agricultural economics.

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 maize MON 88017 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 *other than maize* (i.e., for beet and oilseed rape). They predicted a worst-case decline in soil seedbanks of 7 % per annum for a 5-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 7-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. By contrast, glufosinate-ammonium tolerant maize would be beneficial to farmland birds. 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). The lower density of arable weeds on maize plots treated with glyphosate does 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 in herbicide-treated plots, whereas phytophagous thrips were less abundant. Among predators, *Orius* spp., spiders, and trombidids were more abundant on 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 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).

Table 4. Major factors affecting the risk of reducing in-field botanical diversity based on expert judgment and historical experience

Management option	Risk of reducing in-field botanical diversity		
	Low	Moderate	High
Crop rotation	> four years, presence of functionally distinct crops (e.g., cereals/industrial crops/pulses) 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 in cropping system	Cultural, mechanical and chemical	Cultural and chemical, or mechanical and chemical	Mainly chemical
Use of same mode of action per season	Once	More than once	Many times
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	Presence	Limited presence	No presence

The EFSA GMO Panel concludes that indirect effects associated with the use of the complementary glyphosate-based herbicide regimes 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 (see Table 4, above), which include the efficacy of the applied herbicide regimes in controlling weeds, crop rotations, and the level of farmland biodiversity sustained in receiving environments. In particular, the repeated use of glyphosate-based herbicides at recommended application rates on continuous maize MON 88017 may result in reductions in botanical diversity and/or weed density in maize fields to a level that might adversely affect food chains and webs, but not necessarily biological control functions, at the field and landscape level (see Table 4, above). Such a reduction in 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.

The EFSA GMO Panel conclusion on potential impacts of specific cultivation, management and harvesting techniques associated with the cultivation of maize MON 88017 is consistent with that of the BE CA. In its evaluation, the BE CA identified potential adverse effects of the herbicide used on maize MON 88017 on the environment, and they considered that "*the use of glyphosate 'over the top of the crop' must not interfere with biological functions of non-target organisms (such as biological control and decomposition)*" (section 2.8 of the environmental risk assessment report of the BE CA). In its evaluation the BE CA also acknowledged "*the difficulty to predict the range of farming practices that will be deployed with the GM crop and the consequences for biological functions*" because "*management and utilisation of a GM crop may vary from region to region, farm to farm and over time*". "*The risk assessment should have taken this unpredictability of farm management and its consequences for biological functions better into account, e.g. by relating this to monitoring*" (section 2.8 of the environmental risk assessment report of the BE CA). They were of the opinion that "*the applicant should have linked the latter ERA issue better to monitoring*" (see overall conclusions of the environmental risk assessment report of the BE CA).

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). There is evidence from cultivation of GMHT crops that the repeated, continuous and exclusive use of glyphosate in no- or reduced-tillage systems causes changes in weed flora, and favours the selection of more tolerant or resistant weed communities (Fernandez-Cornejo et al., 2002; Tingle and Chandler, 2004; Johnson et al., 2009; Kruger et al., 2009; Powles, 2008, 2010; Gressel, 2009; NRC, 2010; Owen et al., 2010, 2011; Powles and Yu, 2010; Waltz, 2010; Webster and Sosnoskie, 2010; Beckie, 2011; Heap, 2011; Shaner et al., 2011). 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, particularly pre-sowing applications, become more important (Givens et al., 2009a). Moreover, no- or reduced tillage systems concentrate weed seeds close to the soil surface (Bàrberi and Lo Cascio, 2001; Moonen and Bàrberi, 2004; 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 or 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 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, or by late season weed emergence. 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 maize, cotton and soybean, revealed that no weed shifts were observed in GMHR maize yet, and that this was attributed to the low adoption of GMHR maize (Culpepper, 2006). 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. While Verschwele and Mülleder (2008) showed that risks for weed population

shifts from GMHT crops are no greater than those associated with other herbicides and non-GMHT crops, other studies reported or predicted shifts in weed populations due the increased frequency and rate of glyphosate use in GMHT crops (e.g., Shaner, 2000; Hilgenfeld et al., 2004; Duke, 2005; Owen and Zelaya, 2005; Puricelli and Tuesca, 2005; Culpepper, 2006; Scursoni et al., 2007; Owen, 2000, 2008). In case of GMHT maize, Wilson et al. (2007) found that over a 6-year period in glyphosate-based cropping systems in western USA corn belt, weed populations shifted from a kochia (*Kochia scoparia*) and wild-proso millet (*Panicum miliaceum*) dominated population to predominantly narrowleaf lambsquarters (*Chenopodium desiccatum*) population. Weed shifts may exacerbate weed problems and reduce the effectiveness of weed control (Young, 2006). With the anticipated increase in adoption of GMHR maize as well as application rates of glyphosate, more frequent weed shifts have been predicted (Shaner, 2000).

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[104]. 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). 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, especially following the advent of GMHT crop cultivations (Owen and Zelaya, 2005; Sanderman, 2006; Powles, 2008; Beckie, 2011; Heap, 2011; Green and Owen, 2011; Owen et al., 2011), 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 twelve glyphosate resistant weed species have been identified in the USA, most of which evolved resistance to glyphosate in GMHT cropping systems (Beckie, 2011; Heap, 2011). 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., 2011; Vila-Aiub et al., 2011). The problem of glyphosate resistant weeds is exacerbated by the fact that new resistance mechanisms such as gene amplification are being found (i.e., Gaines et al., 2010). Moreover, the evolution of multiple and cross resistances to herbicides is becoming increasingly more common (Heap, 2011). The overreliance on glyphosate to control 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-

[104] Additional information received on 04/04/2011 / Request 2.2 / Pages 20-26

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., glyphosate tolerant maize monocultures or glyphosate tolerant maize-soybean rotations), little diversity in weed control practices and no mandated herbicide resistance programmes (Waltz et al., 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 usage and subsequent 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 outbreaks 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, and perhaps to global food production (Duke and Powles, 2008a; Powles, 2010; Owen et al., 2011; Ronald, 2011).

The EFSA GMO Panel concludes that the cultivation of maize MON 88017 in monoculture or in rotation with other glyphosate tolerant crops, in conjunction with the repeated and/or exclusive application of glyphosate-based herbicides will cause changes in weed flora, and will favour the evolution and spread of glyphosate resistant weeds due to the selection pressure exerted by glyphosate. This, in turn, may affect food webs, and the functional value of weed vegetation for organisms of higher trophic levels (reduced functional biodiversity). However, where there is more diversity in weed control practices and crop rotation, and where mandated herbicide resistance programmes are put in place, the selection pressure of glyphosate on weeds will be reduced, decreasing the selection of more tolerant or resistant weeds significantly. In general, those management options that lead to a high risk of reduction of botanical biodiversity (see Table 4) also favour resistance evolution.

In its evaluation, the BE CA focused on potential adverse effects of the herbicide use on the environment currently not covered by Directive 91/414/EEC, which was repealed by Regulation (EC) No 1107/2009 on 14 June 2011 (section 2.8 of the environmental risk assessment report of the BE CA). Since the potential for weed resistance evolution is considered under Regulation (EC) No 1107/2009, the environmental effects of the evolution of weed resistance were not taken into account by the BE CA.

Impact on soil microbial communities

While no direct adverse effects of the CP4 EPSPS protein have been reported on non-target organisms, biogeochemical processes and the abiotic environment (see sections 6.2.4.3 and 6.2.6.3, above), the herbicide management associated with the cultivation of maize MON 88017 may under certain circumstances have adverse effects on the biotic environment and biogeochemical processes. As for glyphosate susceptible plants, the application of glyphosate can inhibit the EPSPS protein of some microorganisms and thus decrease or fully inhibit the synthesis of aromatic amino acids (Busse et al., 2001; Zablotowicz and Reddy, 2004). The entry of glyphosate into soil may affect the soil microbial community directly, by providing energy, carbon, nitrogen or phosphorus sources, or by inhibiting growth of glyphosate susceptible bacteria and fungi. Consequently, transient shifts in microbial biomass and/or the structural diversity of the microbial community may occur. Overall, the addition of glyphosate as a technical compound or in it commercial product Roundup Ultra results in increased microbial biomass accompanied by higher soil respiratory activity, giving no rise to conclude on adverse effects on microbial communities (Haney et al., 2000).

Evidence suggests that glyphosate can affect soil and rhizosphere-inhabiting bacteria and fungi, including those capable to live in mycorrhizal relationship, and rhizobia, the latter group is characterised by its capacity to establish symbiosis with specific legumes by mediating nitrogen fixation in root nodules (Busse et al., 2001; Zablotowicz and Reddy, 2004, 2007; Means et al., 2007; Kremer and Means, 2009; Powell et al., 2009a). Potential consequences of frequent glyphosate applications in GMHT cropping systems comprise alterations in the microbial ecology and biological processes carried out in the crop rhizosphere, and may encompass effects on potential phytopathogen antagonist interactions, and interference with plant-growth-promoting rhizobacteria (Lupwayi et al., 2009). 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 high tolerance to glyphosate and the instability of the herbicide in soil, which decreases exposure. In fact, a proportion of glyphosate is sorbed by soil particles where its activity is limited, while free (water-dissolved) glyphosate is degraded by soil microorganisms (Haney et al., 2002; Powell et al., 2009a). It should be noted that sorbed compounds are in equilibrium with their water-dissolved molecules including sorbed glyphosate and thus also expected to decline. Glyphosate may however transiently accumulate. The entry of glyphosate into soil may not only occur directly by spraying of the herbicide, but also indirectly through plant roots of GMHT plants since plants do not metabolize glyphosate but translocate it to actively growing regions, including roots, from which it can then enter the soil. The exudation of glyphosate would cause direct exposure in the rhizosphere where beneficial plant growth promoting microorganisms, including myorrhiza and nitrogen-fixing symbiotic bacteria, compete with potential plant pathogens (Feng et al., 2005; Kremer et al., 2005). Potentially this may generate a bias to the disadvantage of the plant (Powell and Swanton, 2008).

In their field experiment, Liphadzi et al. (2005) found that the use of glyphosate on glyphosate tolerant maize did not affect soil respiration and the diversity of the dominant soil bacteria. Likewise, it was found that neither the diversity of denitrifying bacteria nor that of root-colonizing fungi was affected by glyphosate applied on glyphosate tolerant maize (event DKC3551) (Hart et al., 2009). Weaver et al. (2007) did not observe significant effects of glyphosate on soil microbial communities and its mineralisation in bulk soil or rhizosphere soils, even at concentrations well above recommended field application rates. However, 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 following a field application in a 2 mm soil interaction depth, was shown to induce shifts in soil microbial communities (Lancaster et al., 2009). These and other studies indicate that the single application of glyphosate has only small and transient effects on the soil microbial community (Motavalli et al., 2004; Gomez et al., 2009; Barriuso et al., 2010), if they occur, and that its repeated use may favour those soil microorganisms capable of metabolising glyphosate or tolerant to the herbicide (Lancaster et al., 2009). It should be noted that such temporal responses of the soil microbial biomass to the addition of glyphosate are not unusual when microbiological substrates are added to soil. To the knowledge of the EFSA GMO Panel, a persisting effect of glyphosate on soil microbial communities has not been reported.

Studies on glyphosate interactions with microbial communities in GMHT cropping systems revealed that the most pronounced effects are detected with specific groups (genera or species) of microorganisms (such as fungi of the genera *Fusarium* and *Pythium* or bacteria from the Pseudomonas group) rather than with broader measurements of soil microbial diversity and functions (Kowalchuk et al., 2003; Lupwayi et al., 2007; Means et al., 2007; Powell and Swanton, 2008; Hart et al., 2009a). These microorganisms are often typical inhabitants of crop rhizospheres. Responses of individual fungal species varied depending on their susceptibility to glyphosate; some species express glyphosate-sensitive forms of EPSPS and may not metabolise glyphosate (Morjan et al., 2002). In a laboratory study, growth of *Pythium ultimum* and *F. solani* could be stimulated or inhibited, depending on glyphosate concentration (Kawate et al., 1992). Kremer and Means (2009) observed that *Fusarium* spp. colonisation levels of roots of glyphosate tolerant maize receiving glyphosate were three to ten times higher than those for the atrazine treatment, indicating that glyphosate induces fungal colonisation of maize 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 interactions that occur under actual farming conditions. Moreover, not all *Fusarium* strains respond in the same way; some strains did not grow better in glyphosate-containing 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 glyphosate tolerant 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 may transiently be reduced in glyphosate tolerant soybean due to their reported sensitivity to glyphosate (Zobiole et al., 2011). Glyphosate may enhance fungal root colonisation and potential diseases by stimulating growth of the fungal pathogen and by suppressing bacterial antagonists (Powell and Swanton, 2008). Powell et al. (2009b) have also reported that, depending upon the location of litter placement, glyphosate use can significantly reduce maize litter decomposition.

In soybean, glyphosate has been shown to be preferentially translocated metabolically active, growing plant compartments, including nodules where nitrogen-fixing symbionts (bacteroids) of the bacterial species *Bradyrhizobium japonicum*, which possess a glyphosate sensitive EPSPS protein, can be exposed to it. Upon exposure to glyphosate, bacteroids may accumulate high concentrations of shikimate and certain benzoic acids that could inhibit plant growth. These effects are accompanied by growth inhibition and/or death of bacteroids, depending upon the glyphosate concentration (Cerdeira and Duke, 2006). Zablotowicz and Reddy (2004, 2007) also reported that glyphosate affects root nodulation and nitrogen fixation of Bradyrhizobium compared on conventional soybean in contrast to other herbicides. The consequences of this could be that glyphosate applications may reduce, probably transiently, populations of Bradyrhizobium or other root-nodule forming rhizobia, and thus reduce the natural potential to form symbiosis with nitrogen-fixing legumes (Reddy et al., 2000; King et al., 2001; Reddy and Zablotowicz, 2003; Bohm et al., 2009; Zobiole et al., 2010). In cropping systems, this could reduce the nodulation of the crop and thus increase the need for additional nitrogen fertilisers in nitrogen-depleted soil (Bohm et al., 2009). Agricultural practice yet shows that nodulation of host-plants by rhizobia can be inconsistent, varying unpredictably with the rate and timing of the glyphosate application (Cerdeira and Duke, 2006; Powell et al., 2009a). It was even observed that nitrogen fixation was greater for glyphosate tolerant soybean treated with glyphosate than for untreated plants, but only when glyphosate was applied at the first trifoliate soybean growth stage (Powell et al., 2009a). Nitrogen-fixing Bradyrhizobium or other rhizobia have no importance for maize, as it is not nodulated by these bacteria which are specific for legumes (peas, soybean, etc.). Beside symbiotic nitrogen-fixing rhizobia of legumes, there are several common soil inhabiting bacteria with a potential to fix nitrogen without root nodule formation. Such bacteria may reside in the intracellular space in roots or shoots or in the rhizosphere, but their contribution for supplying the respective crops with nitrogen is under normal agricultural conditions generally very low.

Whilst glyphosate can affect soil bacteria, mycorrhizal fungi and Bradyrhizobium or other rhizobia, such effects have been observed inconsistently, and mostly in other glyphosate tolerant crops than maize. Rearrangements in structural diversity and population abundance of soil microbial communities occur frequently in the agricultural environment. They are typically associated with several sources of variation, caused by natural variability (e.g., soil heterogeneity, weather conditions) and agricultural practices (e.g., soil tillage, crop rotation, irrigation measures) 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 herbicide dose, soil and microorganisms investigated (Gorlach-Lira et al., 1997; Motavalli et al., 2004), and ecological interactions, including whether studies are conducted under laboratory or field conditions (Wardle and Parkinson, 1990, 1992; Busse et al., 2001; Motavalli et al., 2004; Powell and Swanton, 2008; Savin et al., 2009). The EFSA GMO Panel considers that potential effects on soil microbial communities and the ecological functions they provide, due to the use of glyphosate on maize MON 88017 at normal field application rates, if they occur, will be transient and minor, and are likely to be smaller or within the range currently caused by other agronomic and environmental factors. Therefore, the EFSA GMO Panel concludes that the use of glyphosate-based herbicides on maize MON 88017 is unlikely to cause adverse effects to soil microbial communities or beneficial functions mediated by them at normal field

application rates. The EFSA GMO Panel notes that effects of herbicidal active substances on soil microbial communities are considered through functional tests on nitrification and soil respiration under Regulation (EC) No 1107/2009.

In its evaluation, the BE CA considered potential adverse effects of the herbicide use on the environment currently not covered by Directive 91/414/EEC, which was repealed by Regulation (EC) No 1107/2009 on 14 June 2011 (section 2.8 of the environmental risk assessment report of the BE CA). Since potential adverse effects on microbial communities are considered under Regulation (EC) No 1107/2009, these effects were not taken into account by the BE CA.

6.2.8. Conclusion on the environmental risk assessment

Since the scope of the current application covers cultivation, the environmental risk assessment considered the environmental impact of full-scale commercialisation of maize MON 88017.

The BE CA (including its Biosafety Advisory Council) provided to EFSA its report on the environmental risk assessment of maize MON 88017 (dated 28 September 2010) on 6 October 2010 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 BE 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 maize MON 88017 has no altered agronomic and phenotypic characteristics, except for the specific target pest resistance and herbicide tolerance. The likelihood of unintended environmental effects due to the establishment, survival and spread of maize MON 88017 is considered to be extremely low, and will be no different from that of conventional maize 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 *cry3Bb1* and CP4 *epsps* genes from maize MON 88017 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.

The possible resistance evolution to the Cry3Bb1 protein in coleopteran target pests is identified by the EFSA GMO Panel as a concern associated with the cultivation of maize MON 88017, as resistance evolution may lead to altered pest control practices that may cause adverse environmental effects.

Based on the evidence provided by the applicant and relevant scientific literature on maize MON 88017, the EFSA GMO Panel concludes that there are no indications of adverse effects on non-target organisms due to unintended changes in maize MON 88017, and therefore considers *trait*-specific information appropriate to assess whether maize MON 88017 poses a risk to non-target organisms.

The evidence provided by the applicant indicates that the protein sequences of the Cry3Bb1 protein variants of maize MON 88017, MON 863 and MON 853 are similar, and that the biological activity of these Cry3Bb1 protein variants is equivalent. Therefore, the EFSA GMO Panel considers that information generated to evaluate potential adverse effects on non-target organisms due to the expression of the Cry3Bb1 protein in maize MON 863 or MON 853 can be used to inform the environmental risk assessment of maize MON 88017.

The EFSA GMO Panel concludes that potential adverse effects of maize MON 88017 due to the expression of the Cry3Bb1 protein to non-target terrestrial (plant- and ground-dwelling), soil and aquatic arthropods are expected to be negligible in the context of its proposed uses. Rearrangements of species assemblages at different trophic levels in crop stands are commonly associated with any pest management practice, but the EFSA GMO Panel considers that maize MON 88017 will not cause reductions to natural enemies that are significantly greater from those caused by conventional

cultivation where insecticides are used to control corn rootworms. Based on the evidence supplied by the applicant, the EFSA GMO Panel has no reason to believe that maize MON 88017 will adversely affect honeybees. Few studies have assessed the impact of the Cry3Bb1 protein on non-target aquatic arthropods and the fate of the Cry3Bb1 protein in senescent and decaying maize detritus in aquatic environments, but available data indicate it is unlikely that the Cry3Bb1 protein in maize MON 88017 products would cause adverse effects on non-target aquatic arthropods in the context of its proposed uses. In addition, there is no evidence to indicate that maize MON 88017 is likely to cause adverse effects on non-target organisms that are not arthropods in the context of its proposed uses.

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.

On the basis of the data provided by the applicant and those obtained from a literature survey, the likelihood of adverse effects to non-target organisms is foreseen to be very low, and limited to non-target chrysomelid larvae. However, the risk of maize MON 88017 to valued (non-pest) chrysomelid species in the field is likely to be minimal due to their low occurrence and abundance in maize fields and due to the low likelihood of encountering harmful amounts of pollen from maize MON 88017 in and around maize fields. Moreover, the activity of the Cry3Bb1 protein on adult non-target chrysomelid species is expected to be limited.

The EFSA GMO Panel considers that Cry3Bb1 protein concentrations in decaying plant residues from maize MON 88017 decrease rapidly in soil, indicating that non-target soil organisms are exposed to relatively low Cry3Bb1 protein concentrations within a few months after harvest. There is no evidence for accumulation of the Cry3Bb1 protein on agricultural fields cultivated repeatedly with maize MON 88017 or comparable maize events (e.g., MON 863), despite its potential to bind to surface-active particles. Effects of crops on soil microbial communities, which are especially expected in the rhizosphere or on decaying plant material, depend more on their species, variety or age than whether they are genetically modified. Rearrangements in structural diversity and population abundance of non-target soil organisms occur frequently in the agricultural environment. They are typically associated with several sources of variation, caused by natural variability (e.g., soil heterogeneity, weather conditions) and agricultural practices (e.g., soil tillage, crop rotation, irrigation measures) and are thus not necessarily an indication of environmental harm. The EFSA GMO Panel concludes that potential effects on soil microorganisms and microbial communities, as well as the ecological functions they provide, due to the cultivation of maize MON 88017, if they occur, will be transient and minor, and are likely to be smaller or within the range currently caused by other agronomic and environmental factors.

There are no indications that the expression and biological activity of the Cry3Bb1 and CP4 EPSPS proteins expressed in maize MON 88017 are affected by potential interactions between the newly expressed proteins. However, the EFSA GMO Panel took account of crop management in the environmental risk assessment, as interactions between biota may occur under different weed and pest management regimes, irrespective of interactions between the newly expressed proteins.

The EFSA GMO Panel is of the opinion that potential adverse environmental effects of the cultivation of maize MON 88017 are associated with the use of the complementary glyphosate-based herbicide regimes. These potential adverse environmental effects comprise (1) a reduction in farmland biodiversity, (2) changes in botanical diversity due to weed shifts, with the selection of weed communities mostly composed of tolerant species, and (3) the selection of glyphosate resistant weeds. The potential harmful effects could occur at the level of arable weeds, farmland biodiversity, food webs and the ecological functions they provide. The magnitude of these potential adverse environmental effects will depend upon a series of factors, including the specific herbicide and cultivation management applied at the farm level, the crop rotation and the characteristics of receiving environments.

The EFSA GMO Panel considers that the use of glyphosate-based herbicides at recommended field application rates of glyphosate on maize MON 88017 is unlikely to cause adverse effects to soil microbial communities or beneficial functions mediated by them.

The conclusions of the EFSA GMO Panel on the environmental safety of maize MON 88017 are consistent with those of the BE CA. The BE CA concluded that "*based on the information in the application, the additional information received by the applicant, the information found in peer-reviewed studies and the scientific comments raised by the member states, no risks concerning the environment and human and animal health were identified as a result of cultivation of MON 88017, except for potential indirect adverse effects related to the use of glyphosate over the top of the crop*" (see overall conclusions of the environmental risk assessment report of the BE CA). In its evaluation, the BE CA identified potential adverse effects of the herbicide used on maize MON 88017 on the environment, and they considered that "*the use of glyphosate 'over the top of the crop' must not interfere with biological functions of non-target organisms (such as biological control and decomposition)*" (section 2.8 of the environmental risk assessment report of the BE CA). The BE CA did not consider the evaluation of the potential weed resistance evolution was within their remit, as it should be considered under Regulation (EC) No 1107/2009.

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

6.3.1. Risk mitigation measures

6.3.1.1. General aspects of risk 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 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 BE CA and the EFSA GMO Panel evaluated the scientific quality of the 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 risk mitigation

The environmental risk assessment of maize MON 88017 concluded that:

- maize MON 88017 plants are unlikely to cause any direct adverse effects, with the exception of the possible resistance evolution to the Cry3Bb1 protein in coleopteran target pests, as the potential consequences of resistance evolution may cause adverse environmental consequences. Considering that coleopteran target pests will evolve resistance to Cry3Bb1-expressing maize rapidly under conditions of continuous exposure, the applicant proposed to put in place risk mitigation measures to delay the possible evolution of resistance;

- the cultivation of maize MON 88017 may result in adverse environmental effects due to the use of the complementary glyphosate-based herbicides. These potential adverse environmental effects comprise (1) a reduction in farmland biodiversity, (2) changes in botanical diversity due to weed shifts, with the selection of weed communities mostly composed of tolerant species, and (3) the selection of glyphosate resistant weeds. 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 maize MON 88017 will be used in diversified cropping regimes that have similar or reduced environmental impacts compared with conventional maize cultivation.

The specific risks identified in section 6.2.8 (conclusion on the environmental risk assessment), requiring mitigation, are (1) the possible resistance evolution to the Cry3Bb1 protein in coleopteran target pests, (2) a reduction in farmland biodiversity due to novel herbicide regimes, (3) changes in botanical diversity due to weed shifts, with the selection of weed communities mostly composed of tolerant species due to novel herbicide regimes, and (4) the selection of glyphosate resistant weeds due to novel herbicide regimes. The EFSA GMO Panel notes that for risks (2), (3) and (4) the possible environmental effects 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 (see section 6.2.7.1, above); 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 mititgation measures is linked inextricably to Directive 2001/18/EC, subsequent recommendations in this section are based on GMO legislation.

Possible risk mitigation measures, that 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 delay resistance evolution to the Cry3Bb1 protein in coleopteran target pests

Insect resistance management plan proposed by the applicant

In line with the applicants' EU working group on insect resistance management (as referred to by Alcalde et al. (2007)), the applicant proposed to put in place risk mitigation measures to delay the possible resistance evolution in the target insect pests[105]. According to the insect resistance management plan proposed by the applicant, farmers growing more than 5 ha of Cry3Bb1-expressing maize in the EU shall establish refuge areas with non-Cry3Bb1-expressing maize, corresponding to at least 20 % of the area planted with Cry3Bb1-expressing maize. The applicant's reasoning for implementing the *refugia* only on farms where the area of Cry3Bb1-expressing maize is greater than 5 ha is based on (1) the high fragmentation of the European agricultural landscape, (2) the lack of economic feasibility for providing *refugia* on farms with less than 5 ha Cry3Bb1-expressing maize, and on (3) the negligible risk of resistance evolution in areas with Cry3Bb1-expressing maize smaller than 5 ha (Alcalde et al., 2007).

In addition to maintaining an adequate level of refuge areas with non-Cry3Bb1-expressing maize, the insect resistance management plan proposed by the applicant covers the following elements (1) monitoring for any potential evolution of resistance to maize MON 88017 (section 6.3.2, below), (2) the implementation of a comprehensive education programme to aid farmers in understanding the importance of insect resistance management to delay the resistance evolution by planting refuge areas, and (3) the application of a remedial action plan addressing any contingency if resistance should occur.

High dose/refuge strategy

The EFSA GMO Panel considers that appropriate insect resistance management strategies are capable of delaying possible evolution of resistance under field conditions (see also Alstad and Andow, 1995; Andow, 2008; Tabashnik et al., 2008a, 2009; Huang et al., 2011). Resistance management strategies, relying on a 'high dose/refuge strategy', have been endorsed for several Cry-expressing crops in several countries (Bates et al., 2005; Andow, 2008; MacIntosh, 2010; Huang et al., 2011). The 'high dose/refuge strategy' proscribes planting Bt-maize that produces a very high concentration of the insecticidal Cry protein (25 times the amount needed to kill > 99 % of susceptible individuals), so that

[105] Technical dossier / Appendix 1 / Pages 260-293

nearly all target insects that are heterozygous for resistance do not survive on it. In addition, a nearby refuge of non-Bt-maize is required where the target insect pests do not encounter the Cry protein (Ives and Andow, 2002). Under these conditions, most of the rare resistant individuals surviving on Bt-maize will mate with abundant susceptible individuals emerging from nearby refuges to produce heterozygous progeny that is phenotypically susceptible. If inheritance of resistance is recessive, the hybrid progeny from such matings will die on Bt-maize.

The underlying assumptions contributing to the success of the 'high dose/refuge strategy' in delaying resistance evolution are that (1) the Cry protein is expressed in relevant plant tissues at a high dose, (2) initial resistance alleles are rare in the target insect population, so that nearly all resistance alleles will be in heterozygote individuals that cannot survive on the Bt-crop, (3) random mating occurs between resistant insects emerging in Bt-crops and susceptible insects preserved on non-Bt-crops (refuge) at sufficient levels, (4) resistance alleles are partially or fully recessive, and that (5) fitness costs are associated with the resistance.

Whether the underlying assumptions of the 'high dose/refuge strategy' are met for Western corn rootworm and Cry3Bb1-expressing maize is considered below.

(1) *The Cry protein is expressed in relevant plant tissues at a high dose:* The predicted duration of susceptibility of target insect pests to the insecticidal protein is dependent upon many factors (e.g., Tyutyunov et al., 2008), including the dose of the Cry protein in the Bt-crop (Onstad et al., 2001a). It is generally assumed that the expression level in relevant plant tissues must be sufficiently high to kill a high proportion of heterozygous resistant genotypes, so that any resistance allele in the target insect pest population remains functionally recessive (Gould, 1998; Andow, 2008). Instances of field resistance, reported so far (reviewed by Tabashnik et al., 2009; Huang et al., 2011), support model predictions that target insect pests are at greater risk of evolving resistance if managed by Bt-crops that are not high dose (Tabashnik et al., 2004).

The BE CA noted that "*no convincing evidence is given by the applicant to claim 99.9% efficacy of MON 88017*". Reference was made by the BE CA to Hibbard et al. (2010a) who reported that "*the dose (density-independent mortality) of the Cry34Ab1/Cry35Ab1-expressing event DAS-59122 is rather 96.71%* [or 96.48 % as referred to in Hibbard et al., 2010b] *than 99.88%* [or 99.14-99.98 % as referred to in Hibbard et al., 2010b] *as previously calculated by the equation of Storer et al. (2006)*" and Binning et al. (2010) who showed that "*the efficacy of DAS-59122 was lower than the earlier predictions (Storer et al., 2006): in their plant study neonate mortality was determined to be 99.5%*". The average reduction in Western corn rootworm emergence from maize MON 863 reported by Hibbard et al. (2010b) was 98.6 % when averaged across nine environments, and 94.88 % in the mCry3A-expressing maize event MIR604 (see also Hibbard et al., 2011). Maize MON 88017 has been observed to reduce Western corn rootworm populations by 96 % compared with non-Cry3Bb1-expressing maize (Meihls et al., 2008). These findings confirm that (i) current Cry3Bb1-expressing maize events fail to meet the high dose criterion and do not control second or third instars, resulting in some level of adult survival, and that (ii) the expression of the Cry3Bb1 protein in these events is to be considered low-to-moderate (Siegfried et al., 2005; EPA, 2010). Meissle et al. (2009, 2011a) reported that the impact of maize MON 88017 on adult Western corn rootworm is likely to be limited, as compared with first instars.

The ability of heterozygous resistant progeny, resulting from the mating between individuals emerging from the refuge and Bt-maize fields, to survive on Cry3Bb1-expressing maize may diminish the efficacy of the 'high dose/refuge strategy' to delay resistance evolution (Gassmann et al., 2011).

(2) *Initial resistance alleles are rare in the target insect population:* Studies, in which the frequency of resistance alleles to the Cry3Bb1 protein in populations of Western corn rootworm are directly estimated, have not been published in the scientific literature (most likely due to the polygenic

nature of the resistance). Data on the efficacy of Cry3Bb1-expressing maize in controlling Western corn rootworm (Vaughn et al., 2005; Gray et al., 2007; Meihls et al., 2008; Hibbard et al., 2009) and baseline susceptibility of Western corn rootworm populations to the Cry3Bb1 protein (Siegfried et al., 2005; EPA, 2010)[106] provide some indirect indications on the initial resistance allele frequency (EPA, 2010; Onstad and Meinke, 2010). Because resistance monitoring data on baseline susceptibility did not reveal any apparent increases in susceptibility in Western corn rootworm following several years of extensive cultivation of Cry3Bb1-expressing maize in the USA (event MON 863 approved since 2003 and MON 88017 since 2005 (CERA, 2011)), the applicant suggested that "*resistance allele frequencies to the Cry3Bb1 protein may be less than 0.01*". However, EPA (2010) concluded that "*there is too much uncertainty to definitively prove that this is the case*". Moreover, based on the data generated by Meihls et al. (2008) under greenhouse conditions, Onstad and Meinke (2010) calculated that the initial resistance allele frequency may be as high as 0.2, suggesting that initial resistance alleles may be present at a higher frequency under field conditions than initially assumed by the applicant.

(3) *Random mating occurs between resistant insects emerging in Bt-crops and susceptible insects preserved on non-Bt-crops (refuge) at sufficient levels*: The scale of adult movement determines how much mixing and mating can occur between individuals emerging from the refuge and Bt-maize fields. Even though adult Western corn rootworm can move substantial distances (Coats et al., 1986; Toepfer et al., 2006; Carrasco et al., 2009) and perform sustained flights longer than 30 minutes (Coats et al., 1986; Naranjo, 1990), most movements are short-ranged movements within fields or between adjacent fields, especially prior to mating (Naranjo et al., 2001, 2004; Storer, 2003; Meinke et al., 2009; Szalai et al., 2011). The range of measured maize field movement rates of adults was shown to be less than 30 m per day (Nowatzki et al., 2003; Spencer et al., 2003), with an average dispersal rate of approximately 15 m per day (Spencer et al., 2009). A microsatellite marker analysis among Western corn rootworm populations (10 populations; 595 individuals sampled) across nine USA states (from Western Kansas and Texas to New York and Delaware) found that all populations exhibited high levels of genetic diversity, and that adults from adjacent locations exchanged genes more frequently than those from more distant locations, suggesting that local movement is substantial (Kim and Sappington, 2005). The tendency for short-distance dispersal may delay resistance evolution at a landscape level (Caprio and Tabashnik, 1992), but it may contribute to the persistence and intensification of resistance in localised areas (Gassmann et al., 2011).

The pre-mating movement of Western corn rootworm females is generally more limited than that of males, which can be extensive when responding to reproductive females (Meinke et al., 2009). Females are unlikely to disperse before mating, meaning that males are the primary dispersers before mating (Spencer et al., 2003; Marquardt and Krupke, 2009). Mating typically occurs within 24 to 48 hours of female adult emergence within the maize fields they emerged from or nearby. Males normally emerge before females and are capable of mating multiple times (on average two times during their lifespan), though they are less likely to mate as they age, whereas females generally mate only once (Kang and Krupke, 2009a).

Based on a series of laboratory experiments, Kang and Krupke (2009a) argued that the realised mating activity between susceptible males from refuges and potentially resistant females on Bt-maize may be low, because the mating ability of males declines rapidly and adults in Cry3Bb1-expressing maize may emerge later than those in the refuge (Murphy et al., 2010; Pan et al., 2011). Moreover, males have been shown to prefer larger females under laboratory conditions (Kang and Krupke, 2009b), which could result in assortative mating (Hibbard et al., 2011; Murphy et al., 2011). Note that the larval ecological behaviour and adult mating behaviour of Western corn rootworm and their quantitative measurements are complex and that further

[106] Additional information received on 12/05/2010 / Request 1 / Pages 2-4 / Annex: Lang (2009)

investigations are required to sort out the demographics of populations emerging from refuges and Bt-maize (Onstad et al., 2011; Pan et al., 2011).

Given that there is considerable movement of males, EPA (2010) considered planting refuges for maize MON 88017 close to or in the Bt-maize field, preferentially in large blocks or narrow strips, adequate to ensure that males from refuges encounter receptive females on Bt-maize in time to mate.

(4) *Resistance alleles are partially or fully recessive*: Based on reciprocal crosses, Meihls et al. (2008) yielded hybrid progeny of Western corn rootworm that is not completely recessive, and calculated a dominance value (*h*) of 0.285 for larvae and 0.296 for adults (where 0 indicates completely recessive and 1 completely dominant inheritance (Liu and Tabashnik, 1997)). The calculations of *h* point to non-recessive inheritance of resistance under artificial selection experiments, which could lead to rapid response to selection without adequate risk management strategies (Meihls et al., 2008; Gassmann et al., 2011).

(5) *Fitness costs are associated with the resistance:* Modelling results have shown that fitness costs can delay or reverse resistance by selecting against Cry-resistant genotypes in refuges where resistant insects are not exposed to the Cry protein (Carrière and Tabashnik, 2001). Few studies analysed fitness costs associated with resistance to Cry3Bb1-expressing maize in Western corn rootworm (reviewed by Gassmann et al., 2009), but available evidence indicates that fitness costs associated with evolved resistance to Cry3Bb1-expressing maize are minimal (French et al., 2008; Meihls et al., 2008; Bagley et al., 2009).

The evidence discussed above suggests that not all the underlying assumptions contributing to the success of the 'high dose/refuge strategy' in delaying resistance evolution are fulfilled for Western corn rootworm and maize MON 88017. The Cry3Bb1 protein expressed in roots from maize MON 88017 is not expressed at a high dose, and preliminary data indicate that resistance alleles may be present at a higher frequency than initially assumed, Western corn rootworm may mate in a non-random manner, the resistance trait could have non-recessive inheritance, and that fitness costs are not necessarily associated with resistance evolution.

If the high dose requirement is not achieved, model predictions indicate that resistance evolution can be delayed by increasing refuge abundance to compensate for survival of hybrid progeny on Bt-maize (Gould, 1998; Tabashnik et al., 2004), and/or by requiring restrictions on the management of these refuges (Andow, 2008). However, it is also possible that the low-to-moderate dose of maize MON 88017 may slow down resistance evolution (Meihls et al., 2011). In the coleopteran-active maize MIR604, Hibbard et al. (2010b) demonstrated that many or most of Western corn rootworm individuals initially surviving on maize MIR604 after one generation of selection in the field have a susceptible phenotype, suggesting that resistant individuals from the Bt-maize are not only mating with susceptible individuals from refuge areas, but also with susceptible individuals that emerged from the Bt-field. Therefore, Bt-maize itself may act as refuge, yielding susceptible adults that are available to mate with any Western corn rootworm potentially carrying resistance alleles, hence contributing to slow the onset of resistance evolution (Hibbard et al., 2005; Clark et al., 2006).

How larvae are surviving exposure on Cry3Bb1-expressing maize is currently not known precisely. Larvae surviving on Cry3Bb1-expressing maize may do so by grazing on the outside of Cry3Bb1-expressing roots, thereby minimising exposure to the Bt-protein. Based on preliminary information on the feeding behaviour of Western corn rootworm larvae on maize MON 863, the BE CA suggested that "*the emerging adults may not have been exposed to Cry3Bb1 (or only to a limited extent)*", because "*they avoid feeding on root parts expressing Cry3Bb1*". According to Clark et al. (2006), larvae may be able to detect subtle differences in the expression of the Cry3Bb1 protein in the root system (event MON 863) and change their feeding behaviour to find non-toxic or less-toxic root parts, which would facilitate survival to the next instar with relatively normal larval growth (Hibbard et al., 2009). On non-transgenic maize, larvae fed into the root interior, leaving an outer "shell" of 1-2 layers

of epidermal root tissue and continued feeding resulted in larval movement into older and elongated root tissues over time. On maize MON 863, first and second instars began feeding on meristematic tissue, but terminated feeding before entering the root interior. In addition, larvae on maize MON 863 fed less frequently, did not become established at feeding sites, and moved more frequently than same-stage instars on conventional maize. Survival of first and second instars to successful molt on maize MON 863 was approximately 1 %. Larvae that fed and molted to second instar on transgenic roots exhibited the same growth rate as did larvae in a non-transgenic isoline. Moreover, there were no significant differences for the parameters of head capsule width and larval wet and dry weight. These results support the hypothesis that a "repellent factor" in roots or root exudates may contribute to the overall efficacy of maize MON 863 (Hibbard et al., 2008; EPA, 2010; Broekgaarden et al., 2011). However, extrapolating the observations made on maize MON 863 to maize MON 88017 is problematic, as according to the BE CA, "*neither the less heterogeneous Cry3Bb1 expression of MON 88017 compared to MON 863, nor the feeding behaviour of larvae on MON 88017 has been clearly demonstrated*" by the applicant. Moreover, the efficacy of the coleopteran-active maize MIR604 was attributed to antibiosis, rather than larval behavioural factors (antixenosis also called non-preference); larval behavioural analyses with maize MIR604 indicated that the presence of the mCry3A protein in roots did not interfere with larval responses to infochemicals, eliciting the key host location behaviours (attraction, feeding, and host recognition) (El Khishen et al., 2009; Bernklau et al., 2010). The EFSA GMO Panel therefore supports the conclusion of the BE CA that "*uncertainty remains if the larval feeding behaviour observed for MON 863 is the same for MON 88017, as the applicant notes that the latter has a more even distribution of the Cry3Bb1 protein in the roots and provides more consistent root protection compared to MON 863*" (section on monitoring of the environmental risk assessment report of the BE CA).

Due to the remaining scientific uncertainty, the BE CA was "*reluctant to rely on the outcomes of stochastic modelling approaches to support the appropriateness of the 20% refuge strategy* [Storer, 2003; Storer et al., 2006]" (section on monitoring of the environmental risk assessment report of the BE CA). However, the EFSA GMO Panel notes that current models simulating adaptation to low-to-moderate dose Bt-crops account for the fact that many or most of the individuals surviving on Bt-crops have susceptible phenotypes (Onstad et al., 2001a; EPA, 2010; Hibbard et al., 2010b; Pan et al., 2011).

The applicant performed a modelling exercise based on an amended model of Caprio MA (see EPA, 2010) to evaluate the efficacy of a 20 % refuge in delaying resistance evolution[107]. The model relied on a number of highly conservative assumptions such as full adoption rate of maize MON 88017, initial resistance allele frequency of 0.01, allowance for resistance to range from partially recessive to largely dominant, no fitness cost to resistance, levels of control provided by maize MON 88017 as low as 80 %. Using these highly conservative assumptions, the applicant indicated that "*a 20% refuge should be adequate to delay resistance for 7 to 16 years*" (EPA, 2010), or "*likely more than 20 years*" under less conservative conditions[108]. More complex modelling explored a range of efficacy and genetic parameter values and incorporated a spatially-explicit model structure and more realistic data on the biology of Western corn rootworm (Onstad et al., 2001a; Storer, 2003; Onstad, 2006; Storer et al., 2006; Pan et al., 2011). Overall, model predictions indicated that a 20 % refuge can delay resistance evolution for maize events comparable to maize MON 88017 (see above). With a block refuge in an 80 ha maize field and a 20 % refuge planted in the centre every year, Pan et al. (2011) estimated that resistance evolution in Western corn rootworm is delayed by more than 20 years, if the initial resistance allele frequency is 0.001. For an initial resistance allele frequency of 0.01, the resistance allele frequency exceeds 50 % in seven years. In case the 20 % refuge is a block that is relocated annually, then the resistance allele frequency exceeds 50 % in nine and five years, if the initial resistance allele frequencies are 0.001 and 0.01, respectively. Without adequate risk management strategies, the resistance allele frequency exceeds 50 % in five and three years for initial resistance allele frequencies of 0.001 and 0.01, respectively (Pan et al., 2011).

[107] Additional information received on 04/04/2011 / Request 3 / Page 31 / Annex: Ward (2002)
[108] Additional information received on 12/05/2010 / Request 1 / Pages 2-4

The extent with which maize MON 88017 volunteers may affect the rate of resistance evolution is unclear. Krupke et al. (2009) argued that the unpredictable and varying levels of expression of the Cry3Bb1 protein in maize MON 863 volunteers may facilitate more rapid evolution of resistance in Western corn rootworm populations. Larvae may survive exposure simply because the dose is lower, even without any differential feeding behaviour. It is also possible that due to larval movement between refuge and Bt-maize plants (Hibbard et al., 2003) larvae would be exposed to sublethal doses of the Cry3Bb1 protein at later instar stages by feeding on a combination of volunteer and maize MON 88017 plants (Meihls et al., 2008; Krupke et al., 2009; Murphy et al., 2010). However, there is also the possibility that larvae may exhibit a feeding behaviour that minimises exposure to the Cry3Bb1 protein, contributing to slow the onset of resistance evolution. How much each of these mechanisms will contribute to the speed of resistance evolution overall is dependent upon the amount of maize MON 88017 planted, the type of refuge used, and the number of maize volunteers present and the Cry3Bb1 protein expressed by those plants.

Conclusion

The EFSA GMO Panel considers that the applicant provided conservative predictions on the duration of susceptibility of Western corn rootworm to the Cry3Bb1 protein with a 20 % refuge (see also EPA, 2010 for an in depth evaluation), though recognises that all modelling exercises are subject to scientific uncertainty (i.e., Perry et al., 2010), and that caution is recommended when predicting future responses of Western corn rootworm in the EU based on experiences elsewhere, as resistance evolution in target insect pests is dependent upon many factors (Tyutyunov et al., 2008). Moreover, scientific uncertainty related to the appropriateness of the 'high dose/refuge strategy' in delaying resistance evolution in Western corn rootworm remains. Therefore, the EFSA GMO Panel, while agreeing with the 'high dose/refuge strategy', recommends further research is conducted by the applicant to confirm that the underlying assumptions of this strategy are met for the Western corn rootworm, along with the periodic re-evaluation of the adequacy and efficacy of this insect resistance management strategy.

According to the insect resistance management plan proposed by the applicant, only farmers growing more than 5 ha of Cry3Bb1-expressing maize in the EU shall establish refuge areas with non-Cry3Bb1-expressing maize, corresponding to at least 20 % of the surface planted with Cry3Bb1-expressing maize. In practice, this would mean that *refugia* of non-Cry3Bb1-expressing maize would not be implemented on a considerable proportion of farms in certain EU countries, as the area planted to Cry3Bb1-expressing maize on these farms would cover less than 5 ha[109]. In most cases, it is likely that sufficiently large areas of non-Cry3Bb1-expressing maize will remain, providing widely distributed mosaics of both non-Cry3Bb1-expressing and Cry3Bb1-expressing maize at regional scales. Moreover, evidence has shown that several grass species can support the growth of Western corn rootworm larvae (Clark and Hibbard, 2004; Oyediran et al., 2004; Wilson and Hibbard, 2004) and may therefore serve as an additional refuge where these grass species are abundant and appropriately distributed (Chege et al., 2005, 2009; Oyediran et al., 2005). However, if Cry3Bb1-expressing maize was adopted on a larger scale in a region or in a cluster of fields with an aggregate area greater than 5 ha, then the potential for resistance evolution is likely to increase. Therefore, the EFSA GMO Panel recommends that there should be *refugia* equivalent to 20 % of the aggregate area, irrespective of individual field and farm size. The EFSA GMO Panel also recommends that stewardship agreements, as proposed by the applicant, specify the upper limit of the maize MON 88017 surface (e.g., 30 ha) at which separate refuges should be established. For example, *every* 30 ha of maize MON 88017 should be interspersed with a refuge of at least 6 ha of non-Cry3Bb1-expressing maize, especially if the refuge is planted as a separate field adjacent to the Bt-maize field.

To ensure that adequate numbers of susceptible Western corn rootworm individuals are available to mate with resistant ones, EPA (2010) recommended planting refuges for maize MON 88017 adjacent to or in the Bt-maize field, preferentially in large blocks or narrow strips. If the refuge is planted as a

[109] Additional information received on 04/04/2011 / Page 32 / Appendix 6

separate field adjacent to the Bt-maize field, then it should be separated by no more than an alley or road from the maize MON 88017 field[110]. If the refuge is planted as row strips within the maize MON 88017 field, then it should be planted as at least four or more consecutive rows (Hibbard et al., 2003; EPA, 2010). The use of mixtures of maize MON 88017 and conventional seed are not being recommended at this time until further data are gathered on the performance and suitability of this potential refuge option (i.e., Onstad, 2006; Murphy et al., 2010, 2011; Onstad et al., 2011; Pan et al., 2011)[111].

To ensure that adequate numbers of susceptible Western corn rootworm individuals emerge from refuges, the applicant clarified that the type of maize to be planted as refuge should be of a similar hybrid/variety, as close as possible to the Bt-maize, and should be planted at the same time as the Bt-maize[112]. According to the refuge strategy followed in the USA, farmers place the refuge on continuous maize ground if maize MON 88017 is on continuous maize ground. In case maize MON 88017 is planted on rotated ground, then the refuge is to be planted on rotated ground (i.e., Storer, 2003). Since the life cycle of Western com rootworm extends over two consecutive maize growing seasons in the EU, the EFSA GMO Panel considers that areas designed to deliver susceptible Western corn rootworm adults are suitable as refuge only if they have been cropped with non-Cry3Bb1-expressing maize for at least two successive years.

Following a request from the BE CA, the applicant clarified that the refuge and maize MON 88017 areas should be managed using comparable agronomic practices[113]. It is acceptable to treat refuge areas for maize MON 88017 with seed treatments or soil-applied insecticides for the control of Western corn rootworm larvae, as this is not expected to adversely affect adult emergence from the refuge. However, it is not acceptable to treat refuges for adult corn rootworm control since these treatments may diminish the efficacy of the refuge (EPA, 2010). Foliar applications for adult control are an option only if both refuge and maize MON 88017 fields are treated equally, and only if adult population densities are very high. The EFSA GMO Panel considers that microbial Bt-sprays containing the Cry3Bb1 protein should not be used in the refuge maize.

The EFSA GMO Panel pinpoints the importance of implementing educational programs to encourage farmers to establish appropriate refuges and to ensure compliance to the insect resistance management requirements recommended by risk managers. The lack of implementation of the 'high-dose/refuge strategy' has been shown to be an important cause for the evolution of field resistance to Bt-crops (Bourguet et al., 2005; Kruger et al., 2009, 2011a; Andow et al., 2010; Gassmann et al., 2011; Huang et al., 2011; Onstad et al., 2011).

The EFSA GMO Panel advocates the deployment of diversified resistance management strategies, along with more integrated methods to control pests targeted by Bt-crops.

The EFSA GMO Panel conclusions on the potential for target insect resistance evolution and its recommendation to periodically re-evaluate the adequacy of the insect resistance management strategy are consistent with those of the BE CA. The BE CA supported "*the adoption of the proposed 20% refuge strategy as described in the IRM* [insect resistance management] *plan*", but was of the opinion that "*the importance of the refuge in delaying resistance against MON 88017 should be further investigated. If the above assumptions on larval feeding behaviour and Cry3Bb1 expression in MON 88017 are true, it follows that the 20% refuge strategy may be a too highly conservative measure to delay the occurrence of insect resistance*". Therefore, the BE CA noted that "*care should be taken to continuously evaluate and, if needed, adjust the recommended measures in the plan, particularly if large scale adoption of the Bt maize would change existing eradication/containment*

[110] Additional information received on 12/05/2010 / Additional clarifications / Page 7
[111] Additional information received on 04/04/2011 / Pages 4 and 16 / Appendix 6
[112] Additional information received on 12/05/2010 / Additional clarifications / Page 7 // Additional information received on 04/04/2011 / Request 3 / Page 31 / Appendix 6
[113] Additional information received on 12/05/2010 / Additional clarifications / Page 6 // Additional information received on 04/04/2011 / Request 3 / Page 31 / Appendix 6

cropping measures (e.g. crop rotation) possibly affecting abundances of the target population" (section on monitoring of the environmental risk assessment report of the BE CA). "*Given the current knowledge gaps*", the BE CA argued that "*the IRM plan needs further development and continuous updating taken into account the results of ongoing scientific research*" (see overall conclusions of the environmental risk assessment report of the BE CA).

6.3.1.4. Risk mitigation measures to reduce adverse effects due to the use of novel herbicide regimes

The applicant proposed that "*by the time of commercialisation of MON 88017 in the E.U.*", it "*will develop a Technology Use Guide for the European MON 88017 markets. This document is intended to provide more information to the farmer on Monsanto's commitment to stewardship and more detailed weed control recommendations in MON 88017 specific to each region. Monsanto's weed management recommendations in glyphosate-tolerant crop are based on local needs, according to crop rotation, weed species, climate and tillage regime, using Good Agricultural Practices (GAP) as a basis. Therefore, glyphosate will not be the only weed control tool recommended in MON 88017*".

Depending upon protection goals set at Member State level (e.g., EFSA, 2010c,d,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 current levels, and that potential adverse effects on arable weeds, farmland biodiversity, food webs and the ecological functions they provide are limited to the levels currently found in non-GMHT maize. 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 environmental adverse herbicide effects on biodiversity by targeting their main drivers (Butler et al., 2007). Member States may recommend using glyphosate on maize MON 88017 only in regimes that have similar or reduced environmental impacts compared with conventional maize cultivation, and that do not interfere with biological functions currently supported by maize 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 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). Maize has been shown to be a poor crop for biodiversity under European conditions, having the greatest adverse effect on farmland biodiversity compared with oilseed rape and beet (Dewar et al., 2005; Firbank et al., 2005b; Bohan et al., 2007; Smith et al., 2008b). Moreover, maize is frequently not grown in rotation with other crops in the EU (FCEC, 2009), so the repeated use of glyphosate at recommended application rates on continuous maize MON 88017 may result in reductions in botanical diversity and/or weed density in maize fields to a level that might adversely affect food chains and webs. 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. Furthermore, 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 surrounding landscape. It also remains debatable whether increases in crop yield will spare land for biodiversity and hence natural habitats from conversion into arable land in European 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 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; Storkey and Westbury, 2007; Kleijn et al., 2011; Whittingham, 2011). Possible mitigation measures for uncropped land include protecting adjacent habitats from herbicide effects. To limit potential adverse effects due to herbicide drift, the approval for the application of glyphosate on Roundup Ready maize includes recommendations for separation distances of 20 m from certain sensitive areas and measures for the protection of water courses in Germany (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; Cordeau et al., 2011). Headlands and/or field margins, as being part of the field margin complex (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 mixes) or left to regenerate naturally (including naturally regenerated or sown [temporary or long-term] set-aside margins), and (2) cropped, comprising sown arable crops usually under modified management, such as conservation headlands, wild bird cover crops and pollen and nectar mixes. 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 allow less intensive management by reducing 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 relative high degree of weed diversity at field edges.

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 of 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). Such risk mitigation measures can also be applied to glyphosate tolerant maize. A UK field study, conducted in conventional maize with glyphosate using shielded sprayers, revealed that an early over-the-row band spray followed by an overall spray enabled to maintain levels of farmland biodiversity in maize without yield loss (DEFRA, 2004). Another in-crop risk mitigation measure is to rotate crops. Bourassa et al. (2010) reported that rotating maize with oilseed rape had a stronger effect on carabid community than did changing from conventional to GMHT maize, indicating that GMHT maize has little impact on the overall carabid fauna, but that it may influence the activity of certain species through effects on the weed community (see also Bourassa et al., 2008). The choice of crop sequences can be an important tool for manipulating weed communities to select potentially against pernicious species (Squire et al., 2000; Anderson, 2009). Rotating maize with other crops would also be the best preventive strategy against 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., 2011; Sanvido et al., 2011a,c). 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 BE CA was of the opinion that "*the use of glyphosate 'over the top of the crop' must not interfere with biological functions of non-target organisms (such as biological control and decomposition)*" (section 2.8 of the environmental risk assessment report of the BE CA), but did not consider risk mitigation measures to reduce potential adverse effects due to the use of novel herbicide regimes. The BE CA noted "*the applicant's commitment to develop a 'Technology Use Guide for the European MON 88017 markets', describing recommendations for the use of their product, including more detailed weed control recommendations specific to each region and local needs*".

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; Heap, 2011; Owen, 2011). Current maize 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[114] and/or herbicide rotation in cropping system. However, in some parts of the EU, continuous maize is grown, so there is a potentially high risk if

[114] Additional information received on 04/04/2011 / Request 2.2 / Pages 20-26 / Appendix 4

glyphosate is repeatedly used on glyphosate tolerant maize (section 6.2.7, above). Therefore, 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[115].

The EFSA GMO Panel considers it essential that diversified 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, 2011; Shaner et al., 2011). Such measures could 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 could ensure the appropriate management of glyphosate on GMHT maize, so that the evolution of resistant weeds is delayed. Scientific evidence showed that the selection pressure on weeds can be reduced by crop rotation (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 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; Beckie and Reboud, 2009; Busi and Powles, 2009; Gressel, 2009; Gulden et al., 2009; Shaw et al., 2009; Meissle et al., 2010; NRC, 2010; Werth et al., 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., 2011).

In contrast to monocultures, crop rotation allows alternative weed control strategies to be used that may reduce weed population densities and favour a more diverse composition of weed communities, instead of communities that are dominated by one or few weed species. 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. In some studies, the cropping sequence has been reported to be the dominant factor affecting the weed 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.

Alternatively, the efficacy in use of herbicides can be optimised within a cropping system (Wilson et al., 2011). This can be achieved by scouting for weeds, integrating knowledge of weed biology and ecology, using appropriate application technologies, and by applying suitable herbicide regimes (Nazarko et al., 2005; Parker et al., 2006). In this respect, new or existing herbicidal active substances may be targeted to fill the gaps in the activity spectrum of glyphosate. 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; Vasileiadis et al., 2011). A clear advantage of focussing on increased cropping system diversification is that it would increase or conserve farmland biodiversity, and reduce the risk of weed shifts and of 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 (see Table 4, above). It is also advisable that weed resistance management is

[115] Additional information received on 04/04/2011 / Request 2 / Pages 20-30

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., 2011).

6.3.1.5. 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 maize MON 88017 may pose to the environment (section 6.3.1.4 and Table 4, above). 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.

The EFSA GMO Panel considers that the applicant provided conservative predictions on the duration of susceptibility of Western corn rootworm to the Cry3Bb1 protein with a 20 % refuge, though recognises that all modelling exercises are subject to scientific uncertainty, and that caution is recommended when predicting future responses of Western corn rootworm in the EU based on experiences elsewhere, as resistance evolution in target insect pests is dependent upon many factors. Moreover, scientific uncertainty related to the appropriateness of the 'high dose/refuge strategy' in delaying resistance evolution in Western corn rootworm remains. Therefore, the EFSA GMO Panel, while agreeing with the 'high dose/refuge strategy', recommends further research is conducted by the applicant to confirm that the underlying assumptions of this strategy are met for the Western corn rootworm, along with the periodic re-evaluation of the adequacy and efficacy of this insect resistance management strategy.

Since the life cycle of Western com rootworm extends over two consecutive maize growing seasons in the EU, the EFSA GMO Panel considers that areas designed to deliver susceptible Western corn rootworm adults are suitable as refuge only if they have been cropped with non-Cry3Bb1-expressing maize for at least two successive years.

The EFSA GMO Panel conclusions on the potential for target insect resistance evolution and its recommendation to periodically re-evaluate the adequacy of the insect resistance management strategy are consistent with those of the BE CA. "*Given the current knowledge gaps*", the BE CA supported "*the proposed refuge strategy as described in the IRM* [insect resistance management] *plan*", but was of the opinion that "*the IRM plan needs further development and continuous updating taking into account the results of ongoing scientific research*" (see overall conclusions of the environmental risk assessment report of the BE CA).

The EFSA GMO Panel anticipates that the repeated use of glyphosate at recommended application rates on continuous maize MON 88017 and/or other glyphosate tolerant crops grown in rotation may result in reductions in botanical diversity and/or weed density in maize fields to a level that might adversely affect food chains and webs, but not necessarily biological control functions, at the field and landscape level. Such a reduction in 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 on maize MON 88017 is used within diversified cropping regimes that have similar or reduced adverse effects on farmland biodiversity compared with conventional maize cultivation. Possible mitigation measures include protecting adjacent habitats from herbicide drift, (re)introduction and better management of field margins or other 'out of crop' measures, less intense in-crop weed management, and especially rotating crops.

The cultivation of maize MON 88017 in monoculture or in rotation with other glyphosate tolerant crops, in conjunction with the repeated and/or exclusive application of glyphosate-based herbicides

will cause changes in weed flora, and will favour the evolution and spread of glyphosate resistant weeds due to the selection pressure exerted by glyphosate. This, in turn, may affect food webs, and the functional value of weed vegetation for organisms of higher trophic levels (reduced functional biodiversity). 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 post-emergence 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.

With regard to weed resistance management, the BE CA noted that "*a glyphosate resistance management plan was set up* [by the applicant] *in the framework of Directive 91/414/EEC* [which was repealed by Regulation (EC) No 1107/2009 on 14 June 2011] *to address the potential development of resistant weeds*", and "*therefore not reconsidered in his* [its] *evaluation*" (section on monitoring of the environmental risk assessment report of the BE CA).

6.3.2. Post-market environmental monitoring[116]

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, whereas 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.

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).

[116] Technical dossier / Section D9.10

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

With the consideration of risk mitigation measures, the environmental risk assessment of maize MON 88017 concluded that:

- maize MON 88017 plants are likely to cause resistance evolution in coleopteran target pests, and this in turn may cause adverse environmental consequences. Considering that coleopteran target pests will evolve resistance to Cry3Bb1-expressing maize rapidly under conditions of continuous exposure, the applicant proposed to put in place risk mitigation measures to delay the possible evolution of resistance. However, scientific uncertainty related to the appropriateness of the 'high dose/refuge strategy' in delaying resistance evolution in Western corn rootworm remains. Therefore, the EFSA GMO Panel, while agreeing with the 'high dose/refuge strategy', recommends further research is conducted by the applicant to confirm that the underlying assumptions of this strategy are met for the Western corn rootworm, along with the periodic re-evaluation of the adequacy and efficacy of this insect resistance management strategy;

- the cultivation of maize MON 88017 may result in adverse environmental effects due to the use of the complementary glyphosate-based herbicides. These potential adverse environmental effects comprise (1) a reduction in farmland biodiversity, (2) changes in botanical diversity due to weed shifts, with the selection of weed communities mostly composed of tolerant species, and (3) the selection of glyphosate resistant weeds. 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 maize cultivation or to meet the protection goals of different farming regions. However, the practicality and implementation of these measures will vary according to local conditions and so there is scientific uncertainty as to whether they will achieve the desired goals.

6.3.2.3. Case-specific monitoring

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) the possible resistance evolution to the Cry3Bb1 protein in coleopteran target pests, (2) a reduction in farmland biodiversity due to novel herbicide regimes, (3) changes in botanical diversity due to weed shifts, with the selection of weed communities mostly composed of tolerant species due to novel herbicide regimes, and (4) the selection of glyphosate resistant weeds due to novel herbicide regimes. The EFSA GMO Panel notes that for risks (2), (3) and (4) the possible environmental effects 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 (see section 6.2.7.1, above); 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 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, 2009c) 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) the possible resistance evolution to the Cry3Bb1 protein in coleopteran target pests, (2) changes in botanical diversity within fields due to novel herbicide regimes, and (3) resistance evolution to glyphosate in weeds due to novel herbicide regimes.

Monitoring resistance evolution to the Cry3Bb1 protein in coleopteran target pests

The applicant proposed to measure the baseline susceptibility of Western corn rootworm populations to the Cry3Bb1 protein and changes in that susceptibility in the EU. Resistance monitoring, through targeted field sampling in areas where maize MON 88017 adoption is the highest and selection pressure is greatest, should reveal changes in susceptibility of these populations. In this way, changes relative to the baseline susceptibility could be detected in time to enable proactive management before control failures occur (Siegfried et al., 2007; Tabashnik et al., 2008a,b, 2009). The EFSA GMO Panel agrees this approach and considers that susceptibility data can reveal potential changes in resistance levels in Western corn rootworm populations. Such data will also indicate the efficacy of the implemented 'high dose/refuge strategy' in delaying resistance evolution in the target pest species, and reduce the remaining scientific uncertainty related to the adequacy of the insect resistance management plan proposed by the applicant.

The EFSA GMO Panel considers that the overall framework to monitor resistance evolution proposed by the applicant is consistent with those described in the scientific literature (reviewed by Tabashnik et al., 2009). However, scientific uncertainty related to the appropriateness of the 'high dose/refuge strategy' in delaying resistance evolution in Western corn rootworm remains (section 6.3.1.3, below). Therefore, the EFSA GMO Panel suggests the applicant conducts further research to address the remaining scientific uncertainty related to the adequacy of the insect resistance management plan, and to confirm that the underlying assumptions of the 'high dose/refuge strategy' are met for the Western corn rootworm (e.g., determining the initial resistance allele frequency in Western corn rootworm populations, sorting out the demographics of populations emerging from refuges and Bt-maize).

With regard to the monitoring of resistance evolution, the EFSA GMO Panel expects that the use of standard procedures will allow baseline susceptibility testing on small numbers of European populations for an efficient monitoring of resistance evolution to the Cry3Bb1 protein. Therefore, the EFSA GMO Panel recommends:

- utilising appropriate sampling strategies of larvae of Western corn rootworm to set the most adequate and precise susceptibility baselines through random sampling, and to measure changes in susceptibility of populations at greatest risk of resistance evolution through targeted sampling in areas of high adoption rate of maize MON 88017. The sampling strategy should include fields cropped to this Bt-maize and adjacent fields cropped to non-coleopteran-active-Bt-maize or conventional maize, annual sampling during each maize growing season, follow up sampling of the same populations in subsequent seasons and sampling at appropriate times;

- accounting for relevant factors when designing an appropriate sampling strategy (e.g., the abundance, distribution and dispersal behaviour of Western corn rootworm, local variability in susceptibility levels).

In addition to the monitoring of baseline susceptibility and changes in susceptibility, the EFSA GMO Panel considers it relevant that unexpected field damage resulting from Western corn rootworm control failures is monitored and reported (see Gassmann et al., 2011). Such observations may reveal the occurrence of localised resistance before it spreads, and may serve as a trigger for further investigations to detect emerging resistance at an early stage (i.e., maize MON 88017 fields may be followed closely to check if adult Western corn rootworm are found in these fields). The EFSA GMO Panel considers that farmer questionnaires provide a relevant early alert system to report unexpected field damage caused by Western corn rootworm larvae.

"*As no risks concerning the environment and human and animal health were identified as a result of cultivation of MON 88017, except for potential indirect adverse effects related to the use of glyphosate over the top of the crop*", the BE CA supported the applicant's view that "*case-specific monitoring is not considered necessary during the cultivation of MON 88017. However, to delay resistance evolution, an insect resistance management plan was provided by the applicant, comprising case-specific monitoring of the baseline susceptibility of Western corn rootworm populations to the Cry3Bb1 protein*". The BE CA considered that "*the importance of the refuge in delaying resistance against MON 88017 should be further investigated*" (section on monitoring of the environmental risk assessment report of the BE CA). "*Given the current knowledge gaps*", the BE CA argued that "*the IRM plan needs further development and continuous updating taken into account the results of ongoing scientific research*" (see overall conclusions of the environmental risk assessment report of the BE CA).

Monitoring changes in botanical diversity within fields due to novel herbicide regimes

The EFSA GMO Panel recommends that the applicant proposes a detailed stewardship scheme to farmers for the use of glyphosate on maize MON 88017. This scheme should recommend detailed herbicide/cropping regimes that are environmentally sustainable and no more harmful to botanical diversity than the current conventional management practices within each receiving environment, according to local environmental protection goals. 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 safety of 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 botanical odiversity.

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 maize MON 88017 and that they have the proposed efficacy to ensure that any adverse effects on biodiversity are no greater than those caused by conventional management. This may be achieved during the cultivation of maize MON 88017 through a combination of collection of additional information from the farmer questionnaires (see section 6.3.2.4, below) 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 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) both grain and forage maize should be considered, if appropriate, (5) conclusions should be drawn not only for a single season but also at the temporal scale of a complete rotation, (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).

The BE CA considered that "*the applicant should have linked the ERA issue* [potential indirect adverse effects related to the use of glyphosate over the top of the crop] *better to monitoring*", and therefore requested that "*the potential consequences for biological functions of non-target organisms due to the use of glyphosate are better considered in the post-market monitoring plan and that the proposals made in its report are implemented*" (section on overall conclusion of the environmental risk assessment report of the BE CA). "*Given the fact that management and utilisation of a GM crop may vary from region to region, farm to farm and over time*", the BE CA acknowledged "*the difficulty to predict the range of farming practices that will be deployed with the GM crop and the consequences for biological functions*". While the BE CA stated that "*the risk assessment should have taken this unpredictability of farm management and its consequences for biological functions better into account, e.g. by relating this to monitoring*" (section 2.8 of the environmental risk assessment report of the BE CA), it did not require case-specific monitoring of impacts of the specific cultivation, management and harvesting techniques associated with the cultivation of maize MON 88017. The BE CA considered that "*farmer questionnaires are a good tool to detect changes in biological functions, but that the questionnaire should be adapted to cover this issue*" (section 2.8 of the environmental risk assessment report of the BE CA).

Monitoring resistance evolution to glyphosate in weeds due to novel herbicide 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, above), the EFSA GMO Panel advises that the use of glyphosate on GMHT crops, including maize MON 88017, 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 maize MON 88017) as described in section 6.2.7.3, above.

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, below).

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.

The BE CA noted that "*a glyphosate resistance management plan was set up* [by the applicant] *in the framework of Directive 91/414/EEC* [which was repealed by Regulation (EC) No 1107/2009 on 14 June 2011] *to address the potential development of resistant weeds*", and "*therefore not*

reconsidered in his [its] *evaluation*" (section on monitoring of the environmental risk assessment report of the BE CA).

6.3.2.4. General surveillance

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 maize MON 88017 throughout the period of validity of the authorisation. The general surveillance will take into consideration and be proportionate to the extent of cultivation of maize MON 88017 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 programs, in order to identify potential adverse effects associated with the intended uses of maize MON 88017.

Farmer questionnaires

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 maize MON 88017, 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 (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 their or evaluating 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 maize from previous crops (whenever relevant) and feral maize plants (if any) in field margins for the consideration of unanticipated effects on the persistence and invasiveness potential of maize MON 88017;

- in addition to the questions on pest and disease incidences on maize MON 88017, the farmer questionnaire should specifically request information on the occurrence of possible unexpected field damaged maize MON 88017 plants which might be associated with Western corn rootworm control failures, as this information will complement the case-specific monitoring of the possible resistance evolution to the Cry3Bb1 protein in target pests;

- 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, as a

range of weed control strategies could be adopted with different environmental impacts. Moreover, it will deliver relevant information that allows checking adoption of national/regional measures to mitigate the potential reduction of in-field botanical diversity due to use of glyphosate on maize MON 88017. 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 proportion of non-Cry3Bb1-expressing maize compared with maize MON 88017 on the farm, the distance between the refuge area and the monitored maize MON 88017 field in case the refuge is planted as a separate field adjacent to the Bt-maize field, the differences in pest management practices of the refuge, and on whether the refuge has been cropped with non-Cry3Bb1-expressing maize for at least two successive years;

- to consider unexpected effects on beneficial ecological functions provided by soil microbial communities due to the specific use of glyphosate-based herbicides during the growing season of maize MON 88017 or other glyphosate tolerant plants (see Smit et al., 2012 for indirect indicators in terms of yield, fertiliser use, quality).

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 maize MON 88017 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 maize MON 88017, as well as data on impacts on farming systems and the farm environment;

- use a field or group of fields growing maize MON 88017 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 maize MON 88017, on the same farm or in another location. If no comparators are being grown spatially or temporally close to maize MON 88017, 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 adapted, where needed, to each GM plant monitoring on a case-by-case basis by considering additional data requirements relevant for each species/event, its management and its receiving environments;

- are user friendly but also information rich;

- are constructed to encourage independent and objective responses from farmers, land managers and others involved with maize MON 88017 or its transgene 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 maize MON 88017 and where maize MON 88017 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 Bt-maize in the local area and/or the previous years, the receiving environments and the management systems in which maize MON 88017 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 maize MON 88017 and other GM plants should be indicated.

The BE CA considered that "*the applicant should have linked the ERA issue* [potential indirect adverse effects related to the use of glyphosate over the top of the crop] *better to monitoring*", and therefore requested that "*the potential consequences for biological functions of non-target organisms due to the use of glyphosate are better considered in the post-market monitoring plan and that the proposals made in its report are implemented*" (section on overall conclusion of the environmental risk assessment report of the BE CA). The BE CA stated that "*farmer questionnaires are a good tool to detect changes in biological functions, but that the questionnaire should be adapted to cover this issue*" (section 2.8 of the environmental risk assessment report of the BE CA). The BE CA was also of the opinion that the "*current general surveillance plan needs to be adapted to allow identification of unanticipated adverse effects on non-target organisms (see 2.8 and Annex III), and of management regimes that do not have an environmental performance at least as good as current regimes*" (section on monitoring of the environmental risk assessment report of the BE CA). More specifically, the BE CA requested that:

- "*the GS questionnaire should clarify what is meant with insects under 3.8, i.e. whether the term refers to pests or insects other than pests.*

- *insects other than pests, i.e. used in biological control of maize, should be considered separately in the general surveillance plan.*

- *in the farmer questionnaire, insect control treatments applied on the MON 88017 refuges should be requested in order to check the efficacy of the IRM measures*".

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 maize MON 88017 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 maize MON 88017 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, 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, 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 maize MON 88017, existing monitoring networks that monitor herbicide usage, botanical 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 maize MON 88017 cultivation are met.

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 maize MON 88017, 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, 2010f).

Industry stewardship programs

The EFSA GMO Panel welcomes the applicant's proposal to develop stewardship programs for the introduction, marketing, management and stewardship of maize MON 88017, 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 mitigation measures and detailed monitoring plans.

6.3.2.5. Reporting results of post-market environmental monitoring

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 gave its opinion and made 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) the possible resistance evolution to the Cry3Bb1 protein in coleopteran target pests, (2) changes in botanical diversity within fields due to novel herbicide regimes, and (3) resistance evolution to glyphosate in weeds due to novel herbicide regimes. 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 of the applicant (1) to establish farmer questionnaires as a reporting format of any on-farm observations of effects associated with the cultivation of maize MON 88017, (2) to use existing monitoring networks which observe changes in biota and production practices from farm up to regional level to obtain data on environmental impacts in the landscape where maize MON 88017 is cultivated, (3) to review all new scientific, technical and other information pertaining to maize MON 88017, and (4) to develop stewardship programs for the introduction, marketing, management and stewardship of maize MON 88017, but requests that its proposals and those made by the BE CA to strengthen general surveillance are implemented. The EFSA GMO Panel agrees with the reporting intervals and modalities proposed by the applicant.

The evaluation of the BE CA "*was restricted to the scientific quality of the monitoring plans proposed* [by the applicant], *including the IRM* [insect resistance management] *plan and the general surveillance plan*". "*As no risks concerning the environment and human and animal health were identified as a result of cultivation of MON 88017, except for potential indirect adverse effects related to the use of glyphosate over the top of the crop*", the BE CA supported the applicant's view that "*case-specific monitoring is not considered necessary during the cultivation of MON 88017. However, to delay resistance evolution, an insect resistance management plan was provided by the applicant,*

comprising case-specific monitoring of the baseline susceptibility of Western corn rootworm populations to the Cry3Bb1 protein". The BE CA considered that "*the importance of the refuge in delaying resistance against MON 88017 should be further investigated*" (section on monitoring of the environmental risk assessment report of the BE CA). "*Given the current knowledge gaps*", the BE CA argued that "*the IRM plan needs further development and continuous updating taken into account the results of ongoing scientific research*" (see overall conclusions of the environmental risk assessment report of the BE CA).

The BE CA considered that "*the applicant should have linked the ERA issue* [potential indirect adverse effects related to the use of glyphosate over the top of the crop] *better to monitoring*", and therefore requested that "*the potential consequences for biological functions of non-target organisms due to the use of glyphosate are better considered in the post-market monitoring plan and that the proposals made in its report are implemented*" (section on overall conclusion of the environmental risk assessment report of the BE CA). While the BE CA stated that "*the risk assessment should have taken the unpredictability of farm management and its consequences for biological functions better into account, e.g. by relating this to monitoring*" (section 2.8 of the environmental risk assessment report of the BE CA), it did not require case-specific monitoring of impacts of the specific cultivation, management and harvesting techniques associated with the cultivation of maize MON 88017.

The BE CA stated that "*farmer questionnaires are a good tool to detect changes in biological functions, but that the questionnaire should be adapted to cover this issue*" (section 2.8 of the environmental risk assessment report of the BE CA). The BE CA was also of the opinion that the "*current general surveillance plan needs to be adapted to allow identification of unanticipated adverse effects on non-target organisms (see 2.8 and Annex III), and of management regimes that do not have an environmental performance at least as good as current regimes*" (section on monitoring of the environmental risk assessment report of the BE CA).

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Following the submission of an application (Reference EFSA-GMO-CZ-2008-54) 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 insect resistant and herbicide tolerant genetically modified (GM) maize MON 88017 (Unique identifier MON-88Ø17-3) for food and feed uses, import, processing and cultivation. Whilst the scope of this application only covers the cultivation of maize MON 88017, this Scientific Opinion also updates the previous EFSA GMO Panel safety evaluation of the food and feed uses, import and processing of maize MON 88017 and derived products.

In delivering its Scientific Opinion, the EFSA GMO Panel considered the application EFSA-GMO-CZ-2008-54, additional information supplied by the applicant, scientific comments submitted by Member States, the environmental risk assessment report of the BE CA, and relevant scientific publications.

The EFSA GMO Panel evaluated maize MON 88017 with reference to the intended uses and appropriate principles described in its guidelines for 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 for 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 target proteins. An evaluation of the comparative analyses of composition, agronomic and phenotypic characteristics was undertaken, and the safety of the new proteins, both individually and in combination, 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 establish that maize MON 88017 contains one copy of an intact CP4 *epsps* expression cassette and a *cry3Bb1* cassette in a single locus. No other parts of the plasmid

used for transformation are present in the transformed plant. Updated bioinformatic analysis of the open reading frames at the junctions between the inserted DNA and maize genomic DNA did not raise safety concerns. The stability of the inserted DNA and the herbicide tolerance and insect resistance traits was confirmed over several generations. Updated analyses of the levels of newly expressed proteins in various plant parts collected from field trials performed in Europe did not raise safety concerns.

Based on the comparative analysis of maize MON 88017 with its conventional counterpart and other commercial maize varieties, the EFSA GMO Panel concludes that maize MON 88017, as assessed in this application, is compositionally, agronomically and phenotypically not different from its conventional counterpart, except for the newly expressed Cry3Bb1 and CP4 EPSPS proteins. With the exception of the presence of the newly expressed Cry3Bb1 and CP4 EPSPS proteins, maize MON 88017 is also compositionally and agronomically equivalent to conventional maize varieties. In addition, there are no indications of potential toxicity and allergenicity of the Cry3Bb1 and CP4 EPSPS proteins expressed in maize MON 88017. A subchronic (90-day) feeding study revealed no indications of adverse effects in rats fed diets containing grains from maize MON 88017. In addition, a feeding study with broiler chickens provided evidence of nutritional equivalence of maize MON 88017 to conventional maize. The EFSA GMO Panel considers that maize MON 88017 is as safe and as nutritious as its conventional counterpart and reference varieties and that it is unlikely that the overall allergenicity of the whole plant is changed by the genetic modification.

Since the scope of the current application covers cultivation, the environmental risk assessment considered the environmental impact of full-scale commercialisation of maize MON 88017.

The BE CA (including its Biosafety Advisory Council) provided to EFSA its report on the environmental risk assessment of maize MON 88017 (dated 28 September 2010) on 6 October 2010 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 BE 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 maize MON 88017 has no altered agronomic and phenotypic characteristics, except for the specific target pest resistance and herbicide tolerance. The likelihood of unintended environmental effects due to the establishment, survival and spread of maize MON 88017 is considered to be extremely low, and will be no different from that of conventional maize 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 *cry3Bb1* and CP4 *epsps* genes from maize MON 88017 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.

The possible resistance evolution to the Cry3Bb1 protein in coleopteran target pests is identified by the EFSA GMO Panel as a concern associated with the cultivation of maize MON 88017, as resistance evolution may lead to altered pest control practices that may cause adverse environmental effects.

Based on the evidence provided by the applicant and relevant scientific literature on maize MON 88017, the EFSA GMO Panel concludes that there are no indications of adverse effects on non-target organisms due to unintended changes in maize MON 88017, and therefore considers *trait*-specific information appropriate to assess whether maize MON 88017 poses a risk to non-target organisms.

The evidence provided by the applicant indicates that the protein sequences of the Cry3Bb1 protein variants of maize MON 88017, MON 863 and MON 853 are similar, and that the biological activity of these Cry3Bb1 protein variants is equivalent. Therefore, the EFSA GMO Panel considers that information generated to evaluate potential adverse effects on non-target organisms due to the

expression of the Cry3Bb1 protein in maize MON 863 or MON 853 can be used to inform the environmental risk assessment of maize MON 88017.

The EFSA GMO Panel concludes that potential adverse effects of maize MON 88017 due to the expression of the Cry3Bb1 protein to non-target terrestrial (plant- and ground-dwelling), soil and aquatic arthropods are expected to be negligible in the context of its proposed uses. Rearrangements of species assemblages at different trophic levels in crop stands are commonly associated with any pest management practice, but the EFSA GMO Panel considers that maize MON 88017 will not cause reductions to natural enemies that are significantly greater from those caused by conventional cultivation where insecticides are used to control corn rootworms. Based on the evidence supplied by the applicant, the EFSA GMO Panel has no reason to believe that maize MON 88017 will adversely affect honeybees. Few studies have assessed the impact of the Cry3Bb1 protein on non-target aquatic arthropods and the fate of the Cry3Bb1 protein in senescent and decaying maize detritus in aquatic environments, but available data indicate it is unlikely that the Cry3Bb1 protein in maize MON 88017 products would cause adverse effects on non-target aquatic arthropods in the context of its proposed uses. In addition, there is no evidence to indicate that maize MON 88017 is likely to cause adverse effects on non-target organisms that are not arthropods in the context of its proposed uses.

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.

On the basis of the data provided by the applicant and those obtained from a literature survey, the likelihood of adverse effects to non-target organisms is foreseen to be very low, and limited to non-target chrysomelid larvae. However, the risk of maize MON 88017 to valued (non-pest) chrysomelid species in the field is likely to be minimal due to their low occurrence and abundance in maize fields and due to the low likelihood of encountering harmful amounts of pollen from maize MON 88017 in and around maize fields. Moreover, the activity of the Cry3Bb1 protein on adult non-target chrysomelid species is expected to be limited.

The EFSA GMO Panel considers that Cry3Bb1 protein concentrations in decaying plant residues from maize MON 88017 decrease rapidly in soil, indicating that non-target soil organisms are exposed to relatively low Cry3Bb1 protein concentrations within a few months after harvest. There is no evidence for accumulation of the Cry3Bb1 protein on agricultural fields cultivated repeatedly with maize MON 88017 or comparable maize events (e.g., MON 863), despite its potential to bind to surface-active particles. Effects of crops on soil microbial communities, which are especially expected in the rhizosphere or on decaying plant material, depend more on their species, variety or age than whether they are genetically modified. Rearrangements in structural diversity and population abundance of non-target soil organisms occur frequently in the agricultural environment. They are typically associated with several sources of variation, caused by natural variability (e.g., soil heterogeneity, weather conditions) and agricultural practices (e.g., soil tillage, crop rotation, irrigation measures) and are thus not necessarily an indication of environmental harm. The EFSA GMO Panel concludes that potential effects on soil microorganisms and microbial communities, as well as the ecological functions they provide, due to the cultivation of maize MON 88017, if they occur, will be transient and minor, and are likely to be smaller or within the range currently caused by other agronomic and environmental factors.

There are no indications that the expression and biological activity of the Cry3Bb1 and CP4 EPSPS proteins expressed in maize MON 88017 are affected by potential interactions between these two proteins. However, the EFSA GMO Panel took account of crop management in the environmental risk assessment, as interactions between biota may occur under different weed and pest management regimes, irrespective of interactions between the newly expressed proteins.

The EFSA GMO Panel is of the opinion that potential adverse environmental effects of the cultivation of maize MON 88017 are associated with the use of the complementary glyphosate-based herbicide regimes. These potential adverse environmental effects comprise (1) a reduction in farmland

biodiversity, (2) changes in botanical diversity due to weed shifts, with the selection of weed communities mostly composed of tolerant species, and (3) the selection of glyphosate resistant weeds. The potential harmful effects could occur at the level of arable weeds, farmland biodiversity, food webs and the ecological functions they provide. The magnitude of these potential adverse environmental effects will depend upon a series of factors, including the specific herbicide and cultivation management applied at the farm level, the crop rotation and the characteristics of receiving environments.

The EFSA GMO Panel considers that the use of glyphosate-based herbicides at recommended field application rates of glyphosate on maize MON 88017 is unlikely to cause adverse effects to soil microbial communities or beneficial functions mediated by them.

The conclusions of the EFSA GMO Panel on the environmental safety of maize MON 88017 are consistent with those of the BE CA. The BE CA (including its Biosafety Advisory Council) provided to EFSA its report on the environmental risk assessment of maize MON 88017 (dated 28 September 2010) on 6 October 2010 in line with Articles 6.3(c) and 18.3(c) of Regulation (EC) No 1829/2003. The BE CA concluded that "*based on the information in the application, the additional information received by the applicant, the information found in peer-reviewed studies and the scientific comments raised by the member states, no risks concerning the environment and human and animal health were identified as a result of cultivation of MON 88017, except for potential indirect adverse effects related to the use of glyphosate over the top of the crop*" (see overall conclusions of the environmental risk assessment report of the BE CA). In its evaluation, the BE CA identified potential adverse effects of the herbicide used on maize MON 88017 on the environment, and they considered that "*the use of glyphosate 'over the top of the crop' must not interfere with biological functions of non-target organisms (such as biological control and decomposition)*" (section 2.8 of the environmental risk assessment report of the BE CA). The BE CA did not consider the evaluation of the potential weed resistance evolution was within their remit, as it should be considered under Regulation (EC) No 1107/2009.

The EFSA GMO Panel considers that the applicant provided conservative predictions on the duration of susceptibility of Western corn rootworm to the Cry3Bb1 protein with a 20 % refuge, though recognises that all modelling exercises are subject to scientific uncertainty, and that caution is recommended when predicting future responses of Western corn rootworm in the EU based on experiences elsewhere, as resistance evolution in target insect pests is dependent upon many factors. Moreover, scientific uncertainty related to the appropriateness of the 'high dose/refuge strategy' in delaying resistance evolution in Western corn rootworm remains. Therefore, the EFSA GMO Panel recommends further research is conducted by the applicant to confirm that the underlying assumptions of the 'high dose/refuge strategy' are met for the Western corn rootworm, along with the periodic re-evaluation of the adequacy and efficacy of this insect resistance management strategy.

Since the life cycle of Western com rootworm extends over two consecutive maize growing seasons in the EU, the EFSA GMO Panel considers that areas designed to deliver susceptible Western corn rootworm adults are suitable as refuge only if they have been cropped with non-Cry3Bb1-expressing maize for at least two successive years.

The EFSA GMO Panel advocates the deployment of diversified resistance management strategies, along with more integrated methods to control pests targeted by Bt-crops.

The EFSA GMO Panel conclusions on the potential for target insect resistance evolution and its recommendation to periodically re-evaluate the adequacy of the insect resistance management strategy are consistent with those of the BE CA. "*Given the current knowledge gaps*", the BE CA supported "*the proposed refuge strategy as described in the IRM* [insect resistance management] *plan*", but was of the opinion that "*the IRM plan needs further development and continuous updating taking into account the results of ongoing scientific research*" (see overall conclusions of the environmental risk assessment report of the BE CA).

The EFSA GMO Panel anticipates that the repeated use of glyphosate at recommended application rates on continuous maize MON 88017 and/or other glyphosate tolerant crops grown in rotation may result in reductions in botanical diversity and/or weed density in maize fields to a level that might adversely affect food chains and webs, but not necessarily biological control functions, at the field and landscape level. Such a reduction in 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 on maize MON 88017 is used within diversified cropping regimes that have similar or reduced adverse effects on farmland biodiversity compared with conventional maize cultivation. Possible mitigation measures include protecting adjacent habitats from herbicide drift, (re)introduction and better management of field margins or other 'out of crop' measures, less intense in-crop weed management, and especially rotating crops.

The cultivation of maize MON 88017 in monoculture or in rotation with other glyphosate tolerant crops, in conjunction with the repeated and/or exclusive application of glyphosate-based herbicides will cause changes in weed flora, and will favour the evolution and spread of glyphosate resistant weeds due to the selection pressure exerted by glyphosate. This, in turn, may affect food webs, and the functional value of weed vegetation for organisms of higher trophic levels (reduced functional biodiversity). 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 post-emergence cultivation and cover crops. To be most effective, these methods shoud 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.

With regard to weed resistance management, the BE CA noted that "*a glyphosate resistance management plan was set up [by the applicant] in the framework of Directive 91/414/EEC* [which was repealed by Regulation (EC) No 1107/2009 on 14 June 2011] *to address the potential development of resistant weeds*", and "*therefore not reconsidered in his [its] evaluation*" (section on monitoring of the environmental risk assessment report of the BE CA).

The EFSA GMO Panel gave its opinion and made 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, as detailed above, to address (1) the possible resistance evolution to the Cry3Bb1 protein in coleopteran target pests, (2) changes in botanical diversity within fields due to novel herbicide regimes, and (3) resistance evolution to glyphosate in weeds due to novel herbicide regimes. 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 of the applicant (1) to establish farmer questionnaires as a reporting format of any on-farm observations of effects associated with the cultivation of maize MON 88017, (2) to use existing monitoring networks which observe changes in biota and production practices from farm up to regional level to obtain data on environmental impacts in the landscape where maize MON 88017 is cultivated, (3) to review all new scientific, technical and other information pertaining to maize MON 88017, and (4) to develop stewardship programs for the introduction, marketing, management and stewardship of maize MON 88017, but requests that its proposals and those made by the BE CA to strengthen general surveillance are implemented. The EFSA GMO Panel agrees with the reporting intervals and modalities proposed by the applicant.

The evaluation of the BE CA "*was restricted to the scientific quality of the monitoring plans proposed* [by the applicant], *including the IRM* [insect resistance management] *plan and the general surveillance plan*". "*As no risks concerning the environment and human and animal health were identified as a result of cultivation of MON 88017, except for potential indirect adverse effects related to the use of glyphosate over the top of the crop*", the BE CA supported the applicant's view that "*case-specific monitoring is not considered necessary during the cultivation of MON 88017. However, to delay resistance evolution, an insect resistance management plan was provided by the applicant, comprising case-specific monitoring of the baseline susceptibility of Western corn rootworm populations to the Cry3Bb1 protein*". The BE CA considered that "*the importance of the refuge in delaying resistance against MON 88017 should be further investigated*" (section on monitoring of the environmental risk assessment report of the BE CA). "*Given the current knowledge gaps*", the BE CA argued that "*the IRM plan needs further development and continuous updating taken into account the results of ongoing scientific research*" (see overall conclusions of the environmental risk assessment report of the BE CA).

The BE CA considered that "*the applicant should have linked the ERA issue* [potential indirect adverse effects related to the use of glyphosate over the top of the crop] *better to monitoring*", and therefore requested that "*the potential consequences for biological functions of non-target organisms due to the use of glyphosate are better considered in the post-market monitoring plan and that the proposals made in its report are implemented*" (section on overall conclusion of the environmental risk assessment report of the BE CA). While the BE CA stated that "*the risk assessment should have taken the unpredictability of farm management and its consequences for biological functions better into account, e.g. by relating this to monitoring*" (section 2.8 of the environmental risk assessment report of the BE CA), it did not require case-specific monitoring of impacts of the specific cultivation, management and harvesting techniques associated with the cultivation of maize MON 88017.

The BE CA stated that "*farmer questionnaires are a good tool to detect changes in biological functions, but that the questionnaire should be adapted to cover this issue*" (section 2.8 of the environmental risk assessment report of the BE CA). The BE CA was also of the opinion that the "*current general surveillance plan needs to be adapted to allow identification of unanticipated adverse effects on non-target organisms (see 2.8 and Annex III), and of management regimes that do not have an environmental performance at least as good as current regimes*" (section on monitoring of the environmental risk assessment report of the BE CA).

In conclusion, the EFSA GMO Panel considers that the information available for maize MON 88017 addresses the scientific comments raised by Member States and that maize MON 88017, as described in this application, is as safe as its conventional counterpart and commercial maize varieties with respect to potential adverse effects on human and animal health. The EFSA GMO Panel also concludes that maize MON 88017 is unlikely to have any adverse effect on the environment, except for the possible resistance evolution to the Cry3Bb1 protein in coleopteran target pests; resistance evolution may lead to altered pest control practices that may cause adverse environmental effects. The cultivation management of maize MON 88017 could result in environmental harm. The EFSA GMO Panel therefore recommends managing the use of glyphosate on maize MON 88017 within diversified cropping regimes that have similar or reduced environmental impacts compared with conventional maize cultivation. The EFSA GMO Panel advises the deployment of insect resistance management strategies and case-specific monitoring to address (1) the possible resistance evolution to the Cry3Bb1 protein in coleopteran target pests, (2) changes in botanical diversity within fields due to novel herbicide regimes, and (3) resistance evolution to glyphosate in weeds due to novel herbicide regimes. If subjected to appropriate management measures, the cultivation management of maize MON 88017 is unlikely to raise safety concerns for the environment.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the Czech Republic, dated 21 April 2008, concerning a request for placing on the market of maize MON 88017 in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 8 May 2008, from EFSA to the Competent Authority of the Czech Republic.

3. Letter from EFSA to the applicant, dated 9 June 2008, requesting additional information under completeness check of the application.

4. Letter from the applicant to EFSA, received 7 July 2008, providing the additional information requested by EFSA under completeness check of the application.

5. Letter from EFSA to the applicant, dated 12 September 2008, delivering the 'Statement of Validity' for application EFSA-GMO-CZ-2008-54 (placing on the market of maize MON 88017) submitted by Monsanto under Regulation (EC) No 1829/2003.

6. Letter from EFSA (BE CA) to the applicant, dated 10 December 2008, requesting additional information and stopping the clock.

7. Letter from applicant to EFSA (BE CA), received 23 February 2009, providing the additional information.

8. Letter from EFSA (BE CA) to the applicant, dated 8 April 2009, requesting additional information and maintaining the clock stopped.

9. Letter from the applicant to EFSA (BE CA), received 15 June 2009, providing additional information.

10. Letter from EFSA (BE CA) to the applicant, dated 11 September 2009, requesting additional information and maintaining the clock stopped.

11. Letter from the applicant to EFSA (BE CA), received 17 November 2009, providing additional information.

12. Letter from the applicant to EFSA, received 19 January 2010, providing additional information.

13. Letter from EFSA (BE CA) to the applicant, dated 26 January 2010, requesting additional information and maintaining the clock stopped.

14. Letter from the applicant to EFSA (BE CA), received 12 May 2010, providing additional information.

15. Letter from EFSA (BE CA) to the applicant, dated 13 September 2010, requesting additional information and maintaining the clock stopped.

16. Letter from the applicant to EFSA (BE CA), received 17 September 2010, providing additional information.

17. Letter from EFSA (BE CA) to the applicant, dated 13 October 2010, restarting the clock.

18. Letter from EFSA to applicant, dated 20 October 2010, requesting additional information and stopping the clock.

19. Letter from BE CA to EFSA, received 13 December 2010, requesting clarifications.

20. Letter from EFSA to applicant, dated 20 December 2010, requesting additional information and stopping the clock (corrigendum of the letter of 20 October 2010).

21. Letter from EFSA to BE CA, dated 20 December 2010, providing clarifications.

22. Letter from EFSA to applicant, dated 8 March 2011, requesting additional information and stopping the clock.

23. Letter from applicant to EFSA, received 04 April 2011, providing additional information.

24. Letter from EFSA to the applicant, dated 20 April 2011, requesting additional information and maintaining the clock stopped.

25. Letter from the applicant to EFSA, received 23 May 2011, providing the additional information.

26. Letter from EFSA to the applicant, dated 21 June 2011, restarting the clock.

REFERENCES

ACNFP, 1994. Annual Report 1994, Appendix 4. Advisory Committee on Novel Foods and Processes, London, UK.

ACRE, 2007a. 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

ACRE, 2007b. Minutes of the 116th meeting of ACRE at the University of Essex, Colchester, Thursday 6th December 2007, Point 9 Research paper: toxins in trangene crop byproducts may affect headwater stream ecosystems, ACRE/07/P31, http://www.defra.gov.uk/environment/acre/meetings/07/min071206.htm

Ahmad A, Wilde GE, Zhu KY, 2005. Detectability of coleopteran-specific Cry3Bb1 protein in soil and its effect on nontarget surface and below-ground arthropods. Environmental Entomology 34, 385-394.

Ahmad A, Wilde GE, Whitworth RJ, Zolnerowich G, 2006a. Effect of corn hybrids expressing the coleopteran-specific Cry3Bb1 protein for corn rootworm control on aboveground insect predators. Journal of Economic Entomology 99, 1085-1095.

Ahmad A, Wilde GE, Zhu KY, 2006b. Evaluation of effects of coleopteran-specific Cry3Bb1 protein on earthworms exposed to soil containing corn roots or biomass. Environmental Entomology 35, 976-985.

Al-Deeb MA, Wilde GE, 2003. Effect of *Bt* corn expressing the Cry3Bb1 toxin for corn rootworm control on aboveground nontarget arthropods. Environmental Entomology 32, 1164-1170.

Al-Deeb MA, Wilde GE, 2005. Effect of *Bt* corn expressing the Cry3Bb1 toxin on western corn rootworm (Coleoptera: Chrysomelidae) biology. Journal of the Kansas Entomological Society 78, 142-152.

Al-Deeb MA, Wilde GE, Blair JM, Todd TC, 2003. Effect of *Bt* corn for corn rootworm control on nontarget soil microarthropods and nematodes. Environmental Entomology 32, 859-865.

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. In: Romeis J, Meissle M, Sanvido O (Eds), *GMOs in Integrated Plant Production – Ecological impact of genetically modified organisms*, 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. In: Romeis J (Ed), *GMOs in Integrated Plant Production*, 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).

Alcalde E, Amijee F, Blache G, Bremer C, Fernandez S, Garcia-Alonso M, Holt K, Legris G, Novillo C, Schlotter P, Storer N, Tinland B, 2007. Insect resistance monitoring for Bt maize cultivation in the EU: proposal from the industry IRM working group. Journal of Consumer Protection and Food Safety 2(S1), 47-49.

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.

Alstad DA, Andow DA, 1995. Managing the evolution of insect resistance to transgenic plants. Science 268, 1894-1896.

Alston JM, Hyde J, Marra MC, Mitchell PD, 2002. An *ex ante* analysis of the benefits from the adoption of corn rootworm resistant transgenic corn technology. AgBioForum 5, 71-84.

Álvarez-Alfageme F, Bigler F, Romeis J, 2011. Laboratory toxicity studies demonstrate no adverse effects of Cry1Ab and Cry3Bb1 to larvae of *Adalia bipunctata* (Coleoptera: Coccinellidae): the importance of study design. Transgenic Research 20, 476-479.

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

Andow DA, 2008. The risk of resistance evolution in insects to transgenic insecticidal crops. Collection of Biosafety Reviews 4, 142-199.

Andow DA, Alstad DA, 1998. F_2 screen for rare resistance alleles. Journal of Economic Entomology 91, 572-578.

Andow DA, Lövei GL, Arpaia S, 2006. Ecological risk assessment for *Bt* crops. Nature Biotechnology 24, 749-751.

Andow DA, Lövei GL, Arpaia S, 2009. Cry toxins and proteinase inhibitors in transgenic plants do have non-zero effects on natural enemies in the laboratory: rebuttal to Shelton et al. Environmental Entomology 38, 1528-1532.

Andow DA, Farrell SL, Hu Y, 2010. Planting patterns of in-field refuges observed for Bt maize in Minnesota. Journal of Economic Entomology 103, 1394-1399.

APHIS, 2005. Approval of Monsanto Company request 04-125-01p seeking a determination of non-regulated status for corn rootworm resistant MON 88017, http://www.aphis.usda.gov/brs/aphisdocs2/04_12501p_com.pdf

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, 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.

Astini JP, Fonseca A, Clark C, Lizaso J, Grass L, Westgate M, Arritt R, 2009. Predicting outcrossing in maize hybrid seed production. Agronomy Journal 101, 373-380.

Axelsson EP, Hjältén J, LeRoy CJ, Julkunen-Tiitto R, Wennström A, Pilate G, 2010. Can leaf litter from genetically mofidied trees affect aquatic ecosystems? Ecosystems 13, 1049-1059.

Axelsson EP, Hjältén J, LeRoy CJ, Whitham TG, Julkunen-Tiitto R, Wennström A, 2011. Leaf litter from insect-resistant transgenic trees causes changes in aquatic insect community composition. Journal of Applied Ecology, DOI:10.1111/j.1365-2664.2011.02046.x (in press).

Aylor DE, 2002. Settling speed of corn (*Zea mays*) pollen. Journal of Aerosol Science 33, 1599-1605.

Aylor DE, 2003. Rate of dehydration of corn (*Zea mays* L.) pollen in the air. Journal of Experimental Botany 54, 2307-2312.

Aylor DE, 2004. Survival of maize (*Zea mays*) pollen exposed in the atmosphere. Agricultural and Forest Meteorology 123, 125-133.

Aylor DE, Schultes NP, Shields EJ, 2003. An aerobiological framework for assessing cross-pollination in maize. Agricultural and Forest Meteorology 119, 111-129.

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

Bagley M, Oswald K, French BW, Nielson CN, 2009. Fitness of Bt-resistant Western corn rootworm on Mon863 and isoline corn. IWGO Meeting, Munich, Germany, April 5-8, 2009.

Baker HG, 1974. The evolution of weeds. Annual Review of Ecology Systematics 5, 1-24.

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.

Balog A, Szénási A, Szekeres D, Pálinkás Z, 2011. Analysis of soil dwelling rove beetles (*Coleoptera: Staphylinidae*) in cultivated maize fields containing the Bt toxins, Cry34/35Ab1 and Cry1F x Cry34/35Ab1. Biocontrol Science and Technology 21, 293-297.

Bannert M, Stamp P, 2007. Cross-pollination of maize at long distance. European Journal of Agronomy 27, 44-51.

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.

Bardana EJ, 2008. Occupational asthma. Journal of Allergy and Clinical Immunology 121, S408-S411.

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.

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.

Bastiaans L, Paolini R, Baumann DT, 2008. Focus on ecological weed management: what is hindering adoption? Weed Research 48, 481-491.

Bates SL, Zhao J-Z, Roush RT, Shelton AM, 2005. Insect resistance management in GM crops: past, present and future. Nature Biotechnology 25, 57-62.

Baumgarte S, Tebbe CC, 2005. Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. Molecular Ecology 14, 2539-2551.

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

Beachy RN, Fedoroff NV, Goldberg RB, McHughen A, 2008. The burden of proof: A response to Rosi-Marshall et al. Proceedings of the National Academy of Sciences of the United States of America 105, E9.

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.

BEETLE report, 2009. Long-term effects of genetically modified (GM) crops on health and the environment (including biodiversity): prioritization of potential risks and delimitation of uncertainties. German Federal Office of Consumer Protection and Food Safety, BLaU-Umweltstudien and Genius GmbH, http://www.bvl.bund.de/cln_007/DE/06__Gentechnik/00__doks__downloads/05__Inverkehrbringen/beetle__report__2009__long__term__effects,templateId=raw,property=publicationFile.pdf/beetle_report_2009_long_term_effects.pdf

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.

Bern L, 1990. Postcapture particle size selection by *Daphnia cucullata* (Cladocera). Limnology and Oceanography 35, 923-926.

Bernklau EJ, Hibbard BE, Bjostad LB, 2010. Antixenosis in maize reduces feeding by Western corn rootworm larvae (Coleoptera: Chrysomelidae). Journal of Economic Entomology 103, 2052-2060.

Bhatti M, Duan J, Head G, Jiang C, McKee M, Nickson T, Pilcher C, Pilcher C, 2005a. Field evaluation of the impact of corn rootworm (Coleoptera: Chrysomelidae)-protected *Bt* corn on ground-dwelling invertebrates. Environmental Entomology 34, 1325-1335.

Bhatti M, Duan J, Head G, Jiang C, McKee M, Nickson T, Pilcher C, Pilcher C, 2005b. Field evaluation of the impact of corn rootworm (Coleoptera: Chrysomelidae)-protected *Bt* corn on foliage-dwelling arthropods. Environmental Entomology 34, 1336-1345.

Bitocchi E, Nanni L, Rossi M, Rau D, Bellucci E, Giardini A, Buonamici A, Vendramin GG, Papa R, 2009. Introgression from modern hybrid varieties into landrace populations of maize (*Zea mays* ssp *mays* L.) in central Italy. Molecular Ecology 18, 603-621.

Binning RR, Lefko SA, Millsap AY, Thompson SD, Nowatzki TM, 2010. Estimating Western corn rootworm (Coleoptera: Chrysomelidae) larval susceptibility to event DAS-59122-7 maize. Journal of Applied Entomology 134, 551-561.

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.

Bitzer R, Rice M, Pilcher C, Pilcher C, Lam WKF, 2005. Biodiversity and community structure of epedaphic and euedaphic springtails (Collembola) in transgenic rootworm *Bt* corn. Environmental Entomology 34, 1346-1375.

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.

Bøhn T, Primicerio R, Hessen D, Traavik T, 2008. Reduced fitness of *Daphnia magna* fed a Bt-transgenic maize variety. Archives of Environmental Contamination and Toxicology 55, 584-592.

Bøhn T, Traavik T, Primicerio R, 2010. Demographic responses of *Daphnia magna* fed transgenic Bt-maize. Ecotoxicology 19, 419-430.

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.

Bonte M, De Clercq P, 2008. Developmental and reproductive fitness of *Orius laevigatus* (Hemiptera: Anthocoridae) reared on factitious and artificial diets. Journal of Economic Entomology 101, 1127-1133.

Bonte M, De Clercq, 2011. Influence of predator density, diet and living substrate on developmental fitness of *Orius laevigatus*. Journal of Applied Entomology, 135, 343-350.

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.

Boriani M, Agosti M, Kiss J, Edwards CR, 2006. Sustainable management of the Western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), in infested areas: experiences in Italy, Hungary and the USA. EPPO Bulletin 36, 531-537.

Bourassa S, Cárcamo HA, Larney FJ, Spence JR, 2008. Carabid assemblages (Coleoptera: Carabidae) in a rotation of three different crops in Southern Alberta, Canada: a comparison of sustainable and conventional farming. Environmental Entomology 37, 1214-1223.

Bourassa S, Cárcamo HA, Spence JR, Blackshaw RE, Floate K, 2010. Effects of crop rotation and genetically modified herbicide-tolerant corn on ground beetle diversity, community structure, and activity density. The Canadian Entomologist 142, 143-159.

Bourguet D, Desquilbet M, Lemarié S, 2005. Regulating insect resistance management: the case of non-*Bt* corn refuges in the US. Journal of Environmental Management 76, 210-220.

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

Bravo A, Soberón M, 2008. How to cope with insect resistance to Bt toxins? Trends in Biotechnology 26, 573-579.

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.

Broderick NA, Raffa KF, Handelsman J, 2006. Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. Proceedings of the National Academy of Sciences of the United States of America 103, 15196-15199.

Broderick NA, Robinson CJ, McMahon MD, Holt J, Handelsman J, Raffa KF, 2009. Contributions of gut bacteria to *Bacillus thuringiensis*-induced mortality vary across a range of Lepidoptera. BMC Biology 7, 1-9.

Broekgaarden C, Snoeren TAL, Dicke M, Vosman B, 2011. Exploiting natural variation to identify insect-resistance genes. Plant Biotechnology Journal 9, 819-825.

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.

Büchs W, Schlein O, Prescher S, 2008. Performance of decomposers and predators under the influence of Diabrotica-resistant Bt-maize (Cry3Bb1) and conventional cultivars. Verhandlungen der Gesellschaft für Ökologie 38, 702.

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.

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.

Campbell ID, 1999. Quaternary pollen taphonomy: examples of differential redeposition and differential preservation. Palaeogeography, Palaeoclimatology, Palaeoecology 149, 245-256.

Caprio MA, Tabashnik BE, 1992. Gene flow accelerates local adaptation among finite populations: simulating the evolution of insecticide resistance. Journal of Economic Entomology 85, 611-620.

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.

Carrasco LR, Harwood TD, Toepfer S, MacLeod A, Levay N, Kiss J, Baker RHA, Mumford JD, Knight JD, 2009. Dispersal kernels of the invasive alien Western corn rootworm and the effectiveness of buffer zones in eradication programmes in Europe. Annals of Applied Biology 156, 63-77.

Carrière Y, Tabashnik BE, 2001. Reversing insect adaptation to transgenic insecticidal plants. Proceedings of the Royal Society B: Biological Sciences 268, 1475-1480.

Carstens K, Anderson J, Bachman B, De Schrijver A, Dively G, Federici B, Hamer M, Gielkins M, Jensen P, Lamp W, Rauschen S, Ridley G, Romeis J, Waggoner A. 2011. Genetically modified crops and aquatic ecosystems: considerations for environmental risk assessment and non-target organism testing. Transgenic Research, DOI:10.1007/s11248-011-9569-8 (in press).

Carter ME, Villani MG., Allee LL, Losey JE, 2004. Absence of nontarget effects of two *Bacillus thuringiensis* coleopteran active delta-endotoxin on the bulb mite, *Rhizoglypus robini* (Claparède) (Acari, Acaridae). Journal of Applied Entomology 128, 56-63.

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.

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

CERA, 2011. GM crop database. ILSI Research Foundation, Washington D.C., http://cera-gmc.org/index.php?action=gm_crop_database

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.

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.

Chambers PA, Duggan PS, Heritage J, Forbes JM, 2002. The fate of antibiotic resistance marker genes in transgenic plant feed material fed to chickens. Journal of Antimicrobial Chemotherapy 49, 161-164.

Chambers CP, Whiles MR, Rosi-Marshall EJ, Tank JL, Royer TV, Griffiths NA, Evans-White MA, Stojak AR, 2010. Responses of stream macroinvertebrates to Bt maize leaf detritus. Ecological Applications 20, 1949-1960.

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.

Chassy B, Carter C, McGloughlin M, McHughen A, Parrott W, Preston C, Roush R, Shelton A, Strauss SH, 2003. UK field-scale evaluations answer wrong questions. Nature Biotechnology 21, 1429-1430.

Chege PG, Clark TL, Hibbard BE, 2005. Alternate host phenology affects survivorship, growth and development of Western corn rootworm (Coleoptera: Chrysomelidae) larval biology. Environmental Entomoloy 34, 1441-1447.

Chege PG, Clark TL, Hibbard BE, 2009. Initial larval feeding on an alternate host enhances Western corn rootworm (Coleoptera: Chrysomelidae) beetle emergence on *Cry3Bb1*-expressing maize. Journal of the Kansas Entomological Society 82, 63-75.

Ciosi M, Miller NJ, Kim KS, Giordano R, Estoup A, Guillemaud T, 2008. Invasion of Europe by the Western corn rootworm, *Diabrotica virgifera virgifera*: multiple transatlantic introductions with various reductions of genetic diversity. Molecular Ecology 17, 3614-3627.

Clark TL, Hibbard BE, 2004. Comparison of nonmaize hosts to support Western corn rootworm (Coleoptera: Chrysomelidae) larval biology. Environmental Entomology 33, 681-689.

Clark PL, Vaughn TT, Meinke LJ, Molina-Ochoa J, Foster JE, 2006. *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) larval feeding behavior on transgenic maize (MON 863) and its isoline. Journal of Economic Entomology 99, 722-727.

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.

Coats SA, Tollefson JJ, Mutchmor JA, 1986. Study of migratory flight in the Western corn rootworm. Environmental Entomology 15, 1-6.

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.

Cox WJ, Hahn RR, Stachowski PJ, 2006. Time of weed removal with glyphosate affects corn growth and yield components. Agronomy Journal 98, 349-353.

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.

Crowder DW, Onstad DW, 2005. Using a generational time-step model to simulate the dynamics of adaptation to transgenic corn and crop rotation by Western corn rootworm (Coleoptera: Chrysomelidae). Journal of Economic Entomology 98, 518-533.

Crowder DW, Onstad DW, Gray ME, Pierce CMF, Hagar AG, Ratcliffe ST, Steffey KL, 2005. Analysis of the dynamics of adaptation to transgenic corn and crop rotation by western corn rootworm (Coleoptera: Chrysomelidae) using a daily time-step model. Journal of Economic Entomology 98, 534-551.

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.

Cummins KW, Wilzbach MA, Gates DM, Perry JB, Taliaferro WB, 1989. Shredders and riparian vegetation. BioScience 39, 24-30.

Curry JP, Schmidt O, 2006. The feeding ecology of earthworms - A review. Pedobiologia 50, 463-477.

Czarnak-Klos M, Rodríguez-Cerezo E, 2010. Best practice documents for coexistence of genetically modified crops with conventional and organic farming. 1. Maize crop production. European Coexistence Bureau (ECoB) report, http://ecob.jrc.ec.europa.eu/documents/Maize.pdf

Daly T, Buntin GD, 2005. Effect of *Bacillus thuringiensis* transgenic corn for lepidopteran control on nontarget arthropods. Environmental Entomology 34, 1292-1301.

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, Marquardt PT, Johnson WG, 2008. Volunteer corn in northern Indiana soybean correlates to glyphosate-resistant corn adoption. Crop Management, DOI:CM-2008-0721-2001-BR.

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 Poza M, Pons X, Farinós GP, López C, Ortego F, Eizaguirre M, Castañera P, Albajes R, 2005. Impact of farm-scale Bt maize on abundance of predatory arthropods in Spain. Crop Protection 24, 677-684.

De Schrijver A, Devos Y, Van den Bulcke M, Cadot P, De Loose M, Reheul D, Sneyers M, 2007. Risk assessment of GM stacked events obtained from crosses between GM events. Trends in Food Science and Technology 18, 101-109.

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.

Deen W, Hamill A, Shropshire C, Soltani N, Sikkema PH, 2006. Control of volunteer glyphosate-resistant corn (*Zea mays*) in glyphosate-resistant soybean (*Glycine max*). Weed Technology 20, 261-266.

DEFRA, 2004. Modifying weed management in a broad row crop (maize) for environmental benefit, http://randd.defra.gov.uk/Document.aspx?Document=AR0412_2071_FRP.doc

DEFRA, 2005. Secretary of State Margaret Beckett's statement on GM policy, http://webarchive.nationalarchives.gov.uk/20080306073937/http://www.defra.gov.uk/environment/gm/fse/

Delage S, Brunet Y, Dupont S, Tulet P, Pinty J-P, Lac C, Escobar J, 2007. Atmospheric dispersal of maize pollen over the Aquitaine region. In: Stein AJ, Rodríguez-Cerezo E (Eds), *Book of abstracts of the third International Conference on Coexistence between Genetically Modified (GM) and non-GM-based Agricultural Supply Chains*, European Commission, pp 302-303.

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.

Devare MH, Jones CM, Thies JE, 2004. Effect of Cry3Bb transgenic corn and tefluthrin on the soil microbial community: biomass, activity and diversity. Journal of Environmental Quality 33, 837-843.

Devare MH, Londoño-R LM, Thies JE, 2007. Neither transgenic Bt maize (MON863) nor tefluthrin insecticide affect soil microbial activity or biomass: a 3-year field analysis. Soil Biology & Biochemistry 39, 2038-2047.

Devos Y, Reheul D, De Schrijver A, 2005. The co-existence between transgenic and non-transgenic maize in the European Union: a focus on pollen flow and cross-fertilization. Environmental Biosafety Research 4, 71-87.

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

Didden W, 1993. Ecology of terrestrial enchytraeidae. Pedobiologia 37, 2-29.

Digiovanni F, Kevan PG, Nasr ME, 1995. The variability in settling velocities of some pollen and spores. Grana 34, 39-44.

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.

Dillen K, Mitchell PD, Tollens E, 2010a. On the competitiveness of *Diabrotica virgifera virgifera* damage abatement strategies in Hungary: a bio-economic approach. Journal of Applied Entomology 134, 508-518.

Dillen K, Mitchell PD, Van Looy T, Tollens E, 2010b. The Western corn rootworm, a new threat to European agriculture: opportunities for biotechnology? Pest Management Science 66, 956-966.

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.

Donovan WP, Rupar MJ, Slaney AC, Malvar T, Gawron-Burke MC, Johnson TB, 1992. Characterization of two genes encoding *Bacillus thuringiensis* insecticidal crystal proteins toxic to Coleoptera species. Applied and Environmental Microbiology 58, 3921-3927.

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.

Douville M, Gagné F, Masson L, McKay J, Blaise C, 2005. Tracking the source of *Bacillus thuringiensis* Cry1Ab endotoxin in the environment. Biochemical Systematics and Ecology 33, 219-232.

Douville M, Gagné F, Blaise C, André C, 2007. Occurrence and persistence of *Bacillus thuringiensis* (Bt) and transgenic Bt corn *cry1Ab* gene from an aquatic environment. Ecotoxicology and Environmental Safety 66, 195-203.

Duan JJ, Head G, McKee MJ, Nickson TE, Martin JW, Sayegh FS, 2002. Evaluation of dietary effects of transgenic corn pollen expressing Cry3Bb1 protein on a non-target ladybird beetle, *Coleomegilla maculata*. Entomologia Experimentalis et Applicata 104, 271-280.

Duan JJ, Paradise MS, Lundgren JG, Bookout JT, Jiang C, Wiedenmann RN, 2006. Assessing nontarget impacts of Bt corn resistant to corn rootworms: tier-1 testing with larvae of *Poecilus chalcites* (Coleoptera: Carabidae). Environmental Entomology 35, 135-142.

Duan JJ, Huesing JE, Teixeira D, 2007. Development of tier-I toxicity assays for *Orius insidiosus* (Heteroptera: Anthocoridae) for assessing the risk of plant-incorporated protectants to nontarget heteropterans. Environmental Entomology 36, 982-988.

Duan JJ, Marvier M, Huesing J, Dively G, Huang ZY, 2008a. A meta-analysis of effects of Bt crops on honey bees (Hymenoptera: Apidae). PLoS ONE 3, e1415.

Duan JJ, Teixeira D, Huesing JE, Jiang CJ, 2008b. Assessing the risk to nontarget organisms from Bt corn resistant to corn rootworms (Coleoptera: Chrysomelidae): Tier-I testing with *Orius insidiosus* (Heteroptera : Anthocoridae). Environmental Entomology 37, 838-844.

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.

Dunlap FG, White PJ, Pollak LM, 1995. Fatty acid composition of oil from exotic corn breeding materials. Journal of the American Oil Chemists' Society 72, 989-993.

Dutton A, Klein H, Romeis J, Bigler F, 2002. Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. Ecological Entomology 27, 441-447.

Eastham K, Sweet J, 2002. Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer. European Environment Agency, http://reports.eea.eu.int/environmental_issue_report_2002_28/en/GMOs%20for%20www.pdf

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

EC, 2009. Commission Decision of 30 October 2009 authorising the placing on the market of products containing, consisting of, or produced from genetically modified maize MON 88017 (MON-88Ø17-3) pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council (notified under document number C(2009) 8384). Official Journal of the European Union L289, 25-28, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:289:0025:0028:EN:PDF

EC, 2010. Commission Decision of 28 July 2010 authorising the placing on the market of products containing, consisting of, or produced from genetically modified maize MON 88017 x MON 810 (MON-88Ø17-3 x MON-ØØ81Ø-6) pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council (notified under document C(2010) 5139). Official Journal of the European Union L201, 46-49, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:201:0046:0049:EN:PDF

Eckert J, Schuphan I, Hothorn LA, Gathmann A, 2006. Arthropods on maize ears for detecting impacts of Bt maize on nontarget organisms. Environmental Entomology 35, 554-560.

EcoStrat, 2000. Review on non target organisms and Bt-plants. Hilbeck A, Meier M, Raps A. GmbH, Zurich, Switzerland (for Greenpeace International, Amsterdam), http://www.greenpeace.org/raw/content/international/press/reports/review-on-non-target-organisms.pdf

EFSA, 2003. Opinion of the Scientific Panel on genetically modified organisms [GMO] on a request from the Commission related to the Notification (Reference CE/ES/00/01) for the placing on the market of herbicide-tolerant genetically modified maize NK603, for import and processing, under Part C of Directive 2001/18/EC from Monsanto. The EFSA Journal 10, 1-13, http://www.efsa.europa.eu/en/efsajournal/doc/10.pdf

EFSA, 2004a. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Notification (Reference C/DE/02/9) for the placing on the market of insect-protected genetically modified maize MON 863 and MON 863 x MON 810, for import and processing, under Part C of Directive 2001/18/EC from Monsanto. The EFSA Journal 49, 1-25, http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/opinion_gmo_06_en1,2.pdf

EFSA, 2004b.Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safety of foods and food ingredients derived from insect-protected genetically modified maize MON 863 and MON 863 x MON 810, for which a request for placing on the market was submitted under Article 4 of the Novel Food Regulation (EC) No 258/97 by Monsanto. The EFSA Journal 50, 1-25, http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/opinion_gmo_07_en1.pd f

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, 2007. Opinion of the Scientific Panel on Genetically Modified Organisms on an applications (references EFSA-GMO-UK-2005-19 and EFSA-GMO-RX-GA21) for the placing on the market of glyphosate-tolerant genetically modified maize GA21, for food and feed uses, import and processing and for renewal of the authorisation of maize GA21 as existing product, both under Regulation (EC) No 1829/2003 from Syngenta Seeds S.A.S. on behalf of Syngenta Crop Protection AG. The EFSA Journal 541, 1-25, http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/gmo_op_ej541_GA21Maize_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

EFSA, 2009a. Scientific Opinion of the Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-CZ-2005-27) for the placing on the market of the insect-resistant and herbicide-tolerant genetically modified maize MON88017, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 1075, 1-28, http://www.efsa.europa.eu/en/scdocs/doc/1075.pdf

EFSA, 2009b. 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, 2009c. 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, 2009d. Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMO-RX-MON810) for the renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON810; (2) feed consisting of and/or containing maize MON810, including the use of seed for cultivation; and of (3) food and feed additives, and feed materials produced from maize MON810, all under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 1149, 1-84, http://www.efsa.europa.eu/en/efsajournal/doc/1149.pdf

EFSA, 2009e. Scientific Opinion of the Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-CZ-2006-33) for the placing on the market of the insect-resistant and glyphosate-tolerant genetically modified maize MON 88017 x MON 810, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 1192, 1-27, http://www.efsa.europa.eu/en/scdocs/doc/1192.pdf

EFSA, 2010a. Scientific Opinion on application (EFSAGMO-NL-2007-39) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MON 89034 x MON 88017 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 1564, 1-27, http://www.efsa.europa.eu/en/scdocs/doc/1564.pdf

EFSA, 2010b. Scientific Opinion on application (EFSA-GMO-CZ-2008-62) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events as present in its segregating progeny, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from

Dow AgroSciences and Monsanto. The EFSA Journal 1781, 1-37, http://www.efsa.europa.eu/en/scdocs/doc/1781.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. 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, 2010g. 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, 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

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

EPA, 2010. Biopesticides registration action document: *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector PV-ZMIR13L) in MON 863 corn (OECD Unique Identifier: MON-ØØ863-5) PC code: 006484 and *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector PV-ZMIR39) in MON 88017 corn (OECD Unique Identifier: MON-88Ø17-3) PC code: 006498, http://www.epa.gov/oppbppd1/biopesticides/pips/cry3bb1-brad.pdf

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

El Khishen AA, Bohn MO, Prischmann-Voldseth DA, Dashiell KE, French BW, Hibbard BE, 2009. Native resistance to Western corn rootworm (Coleoptera: Chrysomelidae) larval feeding: characterisation and mechanisms. Journal of Economic Entomology 102, 2350-2359.

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

Fang M, Kremer RJ, Motavalli PP, Davis G, 2005. Bacterial diversity in rhizosphere of nontransgenic and transgenic corn. Applied Environmental Microbiology 71, 4132-4136.

Fang M, Motavalli PP, Kremer RJ, Nelson KA, 2007. Assessing changes in soil microbial communities and carbon mineralieation in Bt and non-Bt corn residue-amended soils. Applied Soil Ecology 37, 150-160.

FCEC, 2009. Analysis of the economic, social and environmental impacts of options for the long-term EU strategy against *Diabrotica virgifera virgifera* (Western Corn Rootworm), a regulated harmful organism of maize, http://ec.europa.eu/food/plant/organisms/emergency/final_report_Diabrotica_study.pdf

Felke, M, 2006. Untersuchungen zu Nebenwirkungen der Diabrotica–resistenten, transgenen Maislinie MON88017 auf Nicht-Ziel-Chrysomeliden. Deutsche Pflanzenschutztagung 55, 362.

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.

Feng PCC, CaJacob CA, Martino-Catt SJ, Cerny RE, Elmore GA, Heck GR, Huang J, Kruger WM, Malven M, Miklos JA, Padgette SR, 2010. Glyphosate-resistant crops: developing the next generation products. In: Nandula VK (Ed), *Glyphosate Resistance in Crops and Weeds: History, Development, and Management*, John Wiley & Sons, Inc., New York, pp 45-65.

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.

Fernie AR, Tadmor Y, Zamir D, 2006. Natural genetic variation improving crop quality. Current Opinion in Plant Biology 9, 196-202.

Filion M, 2008. Do transgenic plants affect rhizobacteria populations? Microbial Biotechnology 1, 463-475.

Fiorito TM, Icoz I, Stotzky G, 2008. Adsorption and binding of the transgenic plant proteins, human serum albumin, beta-glucuronidase, and Cry3Bb1, on montmorillonite and kaolinite: microbial utilization and enzymatic activity of free and clay-bound proteins. Applied Clay Science 39, 142-150.

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

Flores S, Saxena D, Stotzky G, 2005. Transgenic Bt plants decompose less in soil than non-Bt plants. Soil Biology & Biochemistry 37, 1073-1082.

French BW, Bagley M, Nielson CN, Oswald K, 2008. Fitness costs related to selection for resistance to the Cry3Bb1 protein in a genetically diverse population of non-diapausing Western corn rootworm. Entomological Society of America Annual Meeting, Reno, NV. November 16-19, 2008.

Fried G, Norton LR, Reboud X, 2008. Environmental and management factors determining weed species composition and diversity in France. Agriculture, Ecosystems & Environment 128, 68-76.

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.

Galitsky N, Cody V, Wojtczak A, Ghosh D, Luft JR, Pangborn W, English L, 2001. Structure of the insecticidal bacterial delta-endotoxin Cry3Bb1 of *Bacillus thuringiensis*. Acta Crystallographica Section D: Biological Crystallography 57, 1101-1109.

Garcia-Alonso M, 2010. Current challenges in environmental risk assessment: The assessment of unintended effects of GM crops on non-target organisms. In: Romeis J (Ed), *GMOs in Integrated Plant Production*, IOBC wprs Bulletin 52, 57-63.

Garcia-Alonso M, Jacobs E, Raybould A, Nickson TE, Sowig P, Willekens H, Van der Kouwe P, Layton R, Amijee F, Fuentes AM, Tencalla F, 2006. A tiered system for assessing the risk of genetically modified plants to non-target organisms. Environmental Biosafety Research 5, 57-65.

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.

Gassmann AJ, Carrière Y, Tabashnik BE, 2009. Fitness costs of insect resistance to *Bacillus thuringiensis*. Annual Review of Entomology 54, 147-163.

Gassmann AJ, Petzold-Maxwell JL, Keweshan RS, Dunbar MW, 2011. Fied-evolved resistance to Bt maize by Western corn rootworm. PLoS ONE 6, e22629.

Gathmann A, Wirooks L, Hothhorn LA, Bartsch D, Schuphan I, 2006. Impact of Bt-maize pollen (MON810) on lepidopteran larvae living on accompanying weeds. Molecular Ecology 15, 2677-2685.

Gehring K, Mülleder N, 2004. Vergleichende bewertung von Liberty Link und Roundup Ready zur bekämpfung von problemunkräutern in mais. Journal of Plant Diseases and Protection XIX, 855-862.

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.

Gianessi LP, Silvers CS, Sankula S, Carpenter JE, 2002. Plant biotechnology: current and potential impact for improving pest management in US agriculture. An analysis of 40 case studies June 2002. Herbicide tolerant field corn. National Center for Food and Agricultural Policy, Washington, US, http://www.ncfap.org/documents/SweetCornHT.pdf

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.

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.

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.

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.

Gould F, 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annual Review of Entomology 43, 701-726.

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

Gower SA, Loux MM, Cardina J, Harrison SK, 2002. Effect of planting date, residual herbicide, and postemergence application timing on weed control and grain yield in glyphosate-tolerant corn (*Zea mays*). Weed Technology 16, 448-494.

Gower SA, Loux MM, Cardina J, Harrison SK, Sprankle PL, Probst NJ, Bauman TT, Bugg W, Curran WS, Currie RS, Harvey RG, Johnson WG, Kells JJ, Owen MDK, Regehr DL, Slack CH, Spaur M, Sprague CL, VanGessel M, Young BG, 2003. Effect of postemergence glyphosate application timing on weed control and grain yield in glyphosate-resistant corn: results of a 2-yr multistate study. Weed Technology 17, 821-828.

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.

Gray ME, Steffey KL, Estes RE, Schroeder JB, 2007. Responses of transgenic maize hybrids to variant Western corn rootworm larval injury. Journal of Applied Entomology 131, 386-390.

Gray ME, Sappington TW, Miller NJ, Moeser J, Bohn MO, 2009. Adaptation and invasiveness of Western corn rootworm: intensifying research on a worsening pest. Annual Review of Entomology 54, 303-321.

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, Minton BW, 2006. Supplementary weed control using soil-applied herbicides in glyphosate-resistant maize in Texas. Crop Protection 25, 1071-1074.

Griffiths BS, Caul S, Thompson J, Birch ANE, Scrimgeour C, Andersen MN, Cortet J, Messéan A, Sausse C, Lacroix B, Krogh PH, 2005. A comparison of soil microbial community structure, protozoa, and nematodes in field plots of conventional and genetically modified maize expressing the *Bacillus thuringiensis* Cry1Ab toxin. Plant and Soil 275, 135-146.

Griffiths BS, Caul S, Thompson J, Birch ANE, Scrimgeour C, Cortet J, Foggo A, Hackett CA, Krogh PH, 2006. Soil microbial and faunal community responses to Bt-maize and insecticide in two soils. Journal of Environmental Quality 35, 734-741.

Griffiths BS, Caul S, Thompson J, Birch ANE, Cortet J, Andersen MN, Krogh PH, 2007a. Microbial and microfaunal community structure in cropping systems with genetically modified plants. Pedobiologia 51, 195-206.

Griffiths BS, Heckmann LH, Caul S, Thompson J, Scrimgeour C, Krogh PH, 2007b. Varietal effects of eight paired lines of transgenic Bt maize and near-isogenic non-Bt maize on soil microbial and nematode community structure. Plant Biotechnology Journal 5, 60-68.

Griffiths NA, Tank JL, Royer TV, Rosi-Marshall EJ, Whiles MR, Chambers CP, Frauendorf TC, Evans-White MA, 2009. Rapid decomposition of maize detritus in agricultural headwater streams. Ecological Applications 19, 133-142.

Gruber H, Paul V, Meyer HHD, Müller M, 2011a. Determination of insecticidal Cry1Ab protein in soil collected in the final growing seasons of a nine-year field trial of Bt-maize MON810. Transgenic Research, DOI:10.1007/s11248-011-9509-7 (in press).

Gruber H, Paul V, Guertler P, Spiekers H, Tichopad A, Meyer HHD, Müller M, 2011b. Fate of Cry1Ab protein in agricultural systems under slurry management of cows fed genetically modified maize (*Zea mays* L.) MON810: a quantitative assessment. Journal of Agricultural and Food Chemistry 59, 7135-7144.

Gruber S, Colbach N, Barbottin A, Pekrun C, 2008. Post-harvest gene escape and approaches for minimizing it. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 3, 1-17.

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.

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.

Hadden, 1978. Investigation of the registration of poplar pollen in lake sediments. PhD Thesis, University of Toronto, Toronto.

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.

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. strain 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, 2009a. Separating the effect of crop from herbicide on soil microbial communities in glyphosate resistant corn. Pedobiologia 52, 253-262.

Hart MM, Powell JR, Gulden RH, Levy-Booth DJ, Dunfield KE, Pauls KP, Swanton CJ, Klironomos JN, 2009b. Detection of transgenic *cp4 epsps* genes in the soil food web. Agronomy for Sustainable Development 29, 497-501.

Harwood JD, Wallin WG, Obrycki JJ, 2005. Uptake of Bt endotoxins by nontarget herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem. Molecular Ecology 14, 2815-2823.

Haughton AJ, Bohan DA, 2008. The impacts of novel management on ecosystem dynamics; tales from the UK Farm Scale Evaluations of GMHT crops. In: Romeis J, Meissle M, Sanvido O (Eds), *GMOs in Integrated Plant Production – Ecological impact of genetically modified organisms*, 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.

Head G, Brown CR, Groth ME, Duan JJ, 2001. Cry1Ab protein levels in phytophagous insects feeding on transgenic corn: implications for secondary exposure risk assessment. Entomologia Experimentalis et Applicata 99, 37-45.

Healy C, Hammond B, Kirkpatrick J, 2008. Results of a 13-week safety assurance study with rats fed grain from corn rootworm-protected, glyphosate-tolerant MON 88017 corn. Food and Chemical Toxicology 46, 2517-2524.

Heap I, 2011. 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.

Heard MS, Clark SJ, Rothery P, Perry JN, Bohan DA, Brooks DR, Champion GT, Dewar AM, Hawes C, Haughton AJ, May MJ, Scott RJ, Stuart RS, Squire GR, Firbank LG, 2006. Effects of successive seasons of genetically modified herbicide-tolerant maize cropping on weeds and invertebrates. Annals of Applied Biology 149, 249-254.

Heck GR, Armstrong CL, Astwood JD, Behr CF, Bookout JT, Brown SM, Cavato TA, DeBoer DL, Deng MY, George C, Hillyard JR, Hironaka CM, Howe AR, Jakse EH, Ledesma BE, Lee TC, Lirette RP, Mangano ML, Mutz JN, Qi Y, Rodriguez RE, Sidhu SR, Silvanovich A, Stoecker MA, Yingling RA, You J, 2005. Development and characterization of a CP4 EPSPS-based, glyphosate-tolerant corn event. Crop Science 45, 329-339.

Hemerik L, Busstra C, Mols P, 2004. Predicting the temperature-dependent natural 10 population expansion of the Western corn rootworm, *Diabrotica virgifera*. Entomologia Experimentalis et Applicata 111, 59-69.

Hibbard BE, Duran PN, Ellersieck MR, Ellsbury MM, 2003. Post-establishment movement of Western corn rootworm larvae (Coleoptera: Chrysomelidae) in central Missouri corn. Journal of Economic Entomology 96, 599-608.

Hibbard BE, Vaughn TT, Oyediran IO, Clark TL, Ellersieck MR, 2005. Effect of Cry3Bb1-expressing transgenic corn on plant-to-plant movement by Western corn rootworm larvae (Coleoptera: Chrysomelidae). Journal of Economic Entomology 98, 1126-1138.

Hibbard BE, El Khishen AA, Vaughn TT, 2009. Impact of MON863 transgenic roots is equivalent on Western corn rootworm larvae for a wide range of maize phenologies. Journal of Economic Entomology 102, 1607-1613.

Hibbard BE, Meihls LN, Ellersieck MR, Onstad DW, 2010a. Density-dependent and density-independent mortality of the Western corn rootworm: impact on dose calculation of the rootworm-resistant *Bt* corn. Journal of Economic Entomology 103, 77-84.

Hibbard BE, Clark TL, Ellersieck MR, Meihls LN, El Khishen AA, Kaster V, Steiner H-Y, Kurtz R, 2010b. Mortality of Western corn rootworm larvae on MIR604 transgenic maize roots: field survivorship has no significant impact on survivorship of F1 progeny on MIR604. Journal of Economic Entomology 103, 2187-2196.

Hibbard BE, Frank DL, Kurtz R, Boudreau E, Ellersieck MR, Odhiambo JF, 2011. Mortality impact of Bt transgenic maize roots expressing eCry3.1Ab, mCry3A, and eCry3.1Ab plus mCry3A on Western corn rootworm larvae in the field. Journal of Economic Entomology 104, 1584-1591.

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.

Hofmann F, Epp R, Kruse L, Kalchschmied A, Maisch B, Müller E, Kuhn U, Kratz W, Ober S, Radtke J, Schlechtriemen U, Schmidt G, Schröder W, van den Ohe W, Vögel R, Wedl N, Wosniok W, 2010. Monitoring of Bt-Maize pollen exposure in the vicinity of the nature reserve Ruhlsdorfer

Bruch in northeast Germany 2007 to 2008. Umweltwissenschaften und Schadstoff-Forschung 22, 229-251.

Hönemann L, Nentwig W, 2009. Are survival and reproduction of *Enchytraeus albidus* (Annelida: Enchytraeidae) at risk by feeding on *Bt*-maize litter? European Journal of Soil Biology 45, 351-355.

Hönemann L, Nentwig W, 2010. Does feeding on *Bt*-maize affect the slug *Arion vulgaris* (Mollusca: Arionidae)? Biocontrol Science and Technology 20, 13-18.

Hönemann L, Zurbrügg C, Nentwig W, 2008. Effects of *Bt*-corn decomposition on the composition of the soil meso- and macrofauna. Applied Soil Ecology, 40, 203-209.

Höss S, Nguyen-Thu H, Menzel R, Pagel-Wieder S, Miethling-Graf R, Tebbe CC, Jehle J, Traunspurger W, 2011. Assessing the risk posed to free-living soil nematodes by genetically modified maize expressing the insecticidal Cry3Bb1 protein. Science of the Total Environment 409, 2674-2684.

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.

Huang F, Andow AA, Buschman LL, 2011. Success of the high-dose/refuge resistance management strategy after 15 years of Bt crop use in North America. Entomologia Experimentalis et Applicata 140, 1-16.

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.

Hummel HE, 2003. Introduction of *Diabrotica virgifera virgifera* into the Old World and its consequences: a recently acquired invasive alien pest species on *Zea mays* from North America. Communications in Agricultural and Applied Biological Sciences 68, 45-57.

Hüsken A, Ammann K, Messeguer J, Papa R, Robson P, Schiemann J, Squire G, Stamp P, Sweet J, Wilhelm R, 2007. A major European synthesis of data on pollen and seed mediated gene flow in maize in the SIGMEA project. In: Stein A, Rodríguez-Cerezo E (Eds), *Books of abstracts of the third International Conference on Coexistence between Genetically Modified (GM) and non-GM-based Agricultural Supply Chains*, European Commission, pp 53-56.

Icoz I, Stotzky G, 2007. Cry3Bb1 protein from *Bacillus thuringiensis* in root exudates and biomass of transgenic corn does not persist in soil. Transgenic Research 17, 609-620.

Icoz I, Stotzky G, 2008. Fate and effects of insect-resistant Bt crops in soil ecosystems. Soil Biology & Biochemistry 40, 559-586.

Icoz I, Saxena D, Andow D, Zwahlen C, Stotzky G, 2008. Microbial populations and enzyme activities in soil *in situ* under transgenic corn expressing Cry proteins from *Bacillus thuringiensis*. Journal of Environmental Quality 37, 647-662.

Isenhour DJ, Yeargan KV, 1981. Effect of temperature on the development of *Orius insidiosus*, with notes on laboratory rearing. Annals of the Entomological Society of America 74, 114-116.

Ives AR, Andow DA, 2002. Evolution of resistance to *Bt* crops: directional selection in structured environments. Ecology Letters 5, 792-801.

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.

Jarosz N, Loubet B, Durand B, Foueillassar X, Huber L, 2005. Variations in maize pollen emission and deposition in relation to microclimate. Environmental Science & Technology 39, 4377-4384.

Jan MT, Roberts P, Tonheim SK, Jones DL, 2009. Protein breakdown represents the major bottleneck in nitrogen cycling in grassland soils. Soil Biology & Biochemestry 41, 2272-2282.

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.

Jeebhay MF, Quirce S, 2007. Occupational asthma in the developing and industrialised world: a review. International Journal of Tuberculosis and Lung Disease 11, 122-133.

Jensen PD, Dively GP, Swan CM, Lamp WO, 2010. Exposure and nontarget effects of transgenic *Bt* corn debris in streams. Environmental Entomology 39, 707-714.

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

Johnson WG, Bradley PR, Hart SE, Buesinger ML, Massey RE, 2000. Efficacy and economics of weed management in glyphosate-resistant corn (*Zea mays*). Weed Technology 14, 57-65.

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.

Kaiser-Alexnat R, 2009. Protease activities in the midgut of Western corn rootworm (*Diabrotica virgifera virgifera* LeConte). Journal of Invertebrate Pathology 100, 169-174.

Kang J, Krupke CH, 2009a. Likelihood of multiple mating in *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Journal of Economic Entomology 102, 2096-2100.

Kang J, Krupke CH, 2009b. Influence of weight of male and female Western corn rootworm (Coleoptera: Chrysomelidae) on mating behaviours. Annals of the Entomological Society of America 102, 326-332.

Kawashima S, Nozaki H, Hamazaki T, Sakata S, Hama T, Matsuo K, Nagasawa A, 2011. Environmental effects on long-range outcrossing rates in maize. Agriculture, Ecosystems & Environment 142, 410-418.

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.

Kim C-G, 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.

Kim KS, Sappington TW, 2005. Genetic structuring of Western corn rootworm (Coleoptera: Chrysomelidae) populations in the United States based on microsatellite loci analysis. Environmental Entomology 34, 494-503.

Kiman ZB, Yeargan KV, 1985. Development and reproduction of the predator *Orius insidiosus* (Hemiptera: Anthocoridae) reared on diets of selected plant material and arthropod prey. Annals of the Entomological Society of America 78, 464-467.

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.

Kiss J, Szentkirályi F, Tóth F, Szénási A, Kádár F, Arpás K, Szekeres D, Edwards CR, 2002. Bt corn: impact on non-targets and adjusting to local IPM systems. In: Lelley T, Balázs E, Tepfer M (Eds), *Ecological impact of GMO dissemination in agro-ecosystems*, Facultas, Wien, pp 157-172.

Kiss J, Edwards CR, Berger HK, Cata P, Cean M, Cheek S, Derron J, Festic H, Furlan L, Igrc-Barcic J, Ivanova I, Lammers W, Omelyuta V, Princzinger G, Reynaud P, Sivcec I, Sivicek P, Urek G, Vahala O, 2005. Monitoring of Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) in Europe 1992-2003. In: Vidal S, Kuhlmann U, Edwards CR (Eds), *Western corn rootworm: Ecology and Management*, CABI Publishing, Wallingford, pp 29-39.

Kiss J, Szentkirályi F, Szénási A, Tóth F, Szekeres D, Kádár F, Árpás K, Edwards CR, undated. Effect of transgenic Bt-corn on biodiversity of non-target insects in corn fields. Report on Contract Number QLK3-CT-2000-00547 (project "Bt-BioNoTa") within the 5th EU framework programme. Report was provided by József Kiss.

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.

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

Knecht S, Nentwig W, 2010. Effect of *Bt* maize on the reproduction and development of saprophagous Diptera over multiple generations. Basic and Applied Ecology 11, 346-353.

Knecht S, Romeis J, Malone LA, Candolfi MP, Garcia-Alonso M, Habustova O, Huesing JE, Kiss J, Nentwig W, Pons X, Rauschen S, Szénási A, Bigler F, 2010. A faunistic database as a tool for identification and selection of potential non-target arthropod species for regulatory risk assessment of GM maize. In: Romeis J (Ed), *GMOs in Integrated Plant Production*, IOBC wprs Bulletin 52, 65-69.

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

Kremer RJ, Means NE, Kim S-J, 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.

Kruger M, Van Rensburg JBJ, Van den Berg J, 2009. Perspective on the development of stem borer resistance to Bt maize and refuge compliance at the Vaalharts irrigation scheme in South Africa. Crop Protection 28, 684-689.

Kruger M, Van Rensburg JBJ, Van den Berg J, 2011a. Transgenic Bt maize: farmers' perceptions, refuge compliance and reports of stem borer resistance in South Africa. Journal of Applied Entomology, DOI:10.1111/j.1439-0418.2011.01616.x (in press).

Kruger M, Van Rensburg JBJ, Van den Berg J, 2011b. Resistance to Bt maize in *Busseola fusca* (Lepidoptera: Noctuidae) from Vaalharts. South Africa. Environmental Entomology 40, 477-483.

Krupke C, Marquardt P, Johnson W, Weller S, Conley SP, 2009. Volunteer corn presents new challenges for insect resistance management. Agronomy Journal 101, 797-799.

Lamp W, 2010. Risk assessment of trangenic con debris on non-target arthropods in agricultural stream. Information Systems for Biotechnology July, 5-8.

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.

Langhof M, Rühl G, 2008. Auskreuzungsstudien bei Mais: Überblick, Bewertung. Forschungsbedarf. Berichte über Landwirtschaft 86, 29-67.

Langhof M, Hommel B, Hüsken A, Njontie C, Schiemann J, Wehling P, Wilhelm R, Rühl G, 2010. Coexistence in maize: isolation distance in dependence on conventional maize field depth and separate edge harvest. Crop Science 50, 1496-1508.

Lawhorn CN, Neher DA, Dively GP, 2009. Impact of coleopteran targeting toxin (Cry3Bb1) of *Bt* corn on microbially mediated decomposition. Applied Soil Ecology 41, 364-368.

Lee B, Kim C-G, Park J-Y, Park KW, Kim H-J, Yi H, Jeong C-C, 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.

Lefko SA, Nowatzki TM, Thompson SD, Binning RR, Pascual MA, Peters ML, Simbro EJ, Stanley BH, 2008. Characterizing laboratory colonies of Western corn rootworm (Coleoptera: Chrysomelidae) selected for survival on maize containing event DAS-59122-7. Journal of Applied Entomology 132, 189-204.

Lehman RM, Osborne SL, Rosentrater KA, 2008a. No differences in decomposition rates observed between *Bacillus thuringiensis* and non-*Bacillus thuringiensis* corn residue incubated soil in the field. Agronomy Journal 100, 163-168.

Lehman RM, Osborne SL, Rosentrater KA, 2008b. No evidence that *Bacillus thuringiensis* genes and their products influence the susceptibility of corn residue to decomposition. Agronomy Journal 100, 1687-1693.

Lehman RM, Osborne SL, Prischmann-Voldseth DA, Rosentrater KA, 2010. Insect-damaged corn stalks decompose at rates similar to Bt-protected, non-damaged corn stalks. Plant Soil 333, 481-490.

Lehoczky E, Reisnger P, Nagy S, Komoves T, 2004. Early competition between maize and weeds. Journal of Plant Diseases and Protection XIX, 319-324.

Leopold J, Felke M, 2008. Auswirkungen von Diabrotica-resistentem B.t.-Mais auf Nicht-Ziel-Chrysomeliden. Deutsche Pflanzenschutztagung 56, 452.

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.

Levine E, Oloumi-Sadeghi H, 1991. Management of diabroticite rootworms in corn. Annual Review of Entomology 36, 229-255.

Levine E, Oloumi-Sadeghi H, 1996. Western corn rootworm (Coleoptera: Chrysomelidae) larval injury to corn grown for seed production following soybeans grown for seed production. Journal of Economic Entomology 89, 1010-1016.

Levine E, Spencer JL, Isard SA, Onstad DW, Gray ME, 2002. Adaptation of the Western corn rootworm to crop rotation: evolution of a new strain in response to a management practice. American Entomologist 48, 94-107.

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.

Li Y, Romeis J, 2010. *Bt* maize expressing Cry3Bb1 does not harm the spider mite, *Tetranychus urticae*, or its ladybird beetle predator, *Stethorus punctillum*. Biological Control 52, 337-344.

Li Y, Meissle M, Romeis J, 2008. Consumption of *Bt* maize pollen expressing Cry1Ab or Cry3Bb1 does not harm adult green lacewings, *Chrysoperla carnea* (Neuroptera: Chrysopidae). PLoS ONE 3, e2909.

Li Y, Meissle M, Romeis J, 2010. Use of maize pollen by adult *Chrysoperla carnea* (Neuroptera: Chrysopidae) and fate of Cry proteins in Bt-transgenic varieties. Journal of Insect Physiology 56, 157-164.

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

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.

Lilley AK, Bailey MJ, Cartwright C, Turner SL, Hirsch PR, 2006. Life in earth: the impact of GM plants on soil ecology? Trends in Biotechnology 24, 9-14.

Liu YB, Tabashnik BE, 1997. Inheritance of resistance to *Bacillus thuringiensis* toxin Cry1C in the diamondback moth. Applied Environmental Entomology 63, 2218-2223.

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.

Lövei GL, Andow DA, Arpaia S, 2009. Transgenic insecticidal crop and natural enemies: a detailed review of laboratory studies. Environmental Entomology 38, 293-306.

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

Lundgren JG, Wiedenmann RN, 2002. Coleopteran-specific Cry3Bb toxin from transgenic corn pollen does not affect the fitness of a nontarget species, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae). Environmental Entomology 31, 1213-1218.

Lundgren JG, Wiedenmann RN, 2005. Tritrophic interactions among Bt (Cry3Bb1) corn, aphid prey, and the predator *Coleomegilla maculate* (Coleoptera: Coccinellidae). Environmental Entomology 34, 1621-1625.

Lundgren JG, Razzak AA, Wiedenmann RN, 2004. Population responses and food consumption by predators *Coleomegilla maculata* and *Harmonia axyridis* (Coleoptera: Coccinellidae) during anthesis in an Illinois cornfield. Environmental Entomology 33, 958-963.

Lundgren JG, Huber A, Wiedenmann RN, 2005. Quantification of consumption of corn pollen by the predator *Coleomegilia maculata* (Coleoptera: Coccinellidae) during anthesis in an Illinois cornfield. Agricultural and Forest Entomology 7, 53-60.

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, Meloche F, Wei L, 2009. Agronomic assessment of Bt trait and seed or soil-applied insecticides on the control of corn rootworm and yield. Field Crops Research 111, 189-196.

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.

MacIntosh SC, 2010. Managing the risk of insect resistance to transgenic insect control traits: practical approaches in local environments. Pest Management Science 66, 100-106.

Madliger M, Sander M, Schwarzenbach RP, 2010. Adsorption of transgenic insecticidal Cry1Ab protein to SiO_2. 2. Patch-controlled electrostatic attraction. Environmental Science & Technology 44, 8877-8883.

Madliger M, Gasser CA, Schwarzenbach RP, Sander M, 2011. Adsorption of transgenic insecticidal Cry1Ab protein to silica particles. Effects on transport and bioactivity. Environmental Science & Technology 45, 4377-4384.

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

Mallory-Smith C, Zapiola M, 2008. Gene flow from glyphosate-resistant crops. Pest Management Science 64, 428-440.

Malone LA, Burgess EPJ, 2009. Impact of genetically modified crops on pollinators. In: Ferry N, Gatehouse AMR (Eds), *Environmental Impact of Genetically Modified Crops*, CAB International, pp 199-222.

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.

Marquardt PT, Krupke CH, 2009. Dispersal and mating behaviour of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) in *Bt* cornfields. Environmental Entomology 38, 176-182.

Marvier M, McCreedy C, Regetz J, Kareiva P, 2007. A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. Science 316, 1475-1477.

Masclaux H, Bec A, Kagami M, Perga ME, Sime-Ngando T, Desvilettes C, Bourdier G, 2011. Food quality of anemophilous plant pollen for zooplankton. Limnology and Oceanography 56, 939-946.

Matson PA, Vitousek PM, 2006. Agricultural intensification: will land spared from farming be land spared for nature? Conservation Biology 20, 709-710.

Matten SR, Head GP, Quemada HD, 2008. How governmental regulation can help or hinder the integration of Bt crops into IPM programs. In: Romeis J, Shelton AM, Kennedy GG (Eds), *Integration of Insect-Resistant Genetically Modified Crops within IPM Programs*, Springer Science + Business Media BV, pp 27-39.

Mattila H, Sears MK, Duan JJ, 2005. Response of *Danaus plexippus* to pollen of two new Bt corn events via laboratory bioassay. Entomologia Experimentalis et Applicata 116, 31-41.

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.

McCann MC, Trujillo WA, Riordan SG, Sorbet R, Bogdanova NN, Sidhu RS, 2007. Comparison of the forage and grain composition from insect-protected and glyphosate-tolerant MON88017 corn to conventional corn (*Zea mays* L.). Journal of Agricultural and Food Chemistry 55, 4034-4042.

McManus BL, Fuller BW, Boetel MA, French BW, Ellsbury MM, Head GP, 2005. Abundance of *Coleomegilla maculata* (Coleoptera: coccinellidae) in corn-rootworm-resistant Cry3Bb1 maize. Journal of Economic Entomology 98, 1992-1998.

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.

Meihls LN, Higdon ML, Siegfried BD, Miller NJ, Sappington TW, Ellersieck MR, Spencer TA, Hibbard BE, 2008. Increased survival of Western corn rootworm on transgenic corn within three

generations of on-plants greenhouse selection. Proceedings of the National Academy of Sciences of the United States of America 105, 19177-19182.

Meihls LN, Higdon ML, Ellersieck M, Hibbard BE, 2011. Selection for resistance to mCry3A-expressing transgenic corn in Western corn rootworm. Journal of Economic Entomology 104, 1045-1054.

Meinke LJ, Sappington TW, Onstad DW, Guillemaud T, Miller NJ, Komáromi J, Levay N, Furlan L, Kiss J, Toth F, 2009. Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) population dynamics. Agricultural and Forest Entomology 11, 29-46.

Meissle M, Romeis J 2008. Compatibility of biological control with *Bt* maize expressing Cry3Bb1 in controlling corn rootworms. In: Mason PG Gillespie DR, Vincent C (Eds), *Proceedings of the Third Symposium on Biological Control of Arthropods*, Christchurch, New Zealand, pp 146-160.

Meissle M, Romeis J, 2009a. Insecticidal activity of Cry3Bb1 expressed in *Bt* maize on larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Entomologia Experimentalis et Applicata 131, 308-319.

Meissle M, Romeis J, 2009b. The web-building spider *Theridion impressum* (Araneae: Theridiidae) is not adversely affected by *Bt* maize resistant to corn rootworms. Plant Biotechnology Journal 7, 645-656.

Meissle M, Pilz C, Romeis J, 2009. Susceptibility of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) to the entomopathogenic fungus *Metarhizium anisopliae* when feeding on *Bacillus thuringiensis* Cry3Bb1-expressing maize. Applied and Environmental Microbiology 75, 3937-3943.

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.

Meissle M, Hellmich RL, Romeis J, 2011a. Impact of Cry3Bb1-expressing *Bt* maize on adults of the Western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Pest Management Science 67, 807-814.

Meissle M, Romeis J, Bigler F, 2011b. Bt maize and integrated pest management – A European perspective. Pest Management Science 67, 1049-1058.

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.

Miethling-Graff R, Dockhorn S, Tebbe CC, 2010. Release of the recombinant Cry3Bb1 protein of Bt maize MON88017 into field soil and detection of effects on the diversity of rhizosphere bacteria. European Journal of Soil Biology 46, 41-48.

Miller N, Estoup A, Toepfer S, Bourguet D, Lapchin L, Derridj S, Kim KS, Reynaud P, Furlan L, Guillemaud T, 2005. Multiple transatlantic introductions of the Western corn rootworm. Science 310, 992.

Miller NJ, Guillemaud T, Giordano R, Siegfried BD, Gray ME, Meinke LJ, Sappington TW, 2009. Genes, gene flow and adaptation of *Diabrotica virgifera virgifera*. Agricultural and Forest Entomology 11, 47-60.

Mitchell PD, Gray ME, Steffey KL, 2004. A composed-error model for estimating pest-damage functions and the impact of the Western corn rootworm soybean variant in Illinois. American Journal of Agricultural Economics 86, 332-344.

Moar W, Roush R, Shelton A, Ferré J, MacIntosh S, Leonard BR, Abel C, 2008. Field-evolved resistance to *Bt* toxins. Nature Biotechnology 26, 1072-1074.

Moeser J, Vidal S, 2005. Nutritional resources used by the invasive maize pest *Diabrotica virgifera virgifera* in its new South-east-European distribution range. Entomologia Experimentalis et Applicata 114, 55-63.

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. In: Rossing WAH, Eggenschwiler L, Poehling HM (Eds), *Landscape Management for Functional Biodiversity*, IOBC wprs Bulletin 29, 77-80.

Mooney HA, Cropper A, Reid WV, 2005. Confronting the human dilemma. Nature 434, 561-562.

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, 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.

Mullin CA, Saunders MC, Leslie TW, Biddinger DJ, Fleischer SJ, 2005. Toxic and behavioural effects to Carabidae of seed treatments used on Cry3Bb1- and Cry1Ab/c-protected corn. Environmental Entomology 34, 1626-1636.

Murphy AF, Ginzel MD, Krupke CH, 2010. Effect of varying refuge structure upon root damage, emergence and sex ratios of western corn rootworm on transgenic corn. Journal of Economic Entomology 103, 147-157.

Murphy AF, Seiter NJ, Krupke CH, 2011. The impact of Bt maize as a natal host on adult head capsule width in field populations of western corn rootworm. Entomologia Experimentalis et Applicata 139, 8-16.

Myers MW, Curran WS, VanGessel MJ, Majek BA, Scott BA, Mortensen DA, Calvin DD, Karsten HD, Roth GW, 2005. The effects of weed density and application timing on weed control and corn grain yield. Weed Technology 19, 102-107.

Naranjo SE, 1990. Comparative flight behavior of *Diabrotica virgifera virgifera* and *Diabrotica barberi* in the laboratory. Entomologia Experimentalis et Applicata 55, 79-90.

Naranjo SE, 1991. Movement of corn rootworm beetles, *Diabrotica* spp. (Coleoptera: Chrysomelidae), at cornfield boundaries in relation to sex, reproductive status and crop phenology. Environmental Entomology 20, 230-240.

Naranjo SE, 1994. Flight orientation of *Diabrotica virgifera virgifera* and *D. barberi* (Coleoptera: Chrysomelidae) at habitat interfaces. Annals of the Entomological Society of America 87, 383-394.

Naranjo SE, 2009. Impacts of *Bt* crops on non-target invertebrates and insecticide use patterns. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 4, 1-23.

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 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.

Nguyen HT, Jehle JA, 2009. Expression of Cry3Bb1 in transgenic corn MON88017. Journal of Agricultural and Food Chemistry 57, 9990-9996.

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, 2011. 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, DOI:10.1016/j.scitotenv.2011.05.057 (in press).

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.

Nowatzki TJ, Bradley N, Warren KK, Putnam S, Meinke LJ, Gosselin DC, Harvey FE, Hunt TE, Siegfried B, 2003. In-field labeling of Western corn rootworm adults (Coleoptera: Chrysomelidae) with rubidium. Journal of Economic Entomology 96, 1750-1759.

Nowatzki TM, Zhou X, Meinke LJ, Vaughn T, Siegfried BD, 2006. Effect of *Bacillus thuringiensis* Cry3Bb1 protein on the feeding behaviour and longevity of adult western corn rootworms (Coleoptera: Chrysomelidae). Journal of Economic Entomology 99, 927-930.

Nowatzki TM, Lefko SA, Binning RR, Thompson SD, Spencer TA, Siegfried BD, 2008. Validation of a novel resistance monitoring technique for corn rootworm (Coleoptera: Chrysomelidae) and event DAS-59122-7 maize. Journal of Applied Entomology 132, 177-188.

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 RE, Swanton CL, Tardif F, Sikkema PH, 2006. Weed control and yield are improved when glyphosate is preceded by a residual herbicide in glyphosate-tolerant maize (*Zea mays*). Crop Protection 25, 1174-1179.

Oberhauser KS, Prysby M, Mattila HR Stanley-Horn DE, Sears MK, Dively GP, Olson E, Pleasants JM, Lam WKF, Hellmich RL, 2001. Temporal and spatial overlap between monarch larvae and corn pollen. Proceedings of the National Academy of Sciences of the United States of America 98, 11913-11918.

Obrist LB, Klein H, Dutton A, Bigler F, 2005. Effects of Bt maize on *Frankliniella tenuicornis* and exposure of thrips predators to prey-mediated Bt toxin. Entomologia Experimentalis et Applicata 115, 409-416.

Obrist L, Dutton A, Romeis J, Bigler F, 2006a. Fate of Cry1Ab toxin expressed by Bt maize upon ingestion by herbivorous arthropods and consequences for *Chrysoperla carnea*. BioControl 51, 31-48.

Obrist LB, Dutton A, Albajes R, Bigler F, 2006b. Exposure of arthropod predators to Cry1Ab toxin in Bt maize fields. Ecological Entomology 31, 143-154.

Obrist LB, Klein H, Dutton A, Bigler F, 2006c. Assessing the effects of Bt maize on the predatory mite *Neoseiulus cucumeris*. Experimental and Applied Acarology 38, 125-139.

OECD, 2002. Consensus Document on compositional considerations for new varieties of maize (*Zea mays*): key food and feed nutrients, anti-nutrients and secondary plant metabolites. Series on the Safety of Novel Food and Feeds, No. 6, 1-42, http://www.oecd.org/dataoecd/15/63/46815196.pdf

OECD, 2003. Consensus Document on the biology of *Zea mays* subsp. *Mays* (maize). Series on Harmonisation of Regulatory Oversight in Biotechnology (ENV/JM/MONO(2003)11), No. 27: 1-49, http://www.olis.oecd.org/olis/2003doc.nsf/LinkTo/NT0000426E/$FILE/JT00147699.PDF

OECD, 2007. Consensus Document on the safety information on transgenic plants expressing *Bacillus thuringiensis*-derived insect control proteins/ Series on Harmonisation of Regulatory Oversight in Biotechnology (ENV/JM/MONO(2007)14), No. 42: 1-10, http://www.oecd.org/dataoecd/36/61/46815888.pdf

Oerke EC, 2006. Crop losses to pests. The Journal of Agricultural Science 144, 31-43.

Onstad DW, 2006. Modeling larval survival and movement to evaluate seed mixtures of transgenic corn for control of western corn rootworm (Coleoptera: Chrysomelidae). Journal of Economic Entomology 99, 1407-1414.

Onstad DW, 2008. Insect resistance management: biology, economics, and prediction. Academic Press, London, United Kingdom.

Onstad DW, Meinke LJ, 2010. Modeling evolution of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) to transgenic corn with two insecticidal traits. Journal of Economic Entomology 103, 849-860.

Onstad DW, Joselyn M, Isard S, Levine E, Spencer J, Bledsoe L, Edwards C, Di Fonzo C, Wilson H, 1999. Modeling the spread of western corn rootworm (Coleoptera: Chrysomelidae) populations adapting to soybean-corn rotation. Environmental Entomology 28, 188-194.

Onstad DW, Guse CAG, Spencer JL, Levine E, Gray ME, 2001a. Modeling the dynamics of adaptation to transgenic corn by Western corn rootworm (Coleoptera: Chrysomelidae). Journal of Economic Entomology 94, 529-540.

Onstad DW, Spencer JL, Guse CA, Levine E, Isard SA, 2001b. Modeling evolution of behavioral resistance by an insect to crop rotation. Entomologia Experimentalis et Applicata 100, 195-201.

Onstad DW, Mitchell PD, Hurley TM, Lundgren JG, Porter RP, Krupke CH, Spencer JL, Difonzo CD, Baute TS, Hellmich RL, Buschman LL, Hutchison WD, Tooker JF, 2011. Seeds of change: corn

seed mixtures for resistance management and integrated pest management. Journal of Economic Entomology 104, 343-352.

Oswald KJ, French BW, Nielson C, Bagley M, 2011. Selection for Cry3Bb1 resistance in a genetically diverse population of nondiapausing Western corn rootworm (Coeloptera: Chrysomelidae). Journal of Economic Entomology 104, 1038-1044.

Owen MDK, 2000. Current use of transgenic herbicide-resistant soybean and corn in the USA. Crop Protection 19, 765-771.

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.

Oyediran IO, Hibbard BE, Clark TL, 2004. Prairie grasses as alternate hosts of the Western corn rootworm (Coleoptera: Chrysomelidae). Environmental Entomology 33, 740-747.

Oyediran IO, Hibbard BE, Clark TL, 2005. Western corn rootworm (Coleoptera: Chrysomelidae) beetle emergence from weedy *Cry3Bb1* rootworm-resistant transgenic maize. Journal of Economic Entomology 98, 1679-1684.

Pagel-Wieder S, Hunfeld H, Gessler F, Niemeyer J, Bohnel H, 2006. Adsorption of Cry1Ab and Cry3Bb1 on different soil clay fractions. Mitteilungen-Biologischen Bundesanstalt Fur Land und Forstwirtschaft 400, 359.

Palaudelmàs M, Peñas G, Melé E, Serra J, Salvia J, Pla M, Nadal A, Messeguer J, 2009. Effect of volunteers on mparaize gene flow. Transgenic Research 18, 583-594.

Pan Z, Onstad DW, Nowatzki TM, Stanley BH, Meinke LJ, Flexner JL, 2011. Western corn rootworm (Coleoptera: Chrysomelidae) dispersal and adaptation to single-toxin transgenic corn deployed with block or blended refuge. Environmental Entomology 40, 964-978.

Park KW, Lee B, Kim C-G, Kim DY, Park J-Y, Ko EM, Jeong S-C, Choi KH, Yoon WK, Kim HM, 2010. Monitoring the occurrence of genetically modified maize at a grain receiving port and along transportation routes in the Republic of Korea. Food Control 21, 456-461.

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.

Parrott W, 2008. Study of Bt impact on caddisflies overstates its conclusions: Response to Rosi-Marshall et al. Proceedings of the National Academy of Sciences of the United States of America 105, E10.

Perry JN, 2011. The effect of Bt-maize on butterflies – reckoning the risk. Outlooks on Pest Management 22, 199-205.

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.

Perry JN, Firbank LG, Champion GT, Clark SJ, Heard MS, May MJ, Hawes C, Squire GR, Rothery P, Woiwod IP, Pidgeon JD, 2004. Ban on triazine herbicides likely to reduce but not negate relative benefits of GMHT maize cropping. Nature 428, 313-316.

Perry JN, Devos Y, Arpaia S, Bartsch D, Gathmann A, Hails RS, Kiss J, Lheureux K, Manachini B, Mestdagh S, Neemann G, Ortego F, Schiemann J, Sweet JB, 2010. A mathematical model of exposure of non-target Lepidoptera to Bt-maize pollen expressing Cry1Ab within Europe. Proceedings of the Royal Society B: Biological Sciences 277, 1417-1425.

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.

Pilcher CD, Obrycki JJ, Rice ME, Lewis LC, 1997. Preimaginal development, survival, and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn. Environmental Entomology 26, 446-454.

Pleasants JM, Hellmich RL, Dively GP, Sears MK, Stanley-Horn DE, Mattila HR, Foster JE, Clark TL, Jones GD, 2001. Corn pollen deposition on milkweeds in or near corn field. Proceedings of the National Academy of Sciences of the United States of America 98, 11919-11924.

Pline-Srnic W, 2005. Technical performance of some commercial glyphosate-resistant crops. Pest Management Science 61, 225-234.

Poerschmann J, Gathmann A, Augustin J, Langer U, Gorecki T, 2005. Molecular composition of leaves and stems of genetically modified Bt and near-isogenic non-Bt maize–characterization of lignin patterns. Journal of Environmental Quality 34, 1508-1518.

Poerschmann J, Rauschen S, Langer U, Augustin J, Gorecki T, 2008. Molecular level lignin patterns of genetically modified *Bt*-maize MON88017 and three conventional varieties using tetramethylammonium (TMAH)-induced thermochemolysis. Journal of Agricultural and Food Chemistry 56, 11906-11913.

Poerschmann J, Rauschen S, Langer U, Augustin J, Gorecki T, 2009. Fatty acid patterns of genetically modified Cry3Bb1 expressing Bt-maize MON88017 and its near-isogenic line. Journal of Agricultural and Food Chemistry 57, 127-132.

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.

Porcar M, García-Robles I, Domínguez-Escribà L, Latorre A, 2010. Effects of *Bacillus thuringiensis* Cry1Ab and Cry3Aa endotoxins on predatory Coleoptera tested through artificial diet-incorporation bioassays. Bulletin of Entomological Research 100, 297-302.

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.

Priesnitz KU, 2010. Potential impact of *Diabrotica* resistant Bt-maize expressing Cry3Bb1 on ground beetles (Coleoptera: Carabidae). PhD Thesis, RWTH Aachen.

Prihoda KR, Coats JR, 2008a. Aquatic fate and effects of *Bacillus thuringiensis* Cry3Bb1 protein: towards risk assessment. Environmental Toxicology and Chemistry 27, 793-798.

Prihoda KR, Coats JR, 2008b. Fate of *Bacillus thuringiensis* (Bt) Cry3Bb1 protein in a soil microcosm. Chemosphere 73, 1102-1107.

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.

Ramirez-Romero R, Desneux N, Chaufaux J, Kaiser L, 2008. Bt-maize effects on biological parameters of the non-target aphid *Sitobion avenae* (Homoptera: Aphididae) and Cry1Ab toxin detection. Pesticide Biochemistry and Physiology 91, 110-115.

Raps A, Kehr J, Gugerli P, Moar WJ, Bigler F, Hilbeck A, 2001. Immunological analysis of phloem sap of *Bacillus thuringiensis* corn and of the nontarget herbivore *Rhopalosiphum padi* (Homoptera: Aphididae) for the presence of Cry1Ab. Molecular Ecology 10, 525-533.

Raubuch M, Roose K, Warnstorff K, Wichern F, Joergensen RG, 2007. Respiration pattern and microbial use of field-grown transgenic *Bt*-maize residues. Soil Biology & Biochemistry 39, 2380-2389.

Rauschen S, 2010. A case of "pseudo science"? A study claiming effects of the Cry1Ab protein on larvae of the two-spotted ladybird is reminiscent of the case of the green lacewing. Transgenic Research 19, 13-16.

Rauschen S, Schuphan I, Eber S, 2008. Assessment of possible non-target impacts of the novel Bt-maize event MON88017 resistant against the Western corn rootworm *Diabrotica virgifera virgifera* (LeConte). In: Romeis J, Meissle M, Sanvido O (Eds), *GMOs in Integrated Plant Production – Ecological impact of genetically modified organisms*, IOBC wprs Bulletin 33, 93-99.

Rauschen S, Schultheis E, Pagel-Wieder S, Schuphan I, Eber S, 2009. Impact of *Bt*-corn MON88017 in comparison to three conventional lines on *Trigonotylus caelestialium* (Kirkaldy) (Heteroptera: Miridae) field densities. Transgenic Research 18, 203-214.

Rauschen S, Schaarmschmidt F, Gathmann A, 2010a. Occurrence and field densities of Coleoptera in the maize herb layer: implications for environmental risk assessment of genetically modified *Bt*-maize. Transgenic Research 19, 727-744.

Rauschen S, Schultheis E, Hunfeld H, Schaarschmidt F, Schuphan I, Eber S, 2010b. *Diabrotica*-resistant Bt-maize DKc5143 event MON88017 has no impact on the field densities of the leafhopper *Zyginidia scutellaris*. Environmental Biosafety Research 9, 87-99.

Raybould A, 2007. Ecological versus ecotoxicological methods for assessing the environmental risks of transgenic crops. Plant Science 173, 589-602.

Raybould A, Tuttle A, Shore S, Stone T, 2010. Environmental risk assessment for transgenic crops producing output trait enzymes. Transgenic Research 19, 595-609.

Raybould A, Graser G, Hill K, Ward K, 2011a. Ecological risk assessments for transgenic crops with combined insect-resistance traits: the example of Bt11 x MIR604 maize. Journal of Applied Entomology, DOI:10.1111/j.1439-0418.2010.01601.x (in press).

Raybould A, Higgins LS, Horak MJ, Layton RJ, Storer NP, Manuel De La Fuente JM, Herman RA, 2011b. Assessing the ecological risks from the persistence and spread of feral populations of insect-resistant transgenic maize. Transgenic Research, DOI:10.1007/s11248-011-9560-4 (in press).

Raymond B, Johnston PR, Wright DJ, Ellis RJ, Crickmore N, Bonsall MB, 2009. A mid-gut microbiota is not required for the pathogenicity of *Bacillus thuringiensis* to diamondback moth larvae. Environmental Microbiology 11, 2556-2563

Raynor G, Ogden E, Hayes J, 1972. Dispersion and deposition of corn pollen from experimental sources. Agronomy Journal 64, 420-427.

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.

Rice ME, 2004. Transgenic rootworm corn: Assessing potential agronomic, economic, and environmental benefits. Plant Health Progress, http://www.plantmanagementnetwork.org/pub/php/review/2004/rootworm/

Ricroch A, Bergé JB, Messéan A, 2009. Revue bibliographique sur la dispersion des transgènes à partir du maïs génétiquement modifié. Comptes Rendus Biologies 332, 861-875.

Ricroch A, Bergé JB, Kuntz M, 2010. Is the German suspension of MON810 maize cultivation scientifically justified? Transgenic Research 19, 1-12.

Riesgo L, Areal FJ, Sanvido O, Rodríguez-Cerezo E, 2010. Distances needed to limit cross-fertilization between GM and conventional maize in Europe. Nature Biotechnology 28, 780-782.

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, 2011. 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, DOI:10.1080/10408398.2010.499480 (in press).

Robinson RA, Sutherland WJ, 2002. Post-war changes in arable farming and biodiversity in Great Britain. Journal of Applied Ecology 39, 157-176.

Rodríguez 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.

Romeis J, Meissle M, 2011. Non-target risk assessment of *Bt* crops – Cry protein uptake by aphids. Journal of Applied Entomology 135, 1-6.

Romeis J, Meissle M, Bigler F, 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. Nature Biotechnology 24, 63-71.

Romeis J, Bartsch D, Bigler F, Candolfi MP, Gielkens M, Hartley SE, Hellmich RL, Huesing JE, Jepson PC, Layton R, Quemada H, Raybould A, Rose RI, Schiemann J, Sears MK, Shelton AM, Sweet J, Vaituzis Z, Wolt JD, 2008a. Nontarget arthropod risk assessment of insect-resistant GM crops. Nature Biotechnology 26, 203-208.

Romeis J, Van Driesche RG, Barratt BIP, Bigler F, 2008b. Insect-resistant transgenic crops and biological control. In: Romeis J, Shelton AM, Kennedy GG (Eds), *Integration of insect-restistant genetically modified crops within IPM programs*, Springer Science + Business Media BV, pp 87-117.

Romeis J, Hellmich RL, Candolfi MP, Carstens K, De Schrijver A, Gatehouse AMR, Herman RA, Huesing JE, McLean MA, Raybould A, Shelton AM, Waggoner A, 2011. Recommendations for the design of laboratory studies on non-target arthropods for risk assessment of genetically engineered plants. Transgenic Research 20, 1-22.

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. In: Romeis J, Bigler F (Eds), *GMOs in Integrated Plant Production – Ecological impact of genetically modified organisms*, 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.

Rosi-Marshall EJ, Tank JL, Royer TV, Whiles MR, Evans-White M, Chambers C, Griffiths NA, Pokelsek J, Stephen ML, 2007. Toxins in transgenic crop by products may affect headwater stream ecosystems. Proceedings of the National Academy of Sciences of the United States of America 104, 16204-16208.

Rose RI, 2007. White paper on tier-based testing for the effects of proteinaceous insecticidal plant-incorporated protectants on non-target invertebrates for regulatory risk assessment. USDA-APHIS and US Environmental Protection Agency, Washington, DC, http://www.epa.gov/pesticides/biopesticides/pips/non-target-arthropods.pdf

Rossi F, Morlacchini M, Fusconi G, Pietri A, Mazza R, Piva G, 2005. Effect of Bt corn on broiler growth performance and fate of feed-derived DNA in the digestive tract. Poultry Science 84, 1022-1030.

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.

Sanahuja G, Banakar R, Twyman RM, Capelle T, Christou P, 2011. *Bacillus thuringiensis*: a century of research, development and commercial applications. Plant Biotechnology Journal 9, 283-300

Sander M, Madliger M, Schwarzenbach RP, 2010. Adsorption of transgenic insecticidal Cry1Ab protein to SiO_2. 1. Forces driving adsorption. Environmental Science & Technology 44, 8870-8876.

Sandermann H, 2006. Plant biotechnology: ecological case studies on herbicide resistance. Trends in Plant Science 11, 324-328.

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, Widmer F, Winzeler M, Streit B, Szerencsits E, Bigler F, 2008. Definition and feasibility of isolation distances for transgenic maize. Transgenic Research 17, 317-355.

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, DOI:10.1007/s11248-011-9524-8. (in press)

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, 2011c. Evaluating environmental risks of genetically modified crops – ecological harm criteria for regulatory decision-making. Environment & Science Policy, DOI:10.1016/j.envsci.2011.08.006 (in press).

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.

Saxena D, Stotzky G, 2001. Bt corn has a higher lignin content than non-Bt corn. American Journal of Botany 88, 1704-1706.

Saxena D, Flores S, Stotzky G, 2002. Bt toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events. Soil Biology & Biochemistry 34, 133-137.

Saxena D, Stewart CN, Altosaar I, Shu Q, Stotzky G, 2004. Larvicidal Cry proteins from *Bacillus thuringiensis* are released in root exudates of transgenic *B. thuringiensis* corn, potato, and rice but not of *B. thuringiensis* canola, cotton, and tobacco. Plant Physiology and Biochemistry 42, 383-387.

Sayed A, Wiechman B, Struewing I, Smith M, French W, Nielsen C, Bagley M, 2010. Isolation of transcripts from *Diabrotica virgifera virgifera* LeConte responsive to the *Bacillus thuringiensis* toxin Cry3Bb1. Insect Molecular Biology 19, 381-389.

Schäfer ML, Lundkvist E, Landin J, Persson TZ, Lundström JO, 2006. Influence of landscape structure on mosquitoes (Diptera: Culicidae) and dytiscids (Coleoptera: Dytiscidae) at five spatial scales in Swedish wetlands. Wetlands 26, 57-68.

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.

SCP, 1998a. Opinion of the Scientific Committee on Plants regarding the genetically modified cotton, tolerant to glyphosate herbicide notified by the Monsanto Company (notification C/ES/97/01), http://ec.europa.eu/food/fs/sc/scp/outcome_gmo_en.html

SCP, 1998b. Opinion of Scientific Committee on Plants regarding submission for placing on the market of fodder beet tolerant to glyphosate notified by DLF-Trifolium, Monsanto and Danisco Seed (notification C/DK/97/01), http://ec.europa.eu/food/fs/sc/scp/outcome_gmo_en.html

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, 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.

Sessitsch A, Smalla K, Kandeler E, Gerzabek MH, 2004. Effects of transgenic plants on soil microorganisms and nutrient dynamics. In: Gillings M, Holmes A (Eds), *Plant Microbiology*, BIOS Scientific Publishers, London and New York, pp 55-75.

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, 2011. What have the mechanisms of resistance to glyphosate taught us? Pest Management Science, DOI:10.1002/ps.2261 (in press).

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.

Shelton AM, Naranjo SE, Romeis J, Hellmich RL, Wolt JD, Federici BA, Albajes R, Bigler F, Burgess EPJ, Dively GP, Gatehouse AMR, Malone LA, Roush R, Sears M, Sehnal F, 2009a. Setting the record straight: a rebuttal to an erroneous analysis on transgenic insecticidal crops and natural enemies. Transgenic Research 18, 317-322.

Shelton AM, Naranjo SE, Romeis J, Hellmich RL, Wolt JD, Federici BA, Albajes R, Bigler F, Burgess EPJ, Dively GP, Gatehouse AMR, Malone LA, Roush R, Sears M, Sehnal F, Ferry N, Bell H, 2009b. Appropriate analytical methods are necessary to assess nontarget effects of insecticidal proteins in GM crops through meta-analysis (Response to Andow et al. 2009). Environmental Entomology 38, 1533-1538.

Shim SM, Choi MH, Park SH, Gu YU, Oh JM, Kim S, Kim HY, Kim GH, Lee YS, 2010. Assessing the digestibility of genetically modified soybean: Physiologically based *in vitro* digestion and fermentation model. Food Research International 43, 40-45.

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.

Shirai Y, 2006. Laboratory evaluation of effects of transgenic Bt corn pollen on two non-target herbivorous beetles, *Epilachna vigintioctopunctata* (*Coccinellidae*) and *Galerucella vittaticollis* (*Chrysomelidae*). Applied Entomology and Zoology 41, 607-611.

Siegfried BD, Vaughn TT, Spencer T, 2005. Baseline susceptibility of Western corn rootworm (Coleoptera: Chrysomelidae) to Cry3Bb1 *Bacillus thuringiensis* toxin. Journal of Economic Entomology 98, 1320-1324.

Siegfried BD, Spencer T, Crespo AL, Storer NP, Head GP, Owens ED, Guyer D, 2007. Ten years of Bt resistance monitoring in the European corn borer: what we know, what we don't know, and what we can do better. American Entomologist 53, 208-214.

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.

Soberón M, Gill SS, Bravo A, 2009. Signaling versus punching hole: how do *Bacillus thuringiensis* toxins kill insect midgut cells? Cellular and Molecular Life Science 66, 1337-1359.

Sochova I, Hofman J, Holoubek I, 2006. Using nematodes in soil ecotoxicology Environment International 32, 374-383.

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.

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.

Spencer JL, Mabry TR, Vaughn TT, 2003. Use of transgenic plants to measure insect herbivore movement. Journal of Economic Entomology 96, 1738-1749.

Spencer JL, Hibbard BE, Moeser J, Onstad DW, 2009. Behavior and ecology of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte). Agricultural and Forest Entomology 11, 9-27.

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.

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.

Storer NP, 2003. A spatially explicit model simulating Western corn rootworm (Coleoptera: Chrysomelidae) adaptation to insect-resistant maize. Journal of Economic Entomology 96, 1530-1547.

Storer NP, Babcock JM, Edwards JM, 2006. Field measures of Western corn rootworm (Coleoptera: Chrysomelidae) mortality caused by Cry34/35Ab1 proteins expressed in maize event 59122 and implications for trait durability. Journal of Economic Entomology 99, 1381-1387.

Storer NP, Babcock JM, Schlenz M, Meade T, Thompson GD, Bing JW, Huckaba RM, 2010. Discovery and characterization of field resistance to Bt maize: *Spodoptera frugiperla* (Lepidoptera: Noctuidae) in Puerto Rico. Journal of Economic Entomology 103, 1031-1038.

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, 2011. The impact of agricultural intensification and land-use change on the European arable flora. Proceedings of the Royal Society B: Biological Sciences, DOI:10.1098/rspb.2011.1686 (in press).

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.

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.

Svobodová Z, Habuštova O, Hussein HM, Půža V, Sehnal F, 2012. Impact of genetically modified maize expressing Cry3Bb1 on non-target arthropods: first year results of a field study. In: Romeis J, Meissle M, Álvarez-Alfageme (Eds), *GMOs in Integrated Plant Production*, IOBC wprs Bulletin 73, 105-117 (in press).

Swan CM, Jensen PD, Dively GP, Lamp WO, 2009. Processing of transgenic crop residues in stream ecosystems. Journal of Applied Ecology 46, 1304-1313.

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.

Szalai M, Köszegi J, Toepfer S, Kiss J, 2011. Colonisation of first-year maize fields by Western corn rootworm (*Diabrotica virgifera virgifera*) from adjacent infested maize fields. Acta Phytopathologica et Entomologica Hungarica 46, 215-226.

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? In: Romeis J, Meissle M, Sanvido O (Eds), *GMOs in Integrated Plant Production – Ecological impact of genetically modified organisms*, IOBC wprs Bulletin 33, 105-110.

Széll E, Zsellér I, Ripka G, Kiss J, Princzinger G, 2005. Strategies for controlling Western corn rootworm (*Diabrotica virgifera virgifera*). Acta Agronomica Hungarica 53, 71-79.

Tabashnik BE, 1994. Evolution of resistance to *Bacillus thuringiensis.* Annual Review of Entomology 39, 47-79.

Tabashnik BE, 2008. Delaying insect resistance to transgenic crops. Proceedings of the National Academy of Sciences of the United States of America 105, 19029-19030.

Tabashnik BE, Gould F, Carrière Y, 2004. Delaying evolution of insect resistance to transgenic crops by decreasing dominance and heritability. Journal of Evolutionary Biology 17, 904-912.

Tabashnik BE, Gassmann AJ, Crowder DW, Carrière Y, 2008a. Insect resistance to *Bt* crops: evidence versus theory? Nature Biotechnology 26, 199-202.

Tabashnik BE, Gassmann AJ, Crowder DW, Carrière Y, 2008b. Reply to Field-evolved resistance to *Bt* toxins. Nature Biotechnology 26, 1074-1076.

Tabashnik BE, Van Rensburg JBJ, Carrière Y, 2009. Field-evolved insect resistance to *Bt* crops: definition, theory and data. Journal of Economic Entomology 102, 2011-2025.

Tank JL, Rosi-Marshall EJ, Royer TV, Whiles MR, Griffiths NA, Frauendorf TC, Treering DJ, 2011. Occurrence of maize detritus and a transgenic insecticidal protein (Cry1Ab) within the stream network of an agricultural landscape. Proceedings of the National Academy of Sciences of the United States of America, DOI:10.1073/pnas.1006925107 (in press).

Tarkalson DD, Kachman SD, Knops JMN, Thies JE, Wortmann CS, 2008. Decomposition of Bt and non-Bt corn hybrid residues in the field. Nutrient Cycling in Agroecosystems 80, 211-222.

Taylor RL, Maxwell BD, Boik RJ, 2006. Indirect effects of herbicides on bird food resources and beneficial arthropods. Agriculture, Ecosystems & Environment 116, 157-164.

Teasdale JR, Cavigelli MA, 2010. Subplots facilitate assessment of corn yield losses from weed competition in a long-term systems experiment. Agronomy for Sustainable Agriculture 30, 445-453.

Tharp BE, Kells JJ, Bauman TT, Harvey RG, Johnson WG, Loux MM, Martin AR, Maxwell DJ, Owen MDK, Regehr DL, Warnke JE, Wilson RG, Wrage LJ, Young BG, Dalley CD, 2004. Assessment of weed control strategies for corn in the North-Central United States. Weed Technology 18, 203-210.

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.

Thomas WE, Burke IC, Wilcut JW, 2004. Weed management in glyphosate-resistant corn with glyphosate, halosulfuron, and mesotrione. Weed Technology 18, 826-834.

Thomas WE, Everman WJ, Allen J, Collins J, Wilcut JW, 2007. Economic assessment of weed management systems in glufosinate-resistant, glyphosate-resistant, imidazolinone-tolerant, and nontransgenic corn. Weed Technology 21, 191-198.

Tingle CH, Chandler JM, 2004. The effect of herbicides and crop rotation on weed control in glyphosate-resistant crops. Weed Technology 18, 940-946.

Toepfer S, Levay N, Kiss J, 2006. Adult movements of newly introduced alien *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) from non-host habitats. Bulletin of Entomological Research 96, 327-335.

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.

Torres JB, Ruberson JR, 2008. Interactions of *Bacillus thuringiensis* Cry1Ac toxin in genetically engineered cotton with predatory heteropterans. Transgenic Research 17, 345-354.

Torres JB, Ruberson JR, Adang MJ, 2006. Expression of *Bacillus thuringiensis* Cry1Ac protein in cotton plants, acquisition by pests and predators: a tritrophic analysis. Agricultural and Forest Entomology 8, 191-202.

Tuska T, Kiss J, Edwards CR, Szabó Z, Ondrusz I, Miskucza P, Garai A, 2002. Determination of economic threshold for silk feeding by western corn rootworm (*Diabrotica virgifera virgifera* LeConte) adults in seed corn. Növényvédelem 38, 505-511.

Tyutyunov Y, Zhadanovskaya E, Bourguet D, Arditi R, 2008. Landscape refuges delay resistance of the European corn borer to Bt-maize: a demo-genetic dynamic model. Theoretical Population Biology 74, 138-146.

van de Wiel CCM, Lotz LAP, 2006. Outcrossing and coexistence of genetically modified with (genetically) unmodified crops: a case study of the situation in the Netherlands. Netherlands Journal of Agricultural Science 54, 17-35.

van de Wiel CCM, Groeneveld RMW, Dolstra O, Kok EJ, Scholtens IMJ, Thissen JTNM, Smulders MJM, Lotz LAP, 2009. Pollen-mediated gene flow in maize tested for coexistence of GM and non-

GM crops in the Netherlands: effect of isolation distances between fields. Netherlands Journal of Agricultural Science 56, 405-423.

van de Wiel CCM, van den Brink L, Bus CB, Riemens MM, Lotz LAP, 2011. Crop volunteers and climate change. Effects of future climate change on the occurrence of maize, sugar beet and potato volunteers in the Netherlands. COGEM report (CGM 2011-11), http://www.cogem.net/showdownload.cfm?objectId=912F0063-1517-64D9-CC99711A575E5755&objectType=mark.hive.contentobjects.download.pdf

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.

Van Frankenhuyzen K, Liua Y, Tonona A, 2010. Interactions between *Bacillus thuringiensis* subsp. *kurstaki* HD-1 and midgut bacteria in larvae of gypsy moth and spruce budworm. Journal of Invertebrate Pathology 103, 124-131.

Van Rensburg JBJ, 2007. First report of field resistance by the stem borer, *Busseola fusca* (Fuller) to Bt-transgenic maize. South African Journal of Plant and Soil 24, 147-151.

van Rozen K, Ester A, 2010. Chemical control of *Diabrotica virgifera virgifera* LeConte. Journal of Applied Entomology 134, 376-384.

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.

Vasileiadis VP, Sattin M, Otto S, Veres A, Pálinkás Z, Ban R, Pons X, Kudsk P, van der Weide R, Czembor E, Moonen AC, Kiss J, 2011. Crop protection in European maize-based cropping systems: current practices and recommendations for innovative Integrated Pest Management. Agricultural Systems 104, 533-540.

Vaughn T, Cavato T, Brar G, Coombe T, DeGooyer T, Ford S, Groth M, Howe A, Johnson S, Kolacz K, Pilcher C, Purcell J, Romano C, English L, Pershing J, 2005. A method of controlling corn rootworm feeding using a *Bacillus thuringiensis* protein expressed in transgenic maize. Crop Science 45, 931-938.

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.

Vila-Aiub MM, Balbi MC, Distéfano AJ, Fernández L, Hopp E, Yu Q, Powles SB, 2011. Glyphosate resistance in perennial *Sorghum halepense* (Johnsongrass), endowed by reduced glyphosate translocation and leaf uptake. Pest Management Science, DOI:10.1002/ps.2286 (in press).

Vogler A, Wettstein-Bättig M, Aulinger-Leipner I, Stamp P, 2009. The airborne pollen flow of maize (*Zea mays* L.) in a multi-crop designed field plot. Agricultural and Forest Meteorology 10, 1776-1780.

Vojtech E, Meissle M, Poppy GM, 2005. Effects of Bt maize on the herbivore *Spodoptera littoralis* (Lepidoptera: Noctuidae) and the parasitoid *Cotesia marginiventris* (Hymenoptera: Bracondiae). Transgenic Research 14, 133-144.

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.

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, 2009. Effects of glyphosate on soil microbial communities and its mineralization in a Mississippi soil. Pest Management Science 63, 388-393.

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.

Wesseler J, Fall EH, 2010. Potential damage costs of *Diabrotica virgifera virgifera* infestation in Europe – the 'no control'. Journal of Applied Entomology 134, 385-394.

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.

Whalon ME, Mota-Sanchez D, Hollingworth RM, Duynslager L, 2011. Arthropod pesticide resistance database. Michigan State University, 2004-2011, http://www.pesticideresistance.org

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.

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.

Wilson TA, Hibbard BE, 2004. Host suitability of nonmaize agroecosystem grasses for the Western corn rootworm (Coleoptera: Chrysomelidae): Environmental Entomology 33, 1102-1108.

Witmer JE, Hough-Goldstein JA, Pesek JD, 2003. Ground-dwelling and foliar arthropods in four cropping systems. Environmental Entomology 32, 366-376.

Wolfenbarger LL, Naranjo SE, Lundgren JG, Bitzer RJ, Watrud LS, 2008. Bt crop effecs on functional guilds of non-target arthropods: a meta-analysis. PLoS ONE 3, e2118.

Wolt JD, 2011. A mixture toxicity approach for environmental risk assessment of multiple insect resistance genes. Environmental Toxicology and Chemistry 30, 763-772.

Wolt JD, Peterson RKD, 2010. Prospective formulation of environmental risk assessments: probabilistic screening for Cry1A(b) maize risk to aquatic insects. Ecotoxicological and Environmental Safety 73, 1182-1188.

Xue K, Serohijos RC, Devare M, Thies JE, 2011. Decomposition rates and residue-colonizing microbial communities of Bacillus thuringiensis insecticidal protein Cry3Bb-expressing (Bt) and non-Bt corn hybrids in the field. Applied and Environmental Microbiology 77, 839-846.

Young BG, 2006. Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. Weed Technology 20, 301-307.

Yanni SF, Whalen JK, Ma BL, 2011. Field-grown Bt and non-Bt corn: yield, chemical composition, and decomposability. Agronomy Journal 103, 486-493.

Yu Q, Cairns A, Powles S, 2007. Glyphosate, paraquat and ACCase multiple herbicide resistance evolved in a *Lolium rigidum* biotype. Planta 225, 499-513.

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.

Zeilinger AR, Andow DA, Zwahlen C, Stotzky G, 2010. Earthworm populations in a northern US cornbelt soil are not affected by long-term cultivation of Bt maize expressing Cry1Ab and Cry3Bb1 proteins. Soil Biology & Biochemistry 42, 1284-1292.

ZKBS, 2011. Statement of the Central Commission on Biological Safety (ZKBS) on the latest scientific publications on the risk assessment of the maize line MON810, Az. 6790-02-0013, http://www.bvl.bund.de/EN/06_Genetic_Engineering/ZKBS/01_Allg_Stellungnahmen/05_plants/zkbs_plants_maize_MON810_statement_2011.pdf?__blob=publicationFile&v=4

Zobiole LHS, Oliveira Jr RS, Kremer RJ, Constantin J, Yamada T, Castro C, Oliveira FA, Oliveira Jr A, 2010. Effects of glyphosate on symbiotic N_2 fixation and nickel concentration in glyphosate-resistant soybeans. Applied Soil Ecology 44, 176-180.

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.

Zurbrügg C, Nentwig W, 2009. Ingestion and excretion of two transgenic *Bt* corn varieties by slugs. Transgenic Research 18, 215-225.

Zurbrügg C, Hönemann L, Meissle M, Romeis J, Nentwig W, 2010. Decomposition dynamics and structural plant components of genetically modified *Bt* maize. Transgenic Research 19, 257-267.

Zuver KA, Bernards ML, Kells JL, Sprague CL, Medlin CR, Loux MM, 2006. Evaluation of postemergence weed control strategies in herbicide-resistant isolines of corn (*Zea mays*). Weed Technology 20, 172-178.