

SCIENTIFIC OPINION

Request from the European Commission related to the safeguard clause invoked by Austria on maize MON810 and T25 according to Article 23 of Directive 2001/18/EC¹

Scientific Opinion of the Panel on Genetically Modified Organisms

(Question No EFSA-Q-2008-314)

Adopted on 04 December 2008

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SUMMARY

On 10 June 1999 and on 8 May 2000, Austria invoked Article 16 of Directive 90/220/EEC (safeguard clause) to provisionally prohibit the placing on the market of the authorised genetically modified (GM) maize events MON810 and T25 on its territory. In February 2004 and November 2007, Austria provided additional information to support the national safeguard measure to be considered under Article 23 of Directive 2001/18/EC. To define whether the information submitted by Austria comprises new information that would affect the environmental risk assessment for the uses laid down in the corresponding consent, the European Commission requested in a letter, dated 18 April 2008, a scientific opinion from the European Food Safety Authority (EFSA).

Following investigation of the evidence presented in the Austrian submission, the Scientific Panel on Genetically Modified Organisms (GMO Panel) of EFSA concludes that there is no new scientific evidence that would invalidate the previous risk assessments of maize MON810 and T25. Therefore, no specific scientific evidence, in terms of risk to human and animal health and the environment, was provided that would justify the invocation of a

¹ For citation purposes: Scientific Opinion of the Panel on Genetically Modified Organisms on a request from the European Commission related to the safeguard clause invoked by Austria on maize MON810 and T25 according to Article 23 of Directive 2001/18/EC. *The EFSA Journal* (2008) 891, 1-64.

* This opinion is not shared by 0 members of the Panel. / (conflict of interest) 0 members of the Panel did not participate in (part of) the discussion on the subject referred to above because of possible conflicts of interest.

safeguard clause under Article 23 of Directive 2001/18/EC for the marketing of maize MON810 and T25, for its intended uses, in Austria.

Key words: GMOs, maize (*Zea mays*), MON810, T25, Austria, safeguard clause, human health, animal health, environment, Directive 90/220/EEC, Directive 2001/18/EC

TABLE OF CONTENTS

| | |
|---------------------------------------------------------------------------------------------|----|
| Panel Members | 1 |
| Summary | 1 |
| Table of Contents | 3 |
| Background | 4 |
| Terms of reference as provided by the European Commission | 4 |
| Acknowledgements | 4 |
| Assessment | 6 |
| 1. Introduction | 6 |
| 2. Assessment of documents provided by Austria | 6 |
| 2.1. Food and feed safety issues related to maize MON810 | 7 |
| 2.1.1. Comparative assessment | 7 |
| 2.1.2. Toxicological assessment | 8 |
| 2.1.3. Allergenicity assessment | 9 |
| 2.2. Food and feed safety issues related to maize T25 | 11 |
| 2.2.1. Health effects | 11 |
| 2.3. Environmental safety issues related to maize MON810 | 11 |
| 2.3.1. Potential for gene transfer | 11 |
| 2.3.2. Interactions between the GM plant and target organisms | 13 |
| 2.3.3. Interactions of the GM plant with non-target organisms: Lepidoptera | 17 |
| 2.3.4. Interactions of the GM plant with non-target organisms: entomofauna | 20 |
| 2.3.5. Interactions of the GM plant with non-target organisms: predators | 21 |
| 2.3.6. Interactions of the GM plant with non-target organisms: parasitoids | 23 |
| 2.3.7. Interactions of the GM plant with non-target organisms: Hymenoptera | 25 |
| 2.3.8. Interactions of the GM plant with non-target organisms: water-dwelling insects | 26 |
| 2.3.9. Interactions of the GM plant with non-target organisms: soil organisms | 27 |
| 2.4. Environmental safety issues related to maize T25 | 34 |
| 2.4.1. Risks for weed communities | 34 |
| 2.5. Post-market environmental monitoring plan | 36 |
| 2.5.1. Case-specific monitoring | 36 |
| 2.5.2. General surveillance | 37 |
| Overall Conclusions and Recommendations | 38 |
| Documentation provided to EFSA | 38 |
| References | 38 |
| Reference list of Austrian report | 58 |

BACKGROUND

On 10 June 1999 and on 8 May 2000, Austria invoked Article 16 of Directive 90/220/EEC (safeguard clause) to provisionally prohibit the placing on the market of the authorised genetically modified (GM) maize events MON810 and T25, respectively, on its territory. In their respective scientific opinions, both the Scientific Committee on Plants in 1999 and 2000 and the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) in 2004 concluded that, based on the information submitted by Austria, maize MON810 and T25 do not constitute a risk to human and animal health or the environment.

On 24 June 2005, the Environment Council indicated its opposition to the Commission proposal requesting Austria to repeal its national safeguard clause. Despite the re-examination of the Commission proposal, the Environment Council maintained its position on 18 December 2006 and indicated that “*the different agricultural structures and regional ecological characteristics in the European Union need to be taken into account in a more systematic manner in the environmental risk assessment*”. In this respect, on 11 September 2007, Austria was asked by the European Commission (DG ENV) to provide ongoing additional studies related to the cultivation of maize MON810 and T25, and to explain to what extent the objective of these studies correspond to the motivation of the Council of 18 December 2006 (in particular to recital 3 referring to “*the different agricultural structures and regional ecological characteristics*” in Austria). Following the request of the European Commission, on 21 November 2007, the Permanent Representation of Austria provided to the European Commission an Austrian study entitled “*Supplementary risk assessment on GMO maize MON810 (with consideration of maize T25)*”. The aim of the Austrian study was to summarise Austria’s arguments in response to the decision of the World Trade Organisation Panel ‘European Communities – Measures affecting the approval and marketing of biotech products’, because part of the measures deals with the Austrian safeguards concerning the import and use of maize MON810 and T25.

On 18 April 2008, EFSA has been requested by the European Commission to provide a scientific opinion on the statement and documents submitted by Austria in the context of the invoked safeguard clause.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA has been asked, under Article 29(1) and in accordance with Article 22(2) and 22(5)(c) of Regulation (EC) No 178/2002, “*to assess whether the information submitted by Austria comprises information affecting the environmental risk assessment of existing information on the basis of new scientific knowledge such that detailed grounds exist to consider that the above authorised GMOs, for uses laid down in the corresponding consent, constitute a risk to the environment*”.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the GMO Panel for the preparation of this opinion: Hans Christer Andersson, Salvatore Arpaia, Detlef Bartsch, Josep Casacuberta, Howard Davies, Patrick du Jardin, Lieve Herman, Niels Hendriksen, Sirpa Kärenlampi, Jozsef Kiss, Gijs Kleter, Ilona Kryspin-Sørensen, Harry Kuiper, Ingolf Nes, Nickolas Panopoulos, Joe Perry, Annette Pöting, Joachim Schiemann, Willem Seinen, Jeremy

Sweet and Jean-Michel Wal; as well as the following members of its Environment Working Group for the preparation of this opinion: Rosie Hails, Sue Hartley, Gerd Neemann, Angela Sessitsch, and Marco Nuti; as well as the following members of its staff: Yann Devos and Sylvie Mestdagh.

ASSESSMENT

1. Introduction

Directive 2001/18/EC provides the possibility for Member States to invoke safeguards on specific GMOs in the case where new or additional information, made available since the date of the consent, would affect the risk assessment of an authorised GMO. Provisions foreseen by Austria seek to provisionally prohibit the marketing of maize MON810 and T25 for its intended uses on the Austrian territory.

The GMO Panel examined the set of supporting documents submitted by Austria. In this respect, the GMO Panel assessed whether the submitted documents comprise new scientific information that would change the outcome of previously performed risk assessments, and if detailed grounds exist to consider that the authorised maize MON810 and T25, for its intended uses, constitute a risk to human and animal health or the environment.

The GMO Panel looked for evidence for GMO-specific risks – including long-term effects – taking into consideration the EFSA guidance document for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006) as well as any related risk assessments carried out in the past. In addition, the GMO Panel considered the relevance of issues raised by Austria in the light of the most recent scientific data and relevant peer-reviewed publications.

2. Assessment of documents provided by Austria

A supporting report, entitled “*Supplementary risk assessment for GM maize MON810 with regard to the conclusions of the WTO-Panel in the case of ‘EC biotech’ on Austrian safeguard measures for GM maize*” was accompanying the mandate of the European Commission (see Terms of Reference as provided by the European Commission).

Several issues were identified related to the risk assessment of maize MON810 and T25, but some of these issues fall outside the current remit of the GMO Panel, and therefore are not addressed in this scientific opinion. The use and risk assessment of pesticides on conventional maize, the assessment of economic consequences due to outcrossing and the adventitious presence of GM material in non-GM crop products fall outside the remit of the GMO Panel. The GMO Panel notes that it only gives its opinion on the scientific quality of post-market environmental monitoring activities proposed by applicants, whilst the final endorsement thereof is done by risk managers.

During its assessment, the GMO Panel identified issues raised by the Austrian authorities that would require further clarifications. To present and clarify the provided set of data, a bilateral meeting was held between Austrian scientists and representatives, and several experts of the GMO Panel and EFSA on 2 December 2008. Representatives of the European Commission attended the meeting as observers. At this meeting, the Austrian delegation presented recently published data on a reproduction study with mice fed maize NK603xMON810 (Velimirov et al., 2008) and a study on the intestinal immune system in rats fed maize MON810 (Finamore et al., 2008) that were not part of the initial data package submitted to the European Commission.

In its risk assessment and in addition to the information supplied by Austria on maize MON810 and T25, the GMO Panel reviewed all relevant and most recent scientific publications. The assessment carried out by the GMO Panel and subsequently the present scientific opinion follow the order of issues listed in the Austrian report, excluding the ones falling outside the remit of the GMO Panel.

In its consideration of maize MON810, the GMO Panel also studied information available from other GM maize events expressing Cry1Ab proteins, in particular maize Bt176 and Bt11. Due to the use of a different promoter, pollen from maize Bt176 contains much higher concentrations of the biologically active Cry protein than pollen of maize Bt11 and MON810 (~40x from Mendelsohn et al. (2004)). In maize Bt11 and MON810, the promoters used were almost inactive in pollen, resulting in very low levels of gene products (Hellmich et al., 2001; Gatehouse et al., 2002; Mendelsohn et al., 2004). In green tissues of MON810 maize plants, the amount of biologically active Cry protein is similar to that of maize Bt11 and Bt176. The GMO Panel indicates in its opinion where information derived from maize Bt176 and Bt11 is used in its assessment on potential impacts of maize MON810.

2.1. Food and feed safety issues related to maize MON810

2.1.1. Comparative assessment

2.1.1.1. Austrian claims

In the Austrian report, it is concluded that *“the set of parameters assessed for compositional analysis is very narrow and does not follow international guidelines. Even the results showing significant differences to non-GM-plants did not lead to further investigation”*. According to Austria, *“the results for substantial equivalence for MON810 are based on a compositional analysis that has several shortcomings: The range of parameters measured is very narrow. Only proximates, amino acids and fatty acids are included in the comparative analysis. Micronutrients and other important ingredients are not considered. Furthermore, even these data do not always include fibres, ADF and NDF (in the case of 1994 field trials). This investigated set of assessed components must be considered as too narrow when compared to the OECD (2002) and to the EUROPABIO (2001) consensus documents. Differences are detected between MON810 and the control, e.g. for glutamine, leucine, proline, ADF, NDF, C18:1 and C18:2 fatty acids, starch, protein. These differences are not considered relevant, as still within literature ranges. In one case the literature range used was exceeded (protein, US trials). The applicant used an older literature range (1976) in order to “normalise” the deviation. None of the differences were considered a reason to repeat or extend the comparative analysis. Last not least it appears that no isogenic control line was used. If compositional analysis is used as an indicator for unintended effects, the number of substances, for which data are presented, is too small. If used for nutritional assessment, certain proximates and micronutrients are missing”*.

2.1.1.2. Conclusion

The arguments provided by the Austrian authorities do not indicate that a specific risk has been identified in maize MON810 since its approval under Directive 90/220/EC, which was preceded by an evaluation of the safety of MON810 by the Scientific Committee on Plants in

1998 (SCP, 1998a). This Committee concluded, amongst others, that “*the transgenic maize line is substantially equivalent to non-transgenic maize except for the transferred trait*”. The Austrian authorities indicated that additional details of the compositional analysis of maize MON810 and the background data should have been provided. However, the publications that Austria mentions appeared after the approval of maize MON810, including the OECD consensus document and would not have been available at the time of the application. In addition, these publications are general in nature and do not highlight any specific risk that might be related to the consumption of maize MON810. Moreover, the GMO Panel has evaluated safety data on various stacked events containing the maize event MON810 combined with other transgenic events, including MON863xMON810 (EFSA, 2005a), MON863xNK603xMON810 (EFSA, 2005b) and NK603xMON810 (EFSA, 2005c). These data included compositional analysis of these stacks, MON810, and other comparators. The evaluations were carried out according to the GMO Panel’s principles as has also been laid out in its Guidance Document (EFSA, 2006). The evaluated data did not indicate any adverse effects.

2.1.2. Toxicological assessment

2.1.2.1. Austrian claims

In the Austrian report, it is concluded that “*the assumptions made by the applicant concerning toxicological and allergenic properties of MON810 maize are based on acute toxicity studies using isolated, bacterial derived proteins, as well as homology and in-vitro digestibility studies. This cannot be considered sufficient as there may be structural differences between plant- and bacteria-derived proteins; any chronic ad sub-chronic effects cannot be assessed by the approach used*”. According to Austria, “*toxicity assessment in the original dossier basically relies on an acute toxicity test and on in-vitro digestibility studies using a bacterial CryIA(b) Protein. In addition, the applicant provided homology comparisons with known toxic proteins and argued with the safe history of Bt toxins. Results obtained from acute toxicity tests of the CryIA(b) protein on rodents cannot be extrapolated for sub-chronic and chronic effects. The assumption that proteins can only act via acute mechanisms is not backed up by a solid empirical basis. This has meanwhile been acknowledged by recent guidance documents, which ask for 28-day repeated-dose sub-acute test (EFSA 2006 and NL Biosafety Council 2003). For conducting studies on toxic as well as allergenic properties of novel proteins test proteins were produced from bacteria. Using test proteins from microbes would in principle be acceptable if the proteins produced in the GM crop and the microbe would be identical or at least equivalent with respect to properties investigated in the test. However, several differences that might occur to the protein in cases the same gene is expressed in plants and microbes have been pointed out (Gurian-Sherman 2003b, Freese and Schubert 2004). Differences might occur at the level of DNA sequence during transformation and in RNA splicing, eventually resulting in an altered amino acid sequence. Posttranslational processing including proteolytic processing, glycosylation, acetylation, phosphorylation, methylation and folding might also differ between plants and microbes. Bacterial proteins were also used for in vitro digestibility studies. Whole plant feeding studies were not performed. It has to be concluded that the assessment of toxic properties of MON810 and the produced Bt-toxin is based on only a few results, which sometimes are obtained by methods which have been questioned in the scientific literature recently. Therefore we believe that the data provided do not support the safety-assumption for MON810 maize sufficiently*”.

2.1.2.2. Conclusion

Austria raised 2 points regarding the assessment of potential toxicity of the transgenic protein expressed in maize MON810 (Cry1Ab or CryIA(b) according to old nomenclature), performed by the applicant. These 2 points are the type of testing that has been performed and the test substrate that has been used, in particular the latter's representativeness for the plant-expressed transgenic Cry1Ab protein. The publications that Austria refers to in order to support their claims of perceived lack of data were published after the approval of maize MON810, and do not provide specific data on the safety MON810. None of the arguments that have been provided by Austria indicate that a specific risk has been identified for Cry1Ab or for MON810 after the evaluation of its safety by the Scientific Committee on Plants in 1998 (SCP, 1998a). Moreover, the GMO Panel has evaluated safety data on various stacked events containing the maize event MON810 combined with other transgenic events, including MON863xMON810 (EFSA, 2005a), MON863xNK603xMON810 (EFSA, 2005b) and NK603xMON810 (EFSA, 2005c). In the light of these applications, new data on the safety of MON810 and its derived stacked events had been provided, including a 90-day toxicity study with MON810, MON863xMON810, and MON863xNK603xMON810. The evaluations were carried out according to the GMO Panel's principles as has also been laid out in its Guidance Document (EFSA, 2006). These studies did not indicate any adverse effects.

With regard to the recent data presented at the bilateral meeting between Austrian scientists and representatives, and several experts of the GMO Panel and EFSA (Velimirov et al., 2008), the GMO Panel concludes that the methods used for these investigations are not routinely used for the safety assessment of whole foods and feeds, and that therefore neither experience with these models nor data on background variability in the tested parameters exists. Moreover, the GMO Panel identified various deficiencies in data reporting, methodologies and statistical calculations, which do not allow any interpretation². Therefore, the GMO Panel considers that these data do not invalidate the conclusions of the GMO Panel on the safety of MON810 maize.

2.1.3. Allergenicity assessment

2.1.3.1. Austrian claims

In the Austrian report, it is concluded that *“the assumptions made by the applicant concerning toxicological and allergenic properties of MON810 maize are based on acute toxicity studies using isolated, bacterial derived proteins, as well as homology and in-vitro digestibility studies. This cannot be considered sufficient as there may be structural differences between plant- and bacteria-derived proteins; any chronic ad sub-chronic effects cannot be assessed by the approach used”*. According to Austria, *“allergenicity testing in case of the MON810 dossiers is limited to the introduced CryIA(b) protein and consists of in-vitro-digestibility tests and homology comparisons to known allergens. History of safe use of Bt proteins in general and low expression levels are also mentioned to support the safety claim. As discussed in detail in Spök et al. (2005) these methods do not provide any direct evidence of allergic properties and not at all on sensitizing properties. Furthermore, the methods and evidence used cannot be considered as reliable indicators of allergenic properties. A detailed review of the shortcomings of the allergenicity assessment provided by the applicant with regard to MON810 maize is given in Dolezel et al. (2007). The following punctuation lists the*

² http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902199319.htm

main points of criticism: • Scientific studies investigating allergenic properties of proteins in connection with their digestibility/stability could not find a correlation at all (Kenna & Evans 2000, Fu et al. 2002). Therefore, if allergenic properties are only concluded from the stability of possible allergens, which is investigated using in-vitro digestibility studies, false positive and false negative results in safety testing might be possible. Furthermore, the differences in the design of in-vitro studies cast considerable doubt whether these experiments provide meaningful data at all. • Routinely used sequence comparison technologies such as FASTA and BLAST (Pearson 2000, Altschul et al. 1990a, b) as well as new methods developed more specifically for predicting the allergenic potential of a given protein would provide false positive and false negative results in many cases as results of the comparisons might differ depending on the parameters set (e.g. substitution matrix and gap penalties) (Börklund et al. 2005). • The homology comparison referred to in the MON810 dossier dates back to 1990 and 1995 respectively. Given the pace of immunological research a more recent comparative analysis would have included five times more sequences from allergenic proteins (Mari 2005). • Furthermore, expression levels of proven allergens may greatly vary in different strains, tissues and developmental stages, and can be influenced by a variety of factors. This has also been acknowledged by a Joint FAO/WHO expert consultation which concluded that it is not possible to link potential allergenicity of a given protein to its expression level (FAO/WHO 2001). • Equally important, allergenicity assessment of the introduced protein should be complemented by an assessment of the whole-plant as described in Spök et al. 2005”.

2.1.3.2. Conclusion

The Austrian arguments are criticisms of how the applicant has performed the assessment of potential allergenicity of the transgenic Cry1Ab protein expressed in maize MON810. These comments do not feature new data indicating that the transgenic protein in maize MON810 would constitute a potential allergenic risk. The Austrian authorities highlighted what they perceive as shortcomings in the methodology that the applicant has followed. They base their comments on publications that have appeared after the approval of maize MON810 under Directive 90/220/EC. However, they do not show any specific indications of potential risks linked to MON810. Moreover, the Austrian comments appear to diverge from the internationally harmonized ‘weight-of-evidence’ approach recommended by Codex Alimentarius’ guidelines for the safety assessment of GM crops for food use (Codex Alimentarius, 2003).

The GMO Panel has evaluated data on the potential allergenicity of various stacked events containing the maize event MON810 combined with other transgenic events, including MON863xMON810 (EFSA, 2005a), MON863xNK603xMON810 (EFSA, 2005b) and NK603xMON810 (EFSA, 2005c). These evaluations were carried out according to the GMO Panel’s principles as laid out in its Guidance Document (EFSA, 2006). These data did not reveal any potential allergic effect.

2.2. Food and feed safety issues related to maize T25

2.2.1. Health effects

2.2.1.1. Austrian claims

According to the Austrian report, “*the risk assessment for GM maize T25 is also addressed, but in a less detailed manner. Nevertheless it can be concluded that the risk assessment provided by the applicant does not take in to account all relevant issues according to the state-of-the-art of scientific knowledge. In summary, from the data provided in the dossier of maize T25 and in the light of recent evidence from scientific literature, it is neither possible to fully verify all aspects of the risk assessment conducted by the applicant nor to conclude a sufficient degree of safety*”. According to Austria, “*the following paragraphs summarize the Austrian findings with regard to the application for placing on the market of the GM maize T25. More details and references to the scientific literature are given in Dolezel et al. (2007). The assessment of the data provided by the applicant with regard to the authorization of the genetically modified maize T25 reveals a number of shortcomings and a lack of verifiability. Toxicity assessment does not consider effects beyond a 14-day study of the introduced protein. All studies are carried out on isolated proteins. Possible toxic properties of the whole-plant are not considered at all. The assessment of the allergenic potential is based on methods and evidence that cannot be considered sufficiently reliable. The approach used is even less appropriate to assess any de-novo sensitizing properties. The possibility of allergenic properties of the whole plant is not considered at all. Field trials and compositional analysis are not fully verifiable and it is not clear whether they have been properly conducted. In light of the most recent guidance provided, the information included in the dossier would also not be sufficient for a market authorisation under Directive 2001/18/EC or Regulation (EC) 1829/2003*”.

2.2.1.2. Conclusion

The Austrian comments relate to the safety assessment of maize T25 as carried out by the applicant and evaluated by the Scientific Committee on Plants in 1998 (SCP, 1998b). The Austrian authorities have not provided evidence of health risks associated with maize T25.

The GMO Panel has previously evaluated the safety data of a number of other GM crops (maize, oilseed rape, cotton, rice, soy) containing a similar genetic modification as T25, i.e. the introduction of the phosphinothricin acetyltransferase (PAT) enzyme conferring tolerance to glufosinate-ammonium-based herbicides. For none of these crops did the evaluation by the GMO Panel indicate any effect linked to products of this modification that could raise concerns for human and animal health.

2.3. Environmental safety issues related to maize MON810

2.3.1. Potential for gene transfer

2.3.1.1. Austrian claims

In the Austrian report, it is concluded that “*data indicate that gene flow from GM maize MON810 (or GM maize T25 respectively) through outcrossing to neighbouring non-modified*

varieties is likely and has relevant environmental and agricultural consequences in Austria. A likely decrease in the income of organic and conventional farmers is caused by out-crossing from GM-maize fields and the consequentially decreased value of their harvest". Austria stated that "the regional conditions for maize cultivation in Austria and the implication of these conditions for out-crossing and gene transfer were not considered".

2.3.1.2. GMO Panel evaluation

Since substantial literature shows that vertical gene flow characteristics of maize MON810 are similar to those of non-GM maize, the GMO Panel does not consider pollen dispersal and consequent cross-pollination as environmental hazards in themselves. The GMO Panel is primarily concerned with assessing the environmental consequences of transgene flow on ecosystems by assessing the spread and fitness of hybrids and backcross progeny as well as exposure to non-target organisms.

Theoretically, seeds originating from the cross-pollination of certain cross-compatible wild/weedy relatives can mediate the potential spread and establishment of hybrids and backcross progeny (Wilkinson et al., 2003; Devos et al., 2008b; Morales and Traveset, 2008). However, in the EU, there are no cross-compatible wild/weedy relatives with which maize can hybridise and form backcross progeny (Eastham and Sweet, 2002). The only recipients of cross-pollinated transgenes from maize are other cultivated maize varieties and types (Devos et al., 2005, 2008c; van de Wiel and Lotz, 2006; Hüsken et al., 2007; Sanvido et al., 2008b; Bitocchi et al., in press). Thus cross-pollination in maize is not considered an environmental risk, but is an agricultural management and coexistence issue and is not within the remit of the GMO Panel. Moreover, even though accidental seed dispersal of maize MON810 in Europe is occurring during its cultivation in many countries, the seed-mediated establishment of maize MON810 and its survival outside of cultivation has not been reported in spite of extensive cultivation and accidental seed dispersal. Maize plants have lost their ability to release seeds from the cob so that most seed dispersal is due to harvesting and post-harvest activities of farmers. Maize seeds and seedlings do not generally survive away from cultivated land and are only winter hardy in southern European countries. In Mediterranean regions, maize kernels remaining on the soil after harvest can germinate, grow and flower, and can locally cross-pollinate neighbouring maize plants (Melé et al., 2007; Gruber et al., 2008). However, the survival of maize is limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to diseases, herbivory and cold climate conditions. Studies conducted by the applicant, published literature on the cultivation of numerous varieties of maize MON810 and monitoring observations indicate that this maize behaves like non-GM maize in its ability to establish volunteers or survive over subsequent seasons and is very unlikely to establish feral populations under European environmental conditions.

Consequences of exposure of non-target organisms to pollen of maize MON810 are addressed in the section 2.2.3.

2.3.1.3. Conclusion

The GMO Panel is of the opinion that the information and arguments supplied by Austria, including regional conditions for maize cultivation in Austria, do not provide new or additional scientific evidence on pollen or seed dispersal and its consequences that would

alter the previous risk assessments of maize MON810.

2.3.2. Interactions between the GM plant and target organisms

2.3.2.1. Austrian claims

In the Austrian report, it is stated that “*the insect resistance management plan is insufficient because there is no information on baseline data, a lack of information regarding the implementation and a questionable assumption with regard to the adoption speed of GM maize MON810 in the European Union, which was estimated to be unrealistically low*”. According to Austria, “*the effectiveness of the refuge strategy cannot be deduced from the information submitted by the notifier, as no exact information on the implementation details of this program is given*” and “*resistance management is the responsibility of each farmer who uses Bt maize and each farmer should be required to implement measures such as setting up of refuges (see e.g. Andow in WTO, 2006 for reference)*”.

2.3.2.2. Resistance development in lepidopteran target pests

Resistance development generally refers to a genetically-based decrease in a population's susceptibility to a toxin and can be evaluated with laboratory bioassays estimating the resistance ratio, which is the LC₅₀ (concentration of toxin killing 50% of the larvae) of a field-derived strain divided by the LC₅₀ of the susceptible strain (Saeglitz et al., 2006; Andow, 2008; Bravo and Soberón, 2008). Susceptibility is usually measured by sampling insects from a field population and determining how their progeny respond to the toxin in laboratory experiments (Tabashnik et al., 2008a). Because insect pests have been able to develop resistance to chemical insecticides applied to control them (Whalon et al., 2008), the potential development of insect resistance to Cry toxins constitutively expressed in Bt-crops is considered as a relevant issue by the GMO Panel.

Major lepidopteran target pests of the Cry1Ac expressing cotton and Cry1Ab expressing maize (such as MON810) have been monitored worldwide for potential resistance development against specific Cry proteins. A recent meta-analysis of available monitoring data indicated that neither in the European Union (EU), nor in the United States (US), have populations of resistant European corn borer (*Ostrinia nubilalis*) and Mediterranean corn borer (*Sesamia nonagrioides*) been found in regions where Cry1Ab expressing maize is grown (Tabashnik et al., 2008a), confirming previous observations (Andow et al., 2000; Bourguet et al., 2003; Farinós et al., 2004; Eizaguirre et al., 2006; Schuphan, 2006; Stodola et al., 2006; Andreadis et al., 2007). In Spain, for instance, after many years of field exposure of corn borer populations to Cry1Ab expressing maize, no indications of resistance development were found (Farinós et al., 2004; Eizaguirre et al., 2006; Andreadis et al., 2007). So far, F₂ screenings (Andow and Alstad, 1998) performed on mated females collected from the field across Mediterranean EU countries and their progeny reared under confined conditions failed to detect major resistance alleles in corn borer populations (Bourguet et al., 2003; Schuphan, 2006; Andreadis et al., 2007). These data indicate that dominant resistance alleles are extremely rare in populations of corn borers and also that the initial frequency of recessive resistance alleles is low (Andow et al., 1998, 2000; Bourguet et al., 2003; Schuphan, 2006; Stodola et al., 2006; Andreadis et al., 2007). In contrast, laboratory selections for resistance with Cry1Ab toxins have yielded partial resistance levels in some corn borer strains after many generations (Chaufaux et al., 2001; Huang et al., 2002; Farinós et al., 2004; Alves et al.,

2006; Schuphan, 2006). While resistance levels fluctuated between generations for each strain, toxin susceptibility decreased significantly over generations for all selected strains. However, none of the laboratory-selected resistant corn borer larvae studied by Farinós et al. (2004) survived on Bt-maize seedlings. It is thus questionable whether these levels of resistance will reflect potential resistance development upon exposure of field populations to Bt-crops (e.g., Bourguet, 2004). Moreover, even though partial resistance has been shown to be reasonably common in some European corn borer populations (Bourguet et al., 2003), the polygenic nature of resistance in tested laboratory strains suggests that major genes for resistance to the Cry1Ab protein are rare in founding populations of the European corn borer (Alves et al., 2006).

Similar observations have been made in other maize target pests that are not representative of the European fauna. Huang et al. (2007), for instance, did not detect major resistance alleles in F₂ populations of the Southwestern corn borer (*Diatraea grandiosella*), which is a major maize stalk borer pest in central and southern parts of the US and in Mexico. However, a level of 'resistance' to maize MON810 has been reported in a Bt-maize-derived population of the African stem borer (*Busseola fusca*) in South Africa where some larvae were able to survive in the presence of the Bt-toxin, but had reduced larval growth rate (Van Rensburg, 2007). Another example of field-evolved resistance in Bt-maize concerns resistance of fall armyworm, *Spodoptera frugiperda*, to the Cry1F protein. Larvae surviving on Cry1F expressing maize in 2 fields in Puerto Rico (US) were collected and exposed to high concentrations of the Cry1F protein in laboratory bioassays, showing no mortality at these concentration levels (Moar et al., 2008; Tabashnik, 2008; Tabashnik et al., 2008b).

2.3.2.3. High dose/refuge strategy and its assumptions

In his latest review, Andow (2008) identified resistance development in target pests as a potential risk, and indicated that this risk can be managed. To delay or prevent the potential development of insect resistance to Bt-crops, a resistance management tactic, relying on a 'high dose/refuge strategy', has been endorsed in the US and EU (Bates et al., 2005; Andow, 2008; Bravo and Soberón 2008). The high dose/refuge strategy intends to reduce the selection pressure for resistance alleles by combining Bt-maize that produces a high dose of toxin with non-Bt-maize plants that are grown nearby as a refuge (Ives and Andow, 2002). To ensure that individuals heterozygous for a resistance allele are killed by the Cry1Ab protein produced in plant tissues, the increase in fitness conferred by resistance alleles must be recessive. The second assumption of the high dose/refuge strategy is that resistance alleles must be rare, so that only few homozygotes survive on Bt-crops. Finally, it is assumed that the few resistant insects emerging in Bt-crops must mate randomly or preferentially with the larger pool of susceptible insects preserved on non-Bt-crops (Alstad and Andow, 1995; Andow, 2008).

2.3.2.4. Insect resistance management plan proposed by applicants

According to the harmonised insect resistance management plan developed by the applicants' EU working group on insect resistance management (as referred to by Alcalde et al. (2007)), farmers growing more than 5ha of maize MON810 in the EU, need to establish refuge areas with maize not expressing the Cry1Ab protein corresponding to at least 20% of the surface planted with maize MON810. The working group's reasoning for only implementing the *refugia* on farms where the Bt-maize area is greater than 5ha 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 5ha Bt-maize; and (3) the negligible risk of resistance development in Bt-maize areas smaller than 5ha.

2.3.2.5. GMO Panel evaluation

The GMO Panel recognizes that the large scale cultivation of Bt-maize such as MON810 over several years will increase the selection pressure on corn borers, which could result in the potential development of resistance to certain Cry toxins. An analysis of global monitoring data, collected in Australia, China, Spain and the US, has revealed an increased frequency of resistance alleles in some field populations of *Helicoverpa zea* (a pest of cotton) to the Cry1Ac protein (Tabashnik et al., 2008a). Field-evolved resistance has also been documented for 2 maize pests that are not representative of the European fauna: *S. frugiperda* (Moar et al., 2008; Tabashnik, 2008; Tabashnik et al., 2008b) and *B. fusca* (Van Rensburg, 2007; Tabashnik, 2008). However, no field-evolved resistance has been reported to Bt-proteins for other lepidopteran pests (*Helicoverpa armigera*, *Heliothis virescens*, *O. nubilalis*, *Pectinophora gossypiella* and *S. nonagrioides*) (Ferré et al., 2008; Tabashnik et al., 2008a).

The GMO Panel considers that the likelihood of resistance development is low in corn borer populations if appropriate resistance management is implemented. No resistance has been reported for maize MON810 toxins following several years of extensive cultivation in Spain. However, the cultivation of Bt-maize in the EU is currently on a limited scale in a few geographic regions. In addition, as potential resistance development is dependent upon multiple environmental and biological factors, one should be cautious of predicting future responses of corn borer populations in the EU based on experiences elsewhere (Tyutyunov et al., 2008). Adult moth dispersal distances, for instance, have been shown to be influenced by plant size, weather conditions during the flight, phomonal patterns in the field and the timing of the flight (Hunt et al., 2001; Engels et al., 2008). Therefore, the GMO Panel advises that the potential development of resistance in target pests continues to be monitored in order to detect potential changes in resistance levels in pest populations. Applicants are generally requested to monitor resistance development in target pests under case-specific monitoring as part of their insect resistance management requirements (Alcalde et al., 2007) and to consider it under general surveillance through farmer questionnaires (Tinland et al., 2007; Schmidt et al., 2008).

The GMO Panel considers that the available scientific data support and validate the 3 assumptions on which the high dose/refuge strategy is based (see section 2.3.2.3):

- (1) *Resistance alleles are rare*: It has been shown that alleles conferring resistance to the Cry1Ab protein are sufficiently rare in European populations of corn borers for the high dose/refuge strategy to successfully delay resistance development (Bourguet et al., 2003; Schuphan, 2006; Andreadis et al., 2007). In field populations of the European corn borer, no resistant homozygotes were found for major resistance genes, and the estimated frequency of Cry1Ab resistance alleles were low (Andow et al., 1998, 2000; Bourguet et al., 2003; Stodola et al., 2006; Andreadis et al., 2007). In European populations of corn borers, the frequency of resistance alleles was estimated as <0.0009 for European corn borer populations from France (Bourguet et al., 2003) and as <0.0097 for Mediterranean corn borer populations from Greece and Spain (Andreadis et al., 2007).

- (2) *Mating occurs between resistant insects emerging in Bt-crops and susceptible insects preserved on non-Bt-crops (refuge) at sufficient levels:* The EU research project ProBenBt, in which various aspects of European and Mediterranean corn borer genetics and Bt-resistance in target lepidopteran pest species were studied, revealed that gene flow among European populations of both pest species is likely to be high enough to delay resistance development to Bt-toxins in maize (Schuphan, 2006). Likewise, Bourguet et al. (2000a,b) reported a high level of gene flow within and between European corn borer populations feeding on maize in France. In contrast, host plants other than maize have been shown to constitute an ineffective refuge to support sufficient numbers of susceptible European corn borers that would mate freely with adults emerging from maize (Bourguet et al., 2000b; Losey et al., 2001, 2002; Leniaud et al., 2006).

The fact that some adults of the European corn borer mate at a more restricted spatial scale (Hunt et al., 2001; Qureshi et al., 2005; Dalecky et al., 2006; Bailey et al., 2007) than previously assumed in the high-dose/refuge strategy might under certain circumstances (e.g., crop rotated landscape) decrease its efficiency (Dalecky et al., 2006; Schuphan, 2006). However, predictions generated by a recently developed demo-genetic dynamic model confirm that applying the high-dose/refuge strategy is likely to maintain the sensitivity to Bt-toxins in the European corn borer (Tyutyunov et al., 2008). Similar modelling work has not been performed on the Mediterranean corn borer so far. It remains thus difficult to extrapolate the predictions obtained for the European corn borer to the Mediterranean corn borer, especially due to its less polyphagous and more sedentary behaviour as well as its lower susceptibility to the Cry1Ab protein (Eizaguirre et al., 2006).

- (3) *Resistance alleles are recessive:* Because no resistant corn borer strains are known from the field, it has not been possible to define if the resistance in corn borer populations to Bt-maize would be recessive. However, it is considered a valid assumption that resistance alleles are recessive. Despite intensive Bt-maize cropping in various areas, no resistant corn borers have been found, and resistance against Bt-crops known from other insects has been shown to be recessive (Andow, 2008).

The GMO Panel agrees with the insect-resistance management plan proposed by the applicants' EU working group on insect resistance management. According to this insect resistance management plan, non-Bt-maize *refugia* would not be implemented on a considerable proportion of farms in certain EU countries, as the area planted to Bt-maize on these farms would cover less than 5ha. Considering experiences in Spain and other EU countries, this would not pose a risk, as Bt-maize would not be widely adopted in a given region. The Spanish experience illustrates that only in regions where pest infestation is high, does the adoption rate of Bt-maize reach 60% (Gómez-Barbero et al., 2008). Therefore, it is likely that sufficiently large areas of non-Bt-maize will remain available to provide a mosaic structure in the refuge strategy at the regional scale. However, if Bt-maize was adopted on a larger scale in a region, the risk of resistance development is likely to increase requiring specific refuge management measures. Since risk management is outside the remit of the GMO Panel, it is the responsibility of appropriate competent authorities in Member States to approve insect resistance management plans that are consistent with the environmental protection goals and biodiversity action plans in each Member State.

The GMO Panel notes that baseline information for estimating potential shifts in susceptibility to the Cry1Ab protein of corn borer populations that might result from the

selection and exposure to maize MON810 is available in the EU. In the frame of the EU research project, ProBenBt, baseline susceptibility to the Cry1Ab protein of European and Mediterranean corn borer populations has been tested in geographically distinct populations in the EU (Schuphan, 2006). Baseline susceptibility was determined for European corn borer larvae from laboratory and field populations from France, Germany, Greece, Italy, Slovakia and Spain, and for Mediterranean corn borer larvae from Greece and Spain. Even though differences were reported in observed susceptibility among corn borer populations, the magnitude of differences was small in comparison with already available data (Marçon et al., 1999; Gonzales-Nunez et al., 2000; Farinós et al., 2004) and data generated within the ProBenBt project (Saeglitz et al., 2006; Schuphan, 2006).

2.3.2.6. Conclusion

The GMO Panel is of the opinion that the claims and document supplied by Austria do not provide any new or additional scientific evidence that would invalidate previous risk assessments of maize MON810, the management of insect resistance development, or findings from post-market environmental monitoring of maize MON810.

2.3.3. Interactions of the GM plant with non-target organisms: Lepidoptera

2.3.3.1. Austrian claims

The Austrian report states that *“there is a risk to non-target organisms. This conclusion is based on the scientific literature and data relevant for the Austrian situation, like population densities of lepidoptera in agricultural environments, including maize fields, and their classification as “endangered species”. As it has been shown that Bt-toxins also affect non-target lepidopteran species, it is likely that the use of Bt plants will negatively affect populations of lepidoptera living in agricultural environments”*. Austria concluded that *“we expect it to be likely that butterfly species which are already endangered would be additionally affected by cultivation of GM maize MON810. Since additional impacts on endangered species should be minimised the cultivation of GM maize MON810 is expected to have negative effects”*.

2.3.3.2. Effects on lepidopteran species

Although maize is not considered an important resource of food for indigenous lepidopteran species in the EU, larvae of lepidopteran species consuming the Bt-plant or its products can be exposed to the Cry1Ab protein. In the vicinity of Bt-maize fields, larvae can be exposed to the Bt-protein when feeding on their host plant leaves naturally dusted with pollen and anthers of Bt-maize during anthesis. In a theoretical exposure assessment, Schmitz et al. (2003) estimated that approximately 7% of German macrolepidopteran species (butterflies and nocturnal species) occur in farmland areas where maize is grown and thus could be potentially affected by exposure to Bt-maize pollen. In this respect, there is no difference between Germany and Austria due to the fact that in both countries non-target Lepidoptera can be exposed to maize MON810 in field margins. Traxler et al. (2005) reported, that of the 215 butterfly species occurring in Austria, 152 appear in agricultural landscapes and a proportion of these are in potential contact with maize pollen.

Larvae of a range of lepidopteran species are susceptible to the Cry1Ab protein and can be adversely affected by the toxin after ingestion of significant amounts (Losey et al., 1999; Jesse and Obrycki, 2000; Hellmich et al., 2001; Felke et al., 2002; Anderson et al., 2004, 2005; Dutton et al., 2005; Lang and Vojtech, 2006; Prasifka et al., 2007). Dutton et al. (2005) showed that the pest species, *Spodoptera littoralis*, fed either on Cry1Ab expressing plant material (event Bt11) or Bt-sprayed (Dipel) plants is adversely affected, with young *S. littoralis* larvae being the most sensitive to the Bt-toxin. Compared to larvae maintained on control plants, larvae maintained on transgenic or sprayed plants had a higher mortality and a slower development time, confirming that certain herbivore Lepidoptera, including *S. littoralis*, are sensitive to the Cry1Ab toxin (Dutton et al., 2005). Sensitivity to the Cry1Ab protein was also shown for the stored-product moth pest species, *Ephestia kuehniella*, *Ephestia elutella*, *Cadra cautella* and *Plodia interpunctella* (Hubert et al., 2008). The anticipated effects of Bt-maize on secondary Lepidoptera pests largely depend upon the maize event, its expression pattern, the type of ingested plant material, and exposure due to the development stage and phenology of the species in field conditions.

In laboratory studies, lethal and sublethal effects of Bt-maize pollen consumption by larvae have been demonstrated for several non-target lepidopteran species, with the magnitude of effects depending upon the GM maize event and lepidopteran species used, as well as the amount of pollen consumed and toxin amounts contained in it. Concentrations of the biologically active Cry1Ab protein in pollen of maize Bt11 and MON810 were shown to be similar and relatively low resulting in similar toxicological effects on non-target lepidopteran populations exposed to pollen from these maize events (Mendelsohn et al., 2004). By contrast, maize Bt176 pollen contains much higher concentrations of the Cry1Ab protein (Hellmich et al., 2001). A laboratory assay revealed toxicity to monarch butterfly (*Danaus plexippus*) larvae that consumed Bt-maize pollen deposited on milkweed (*Asclepias* spp.) leaves compared to those reared on leaves dusted with non-transformed maize pollen or on leaves without pollen (Losey et al., 1999). Larvae of the common species, *Pieris brassicae*, *Pieris rapae* and *Plutella xylostella* also fed less, grew more slowly and showed a higher mortality when they ingested food plant material containing pollen of maize Bt176, compared to larvae of an untreated control group (Felke et al., 2002).

Toxicity of pollen from maize Bt176 has also been tested on butterfly species of conservation concern in some EU Member States, such as the common swallowtail (*Papilio machaon*) and the peacock butterfly (*Inachis io*). Lang and Vojtech (2006) reported a lower survival rate of larvae of *P. machaon*, exposed to the highest levels of Bt-maize pollen densities (event Bt176) that might be experienced under field conditions. The ingestion of Bt-maize pollen led to reduced plant consumption, lower body weight, longer development time of larvae, and smaller wing size as adults. Felke and Langenbruch (2005) revealed that the ingestion of a small number (10) of pollen grains of maize Bt176 reduced the larval speed development of *I. io* and resulted in a significant reduction in average weight, as compared to individuals that received pollen from non-Bt-maize. However, pollen of MON810 showed no effect on mortality on larvae of *P. xylostella*, which is known to be a more sensitive species to Cry1Ab (Felke and Langenbruch, 2005).

Besides the assessment of the impact of Bt-maize pollen on Lepidoptera, an exposure assessment is needed for assessing potential risks for a given lepidopteran species. An extensive study of field experiments conducted in the US reported that the risk of Bt-maize pollen to monarch butterfly populations is likely to be negligible for maize MON810 (Hellmich et al., 2001; Oberhauser et al., 2001; Pleasants et al., 2001; Sears et al., 2001;

Stanley-Horn et al., 2001; Oberhauser and Rivers, 2003; Wolt et al., 2003). Lethal and sublethal effects were only observed when monarch butterfly larvae consumed a very high number of maize MON810 pollen (Sears et al., 2001; Stanley-Horn et al., 2001; Dively et al., 2004). Because the proportion of the monarch butterfly larvae population exposed to toxic levels of Bt-pollen is small (e.g., due to the lack of temporal overlap between larval development and pollen shed (Oberhauser et al., 2001)) and the amount of toxin contained in maize MON810 pollen is low as compared to maize Bt176 (Hellmich et al., 2001), it was concluded that impacts on *D. plexippus* populations are negligible (Sears et al., 2001; Dively et al., 2004). Pollen concentrations exceeding the toxicity level mainly occur on leaf surfaces in Bt-maize fields and within 1-3m of the edge of the Bt-maize field (Jesse and Obrycki, 2000; Pleasants et al., 2001; Zangerl et al., 2001; Wolt et al., 2003; Dively et al., 2004; Lang et al., 2004; Felke and Langenbruch, 2005), whilst susceptibility to the Bt-toxin declines with older instars (Hellmich et al., 2001; Felke et al., 2002). Even though Dively et al. (2004) detected a higher mortality and a decreased fitness to monarch larvae consuming MON810 pollen in laboratory and semi-field tests, these sublethal effects on the monarch population due to long-term exposure to Bt-maize pollen were considered small (~0.6% to 2.5%) by the authors and much lower than those attributed to natural variability.

Decreased larval feeding and weight of monarch butterfly larvae have been reported after exposure in the laboratory to a high density of Cry1Ab-expressing anthers (MON810) as compared to larvae exposed to milkweed leaf disks with no anthers or non-Bt-anthers (Hellmich et al., 2001; Anderson et al., 2004, 2005). However, an examination of anthers in and near maize fields showed that toxic levels of anthers rarely occur under normal field conditions, so that exposure of monarch butterflies to toxins from intact anthers from Bt-maize alone or in combination with pollen from Bt-maize is likely to be very low (Anderson et al., 2004). Although Anderson et al. (2004) and Prasifka et al. (2007) reported a reduction in feeding and weight gain due to behavioural changes under laboratory conditions, a point that still remains to be explained is how this change might translate to the field. Under field conditions early instar larvae, which are most susceptible to the Cry1Ab protein, are less exposed, as they mainly feed on the upper third of milkweed plants where the lowest densities of anthers occur (Pleasants et al., 2001; Anderson et al., 2004). In addition, larvae can move to the underside of leaves where they would avoid any contact with anthers (Pleasants et al., 2001; Jesse and Obrycki, 2003).

The extrapolation of observations made on certain non-target lepidopteran species to others remains difficult due to the variability in acute sensitivity among lepidopteran species to the Cry1Ab protein (as determined in artificial diet studies reported in Wolt et al., 2003) and due to the different biology among lepidopteran species. In addition, there are few studies on the distribution and hence the exposure of European lepidopteran species in agricultural landscapes on a population level (Schmitz et al., 2003; Anonymous, 2006; Gathmann et al., 2006a,b). In this respect, a 3-year field study performed in Germany, revealed no difference in abundance of larvae of the lepidopteran species *P. rapae* and *P. xylostella* between the Bt-based treatment (event MON810) and control treatment on weed strips artificially sown in maize field plots (Gathmann et al., 2006b). Although 7 other lepidopteran species were observed in the study, their low abundance did not enable suitable statistical analysis. Studying all lepidopteran species that could be potentially exposed to Bt-maize pollen is difficult in practice, especially if potential small effects are to be detected (Lang, 2004; Gathmann et al., 2006b) against a wide range of existing environmental and agronomic stressors currently influencing lepidopteran populations (Aviron et al., 2006; Gathmann et al., 2006b).

2.3.3.3. Conclusion

The GMO Panel concludes that no new scientific data regarding exposure of non-target lepidopteran species to maize MON810 were presented in the Austrian report that would alter the environmental risk assessment on lepidopteran species of this event.

2.3.4. Interactions of the GM plant with non-target organisms: entomofauna

2.3.4.1. Austrian claims

The Austrian report states that “*for all kinds of Cry1A(b) expressing GM maize varieties the overall mean abundance of non-target invertebrates was significantly lower for the GM-varieties compared to control of non-GM maize varieties which were not treated with insecticides. This form of comparison is relevant to assess any differences of the potential cultivation of GM maize MON810 with conventional varieties grown under conditions of organic agriculture and reduced insect management by insecticides. These management practices are favoured by the Austrian national plans for implementation of eco-friendly agriculture. Specific additional funding to farmers in Austria is connected to application of such management practices*”.

2.3.4.2. Effects on entomofauna

Nine years of experience of Cry1Ab maize cultivation in Spain revealed no adverse effects on non-target arthropods (de la Poza et al., 2005; Pons et al., 2005; Eizaguirre et al., 2006; Farinós et al., 2008). Two different field studies in which the potential impact of Bt-maize (event Bt176) on predatory arthropods was studied over at least 3 consecutive years in Spain did not show significant differences in predatory arthropod abundance among Bt-maize and the isogenic counterpart, though their abundance varied between years and sites (de la Poza et al., 2005; Eizaguirre et al., 2006). Focussing on effects of Bt-maize in species richness, diversity and seasonal phenology of ground-dwelling arthropods, Farinós et al. (2008) reported that no significant differences among the most abundant arthropod groups (e.g., spiders, ground beetles, rove beetles) could be attributed to Bt-maize. Both Pons et al. (2005) and Eizaguirre et al. (2006) showed that Cry1Ab expressing maize did not have an adverse impact on non-target pest species in the field: overall, more aphids and leafhoppers were found in Bt-maize fields as compared to non-Bt-maize fields, whilst numbers of cutworms (*Agrotis segetum*) and wireworms (larvae of click beetle *Agriotes lineatus*) remained similar.

In a field monitoring study performed in Germany from 2000 to 2005, field pairs (half-fields) planted with Bt-maize (event MON810) and a conventional maize variety were followed to determine densities of arthropod taxa on plants, activity densities and diversity of ground-dwelling arthropods (Schorling and Freier, 2006). Density comparisons of different taxa (such as aphids, thrips, heteropterans, aphid specific predators, spiders and carabids) revealed a few significant differences for specific taxa between Bt and conventional maize fields, but no general tendencies over the 6 years. No effects due to the growing of maize MON810 on non-target communities including lepidopteran larvae were observed during a field study performed in Germany over 3 consecutive years (Gathmann et al., 2006b; Eckert et al., 2006; Toschki et al., 2007). In another study, monitoring of foliage-dwelling spiders was carried out in Bt-maize fields and adjacent margins over 3 successive years in Germany (event Bt176) as compared to non-Bt-maize fields. Results did not reveal consistent adverse effects on

individual numbers, species richness and guild structure of spiders due to the cultivation of Bt-maize (Ludy and Lang, 2006a). Ludy and Lang (2006b) also reported that web-building spiders such as the garden spider (*Araneus diadematus*) can be exposed to and thus ingest high amounts of Bt-maize pollen via recycling of pollen-dusted webs. However, a laboratory study showed that the garden spider is not affected in its weight, survival, moult frequency, reaction time and various web variables following consumption of high amounts of Bt-maize pollen.

Results of a meta-analysis of 42 independent field experiments carried out across different continents by Marvier et al. (2007) indicated that non-target invertebrates are generally more abundant in near-isogenic control fields where no insecticide treatments are applied, than in fields cropped with Bt-cotton or Bt-maize (events MON810, Bt176 and MON863) mainly due to a lower abundance of Bt-susceptible (target) pest species, which are prey/hosts for natural enemies. However, when non-Bt-cotton or maize fields are managed conventionally with the application of insecticides, non-target taxa were shown to be less abundant than in fields cropped with Bt-cotton or maize.

A more recent meta-analysis of published field studies on non-target effects of Bt-crops made the differentiation among functional guilds of non-target arthropods. The abundance of predators, parasitoids, omnivores, detritivores and herbivores was compared under scenarios where neither, only the non-Bt-crops, or both Bt and non-Bt-crops received insecticide treatments. Different effects of Bt-maize on functional guilds of non-target arthropods were shown (Wolfenbarger et al., 2008). As expected, fewer specialist parasitoids of the target pest occurred in Bt-maize fields, as compared to unsprayed non-Bt-controls, but no significant reduction was detected for other parasitoids. In comparison to sprayed non-Bt-controls, numbers of predators and herbivores were higher in Bt-crops, with the magnitude of the difference being influenced by the type of insecticide. Due to reductions of their predators in sprayed non-Bt-maize, omnivores and detritivores were more abundant in insecticide-treated controls. However, no differences in abundance were found when both Bt and non-Bt-crops were sprayed. Predator-to-prey ratios were unchanged by either Bt-crops or the use of insecticides; ratios were higher in Bt-maize relative to the sprayed non-Bt-control. These data indicate that a decreased abundance of some target and non-target invertebrate taxa in maize agro-ecosystem might be observed in areas of cultivation where no alternative pest control measures are adopted. However, the use of and type of insecticides influence the magnitude and direction of observed effects, and insecticide effects were reported to be larger than those of Bt-crops. Therefore, specific pest control practices on conventional maize in the area of GMO deployment would need to be evaluated in order to evaluate the relative effects of maize MON810.

2.3.4.3. Conclusion

In conclusion, the GMO Panel considers that there is no new evidence that indicates a specific risk to non-target organisms linked to the maize agro-ecosystem in Austria.

2.3.5. Interactions of the GM plant with non-target organisms: predators

2.3.5.1. Austrian claims

The Austrian report states that “*Bt toxin exposition of non-target organisms of higher trophic levels like predators has been documented in recent studies. Any large-scale application of*

GM maize MON810 would thus lead to exacerbated effects on specific natural enemies of maize pest insects”.

2.3.5.2. Trophic chain effects on predators

Invertebrate predators can be exposed to the Cry1Ab protein not only by feeding on plant material or on honeydew excreted from sap-sucking species, but also by feeding on their prey organisms that have previously fed on Bt-maize (Romeis et al., 2008a,b). Harwood et al. (2005), cited by Austria, studied exposure to the Cry1Ab toxin (event Bt11) for certain groups of non-target organisms, namely Diptera, Hymenoptera, Coleoptera (including predatory Coccinellidae), Hemiptera, Homoptera, Neuroptera, Heteroptera (including herbivore species), Orthoptera, Collembola, Lepidoptera, Dictyoptera and Araneae. The authors reported levels of Bt-toxin observed within non-target herbivores and their natural enemies such as spiders and predatory insects under field conditions, showing that significant quantities of the Cry1Ab protein can move into higher trophic levels. Similarly, Obrist et al. (2006a) investigated the transmission of the Cry1Ab protein through the food chain and thus the exposure of predatory species to the Cry1Ab toxin (event Bt176). These studies showed that the Cry1Ab protein from Bt-maize passed along trophic chains up to the third trophic level, and that in some cases it accumulated in concentrations that were higher than in leaves. The Cry1Ab protein was detected in certain predators (such as *Orius* spp., *Chrysoperla* spp. and *Stethorus* sp.), whilst its presence was negligible in others (e.g., hemerobiids, *Nabis* sp., *Hippodamia* sp., *Demetrias* sp.). Another tritrophic study performed by Obrist et al. (2006b) not only confirmed protein uptake by larvae of the green lacewing, *Chrysoperla carnea*, via its herbivore preys, *Tetranychus urticae* and *Spodoptera littoralis*, after Bt-maize consumption (see also Dutton et al., 2002), but also confirmed maintenance of the biological activity of the Cry1Ab protein after ingestion by both herbivore species. Harwood et al. (2007) showed the presence of the Cry1Ab protein in gut samples of certain predatory coccinellids (e.g., *Coleomegilla maculate*, *Harmonia axyridis*, *Cycloneda munda*, *Coccinella septempunctata*). The fact that the presence of the Cry1Ab protein was not always confined to periods of anthesis suggested that tritrophic linkages in the food chain facilitated the transfer of the Cry1Ab protein into higher order predators.

The uptake of the Cry1Ab protein by predators will not only occur by direct feeding on Bt-expressing plant material (such as pollen), but also indirectly through the consumption of arthropod prey that contains the Bt-protein, especially for species preying on spider mites (e.g., Andow et al., 2006; Romeis et al., 2008a,b). Spider mites were shown to contain the highest amounts of the Cry1Ab protein (on average 5.56µg toxin per fresh weight; *Tetranychus urticae*) when kept on maize event Bt11, compared to thrips (0.91µg; *Frankliniella tenuicornis*) and leafhoppers (0.20µg; *Zyginiidia scutellaris*) (Dutton et al., 2004). The exposure to the Cry1Ab protein might be thus very different between predatory taxa due to variability in phenology and feeding habits.

Potentially toxic effects on predators fed with preys containing levels of the Cry1Ab protein might occur when predators are sensitive to the protein. However, direct toxic effects on predators are unlikely due to the specific toxicity of the Cry1Ab protein to Lepidoptera. Based on the current literature, Romeis et al. (2006) suggested that there are little or no indications of direct adverse effects of Cry1Ab expressing maize on natural enemies. Hence, several studies confirm that the Cry1Ab protein is not toxic to non-target organisms less closely related to targeted pests. Meissle et al. (2005) related the adverse effects on the

generalist predator, *Poecilus cupreus*, fed *S. littoralis* larvae, which had been raised on Bt-maize (event MON810) to the nutritional quality of the prey and not to the direct effect of the Cry1Ab protein. In another study, the presence of Cry1Ab in both prey *T. urticae* and ladybird *Stethorus punctillum* predator collected from commercial fields of maize MON810 had neither an adverse effect on survival of the predator, nor on the developmental time through to adulthood. Furthermore, no subsequent effects on ladybird fecundity were observed (Alvarez-Alfageme et al., 2008).

Hilbeck et al. (1998a,b, 1999) indicated significantly prolonged larval development and increased mortality when *C. carnea* larvae were fed lepidopteran larvae reared on Cry1Ab expressing maize under laboratory conditions. However, key experiments on what caused the significantly higher mortality in Bt-exposed lacewings larvae in these studies are still missing to date. Because Rodrigo-Simón et al. (2006) reported that the Cry1Ab protein does not show specific binding *in vitro* to brush border membrane vesicles from the midgut of *C. carnea* larvae, which is a prerequisite for toxicity, the higher mortality is likely to be a consequence of the lepidopteran prey apparently being of lower nutritional quality (Romeis et al., 2004, 2006). This conclusion is supported by data showing that *C. carnea* larvae are unaffected when feeding on non-susceptible *T. urticae* containing large amounts of biologically active Cry1Ab protein (Dutton et al., 2002). In addition, *C. carnea* larvae in the field are known to feed mainly on aphids, whereas lepidopteran larvae are not considered an important prey, especially after their first moult (Romeis et al., 2004). Because aphids do not accumulate the Cry1Ab protein (Head et al., 2001; Raps et al., 2001; Dutton et al., 2002), the risk they pose on *C. carnea* larvae can be regarded as negligible. Even though chronic effects cannot be excluded completely, the continuous exposure of *C. carnea* to diets exclusively based on lepidopteran larvae is unlikely under field conditions where a variety of prey is available (Canard, 2001; Dutton et al., 2003). In addition, Li et al. (2008) demonstrated that adults of *C. carnea* are not affected by Bt-maize pollen and are not sensitive to the Cry1Ab protein at concentrations exceeding those observed in pollen of Bt-maize.

2.3.5.3. Conclusion

The GMO Panel is of the opinion that the information and documents provided by Austria do not provide any new or additional scientific evidence that would invalidate the previous risk assessments of maize MON810 for the non-target organisms.

2.3.6. Interactions of the GM plant with non-target organisms: parasitoids

2.3.6.1. Austrian claims

The Austrian report states that “*Bt toxin exposition of non-target organisms of higher trophic levels like parasitoids has been documented in recent studies. Any large-scale application of GM maize MON810 would thus lead to exacerbated effects on specific natural enemies of maize pest insects*”.

2.3.6.2. Trophic chain effects on parasitoids

In general, invertebrate parasitoids appear to be more sensitive to diets that contain Cry proteins than predators (Lövei and Arpaia, 2005), though effects are possibly associated with the poor quality of their hosts. Parasitoids can be exposed to the Cry1Ab protein through one

or more trophic levels (e.g., their host organisms feeding on Bt-plant tissue). Indirect host-mediated effects were observed when effects of Bt-maize on the non-target lepidopteran herbivore, *S. littoralis*, and on the hymenopteran parasitic wasp, *Cotesia marginiventris*, were investigated. *C. marginiventris* survival, developmental times and cocoon weights were significantly adversely affected when their *S. littoralis* host larva had been fed Bt-maize. Because *S. littoralis* larvae are significantly affected by the Cry1Ab expressing maize in terms of development time and survival (e.g., Dutton et al., 2002, 2005; Vojtech et al., 2005), it is likely that these slower developing hosts might not provide sufficient nutrients for the normal development of parasitoid larvae. Even though direct effects to parasitoid larvae cannot be excluded, as host larvae contained the Cry1Ab protein, these direct toxic effects seemed unlikely due to the specificity of the Cry1Ab protein (Vojtech et al., 2005). However, Ramirez-Romero et al. (2007) suggested that the Cry1Ab protein present in the host, *Spodoptera frugiperda*, fed Bt-maize may have a direct effect on *C. marginiventris*. They observed that the exposure to Cry1Ab protein via hosts fed Bt-maize tissue affected parasitoid developmental times, adult size, and fecundity, but not cocoon-to-adult mortality and sex ratio. These effects occurred even when concentrations of the Cry1Ab protein were low in hosts. The fact that *C. marginiventris* females were smaller and less fecund when fed Cry1Ab containing hosts as compared to conventional maize, led the authors to suggest a direct effect of the Cry1Ab protein, though effects on parasitoids of direct exposure to the Cry1Ab protein were not studied (Ramirez-Romero et al., 2007). The authors stated that they were also able to demonstrate the importance of the plant in causing negative effects at the third trophic level, since no negative results were observed when pure protein-containing diet was used in the tritrophic experiments. However, the specific toxicity of the purified Cry1Ab protein batch used by Ramirez-Romero et al. (2007) was not measured and compared to the effectiveness of Bt-plant material to susceptible target organisms. The effectiveness of different purified Cry1Ab batches can vary considerably from source to source by a factor of 10 (Saeglitz et al., 2006), so that the influence of host quality can not be excluded in the Ramirez-Romero et al. (2007) study.

By contrast, the performance of *C. marginiventris* feeding on aphid honeydew was observed to increase due to positive effects of Bt-maize (events Bt11, MON810 and Bt176) on the performance of the maize leaf aphid, *Rhopalosiphum maidis* (Faria et al., 2007). Even though aphid performance was within the normal variation observed among conventional maize varieties, different studies reported that aphids perform better on Bt-maize than on near isogenic counterparts (e.g., Bourguet et al., 2002; Dutton et al., 2002; Lumbierres et al., 2004; Pons et al., 2005; Eizaguirre et al., 2006). With the larger colony densities of aphids on Bt-maize, more honeydew was produced, in turn increasing parasitoid longevity and rate of parasitism. Based on the observations made, Faria et al. (2007) concluded that as long as aphid numbers do not reach pest status, the increase in Bt-maize susceptibility to aphids may pose an advantage in maintaining beneficial insect fauna in Bt-maize. Because phloem sap of Cry1Ab expressing maize does not contain the Cry1Ab protein, the protein is not ingested or excreted by sap-sucking species (such as *R. maidis* and *Rhopalosiphum padi*) (Head et al., 2001; Raps et al., 2001; Dutton et al., 2002). Parasitoid species feeding on honeydew excreted by sap-sucking species are thus not likely to be exposed to the Cry1Ab protein (Romeis et al., 2008b).

Field studies have revealed that parasitoid abundance is generally similar in Bt and non-Bt-maize with a few exceptions as referenced in the review of Romeis et al. (2006). Fewer specialist parasitoids of the target pest occur in Bt-maize fields compared to unsprayed non-

Bt-controls, but no significant reduction has been detected for other parasitoids (Wolfenbarger et al., 2008).

2.3.6.3. Conclusion

The GMO Panel is of the opinion that the information and documents provided by Austria do not provide any new or additional scientific evidence that would invalidate the previous risk assessments of maize MON810 for the non-target organisms.

2.3.7. Interactions of the GM plant with non-target organisms: Hymenoptera

2.3.7.1. Austrian claims

The Austrian report states that “*significant effects of Cry1A(b) toxin expressing GM maize are found specifically for hymenoptera*”.

2.3.7.2. Effects on hymenoptera

In the absence of references to specific studies from Austria, the GMO Panel focuses on honeybees, the most studied hymenopteran insect in relation to the toxicity of the Cry1Ab protein. Maize pollen can be collected, stored and consumed by honeybees, especially in regions where there are limited sources of pollen when maize is flowering. Pollen feeding is a route of exposure of honeybees to Cry1Ab toxin expressed in maize MON810, and potential adverse effects have been considered in previous scientific opinions of the GMO Panel.

Reviewing available scientific data on potential adverse effects on honeybees of the Cry1Ab toxin or Bt-pollen of maize gathered either under laboratory or semi-field conditions, Malone (2004) concluded that none of the Bt-plants commercially available at the time of the publication have significant impacts on the health of honeybees. Other feeding studies performed in controlled conditions with honeybees being fed either with Bt-pollen or mixtures of honey or sugar syrup containing purified δ -endotoxin have indicated no direct adverse effects on larvae and adult survival (Malone and Pham-Delègue, 2001; Ramirez-Romero et al., 2005, 2008; Rose et al., 2007). Based on a meta-analysis of 25 independent laboratory studies assessing direct effects on honeybee survival of Cry proteins from currently commercialised Bt-crops, Duan et al. (2008) concluded that the assessed Cry proteins do not negatively affect the survival of either honeybee larvae or adults in laboratory settings. However, Duan et al. (2008) considered that in field settings, honeybees might face additional stresses, which could theoretically affect their susceptibility to Cry proteins or generate indirect effects.

Since exposure to Bt-pollen could have potential indirect adverse effects on the development of the whole honeybee colony, some studies focused on the hypopharyngeal gland development in honeybees. Hypopharyngeal glands are considered an important indicator of bee life history and thus for colony development, as worker (nurse) bees use their hypopharyngeal gland to prepare brood food (jelly) for the larvae. In this respect, Babendreier et al. (2005) fed young adult bees for 10 days with Bt-maize pollen expressing Cry1Ab toxin (event MON810) or with purified Cry1Ab toxin solubilized in sugar solutions. No significant differences either in diameter or weight development of hypopharyngeal glands of control bees and bees fed Bt-pollen or Bt-containing sugar solutions were found. By contrast,

protease inhibitors caused significant differences which indicated the sensitivity of the method.

In a field study where colonies foraged on Cry1Ab expressing maize (event Bt11) and were fed Bt-pollen cakes for 28 days, Rose et al. (2007) did not observe adverse effects on bee weight, foraging activity, and colony performance. Similarly, in a flight cage study maintained in controlled conditions, no significant differences were reported in honeybee mortality, syrup consumption and olfactory learning performance when honeybee colonies were exposed to different syrups containing Cry1Ab protoxin (Ramirez-Romero et al., 2005). In this respect, Ramirez-Romero et al. (2008) recently concluded that negative effects of the Cry1Ab protein on foraging behaviour and olfactory learning performance of honeybees are unlikely in natural conditions. Feeding behaviour and olfactory learning performance were disturbed only when honeybees were exposed to extremely high concentrations of Cry1Ab protein (5000ppb), which do not occur under normal apicultural or field conditions (Ramirez-Romero et al., 2008).

As pollen shedding in a given maize field usually takes place for approximately 10 days each season, potential bee exposure to pollen from maize MON810 will be limited under normal apicultural conditions. In most cases, the proportion of maize pollen as a total of all pollen collected and fed to larvae during a summer will be low. Babendreier et al. (2004), for instance, reported that fully grown worker bee larvae contain between 1720 and 2310 maize pollen grains in their gut before defecation, corresponding to 1.52-2.04mg of pollen consumed per larva. On average, 74.5% of pollen grains were completely digested, while 23.3% were partially digested and 2.2% remained undigested. Since pollen consumption of honeybee larvae is minimal when compared to adults, larval stages are far less exposed to Bt-toxins: Babendreier et al. (2004) indicated that the contribution of the protein by directly feeding larvae with pollen is less than 5% in relation to the total amount of protein necessary for complete larval development. Moreover, due to the low concentration of Cry1Ab in MON810 pollen, honeybees will only be exposed to very low concentrations of the toxin.

2.3.7.3. Conclusion

The GMO Panel considers that the low exposure level of Cry1Ab containing pollen combined with its low toxicity is unlikely to result in any adverse effects on honeybees under normal apicultural conditions. In addition, available scientific evidence gathered from laboratory and semi-field studies does not demonstrate impacts of maize MON810 pollen on honeybees. Claims and documents provided by Austria do not provide any new or additional scientific evidence that would invalidate previous risk assessments of maize MON810.

2.3.8. Interactions of the GM plant with non-target organisms: water-dwelling insects

2.3.8.1. Austrian claims

In its report, Austria states that “*any undesired impairment of aquatic habitats should be avoided and that the potential effect of GM maize MON810 on these ecosystems has to be regarded as relevant. According to new scientific results, aquatic non-target organisms, like trichoptera are likely to be negatively affected*”.

2.3.8.2. Effects on water-dwelling organisms

Based on findings reported by Rosi-Marshall et al. (2007), Austria expressed concerns about the transport of Bt-maize byproducts (e.g., pollen, detritus) to downstream water bodies and their potential toxic effects on non-target aquatic organisms following consumption.

Rosi-Marshall et al. (2007) reported that byproducts of Cry1Ab expressing maize entered headwater streams and claimed that this would reduce growth and increase mortality of some non-target stream insects such as Trichoptera. This study quantified maize biomass (Bt or non-Bt) in headwater streams, measured degradation rates in aquatic systems, but found no difference between Bt and non-Bt-maize plant material. Concentrations of the Cry1Ab protein in leaves and pollen were not measured, so no dose-response relationship with the Bt-protein can be made. It is thus unclear how the degradation rate of the Bt-protein is related to that of plant material. In addition, the identity of the Bt-maize event used in the feeding test is not clear and no isogenic controls to compare with the GM material were used. Also, there is no detailed information given on the amount of maize material fed to test organisms, and effects reported are relatively minor in comparison with known toxic chemicals. Finally, there is no information on reproducibility of the feeding test.

2.3.8.3. Conclusion

The GMO Panel considers that important background information on levels of exposure and plant material used is missing and that the conclusions made by Rosi-Marshall et al. (2007) are not supported by the data presented in the paper. Similar views were also expressed by ACRE (2007), Beachy (2008) and Parrott (2008).

It could be concluded that a potential hazard for Trichoptera has been identified under laboratory conditions when exposed to high doses of Cry toxins. However, due to the low level of Cry toxins in aquatic systems reported by Douville et al. (2005), exposure of Trichoptera in aquatic ecosystems is likely to be extremely low (Chambers et al., 2007), so that the GMO Panel considers it unlikely that Bt-toxins in maize MON810 products would cause adverse effects to Trichoptera populations in water courses.

2.3.9. Interactions of the GM plant with non-target organisms: soil organisms

2.3.9.1. Austrian claims

In its report, Austria is of the opinion that *“a range of information related to risks to soil organisms and soil function through degradation of GM plant material was left unconsidered in previous assessments”* and concludes that *“evidence is available to show that the risk for soil organisms (such as symbiotic fungal communities, earthworms, nematodes, isopods, collembolans) is a relevant under regional Austrian conditions. Since healthy soils are a prerequisite for low-input agriculture and organic agriculture, the potential effects of the cultivation of GM maize MON810 are likely leading to a negative impact on soil quality”*.

2.3.9.2. Persistence of Bt-proteins in soil

In order to assess the potential adverse impact of Bt-crops on soil organisms, both exposure and sensitivity of non-target soil organisms to the Cry1Ab protein need to be established. It is

well-documented that during plant growth Bt-maize can contribute to the presence and persistence of plant-produced Cry proteins in soil via root exudation (e.g., Saxena et al., 2002, 2004). A second route for potential accumulation and persistence of Bt-proteins in soil relates to dead plant material remaining in fields after harvest which is incorporated into the soil during tillage operations (Stotzky, 2004).

The persistence of the Cry1Ab protein in soil is dependent upon multiple factors, varying among different environmental conditions (e.g., type of crop, soil characteristics, microbial activity, temperature) and the method used for quantification of the protein. In a recent review paper, Icoz and Stotzky (2008) discuss the variability in persistence of the Cry1Ab protein in soils. Half-lives (the time until the amount of a substance remaining is 50% of the original amount) of the Cry1Ab protein ranged from 1.6 days in a soil amended with biomass of Bt-maize (Sims and Holden, 1996) up to 34 days in soil amended with biomass of and planted to Bt-rice (Wang et al., 2006). Schrader et al. (2008) observed a strong decline of immunoreactive Cry1Ab in plant residues of maize MON810 in microcosm experiments: after 5 weeks, in leaf material, it was reduced to 14.1% and in root material to 12.8% of the initial concentration, which was approximately 5µg/g.

Although Bt-toxins are degraded or inactivated in soil within weeks, a small fraction can persist far longer under certain conditions. Laboratory studies have shown that the Cry1Ab protein can bind on clay minerals and humic substances in soil, thereby reducing its availability to microorganisms. This reduced availability decreases degradation of the Cry1Ab protein, so the insecticidal activity is retained during the growing season (e.g., Tapp et al., 1994; Tapp and Stotzky, 1995; Crecchio and Stotzky, 2001). In this respect, Zwahlen et al. (2003a) showed that the Cry1Ab protein is still detectable in decaying maize material after a soil exposure in litter bags for 200-240 days. Cry1Ab protein in low concentrations was detected for up to 56 days in soil amended with purified protein or biomass of Bt-cotton (Donegan et al., 1995), 234 days in soil amended with purified protein (Tapp and Stotzky, 1998) or for up to 180 to 350 days in soil amended with biomass of or planted to Bt-maize residues of Bt-maize (Saxena and Stotzky, 2002). Stotzky (2004) reported that Cry1Ab protein released in root exudates and from biomass of Bt-maize persisted in low concentrations in soil microcosms for at least 180 days and 3 years, respectively.

The potential accumulation of plant-produced Cry1Ab proteins in soil following repeated and large-scale cultivation of Bt-maize has been studied. Unbound Cry1Ab protein was recorded in soil during 4 consecutive years of Bt-maize cultivation, and no accumulation was observed (Icoz et al., 2008). In addition, Baumgarte and Tebbe (2005) and Andersen et al. (2007) reported that concentrations of the Cry1Ab protein found in soil were higher in a given season for plots planted with varieties derived from the maize MON810 in comparison with non-Bt-maize varieties, but concentrations did not seem to increase from year to year. Hopkins and Gregorich (2003, 2005) and Dubelman et al. (2005) also reported that Cry1Ab proteins from GM plants do not persist in soil 3 months after harvest, and they found no evidence of accumulation of the Cry1Ab protein in soil from fields planted for at least 3 consecutive years with Bt-maize, regardless of soil type, geographic regions and climatic conditions (Dubelman et al., 2005). Despite the fact that Cry proteins can bind rapidly on clay minerals and humic substances, there is no evidence for accumulation of the Cry1Ab protein in soils in the field, even after 3 years of continuous cultivation of Bt-crops (e.g., Baumgarte and Tebbe, 2005; Marchetti et al., 2007; Hönemann et al., 2008).

Reviews of the literature indicate that possible exposure of non-target soil organisms to Cry1Ab protein is likely to be variable and case-specific. In an assessment of environmental risks, exposure has to be combined with a hazard assessment. In this respect, the focus of the GMO Panel is on the assessment of the susceptibility of non-target soil fauna to the Cry1Ab protein, effects on microorganisms and impacts on soil organism diversity and functions. These aspects are discussed in the following sections.

2.3.9.3. Microbiological effects in soil

Due to the close interaction between crops and microbe-mediated soil processes, soil organisms in the rhizosphere are likely to be exposed to the Cry1Ab protein released from Bt-maize as root exudates. Some studies demonstrated consistent significant differences in relation to microorganisms between soils with Bt and non-Bt-maize. Root exudates of Bt-maize (event Bt176) were shown to reduce presymbiotic hyphal growth of the arbuscular mycorrhizal fungus, *Glomus mosseae*, as compared with those of another Bt-maize (event Bt11) and control maize (Turrini et al., 2004). Castaldini et al. (2005) also reported consistent differences in rhizosphere heterotrophic bacteria and mycorrhizal colonization (including *G. mosseae*) between Bt-maize (event Bt176) and its conventional counterpart. According to the authors, the genetic modification in maize Bt176 might have led to changes in plant physiology and composition of root exudates, which in turn may have affected symbiotic and rhizosphere microorganisms. In this respect, Widmer (2007) suggested that effects observed on symbiotic microorganisms will only be disadvantageous for the crop itself, without representing a concern for the ecosystem. In addition, a number of other studies (reviewed by Widmer, 2007; Filion, 2008; Icoz and Stotzky, 2008), performed under laboratory, glasshouse or field conditions covering a large array of classical and more recent analytical tools, revealed only some minor changes in soil microbial community structure with Bt-maize compared to non-Bt-maize (Blackwood and Buyer, 2004; Brusetti et al., 2004; Griffiths et al., 2006; Mulder et al., 2006) or generally show no adverse effects of the Cry1Ab protein released by Bt-maize in root exudates or from biomass incorporated into soil microorganisms or microorganism-mediated processes (Saxena and Stotzky, 2001a; Flores et al., 2005; Anonymous, 2006; Hönemann et al., 2008; Icoz et al., 2008). Where effects 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 and soil type (Fang et al., 2005, 2007; Griffiths et al., 2005, 2006; Lilley et al., 2006; Filion, 2008; Icoz and Stotzky, 2008). Factors such as plant growth stage and field heterogeneities produced larger effects on soil microbial community structure than maize MON810 (Baumgarte and Tebbe, 2005; Griffiths et al., 2007b).

Mulder et al. (2006) reported short-term effects of maize MON810 which induced ecological shifts in microbial communities of cropland soils in laboratory tests. However, differences in agronomic and compositional characteristics between the tested Bt-maize and the near isogenic comparator may have caused the shift in microbial communities, so that no conclusions on the impact of the genetic modification can be made. Microbial activity could have been mainly affected by, for instance, sugar content (Biavati and Sorlini, 2007) rather than the Cry1Ab protein. Percentage differences in sugar content were relatively higher than those observed in levels of the Cry1Ab protein. The highly enhanced soil respiration reported during the first 72 hours after the addition of Bt-maize residues in Mulder et al. (2006) can be interpreted as being related to the presence of other macronutrient crop residues. However, 3 weeks after the addition of the maize residues to the soil, no differences were detected

between the activity of specific bacterial guilds in soils amended with transgenic maize and in soils amended with conventional maize.

Studies in which the decomposition of Bt-maize was compared with that of non-Bt-isogenic lines mostly showed that Cry1Ab expressing maize does not affect decomposition rate or mass of carbon remaining over time (e.g., Cortet et al., 2006; Tarkalson et al., 2008). Litter-bag experiments with Bt-maize (Bt11) reported by Zwahlen et al. (2007) did not reveal major changes in the decomposition rate of Bt-maize residues. Similarly, various studies on maize MON810 found no evidence of effects related to the genetic modification when examining the decomposition rate of Bt-maize (Griffiths et al., 2007b; Hönemann et al., 2008; Lehman et al., 2008; Tarkalson et al., 2008). These recent findings confirm that previously reported decreases in decomposition rate (e.g., Saxena and Stotzky, 2001b; Flores et al., 2005; Fang et al., 2007; Raubuch et al., 2007) do not result from an inhibition of soil microorganisms by the Cry1Ab protein, but more likely from increased lignin contents in certain maize varieties. Altered lignin content in maize varieties has been shown not to be a generic effect of the *cry1Ab* gene insertion (Griffiths et al., 2007b).

2.3.9.4. Biological effects in soil

Multi-year experiments conducted with GM maize at 4 sites across 3 European climatic zones in the context of the EU-funded ECOGEN project (Andersen et al., 2007; Krogh and Griffiths, 2007) showed that no or only few effects on snails, microarthropods or mycorrhizal fungi could be attributed to Bt-maize (event MON810) (Cortet et al., 2007; de Vaufleury et al., 2007; Griffiths et al., 2007a; Krogh et al., 2007). Field experiments revealed that Bt-maize could have a significant, but small and transient, effect on soil protozoa, nematodes and microorganisms (Griffiths et al., 2005, 2007a). Even though the presence of the Cry1Ab protein in snail faeces was identified as a novel route of exposure into the soil food web (de Vaufleury et al., 2007), no direct effects could be detected related to maize MON810 in mesocosm experiments. The ECOGEN experiments allowed for a comparison of results ensuing from different scales and for an assessment of their utility since the same organisms and soils were studied in laboratory, glasshouse and field. Although useful information and insights from each of the experimental approaches and scales were gathered, predicting outcomes to one scale from results obtained from another still remains difficult (Birch et al., 2007). Based on the ECOGEN analyses, the authors concluded that Bt-maize does not have adverse effects on soil biota, since effects observed were most likely to be caused by season, soil type, tillage, crop type or variety (Cortet et al., 2007; de Vaufleury et al., 2007; Griffiths et al., 2007a; Krogh et al., 2007). Similarly, effects on soil microbial community structure, microarthropods and larvae of a non-target root-feeding Dipteran (*Delia radicum*) observed in a glasshouse experiment were most likely due to soil type and plant growth stage, rather than Bt-maize (event MON810). Although statistically significant effects of Bt-maize on soil microfauna populations (e.g., overall increase in protozoa (amoebae) and nematode numbers) were observed, these effects were relatively small, especially when compared with effects of soil type, plant growth stage, insecticide application and variety (Griffiths et al., 2006, 2007b).

Several other studies did not show any consistent effect of Bt-maize on soil species. For example, in an 8 month field study consisting of litter-bag experiments with Bt-maize (Bt11), Zwahlen et al. (2007) did not detect major changes in the composition of the soil fauna community, collembolans, mites and annelids, during the experiment. Similar conclusions

were drawn by Hönemann et al. (2008) who observed similar meso and macrofauna soil communities between the tested maize varieties (including 2 varieties containing event MON810).

2.3.9.5. Effects on earthworms

Earthworms can be exposed to Cry1Ab protein through root exudates and decomposing plant material. However, laboratory and field studies performed on some earthworm species, such as *Aporrectodea caliginosa* (Vercesi et al., 2006; Schrader et al., 2008), *Eisenia foetida* (Clark and Coats, 2006) and *Lumbricus terrestris* (Saxena and Stotzky, 2001a; Zwahlen et al., 2003b; Schrader et al., 2008) did not reveal significant adverse effects on earthworm survival, growth and reproduction following Cry1Ab ingestion. The detection of the Cry1Ab protein in the gut and faeces of earthworms confirmed protein ingestion (reviewed by Icoz and Stotzky, 2008).

Based on laboratory experiments, Saxena and Stotzky (2001a) concluded that the uptake of the Cry1Ab protein (event MON810) by earthworms is of no safety concern, since no adverse effects on mortality or weight were observed on *L. terrestris* exposed to soil planted to or amended with plant material from Cry1Ab expressing maize after 40 or 45 days, respectively, compared to non-Bt-maize. However, as pointed by Clark et al. (2005), growth is probably not an appropriate assessment endpoint: individuals used by Saxena and Stotzky (2001a) were already mature, with fully developed clitella, and thus less likely to exhibit changes in growth. Zwahlen et al. (2003b) investigated mortality and growth of *L. terrestris* in laboratory and field experiments by exposing juveniles and adults to maize Bt11 (expressing the Cry1Ab protein) during a period of 200 days. Field experiments did not reveal any differences in growth rate between Bt-based and near isogenic maize material exposure. In laboratory experiments, the growth of adults, expressed as mean fresh weight, was similar for 160 days, but declined thereafter in Bt-exposed earthworms up to 200 days. Experimental conditions in the laboratory were quite different from those encountered under field conditions, and it is difficult to attribute this biological effect to the life stage, Cry protein or to unanticipated changes in plant characteristics that could have altered microbial composition in such confined soil samples. Moreover, earthworm reproductive activity was recorded, but not quantified and therefore it is not possible to make any inference on long-term effects on natural populations. Lower earthworm biomass could have been attributed to, for instance, differences in timing or production of cocoons in the Bt-maize treatment.

Laboratory toxicity studies, in which *E. foetida* were fed leaf material from Bt-maize (events Bt11 and MON810) or the isogenic counterpart in a soil system and monitored for 28 days, did not reveal adverse effects on survival or reproduction due to the ingestion of Bt-maize leaf material. However, differences in nutritional parameters of Bt-maize lines and isolines were anticipated to lead to differences in effects on earthworms (Clark and Coats, 2006).

Vercesi et al. (2006) studied effects of maize MON810 on important life-history traits (survival, reproduction and growth) of *A. caliginosa* under various experimental conditions. In a series of experiments, the authors investigated the growth of juveniles until maturity as well as cocoon production and hatchability. Finely ground leaves of maize MON810 added to soil had no adverse effects on these life-history traits in *A. caliginosa*, even if they were exposed to high worst-case scenario concentrations. In addition, growth of juvenile *A. caliginosa* was unaffected when they were kept in pots with a growing Bt-maize plant for 4

weeks. Only when considering cocoon hatchability, a slight, but statistically significant, negative effect of high concentration of finely ground Bt-maize residues was observed. However, due to the addition of high concentrations of finely ground Bt-maize residues, Vercesi et al. (2006) questioned whether the negative effect would have any ecological significance under field conditions. In experiments performed by Schrader et al. (2008), the 2 tested earthworm species, *A. caliginosa* and *L. terrestris*, survived incubation for 5 weeks, irrespective of whether they received MON810 or non-transgenic maize material.

Other papers (e.g., Krogh et al., 2007) confirmed that no effects on earthworms were detected in field surveys during the cultivation of Bt-maize expressing the Cry1Ab protein. No significant differences were reported in the population density or biomass of *Lumbricidae* between soils with Bt (events MON810 and Bt176) and non-Bt maize and between soils with maize treated with or without insecticide at 5 sites during 4 years of maize cultivation in field, though both the site and sampling years had a significant influence on both assessment endpoints (Anonymous, 2006).

2.3.9.6. Effects on nematodes

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 (Anonymous, 2006; Icoz and Stotzky, 2008).

A recent review on the effects of Bt-crops on soil ecosystems illustrated that, depending upon experimental conditions, the Cry1Ab protein might have different effects on nematodes (Icoz and Stotzky, 2008). Saxena and Stotzky (2001a) found no significant differences in the number of nematodes in the rhizosphere soil of Bt and non-Bt-maize grown in a plant-growth chamber or between soil amended with biomass of Bt and non-Bt-maize. An overall comparison of MON810 versus non-Bt-maize across 3 different field sites in different European regions revealed a significant, but transient, reduction in numbers of nematodes under Bt-maize as compared with non-Bt-maize (Griffiths et al., 2005). Nematode community structure was different at each site and the effect of Bt-maize was not confined to specific nematode taxa. The authors concluded that the effect of Bt-maize was small and within the normal variation range expected in the considered agricultural systems. In contrast, Griffiths et al. (2006) reported significantly higher nematode populations of *Acrobeloides* spp. and *Pratylenchus* spp. under Bt-maize (event MON810) than non-Bt-maize in a greenhouse study. There was an overall increase in nematode numbers under Bt-maize when all data were pooled, but no significant effect at any individual plant growth stage or in any particular soil type. The difference in environmental conditions in the greenhouse and the field might have affected interactions between plants and soil organisms (Griffiths et al., 2006; Birch et al., 2007). In addition, based on a glasshouse study involving 8 different paired varieties of maize (Bt – including event MON810 – and near-isogenic), Griffiths et al. (2007b) reported that (1) nematode abundance varied mainly between maize varieties, rather than between Bt and non-Bt maize, and that (2) differences in previously published soil nematode studies under Bt-maize were smaller than varietal effects.

Effects of Bt-maize (events MON810 and Bt176) on 2 nematode species, plant-parasitic *Pratylenchus* spp. and the bacteriovorous *Caenorhabditis elegans*, have also been studied in field trials in Germany (Anonymous, 2006). No adverse Bt-effects were observed with

respect to population density of *Pratylenchus* spp., whilst growth, number of eggs and reproduction rate of *C. elegans* were negatively affected. In a laboratory bioassay, Höss et al. (2008) studied potential toxic effects of the Cry1Ab protein on *C. elegans* either by exposing *C. elegans* to rhizosphere and bulk soil from experimental fields cultivated with Bt-maize (event MON810) or to different solutions of the Cry1Ab protein expressed in *Escherichia coli*. Nematode reproduction and growth were significantly reduced in rhizosphere and bulk soil of Bt-maize as compared with soil from isogenic maize, and were significantly correlated with concentrations of the Cry1Ab protein in soil samples. However, because concentrations of the Cry1Ab protein measured in soil samples from Bt-maize were low and not sufficiently high to produce direct toxic effects on *C. elegans* (see also Baumgarte and Tebbe, 2005), adverse effects on the reproduction and growth of *C. elegans* were assigned to indirect effects. Höss et al. (2008) concluded that further investigations are needed to assess whether there are potential indirect effects of the protein on reproduction and growth of *C. elegans* and to clarify the causes. Any observed effects would then have to be compared with other factors limiting populations such as cultivation and other fluctuations in the physical soil environment.

Experiments conducted in the context of the ECOGEN project showed that changes to nematode communities due to Bt-maize (event MON810) were small and transient, and smaller than those induced by seasonal, soil type, tillage, crop type or varietal effects (Griffiths et al., 2007a). Reduced abundance of nematodes was only observed at the field site in Denmark in October 2005 and not at the other sampling occasions. Rearrangements of nematode populations, which are normally associated to several sources of variation in the agricultural environment, occur frequently and are not necessarily an indication of environmental harm.

2.3.9.7. Effects on isopods

Woodlice (*Porcellio scaber*), considered a model decomposer organism, have been used in laboratory feeding studies for detecting potential adverse impacts related to exposure to plant material from Cry1Ab expressing maize. Exposure to and assimilation of the Cry1Ab protein by *P. scaber* were demonstrated by lower concentrations of the protein in faeces than in the consumed plant material (Wandeler et al., 2002; Pont and Nentwig, 2005). No adverse effects of the Cry1Ab protein on consumption, survival and growth of *P. scaber* were observed when fed plant material of Bt-maize expressing the Cry1Ab protein and non-Bt-maize (Escher et al., 2000). The survival and growth of *Trachelipus rathkii* and *Armadillidium nasatum*, 2 abundant isopods in maize growing regions, were not adversely affected after exposure to the purified Cry1Ab protein or leaves of Bt-maize (events Bt11 and MON810) under laboratory conditions for 8 weeks (Clark et al., 2006). Detected differences in mortality, weight gain and consumption by isopods and in digestibility of plant material were generally attributed to differences in the nutritional quality of maize varieties used (Escher et al., 2000; Wandeler et al., 2002; Pont and Nentwig, 2005; Clark et al., 2006).

2.3.9.8. Effects on Collembola

Because collembolans are important in the breakdown and recycling of crop residues, they are key indicator species of soil fertility and health. In general, no negative effects of the Cry1Ab protein on collembolans have been observed (reviewed by Icoz and Stotzky, 2008). The addition of 4 purified Bt insecticidal proteins (Cry1Ab, Cry1Ac, Cry2A, and Cry3A) at

concentrations of 200mg g⁻¹ to the diet of the collembolans, *Folsomia candida* and *Xenylla grisea*, for 21 days did not affect their survival or reproduction compared with the unamended diet (Sims and Martin, 1997). No deleterious effects on survival and reproduction of *F. candida* were observed when fed leaves of Bt-maize expressing the Cry1Ab protein compared with leaves of non-Bt-isolines (Clark and Coats, 2006). While Bakonyi et al. (2006) showed that Bt-maize was less preferred as food by *F. candida* than near-isogenic non-Bt-maize, this effect was not observed for *Heteromurus nitidus* and *Sinella coeca*. *F. candida* defecated 30% less around Bt-maize, but did not show preference to stay on any plant material. Preference was not linked to consumption, so the tendency to stay on the plant material was not linked to palatability. For well-fed *F. candida*, the consumption was 30% less on Bt-diet, but when they were starved, they indiscriminately consumed both diets. An interpretation of the study in toxicological terms relies on the value of an avoidance of toxic substances for predicting the toxic potential in a realistic field situation. Hitherto the Cry1Ab protein has not been shown to be toxic to Collembola. In addition to the presence of the assumed toxicant (cf., the Cry1Ab protein), there were differences in C/N ratio in the plant material. Such differences are common because Bt-maize is a F₁ hybrid and comparators are of similar hybrid origin or single lines and therefore not fully isogenic. Different varieties have been shown previously to elicit various responses related to their background genetic composition and not to the GM event or its products (Griffiths et al., 2007b). The different consumption of Bt-maize may be due to nutritional differences, as suggested by the C/N ratio. The study shows that *F. candida*, which responded with a lower consumption of the Bt-toxin, did not discriminate between the 2 diets under starved condition. Heckmann et al. (2006) reported that the growth and reproduction of the collembolan, *Protaphorura armata*, reared on ground roots of Bt-maize expressing the Cry1Ab protein were not significantly different from those reared on ground roots of non-Bt-maize for 4 weeks. *P. armata* performed significantly better on a diet of yeast amended with purified Cry1Ab protein than on ground root tissue of Bt and non-Bt-maize. No significant differences in the population density of collembolans were found in soils cultivated with Bt and non-Bt-maize and between the application of an insecticide (Baythroids) and no insecticide (Anonymous, 2006).

2.3.9.9. Conclusion

The GMO Panel concludes that no new data were presented to show that maize MON810 would pose a risk to non-target soil microorganisms and fauna.

2.4. Environmental safety issues related to maize T25

2.4.1. Risks for weed communities

2.4.1.1. Austrian claims

In the Austrian report, it is stated that “changes in weed management are to be expected with introduction of GM maize T25” and that “a proper assessment of the effects on weed communities is required based on a in-depth analysis of weeds and interactions between the GMO and target organisms of the GM maize T25 as required both under Directive 90/220/EEC (Annex II, IV. C.3. and C.4) and Directive 2001/18/EC (Annex IIIB, D.). The insufficient control of certain weeds provided by glufosinate-ammonium and the resulting shift in weed communities has to be considered adequately. Furthermore, long term effects of

the herbicide tolerant plant cannot be evaluated independently from the respective herbicide use and effects of glufosinate-ammonium in combination with GM maize T25 on weed communities need to be addressed by such a monitoring plan”.

2.4.1.2. Effects on weed communities

Like any other extensively used weed management approach, herbicide regimes used with genetically modified herbicide tolerant (GMHT) crops have the potential to alter the composition, richness and diversity of weed communities. The selection pressure induced by glyphosate has been shown to cause weed shifts from susceptible towards more tolerant plants in certain GMHT cropping systems (Shaner, 2000; Owen and Zelaya, 2005; Puricelli and Tiesca, 2005; Scursioni et al., 2007; Owen, 2008). While a survey of weed scientists conducted across the US revealed that weed shifts have not been observed in GMHT maize so far, this was attributed to the low adoption of GMHT maize (Culpepper, 2006). Glyphosate-induced weed shifts are generally caused by the natural tolerance of a particular species to glyphosate or the evolution of glyphosate resistance within the weed population. Recurrently applying the same herbicide regime during the growing season or in the rotation might thus favour a few dominant weed species, and eventually select for species that are inherently tolerant to the active substance used. Therefore, weed shifts can also be anticipated in cropping systems that rely on glufosinate-ammonium-based herbicide regimes due to its limited efficacy on certain perennial weeds such as some *Viola* species and grass species.

A decline of the long-term persistence of arable weeds in the seedbank due to the use of non-selective herbicides is considered as a relevant issue by the GMO Panel. Reduced food resources and/or foraging and nesting habitat might threaten invertebrates, small mammals and seed-eating birds (Watkinson et al., 2000; Butler et al., 2007). Effects on farmland biodiversity associated with the use of non-selective herbicides in GMHT cropping systems have been demonstrated in the UK Farm-Scale Evaluation trials (Firbank et al. 2003). Results showed that herbicide regimes used with maize T25 resulted in increased botanical diversity than standard herbicide programmes used on maize crops. In the maize growing season, the weed density in GMHT maize was approximately 2 to 3-fold higher throughout the season, and biomass was 1.85-fold higher than in conventionally managed maize. Biomass of dicotyledonous weeds and counts of their seed-rain were greater in GMHT maize due to the greater weed control exerted by conventional herbicide regimes compared with those used with GMHT maize (Heard et al., 2003a,b).

There were few effects on major groups of invertebrates, though there were more ground-dwelling detritivores in GMHT 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 GMHT maize fields (Brooks et al., 2003). Consumer-resource ratios were similar between herbicide regimes, except that there were more invertebrate predators per herbivore in GMHT maize. In GMHT maize, the rain of weed seeds important in diets of 17 granivorous bird species was higher than in conventionally managed maize, though the difference was only significant for the following 7 species: *Pedrix pedrix*, *Columba oenas*, *Columba palumbus*, *Carduelis chloris*, *Pyrrhula pyrrhula*, *Emberiza schoeniclus* and *Emberiza cirrus* (Gibbons et al., 2006). In subsequent conventional crops, the beneficial effect of herbicide regimes was detectable in the weed seedbank. Seedbanks following GMHT maize were 1.23-fold higher than following non-GMHR maize for both the first and second years (Firbank et al., 2005). Although cumulative effects due to the continuous cultivation of

GMHT crops were predicted (Heard et al., 2005), such effects have been difficult to detect. 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. On the one hand, herbicide regimes applied in non-GMHT maize included the triazine herbicides atrazine, simazine and cyanazine (Champion et al., 2003). Considering that these herbicides are withdrawn from approved lists of EU chemicals nowadays, data obtained in the Farm-Scale Evaluation trials were reanalysed. This reanalysis revealed that the replacement of triazine herbicides by less efficient conventional herbicides slightly reduced the beneficial effect of herbicide regimes, but did not eliminate it (Perry et al., 2004; Brooks et al., 2005). On the other hand, herbicide regimes used with GMHT maize might not fully reflect real agricultural practice, as the application of glufosinate-ammonium was limited to 1 spray applied at dose rates lower than 0.800kg/ha in most cases (Champion et al., 2003). In practice, other herbicide regimes – with respect to the number of applications (single vs. sequential), dose, and to the presence of a residual herbicide – than the one used in the Farm-Scale Evaluation trials are more plausible, resulting in a different impact on farmland biodiversity (Sanvido et al., 2007; Devos et al., 2008a).

2.4.1.3. Conclusion

Since mainly novel herbicide management regimes used in maize cropping systems are determining the environmental impact of GMHT crops, the GMO Panel encourages both applicants and appropriate competent authorities in Member States establish and implement herbicide management systems for GMHT crops that do no more environmental harm than conventional systems and which are consistent with the environmental protection goals and biodiversity action plans in each Member State (EFSA, 2008).

2.5. Post-market environmental monitoring plan

2.5.1. Case-specific monitoring

2.5.1.1. Austrian claims

In the Austrian report, it is stated that “*the absence of effects on non-target organisms, which was stated by the notifier in the risk assessment, as well as the possible occurrence of secondary pests should be subject to a monitoring in line with the requirements laid down in Directive 2001/18/EC as well as the Guidance by the EFSA GMO Panel*”. Because “*secondary pests were neither considered in the risk assessment nor in a monitoring plan*”, “*effects of Bt crop cultivation on other pests and the development of secondary pests and consequently the additional use of synthetic plant protection products should be monitored for applications of Bt crops*”.

2.5.1.2. GMO Panel assessment

Theoretically, the increase of (secondary) pest densities may occur if the number and spectrum of insecticide applications are reduced in cropping systems. Reduced or no insecticide applications in Bt-crops expressing selective Bt-toxin(s) provide an opportunity

for secondary pest species, previously controlled by insecticides used against key target pests, to reach damaging levels in Bt-crops (Sanvido et al., 2007; Fitt, 2008; Kennedy, 2008; Naranjo et al., 2008). Natural enemies failing to fully control secondary pest species, and reduced competition with target pests might also play a role in the occurrence of secondary pest outbreaks (Romeis et al., 2008b; Sanvido et al., 2008a).

The cultivation experience of maize MON810 in the EU and elsewhere does not provide evidence for secondary pest outbreaks in Cry1Ab expressing maize. Differences in densities of herbivorous arthropods between maize MON810 and near isogenic counterparts were not attributed to the transgenic trait itself, but were in the range of variation among varieties (Eizaguirre et al., 2006). While some studies reported that aphids perform better on Bt-maize than on near isogenic counterparts (e.g., Bourguet et al., 2002; Dutton et al., 2002; Lumbierres et al., 2004; Pons et al., 2005; Eizaguirre et al., 2006), aphid performance was within the normal variation observed among conventional maize varieties.

2.5.1.3. Conclusion

The GMO Panel notes that it is currently assessing the post-environmental monitoring (PMEM) plan proposed by the applicant in the context of the application (EFSA-GMO-RX-MON810 20.1.a) for renewal of the authorisation for continued marketing of existing maize MON810 products that were authorised under Directive 90/220/EEC (Commission Decision 98/294/EC) and subsequently notified according to Article 20(1)(a) of Regulation (EC) No 1829/2003 on genetically modified food and feed. The currently valid authorization of MON810 maize does not request the applicant to carry out a PMEM according to Annex VII of Directive 2001/18/EC. Conclusions on the scientific quality of case-specific monitoring activities proposed by the applicant under the current renewal procedure will thus be given in the context of the assessment of the renewal application.

In the context of resistance development in target pests, the GMO Panel maintains its advice that the potential development of resistance in the European and Mediterranean corn borer continues to be monitored in order to detect potential changes in resistance levels in pest populations (see section 2.3.2.5).

2.5.2. General surveillance

2.5.2.1. Austrian claims

In the Austrian report, it is stated that *“the lack of a detailed and effective general surveillance plan must be regarded a major deficiency of the application and inadequate with a view to addressing the concerns that have been put forward against this application”*.

2.5.2.2. Conclusion

As stated above in 2.5.1.3 conclusions on the scientific quality of the PMEM (including general surveillance approach) proposed by the applicant will be given in the context of the assessment of the renewal application (EFSA-GMO-RX-MON810 20.1.a).

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The GMO Panel has investigated the claims and report provided by Austria. In the Austrian report, the GMO Panel did not identify any new data subject to scientific scrutiny or scientific information that would change the previous risk assessments conducted on maize MON810 and T25, which currently have marketing consent in the EU. In addition, the Austrian submission did not supply scientific evidence that the environment or ecology of Austria presents conditions that would require separate risk assessments from those conducted for other regions in the EU.

The GMO Panel concludes that maize MON810 and T25 are unlikely to have adverse effects on human and animal health or on the environment in the context of their proposed uses. The GMO Panel therefore reaffirms its previous conclusions on the safety of maize MON810 and T25.

Having considered the information submitted by Austria and a broad range of scientific literature, the GMO Panel is of the opinion that there is no specific scientific evidence, in terms of risk to human and animal health and the environment, that would justify the invocation of safeguard clause under Article 23 of Directive 2001/18/EC for the marketing of maize MON810 and T25 for its intended uses in Austria. In conclusion, the GMO Panel finds that the scientific evidence currently available does not sustain the arguments provided by Austria and that cultivation of maize MON810 and T25 is unlikely to have an adverse effect on human and animal health and the environment in Austria.

DOCUMENTATION PROVIDED TO EFSA

1. Letter, dated 18 April 2008, including supporting documents from M.P. Carl, Director-General Environment EC, to Catherine Geslain-Lanéelle, Executive Director EFSA (ref ENV/B3/YK/gm D(2008) 3737) requesting for a scientific opinion on the safeguard notification submitted by Austria under Article 23 of Directive 2001/18/EC for maize MON810 and T25 and comprising the following supporting report:
 - Supplementary risk assessment for GM maize MON810 with regard to the conclusions of the WTO-Panel in the case of 'EC biotech' on Austrian safeguard measures for GM maize

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