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SCIENTIFIC OPINION

Request from the European Commission related to the safeguard clause invoked by France on maize MON810 according to Article 23 of Directive 2001/18/EC and the emergency measure according to Article 34 of Regulation No 1829/2003/EC[1]

Scientific Opinion of the Panel on Genetically Modified Organisms

(Question No EFSA-Q-2008-077)

Adopted on 29 October 2008

PANEL MEMBERS[*]

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.

SUMMARY

On 9 February 2008, France notified to the European Commission an Order suspending the cultivation of seed varieties derived from the genetically modified maize event MON810, as well as a safeguard measure invoked under Article 23 of Directive 2001/18/EC to provisionally prohibit the cultivation of the authorised maize MON810 on its territory. An amendment of the Order was further notified to the European Commission on 20 February 2008. In the meantime, on 13 February 2008, France notified to the European Commission a note entitled 'Emergency measure' under Article 34 of Regulation (EC) No 1829/2003. In this respect, the European Commission received from France a written submission consisting of different supporting documents.

On 27 February 2008, the European Commission requested the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority to

[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 France on maize MON810 according to Article 23 of Directive 2001/18/EC and the emergency measure according to Article 34 of Regulation No 1829/2003/EC. *The EFSA Journal* (2008) 850, 1-45.

[*] 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.

assess the package of documents supporting and justifying the French safeguard clause and the duration of the invoked measure.

Having assessed the information package provided by France in support of its safeguard clause and having considered all relevant publications on the subject, the GMO Panel concludes that, in terms of risk to human and animal health and the environment, the provided information package does not present new scientific evidence that would invalidate the previous risk assessments of maize MON810. 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 safeguard clause under Article 23 of Directive 2001/18/EC and an emergency measure under Article 34 of Regulation (EC) No 1829/2003.

Key words: **GMOs, maize (*Zea mays*), MON810, France, safeguard clause, emergency measure, human health, animal health, environment, Directive 2001/18/EC, Regulation 1829/2003/EC**

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BACKGROUND

Maize MON810 (notification reference C/F/95/12-02) was authorised in the European Union (EU) for all uses with the exception of food by the Commission Decision 98/294/EC on 22 April 1998. On 3 August 1998, France granted a final consent. Food use of maize derivatives was notified according to Article 5 of Regulation (EC) No 258/97 on 6 February 1998.

On 9 February 2008, France notified to the European Commission an Order suspending the cultivation of seed varieties derived from the maize event MON810, as well as a safeguard measure invoked under Article 23 of Directive 2001/18/EC to provisionally prohibit the cultivation of the authorised maize MON810 on its territory. An amendment of the Order was further notified to the European Commission on 20 February 2008. In the meantime, on 13 February 2008, France also notified to the European Commission a note entitled 'Emergency measure' under Article 34 of Regulation (EC) No 1829/2003. In this respect, France submitted an information package made of different supporting documents to the European Commission.

Following receipt of the information package, the European Commission requested, on 27 February 2008, the EFSA Scientific Panel on Genetically Modified Organisms (GMO Panel) to assess the package of documents supporting and justifying the French safeguard clause and the duration of the invoked measure.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

On 27 February 2008, EFSA was requested by the European Commission, under Article 29(1) and in accordance with Articles 22(5) and 22(5)(c) of Regulation (EC) No 178/2002, "*To assess:*

1. *the opinion of the "comité de préfiguration" of the High Authority for GMOs, dated 9 January 2008, which is mentioned as reference in the first "considérant" of the Order*[2]*;*

2. *the French position that the justifications presented by Monsanto on 30 January 2008 are not sufficient to invalidate the data of the French Order, as presented in the second "considérant" of the Order;*

3. *the scientific evidence which is presented in the accompanying note of the Order and in the note forwarded to the European Commission under Regulation (EC) No 1829/2003;*

4. *the scientific justification of the duration of the measure, which is linked to the ongoing procedure on the notification for the renewal of MON810, as referred to in Article 1 in the Decree.*"

[2] French Order of 7 February 2008 suspending the cultivation of seed varieties maize MON810.

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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 would affect the risk assessment of an authorised GMO. Provisions foreseen by France seek to provisionally prohibit the marketing of maize MON810 for its intended uses on the French territory.

The GMO Panel examined the set of supporting documents submitted by France. 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, for its intended uses, constitutes a risk to human and animal health or the environment.

The GMO Panel looked for evidence for GMO-specific risks 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 raised concerns in the light of the most recent scientific data and relevant peer-reviewed publications.

2. Assessment of documents provided by France

A set of supporting documents, accompanying the mandate of the European Commission (see *Terms of Reference as provided by the European Commission*), was forwarded to EFSA on 27 February 2008 and supplemented with additional documents on 16 June 2008 upon request of EFSA.

France provided the following documents:

- the opinion of the 'Comité de préfiguration' of the High Authority for GMOs, dated 9 January 2008;
- the analysis by Monsanto, dated 30 January 2008, of the opinion on the dissemination of maize MON810 on the French territory by the 'Comité de préfiguration' of the High Authority for GMOs;
- the French Order of 7 February 2008 suspending the cultivation of maize varieties derived from maize MON810 in France;
- the French Order of 13 February 2008 amending the French Order of 7 February 2008 suspending the cultivation of maize varieties derived from the event MON810 in France;
- the note from the French authorities accompanying the French Order of 7 February 2008 (Object: Safeguard clause);

- the supplementary document transmitted by French authorities[3] (drafted by Prof. Dr. Yvon Le Maho) arguing that the analysis performed by Monsanto on 30 January 2008 is not sufficient to invalidate the data of the French Order and its accompanying note (dated 12 June 2008).

Based on the above listed supporting documents provided by France, 13 issues were identified. Some of these points, however, fall outside the current remit of the GMO Panel. For instance, the French 'Comité de préfiguration' of the High Authority for GMOs states that "*New evidence has revealed that Bt maize affects mycotoxin levels which may be 90%–95% (AFSSA; 2004) lower than levels with conventional hybrids not treated with insecticides; insecticide treatment does not lead to such a large reduction. Levels of fumonisin (classified as probably carcinogenic in humans, CIRC group 2B[4]) for conventional hybrids regularly exceed 2000 ppb in relation to insect attack in the Midi-Pyrénées and Aquitaine*". The use and risk assessment of pesticides on conventional maize and any beneficial and socio-economic aspects related to the cultivation of maize MON810 fall outside the remit of the GMO Panel, and therefore these aspects are not addressed in this scientific opinion. Similarly, ethical concerns related to GM crops, concerns about the coexistence of maize cropping systems, and the definition of tolerance thresholds for the unintentional or technically unavoidable presence of approved GM material in non-GM crop products are not taken into account by the GMO Panel.

In relation to "*biovigilance*", the GMO Panel wishes to clarify that it gives its opinion on the scientific quality of post-market environmental monitoring activities proposed by applicants, whilst its final endorsement is done by risk managers.

[3] The French authority pointed its attention to the fact that this transmitted document does not bind either the interim committee of the High Authority on Genetically Modified Organisms or a fortiori the French authorities.

[4] Translator's note: CIRC group 2B is 'Possibly carcinogenic to humans'; 'Probably carcinogenic to humans' is group 2A.

The GMO Panel considered the following issues related to the risk assessment of maize MON810:

2.1. Environmental safety issues:

2.1.1. Environmental impacts of vertical gene flow

2.1.2. Resistance development in lepidopteran target pests

2.1.3. Exposure and impacts on non-target fauna

2.1.4. Exposure and impacts on pollinating insects

2.2. Food and feed safety issues:

2.2.1. Specific arguments

2.2.2. General impressions

2.2.3. Health effects

During its assessment, the GMO Panel identified issues raised by the French authorities that would require further clarifications from French scientists. To present and clarify the provided set of data, an informal meeting was held between French scientists, several experts of the GMO Panel and the EFSA staff on 9 October 2008. Representatives of the European Commission attended the meeting as observers.

In its risk assessment and in addition to the information package supporting the French national measure on maize MON810, the GMO Panel reviewed all relevant and most recent scientific literature and publications, such as the scientific advices of AFSSA (2008) and COGEM (2008), as well as those considered specific for French receiving environments. The assessment carried out by the GMO Panel and subsequently the present scientific opinion follow the order of the above listed points of issues, 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 maize Bt11. Due to the use of a different promoter, pollen from maize Bt176 contains 40-fold higher concentrations of the biologically active Cry protein than pollen of maize Bt11 and MON810. In maize Bt11 and MON810, the promoters used were almost inactive in pollen, resulting in very low levels of the gene products accumulating (Hellmich et al., 2001; Gatehouse et al., 2002; Mendelsohn et al., 2004). For green tissues of MON810 Bt-maize plants, the amount of biologically active Cry protein is in a similar range compared to maize Bt11 and Bt176. The GMO Panel indicates in its opinion where information derived from the maize Bt176 and Bt11 is used in its assessment on potential impacts of maize MON810.

Where the scientific views of the GMO Panel differ from those expressed in some of the documents provided by France, the divergence of views is specifically discussed in the following sections.

2.1. Environmental safety issues

2.1.1. Environmental impacts of vertical gene flow

In its report, the French 'Comité de préfiguration' of the High Authority for GMOs states that "*New evidence since 1998 concerns the characterisation of pollen dispersal (Klein et al., 2003; Rosi-Marshall et al., 2007; Brunet 2006; Quist and Chapela 2001) over large distances (kilometres) (A. Messéan, 2006) related in particular to climatic conditions and events and to different environments. These results show that it is not possible to exclude cross-pollination between GMO fields and GMO-free fields at the local scale (small agricultural region) (A. Messéan, 2006). The importance of these results was discussed in relation to impact on seed purity, compliance with thresholds of unintended presence / contamination and the rules for coexistence.*"

2.1.1.1. Vertical gene flow: impact assessment

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., 2008; 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. 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 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 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 in France (Delos et al., 2006, 2007) and Spain (Eizaguirre et al., 2006) indicate that this maize behaves like non-GM maize and is unlikely to establish volunteers or survive over subsequent seasons or to establish feral populations under European environmental conditions.

Consequences of exposure of non-target organisms to pollen of maize MON810 are addressed in sections 2.1.3 and 2.1.4.

2.1.1.2. Conclusion

The GMO Panel is of the opinion that the information and documents provided by France 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.

2.1.2. Resistance development in lepidopteran target pests

The French 'Comité de préfiguration' of the High Authority for GMOs states that there is "*No new evidence on the principal insect pests (no demonstrated resistance) but selection of a resistant strain in two secondary target lepidoptera (Huang et al, 2007; Van Rensburg, 2007).*"

2.1.2.1. Assessment of resistance development

The GMO Panel agrees with the French 'Comité de préfiguration' of the High Authority for GMOs that no resistance development in major lepidopteran pests targeted by maize MON810 has been observed in fields in the EU and the United States (US) where resistance management is in place.

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 LC_{50} (concentration of toxin killing 50% of the larvae) of a field-derived strain divided by the LC_{50} of the susceptible strain (Saeglitz et al., 2006; Andow, 2008; Bravo and Soberón 2008). 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.

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 several regions such as the US and Europe (Alstad and Andow, 1995; Bates et al., 2005; Andow, 2008; Bravo and Soberón 2008). In addition, 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 these monitoring data indicated that neither in the EU, nor in the US, populations of resistant European corn borer (*Ostrinia nubilalis*) or Mediterranean corn borer (*Sesamia nonagrioides*) have 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; Tabashnik et al., 2003, Farinós et al., 2004; Schuphan, 2006; Stodola et al., 2006). In Spain, for instance, after 6 years of field exposure of *S. nonagrioides* to Cry1Ab expressing maize, no indications of resistance development were found (Eizaguirre et al., 2006; Andreadis et al., 2007). F_2 screenings, aiming at detecting rare alleles conferring recessive resistance in field-collected mated females and their progeny reared under confined conditions (Andow and Alstad, 1998), have failed to generate resistant *O. nubilalis* strains so far (Bourguet, 2004). In contrast, laboratory selections with Cry1Ab pro/toxins have yielded significant levels of resistance in some European corn borer strains after many generations (Chaufaux et al., 2001; Huang et al., 2002; Alves et al., 2006). However, the polygenic nature of resistance in the tested laboratory strains suggests that major genes for resistance to

Cry1Ab protein are not common 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 and French fauna. Huang et al. (2007), for instance, reported that no major resistance alleles were detected in F_2 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, as pointed out by the 'Comité de préfiguration', 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, though they showed reduced larval growth rate (Van Rensburg, 2007). This African stem borer is not present in the European fauna.

The only example of field-evolved resistance in Bt-maize concerns resistance of fall armyworm, *Spodoptera frugiparda*, 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 et al., 2008b).

Available data indicate that recessive resistance alleles are rare in populations of European and Mediterranean corn borers. Moreover, according to the EU research project ProBenBt in which various aspects of European and Mediterranean corn borer genetics and Bt-resistance in targeted lepidopteran pest species were studied, 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). 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 resistance management strategy is likely to maintain the sensitivity to Bt-toxins in the European corn borer (Tyutyunov et al., 2008).

2.1.2.2. Conclusion

The GMO Panel concludes that the large scale cultivation of maize MON810 over several years will increase the selection pressure on corn borers, which could result in the potential development of resistance. Even though an analysis of global monitoring data, collected in Australia, China, Spain and the US, revealed an increased frequency of resistance alleles in some field populations of both *Helicoverpa zea* (a pest of cotton) to the Cry1Ac protein and *S. frugiparda* (a pest of maize) to the Cry1F protein (Moar et al., 2008; Tabashnik et al., 2008b), no field-evolved resistance has been reported to Bt-proteins for other lepidopteran pests (*Helicoverpa armigera*, *H. virescens*, *O. nubilalis*, *Pectinophora gossypiella* and *S. nonagrioides*) (Tabashnik et al., 2008a). The GMO Panel considers that the likelihood of occurrence is low in corn borer populations if appropriate resistance management is implemented. In fact, under field conditions and after several years of cultivation, no resistance has been reported for maize MON810 yet. 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 factors, predicting future responses of

corn borer populations in Europe is case specific (Tyutyunov et al., 2008). Adult moth dispersal distances, for instance, have been shown to be influenced by plant size, weather conditions during the flight, pheromonal 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 and/or consider it under general surveillance through farmer questionnaires (Schmidt et al., 2008). In his latest review, Andow (2008) identifies resistance development as a potential risk, but indicates that it can be managed.

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

2.1.3. Exposure and impacts on non-target fauna

In its report, the French 'Comité de préfiguration' of the High Authority for GMOs states that "*New evidences confirm the possibility of long-term toxic effects in earthworms (Zwahlen et al. 2003), isopods, nematodes and monarch butterflies (Rhopalocera) (Hardwood et al. 2005, Prasifka et al. 2007; Dutton et al. 2005). Exposure among natural populations of monarchs remains very low (less than 1%), notably for these latter via harmful effects on behaviour. (Marvier et al, 2007). Published articles have shown that Bt toxin may be present in the food chain (Obrist et al, 2006) and persistence of insecticide molecules has been reported in water (Douville et al, 2006; Rosi-Marshall et al, 2007) and in sediment draining from a plot (more than 20–40 days) (Icoz, Stotzky, 2007), in contact with roots and in the soil (Saxena and Stotzky, 2005; Mulder et al. 2006; Castaldini et al, 2005) with exposure of insect populations (Griffith et al, 2006; Johnson et al, 2006) higher up the food chain. A global analysis of nontarget entomofauna (Marvier et al. 2007) demonstrated that Bt maize cultivation does have an effect on some families of invertebrates, although these effects were smaller than those related to treatment with insecticides. The Marvier study did not provide any evidence on direct toxicity.*" The French 'Comité de préfiguration' of the High Authority for GMOs also states that "*the biological and microbiological effects of the observed dispersal or persistence of Bt molecules or of the transgene in the soil (more than 200 days) (Crecchio, Stotzky, 2001) need to be examined*" as the "*dispersal of the Bt toxin and its persistence have been demonstrated and are governed by edaphic, climatic and environmental factors (Icoz and Stotzky, 2007)*".

2.1.3.1. Persistence of Bt-proteins in soil: exposure assessment

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 on fields after harvest and 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, pH, microbial activity, temperature, method used for quantification of the protein). In a recent review paper, Icoz and Stotzky (2008b) 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 5 µg/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. The Cry1Ab protein in low concentrations was detected for up to 56 days in soil amended with purified 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). Stozky (2004) reported that the 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. The 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 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).

The review of the literature indicates that the possible exposure of non-target soil organisms to the Cry1Ab protein is likely to be variable and case-specific. In an assessment of environmental risks, therefore the 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.

Findings reported by Icoz and Stotzky (2008a) – quoted by the French 'Comité de préfiguration' of the High Authority for GMOs – were not related to maize MON810. In the study, maize MON863 expressing the Cry3Bb1 protein was used and the accumulation of the toxin released in root exudates and through decay of plant biomass was analysed over time. The authors concluded that the Cry3Bb1 protein does not persist in soil and is degraded rapidly (21 days) showing some minor differences in rate of degradation as a function of different experimental conditions. The fact that the persistence of Cry proteins is very variable, depending upon soil type and environmental conditions was confirmed. As such, no new scientific evidence directly relating to maize MON810 or maize producing the related Cry1Ab protein were reported in the cited publication. Likewise, the publication of Johnson et al. (2007), quoted by the 'Comité de préfiguration', addresses risk assessment and risk management in general terms, and does not provide new or additional information about exposure or impact of the Cry1Ab protein to non-target soil organisms in support to the French safeguard clause.

2.1.3.2. Biological effects in soil: impact assessment

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).

2.1.3.3. Non-target soil organisms: impact assessment on earthworms

Earthworms can be exposed to the 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 protein ingestion. The detection of the Cry1Ab protein in the gut and faeces of earthworms confirmed protein ingestion (reviewed by Icoz and Stotzky, 2008b).

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 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 (StMUGV, 2006).

2.1.3.4. Non-target soil organisms: impact assessment 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).

2.1.3.5. Non-target soil organisms: impact assessment 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 (StMUGV, 2006; Icoz and Stotzky, 2008b).

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, 2008b). 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 bacteriovorious *Caenorhabditis elegans*, have also been studied in field trials in Germany (StMUGV, 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 varietial 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. No significant differences in nematode abundance in field sites in France were shown. Current scientific information indicates that possible changes in the nematode community structure associated with Bt-maize and their products are likely to be minor compared with effects of agricultural practices, environmental stresses or differences between localities and maize varieties. 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.

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

2.1.3.6. Microbiological effects: impact assessment

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 heterothrophic 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; Icoz and Stotzky, 2008b), 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; StMUGV, 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; Icoz and Stotzky, 2008b). 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 croplands' 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 between the activity of specific bacterial guilds in soils amended with transgenic maize and bacteria in soils amended with conventional maize were detected.

Studies in which the decomposition of Bt-maize was compared with that of non-Bt-isogenic lines mostly show 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).

The GMO Panel is of the opinion that potential effects on soil microorganisms due to maize MON810 if they occur, will be transient, minor and localised in different field settings and are likely to be within the range currently caused by a range of other agronomic and environmental factors.

2.1.3.7. Presence of *cry1Ab* gene and Bt-proteins in water: exposure and impact assessment in aquatic environments

The occurrence and persistence of the *cry1Ab* gene from Bt (*kurstaki*) and Bt-maize (event MON810) have been examined in aquatic environments near fields where Bt-maize was cultivated. Douville et al. (2007) reported that the *cry1Ab* gene persisted for more than 21 and 40 days in surface water and sediment, respectively, and detected the *cry1Ab* gene in surface water samples taken at long distances downstream from the maize plot. However, DNA presence alone is not considered a reliable indicator of toxicity to non-target organisms. A more reliable indicator of toxicity to non-target organisms would be the presence and concentrations of the Cry1Ab protein in surface water and sediment. In a previous study of the same group of researchers, it was reported that the presence of the Cry1Ab protein in water bodies was either absent or just above the detection limit (Douville et al., 2005), suggesting that Cry1Ab protein concentrations would remain far below any toxic level.

Based on findings reported by Rosi-Marshall et al. (2007) and Bøhn et al. (2008), the French 'Comité de préfiguration' of the High Authority for GMOs 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 Trichopterans. This study quantified maize biomass (Bt or non-Bt) in headwater streams, but the GMO Panel (EFSA, 2007a) and other scientists (ACRE, 2007; Beachy, 2008; Parrott, 2008) have indicated that the study is incomplete so that conclusions on environmental impacts cannot be made. The authors measured degradation rates in aquatic systems and 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. Therefore, the GMO Panel is of the opinion that important background

information on levels of exposure and plant material used is missing and considers 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 can be concluded that a potential hazard for Trichopterans 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 in this paper and by Douville et al. (2005), exposure of Trichopterans in aquatic ecosystems is likely to be extremely low, so that the GMO Panel considers that it is unlikely that Bt-toxins in maize MON810 products would cause toxic effects to Trichopterans.

A laboratory experiment performed by Bøhn et al. (2008) revealed that *Daphnia magna* fed with a Bt-maize flour-containing suspension (event MON810) had a higher mortality and a lower proportion of females reached sexual maturity as compared to the non-Bt-maize treatment, suggesting toxic effects of Bt-maize. However, since maize flour is not part of the natural diet of *Daphnia*, the unusual delays in development of *Daphnia* fed non-Bt-maize might have been caused by nutritional deficiencies related to a maize-based diet. Moreover, internationally accepted guidelines for toxicity and reproduction testing of *Daphnia* were not followed. Due to these methodological weaknesses, the GMO Panel doubts that any substantive conclusion on potential risks of maize MON810 can be drawn from the study.

2.1.3.8. Exposure and impacts on non-target lepidopteran organisms

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 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 moths) occur in farmland areas where maize is grown and thus could be potentially affected by exposure to Bt-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 plants (Dipel) 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 the 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 relatively low resulting in similar toxicological effects on non-target lepidopteran populations exposed to pollen from these maize events (Mendelsohn et al., 2004), in contrast to maize Bt176 pollen which contain much higher concentrations of the Cry1Ab protein (Hellmich et al., 2001). A laboratory assay revealed toxicity to monarch butterfly larvae (*Danaus plexippus*) that consumed Bt-maize pollen deposited on milkweed plants (*Asclepias* spp.) compared to those reared on leaves dusted with non-transformed maize pollen or on leaves without pollen (Losey et al., 1999). Larvae of the pest species *Pieris brassicae*, *Pieris rapae* and *Plutella xylostella* also fed less, grew more slowly and showed a higher mortality when they ingested their food plant material dusted with pollen of maize Bt176, compared to larvae of an untreated control group (Felke et al., 2002). Similarly, Lang and Vojtech (2006) reported a lower survival rate of larvae of the European common swallowtail, *Papilio machaon*, exposed to the highest levels of Bt-maize pollen densities (event Bt176) that might be experienced under field conditions. The uptake of Bt-maize pollen led to reduced plant consumption, lower body weight, longer development time of larvae, and smaller wing size as adults. Hence, besides an impact assessment, 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 on 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. plexipus* 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 1-3 m 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), 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.

Although 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), an examination of anthers in and near maize fields showed that toxic levels of anthers are uncommon (Anderson et al., 2004). The GMO Panel concludes that intact Bt-anthers alone or in combination with Bt-pollen are not likely to pose a significant risk to monarch butterflies. 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 GMO Panel agrees with the French 'Comité de préfiguration' of the High Authority for GMOs that extrapolating observations made on certain non-target lepidopteran species to others remains difficult due 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. There are a few studies on the distribution and hence the exposure of European lepidopteran species in agricultural landscapes on a population level (Schmitz et al., 2003; Gathmann et al., 2006a,b; StMUGV, 2006). 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. This confirms that studying all lepidopteran species that could be potentially exposed to Bt-maize pollen may be difficult in practice, especially if potential 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).

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

2.1.3.9. Global analysis of non-target 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 clear 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 the Bt-maize treatment. 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 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, mould 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. Thereby, 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 showed different effects of Bt-maize among functional guilds of non-target arthropods (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.

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 France.

2.1.3.10. Trophic chain effects on predators

Invertebrate predators can be exposed to the Cry1Ab protein through their prey organisms. Harwood et al. (2005), quoted by the French 'Comité de préfiguration', for instance, 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 on 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.

Hence, 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. In addition, other routes of exposure of non-target organisms can occur (e.g., Andow et al., 2006). 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. Literature data on the susceptibility of several groups of natural enemies are available and have been reviewed. In this respect, Lövei and Arpaia (2005) pointed out some shortcomings related to species selection, sample size, statistical power and duration of certain laboratory toxicity studies performed on arthropod natural enemies. 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. In this respect, several studies confirmed 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).

2.1.3.11. 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 Cry 1Ab protein (Vojtech et al., 2005). However, another study suggested that the Cry1Ab protein present in the host, *Spodoptera frugiperda*, fed Bt-maize may have a direct effect on *C. marginiventris*, (Ramirez-Romero et al., 2007). These authors 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 were also able to prove the importance of the plant in causing negative effects at the third trophic level, since negative results were not observed when pure protein-containing diet was used in the tritrophic experiments.

By contrast, the performance of *C. marginiventris* fed 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, *Rhopalosiphim 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 parasitoism. Based on the observations made, Faria et al. (2007) concluded that as a 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.

2.1.3.12. Conclusion

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

2.1.4. Exposure and impacts on pollinating insects

In its report, the French 'Comité de préfiguration' of the High Authority for GMOs states that "*Impact studies need to be carried out on bees in hives kept under normal apiculture conditions, to analyse the cumulative effects*" and argues that "*there was no consensus on this point.*"

2.1.4.1. Impact assessment on pollinating insects

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 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).

2.1.4.2. Exposure assessment on pollinating insects

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 1.52-2.04 mg 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.

2.1.4.3. 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 France do not provide any new or additional scientific evidence that would invalidate previous risk assessments of maize MON810.

2.2. Food and feed safety issues

2.2.1. General impression

The French 'Comité de préfiguration' of the High Authority for GMOs has provided various arguments as to why they consider the safety testing performed with maize MON810 and the Cry1Ab protein has not been sufficient. However, the GMO Panel considers that the arguments provided do not point at specific hazards that would have been identified recently in maize MON810 or the Cry1Ab protein, but are of a more general nature, touching upon the risk assessment approach recommended by the internationally harmonized Codex alimentarius (Codex alimentarius, 2003) and the EFSA Guidance Document (EFSA, 2006), although the latter 2 documents are not explicitly mentioned in the French arguments.

2.2.2. Specific arguments

The French 'Comité de préfiguration' of the High Authority for GMOs states the following:

- "*The proteins produced by Bacillus thuringiensis and that produced by MON810 do not have the same primary sequences;*

- *The protein produced by MON810 can be modified in its spatial conformation by addition of elements, which can have important consequences for its functional characteristics and its potential pathogenic capacity;*

- *The duration of the toxicological tests is insufficient and these have to be conducted in multiple animal models;*

- *The toxicological tests performed for the assessment of transgenic plants do not cover the new domains of health (prion disease, oncology).*"

In addition, it concludes that "*In the absence of long-term tests on the protein in the configuration in which it is really produced by MON810, the precautionary principle should prevail*".

2.2.3. Health effects

2.2.3.1. Allergenicity

The French 'Comité de préfiguration' of the High Authority for GMOs states that "*Emergent allergic problems linked with novel foods or industrial processes need to be taken into account (Wassenberg et al., 2007). It is known in particular that Cry1Ab triggers an immune response in the rat model (Kroghsbo et al., 2008)*".

During the safety assessment of GMOs and as recommended by the EFSA Guidance Document (EFSA, 2006), potential allergenicity of a GMO is considered. In this respect, a 'weight-of-evidence' approach is followed, taking into account multiple factors that provide an indication of the likelihood that the GMO may represent additional allergic hazards over its conventional counterparts. According to the GMO Panel, no indications have been found for the Cry1Ab protein that would raise concerns over any potential allergenicity.

With regard to the notion that the Cry1Ab protein has triggered an immune response in the experiment described by Kroghsbo et al. (2008), it is not uncommon for a protein to act as an antigen. The authors themselves note that "*It is well documented that introduction of a new or 'foreign' protein by the oral route will induce an antigen-specific immune response*". This information can therefore not be taken by the GMO Panel as an indication of any allergic response to the Cry1Ab protein.

2.2.3.2. Toxicity

The French 'Comité de préfiguration' raised questions with regard to modifications of the conformation of proteins. Thereby, it states that "*The toxicology tests applied to the Cry1Ab protein are far from covering the fields of new research that have been revealed by recent studies on prion diseases (CJD, mad cow's disease, scrapie, contaminations and transplantations), which have had an important global impact with destructive effects on human and animal health caused by new processes applied in agriculture, and which are related to modifications of the conformation of proteins. In effect, the Cry1Ab protein has not been tested according to current methods in the domain of research on prions (newborn rats with IC or IP injections; subsequently studies during 120 till 300 days at minimum) (Liberski and Brown, 2007; Unterberger and Voigtlander, 2007). It has to be stressed that such studies*

could have prevented the "mad cow" crisis and, more recently, that of the growth hormone affecting young children (Lewis et al., 2006; Pauli, 2005)."

In accordance with the EFSA Guidance Document (EFSA, 2006) and the Codex alimentarius guidelines (Codex alimentarius, 2003), the GMO Panel considers characteristics of proteins, including structure and functionality and various other relevant physico-chemical and biochemical properties as part of the data routinely assessed for GMOs, as well as their potential toxicity and allergenicity. The GMO Panel is of the opinion that arguments raised by the French authorities are highly speculative and do not reveal new insights that the Cry1Ab protein specifically could act as a prion, particularly the prion involved with TSE/BSE.

In its report, the French 'Comité de préfiguration' argues that "*The toxicological studies also have to consider research on oncogenes. Tests on newborn animals have, since a long time, been applied in viral and non-viral oncology. These tests have enabled the discovery of oncogenes that are the cause of many human cancers (Gelman et al., 1993; Bonham et al., 1992; Hassan et al., 1990; Darlix et al., 2007).*"

The GMO Panel considers that the information does not specifically indicate that the transgenic DNA inside maize MON810 could have oncogenic properties. Moreover, neither is the *cry1Ab* gene a known oncogene, nor does the function and origin of the *cry1Ab* transgene in maize MON810 indicate any role as an oncogene in plants or humans/animals. In a more general sense, the impact of potential horizontal gene transfer of transgenic DNA is also considered by the GMO Panel during the safety assessment of GMOs with respect to potential implications for human and animal health and the environment according to the EFSA Guidance Document.

2.2.3.3. Long-term toxicity tests

In its report, the French 'Comité de préfiguration' argues that "*Independently from the precautions that already have to be taken with regard to the new pathologies caused by yet little understood mechanisms, given that the authorizations currently base themselves on tests performed solely on rats only during 90 days, this limitation is far from creating unanimity in the scientific community. The usual tests for food additives and pesticides are carried out during at least two years, on multiple generations of animals, and on multiple mammalian species. Furthermore, the power of the statistical methods employed is doubtful as they appear to be hardly sensitive for differences, even if some of these [differences] are significant. Actually, instead of being defined at the start of the experiment, the procedure evolves based on the results obtained. For the bodyweight curves, a technique that has been adapted to longitudinal results should have been employed (Lavielle, 2007). In conclusion, the Committee has serious doubts, on one hand on the methodology to decide if a difference is significant or not, on the other hand about the biological interpretation of observed differences.*

Moreover, scientists should have access to the original results of the toxicological tests that have been employed. To block their diffusion, as has been done in the past years with regard to the results of tests on rats fed or not with MON863 maize hinders the progress of scientific knowledge and contradicts European (in particular Directive 2001/18/EC) and French regulations. While re-examining these results, Seralini et al. (2007) have proven differences in weights amongst male and female rats, as well as signs of hepatorenal toxicity. A study commissioned by the enterprise (Doull et al., 2007) subsequently contested this interpretation

arguing that a dose-effect relationship had not been proven and because the results differed in function of gender.

In fact, the protocol of the initial study by the enterprise has not been established in a way that could prove such a dose-effect as it limits itself to two dose levels only. What is more, for metabolic and hormonal disturbances, the response need not being linear. In each case, again, it is needed more than ever before that toxicological tests are performed with a longer duration and not only on rats. It should be reminded that the tragic history of thalidomide and its impact on the fetus was linked to the fact that only two animal models were utilized.

In the absence of long-term tests with the recombinant Cry1Ab protein, its RNA messenger, and MON810, it appears to be important to let the precautionary principle prevail, without biasing future actions to be taken in research and development. Within this context, such tests should be carried out completely independently from the enterprise and double-blind. Once results have been obtained, they should be made public."

The GMO Panel considers that the data provided by the French authorities do not contain any data or other indications for hazards specifically posed by MON810 maize.

The text also discusses the perceived shortcomings of the 90-days rat feeding study performed by the applicant. Whilst the 90-days rat study with whole GM crop products is provided to EFSA with many dossiers it receives, it is not a standard requirement to perform these studies. By contrast, the performance of these studies has to be chosen for on a case-by-case basis, depending on the outcome of the extensive comparative assessment in which a GM crop is compared to its counterpart with regard to molecular characteristics, composition (macronutrients, micronutrients, anti-nutrient, toxins, allergens), and agronomic/phenotypic characteristics in accordance with internationally harmonized guidelines of Codex alimentarius (Codex alimentarius, 2003) and the EFSA Guidance Document (EFSA, 2006). Based upon the biologically relevant changes in characteristics of the GM crop thus identified, further testing may be required. More details including the assessment of long-term effects can be found in the recently published report of the GMO Panel's Working Group on Animal Feeding Trials, which has appeared in a supplement to Food and Chemical Toxicology (EFSA, 2008).

With regard to the comments made by Séralini et al. (2007), this pertains to maize MON863, which is different from maize MON810, the subject of the current safeguard clause. In addition, the GMO Panel has already published a statement on the Séralini et al. (2007) publication (EFSA, 2007b), concluding that these data do not cause it to deviate from its previous opinion on MON863.

The data presented by the French 'Comité de préfiguration' neither provide any new scientific information nor give any other indications that maize MON810 would pose a risk.

2.2.3.4. Characteristics of the Cry1Ab protein

With regard to the Cry1Ab protein, the French 'Comité de préfiguration' points out that "*…The natural protein and that produced by MON810 do not have the same primary sequences. Even more, the one produced by MON810 can possibly be modified by addition of phosphates, N-acetylglucosamine, and hexoses, which can cause a change in the conformation of the protein (Ahmad et al., 2006), in its functional characteristics, also with*

regard to its possible pathogenic potential (Wang et al., 2007; Pang et al., 2007; Chen et al., 2006; Wells et al., 2004; Lüdemannet al., 2005). This is not the case for the natural form of Cry1ab as bacteria are incapable of these possible post-translational modifications (Dennis et al., 2006). However, in its analysis of the opinion of CPHA, the enterprise stayed silent about the important queries raised about these differences."

The GMO Panel considers that the data provided do not point at a hazard that can specifically be linked to the Cry1Ab protein and does not provide any new information on this protein either. The references pertain to the functionality and post-translation modifications of other proteins than Cry1Ab. In addition, it implies that the safety assessment of the Cry1Ab protein would be limited to a consideration of its similarity to the protein produced naturally by *Bacillus thuringiensis*. None of the data on the Cry1Ab protein and similar Cry proteins that have been assessed by the GMO Panel for their safety have indicated any modifications with potential adverse health effects.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The GMO Panel has investigated the claims and documents provided by France. In these documents, the GMO Panel did not identify any new data subject to scientific scrutiny or scientific information that would change previous risk assessments conducted on maize MON810 which currently has marketing consent in the EU.

Having considered the overall information package submitted by France as well as a broad range of relevant 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 a safeguard clause under Article 23 of Directive 2001/18/EC and an emergency measure under Article 34 under Regulation (EC) No 1829/2003.

DOCUMENTATION PROVIDED TO EFSA

1. Letter dated 27 February 2008, with the supporting documents from M.P. Carl, Director-General Environment EC, to Catherine Geslain-Lanéelle, Executive Director EFSA (ref. ENV/B3/YK/gm D(2008) 3460) – Assessment of the scientific studies supporting the suspension of cultivation of MON810 in France - Request for EFSA opinion.
2. Letter dated 11 March 2008 (ref. SR/SM/KL/shv (2008) 2768466), from EFSA to the requestor, European Commission/DG ENV, asking for clarifications and further information.
3. Letter dated 19 March 2008 (ref. ENV/B3/YK/gm D(2008) 4751), from the European Commission/DG ENV, to EFSA responding to EFSA concerns raised in its letter dated 11 March 2008.
4. Note dated 12 June 2008, from French authorities to EFSA, providing EFSA with an additional report.
5. Letter dated 17 July 2008 (ref. RM/PB/SM/shv (2008) 3159835), from EFSA to the European Commission/DG ENV, requesting clarifications on the status of the additional report.
6. Letter dated 25 July 2008 (ref. ENV/B3/YK/gm D(2008) ARES (2008) 17110), from the European Commission/DG ENV, to EFSA responding to EFSA concerns raised in its letter dated 17 July 2008.

7. Letter dated 25 September 2008 (ref. RM/PB/SM/shv (2008) 3275930), from EFSA to the European Commission/DG ENV, thanking for the clarifications.

REFERENCES

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

AFSSA, 2008. Avis de l'Agence française de sécurité sanitaire des aliments relatif à une demande de renouvellement de mise sur le marché du maïs génétiquement modifié MON 810, résistant aux lépidoptères, pour l'importation, la transformation, ainsi que l'utilisation en alimentation humaine et animale de ses produits dérivés, au titre du règlement (CE) n°1829/2003, http://www.afssa.fr/Documents/BIOT2008sa0043.pdf

Alstad, D.A., Andow, D.A., 1995. Managing the evolution of insect resistance to transgenic plants. *Science*, 268: 1894-1896.

Alvarez-Alfageme, F., Ferry, N., Castañera, P., Ortego, F., Gatehouse, A.M.R., 2008. Prey mediated effects of Bt maize on fitness and digestive physiology of the red spider mite predator *Stethorus punctillum* Weise (Coleoptera: Coccinellidae). *Transgenic Research*, 17: 943-954.

Alves, A.P., Spencer, T., Tabashnik, B.E., Siegfried, B.D., 2006. Inheritance of resistance to the Cry1Ab *Bacillus thuringiensis* toxin in *Ostrinia nubilalis* (Lepidoptera: Crambidae). *Journal of Economic Entomology*, 99: 494-501.

Andersen, M.N., Sausse, C., Lacroix, B., Caul, S., Messéan, A., 2007. Agricultural studies of GM maize and the field experimental infrastructure of ECOGEN. *Pedobiologia*, 51: 175-184.

Anderson, P.L., Hellmich, R.L., Sumerford, D.V., Lewis, L.C., 2004. Effects of Cry1Ab-expressing corn anthers on monarch butterfly larvae. *Environmental Entomology*, 33: 1109-1115.

Anderson, P.L., Hellmich, R.L., Prasifka, J.R., Lewis, L.C., 2005. Effects on fitness and behavior or monarch butterfly larvae exposed to a combination of Cry1Ab-expressing corn anthers and pollen. *Environmental Entomology*, 34: 944-952.

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

Andow, D.A., Alstad, D.A., 1998. F2 screen for rare resistance alleles. *Journal of Economic Entomology*, 91: 572-578.

Andow, D.A., Olson, D.M., Hellmich II, R.L., Alstad, D.N., Hutchinson, W.D., 2000. Frequency of resistance alleles to *Bacillus thuringiensis* toxin in an Iowa population of European corn borer. *Journal of Economic Entomology*, 93: 26-30.

Andow, D.A., Lövei, G.L., Arpaia, S., 2006. Ecological risk assessment for *Bt* crops. *Nature Biotechnology*, 24: 749-751.

Andreadis, S.S., Alvarez-Alfageme, F.A., Sánchez-Ramos, I., Stodola, T.J., Andow, D.A., Milonas, P.G., Savopoulou-Soultani, M., Castánera, P., 2007. Frequency of resistance to *Bacillus thuringiensis* toxin Cry1Ab in Greek and Spanish population of *Sesamia nonagrioides* (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, 100: 195-201.

Aviron, S., Sanvido, O., Herzog, F., Baudry, J., Romeis, J., and Bigler, F., 2006. Monitoring effects of GM crops on butterflies: the use of multiscale approaches for general surveillance. *Journal of Consumer Protection and Food Safety*, 1(S1): 85-88.

Babendreier, D., Kalberer, N.M., Romeis, J., Fluri, P., Bigler, F., 2004. Pollen consumption in honey bee larvae: a step forward in the risk assessment of transgenic plants. *Apidologie*, 35: 293-300.

Babendreier, D., Kalberer, N.M., Romeis, J., Fluri, P., Mulligan, E., Bigler, F., 2005. Influence of Bt-transgenic pollen, Bt-toxin and protease inhibitor (SBTI) ingestion on development of the hypopharyngeal glands in honeybees. *Apidologie*, 36: 585-594.

Bailey, R.I., Bourguet, D., Le Pallec, A.-H., Ponsard, S., 2007. Dispersal propensity and settling preferences of European corn borers in maize field borders. *Journal of Applied Ecology*, 44: 385-394.

Bates, S.L., Zhao, J.-Z., Roush, R.T., Shelton, A.M., 2005. Insect resistance management in GM crops: past, present and future. *Nature Biotechnology*, 25: 57-62.

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

Biavati, B., Sorlini, C., 2007. Microbiologia agroambientale. Casa Editrice Ambrosiana, Milano.

Beachy, R.N., Fedoroff, N.V., Goldberg, R.B., McHughen, A., 2008. The burden of proof: A response to Rosi-Marshall et al. *Proceedings of the National Academy of Sciences of the United States of America*, 105: E9.

Birch, A.N.E., Griffiths, B.S., Caul, S., Thompson, J., Heckmann, L.H., Krogh, P.H., Cortet, J., 2007. The role of laboratory, glasshouse and field scale experiments in understanding the interactions between genetically modified crops and soil ecosystems: a review of the ECOGEN project. *Pedobiologia*, 51: 251-260.

Blackwood, C.B., Buyer, J.S., 2004. Soil microbial communities associated with Bt and non-Bt corn in three soils. *Journal of Environmental Quality*, 33: 832-836.

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

Bourguet, D., 2004. Resistance to *Bacillus thuringiensis* toxins in the European corn borer: what chance for *Bt* maize? *Physiological Entomology*, 29: 251-256.

Bourguet, D., Chaufaux, J., Micoud, A., Delos, M., Naibo, B., Bombarde, F., Marque, G., Eychenne, N., Pagliari, C., (2002) *Ostrinia nubilalis* parasitism and the field abundance of non-target insects in transgenic *Bacillus thuringiensis* corn (Zea mays). *Environmental Biosafety Research*, 1: 49-60.

Bourguet, D., Chaufaux, J., Séguin, M., Buisson, C., Hinton, J.L., Stodola, T.J., Porter, P., Cronholm, G., Buschman, L.L., Andow, D.A., 2003. Frequency of alleles conferring resistance to Bt maize in French and US corn belt populations of the European corn borer, *Ostrinia nubilalis*. *Theoretical and Applied Genetics*, 106: 1225-1233.

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

Brusetti, L., Francia, P., Bertolini, C., Pagliuca, A., Borin, S., Sorlini, C., Abruzzese, A., Sacchi, G., Viti, C., Giovanetti, L., Giuntini, E., Bazzicalupo, M., Daffonchio, D., 2004. Bacterial communities associated with the rhizosphere of transgenic Bt 176 maize (*Zea mays*) and its non transgenic counterpart. *Plant and Soil*, 266: 11-21.

Castaldini, M., Turrini, A., Sbrana, C., Benedetti, A., Marchionni, M., Mocali, S., Fagiani, A., Landi, S., Santomassimo, F., Pietrangeli, B., Nuti, M.P., Miclaus, N., Giovanetti, M., 2005. Impact of Bt corn on rhizospheric and soil eubacterial communities and on beneficial mycorrhizal symbiosis in experimental microcosms. *Applied and Environmental Microbiology*, 71: 6719-6729.

Chaufaux, J., Séguin, M., Swanson, J.J., Bourguet, D., Siegfried, B.D., 2001. Chronic exposure of the European corn borer (Lepidoptera: Crambidae) to Cry1Ab *Bacillus thuringiensis* toxin. *Journal of Economic Entomology*, 94: 1564-1570.

Clark, B.W., Coats, J.R., 2006. Subacute effects of Cry1Ab Bt corn litter on the earthworm *Eisenia fetida* and the springtail *Folsomia candida*. *Environmental Entomology*, 35: 1121-1129.

Clark, B.W., Phillips, T.A., Coats, J.R., 2005. Environmental fate and effects of *Bacillus thuringiensis* (Bt) proteins from transgenic crops: a review. *Journal of Agricultural and Food Chemistry*, 53: 4643-4653.

Clark, B.W., Prihoda, K.R., Coats, J.R., 2006. Subacute effects of transgenic Cry1Ab *Bacillus thuringiensis* corn litter on the isopods *Trachelipus rathkii* and *Armadillidium nasatum*. *Environmental Toxicology and Chemistry*, 25: 2653-2661.

Codex alimentarius, 2003. Principles for risk analysis and guidelines for safety assessment of foods derived from modern biotechnology, ftp://ftp.fao.org/codex/Publications/Booklets/Biotech/Biotech_2003e.pdf

COGEM, 2008. Response by COGEM to the French Opinion on MON810, CGM/080131-04, http://www.cogem.net/ContentFiles/CGM08013-04%20advice%20MON810%20-%20English.pdf

Cortet, J., Andersen, M.N., Caul, S., Griffiths, B.S., Joffre, R., Lacroix, B., Sausse, C., Thompson, J., Krogh, P.H., 2006. Decomposition processes under Bt (*Bacillus thuringiensis*) maize: Results of a multi-site experiment. *Soil Biology & Biochemistry*, 38: 195-199.

Cortet, J., Griffiths, B.S., Bohanec, M., Demsar, D., Andersen, M.N., Caul, S., Birch, A.N.E., Pernin, C., Tabone, E., de Vaufleury, A., Xin, K., Krogh, P.H., 2007. Evaluation of effects of transgenic Bt maize on microarthropods in a European multi-site experiment. *Pedobiologia*, 51: 207-218.

Crecchio, C., Stotzky, G., 2001. Biodegradation and insecticidal activity of the toxin from *Bacillus thuringiensis* subsp. *kurstaki* bound on complexes of montmorillonite-humic acids-Al hydroxypolymers. *Soil Biology & Biochemistry*, 33: 573-581.

Dalecky, A., Ponsard, S., Bailey, R.I., Pélissier, C., Bourguet, D., 2006. Resistance evolution to Bt crops: predispersal mating of European corn borers. *PLoS Biology*, 4: 1048-1057 (e181).

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

de Vaufleury, A., Kramarz, P.E., Binet, P., Cortet, J., Caul, S., Andersen, M.N., Plumey, E., Coeurdassier, M., Krogh, P.H., 2007. Exposure and effect assessments of *Bt*-maize on non-target organisms (gastropods, microarthropods, mycorrhizal fungi) in microcosms. *Pedobiologia*, 51: 185-194.

Delos, M., Hervieu, F., Folcher, L., Micoud, A., Eychenne, N., 2006. Biological surveillance programme for the monitoring of crop pests and indicators in France. *Journal of Consumer Protection and Food Safety*, 1(S1): 30-36.

Delos, M., Hervieu, F., Folcher, L., Micoud, A., Eychenne, N., 2007. Biological surveillance programme for the monitoring of crop pests and indicators, French devices and European approach compared. *Journal of Consumer Protection and Food Safety*, 2(S1): 16-24.

Devos, Y., De Schrijver, A., Reheul, D., 2008. Quantifying the introgressive hybridisation propensity between transgenic oilseed rape and its wild/weedy relatives. *Environmental Monitoring and Assessment*, DOI:10.1007/s10661-008-0204-y.

Dively, G.P., Rose, R., Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Calvin, D.D., Russo, J.M., Anderson, P.L., 2004. Effects on monarch butterfly larvae (Lepidoptera: Danaidae) after continuous exposure to Cry1Ab-expressing corn during anthesis. *Environmental Entomology*, 33: 1116-1125.

Donegan, K.K., Palm, C.J., Fieland, V.J., Porteous, L.A., Ganio, L.M., Schaller, D.L., Bucao, L.Q., Seidler, R.J., 1995. Changes in levels, species, and DNA fingerprints of soil microorganisms associated with cotton expressing the *Bacillus thuringiensis* var. *kurstaki* endotoxin. *Applied Soil Ecology*, 2: 111-124.

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

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

Duan, J.J., Marvier, M., Huesing, J., Dively, G., Huang, Z.Y., 2008. A meta-analysis of effects of Bt crops on honey bees (Hymenoptera: Apidae). *PLoS ONE*, 3: 1-6 (e1415).

Dubelman, S., Ayden, B.R., Bader, B.M., Brown, C.R., Jiang, C., Vlachos, D., 2005. Cry1Ab protein does not persist in soil after 3 years of sustained *Bt* corn use. *Environmental Entomology*, 34: 915-921.

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

Dutton, A., Romeis, J., Bigler, F., 2005. Effects of Bt maize expressing Cry1Ab and Bt spray on *Spodoptera littoralis*. *Entomologia Experimentalis et Applicata*, 114: 161-169.

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

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

EFSA, 2006. Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. *The EFSA Journal*, 99: 1-100, [including draft document updated in 2008, http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902010430.htm], http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620775747.htm

EFSA, 2007a. Minutes of the 37th plenary meeting of the Scientific Panel on Genetically Modified Organisms held on 22-23 November 2007 in Brussels, Belgium (adopted on 18 December 2007), http://www.efsa.europa.eu/cs/BlobServer/Event_Meeting/GMO_Minutes_37th_plenmeet.pdf

EFSA, 2007b.Review of statistical analyses conducted for the assessment of the MON 863 90-day rat feeding study, http://www.efsa.europa.eu/cs/BlobServer/Scientific_Document/sc_rep_efsa_stat_review,0.pdf

EFSA, 2008. Safety and nutritional assessment of GM plants and derived food and feed: the role of animal feeding trials. Report of the EFSA GMO Panel Working Group on animal feeding trials. *Food and Chemical Toxicology*, 46: S2-S70,

http://www.efsa.europa.eu/cs/BlobServer/Non_Scientific_Document/gmo_report_feedingtrials.pdf

Eizaguirre, M., Albajes, R., López, C., Eras, J., Lumbieres, B., Pons, X., 2006. Six years after the commercial introduction of Bt maize in Spain: field evaluation, impact and future prospects. *Transgenic Research*, 15: 1-12.

Engels, H., Sinha, A., Schupman, I., Eber, S., 2008. Small-scale dispersal of the European corn borer and its relevance for resistance management in Bt maize. *Journal of Applied Entomology*, 132: 675-680.

Escher, N., Kach, B., Nentwig, W., 2000. Decomposition of transgenic *Bacillus thuringiensis* maize by microorganisms and woodlice *Porcello scaber* (Crustacea: Isopoda). *Basic and Applied Entomology*, 1: 161-169.

Fang, M., Kremer, R.J., Motavalli, P.P., Davis, G., 2005. Bacterial diversity in rhizosphere of nontransgenic and transgenic corn. *Applied Environmental Microbiology*, 71: 4132-4136.

Fang, M., Motavalli, P.P, Kremer, R.J., Nelson, K.A., 2007. Assessing changes in soil microbial communities and carbon mineralieation in Bt and non-Bt corn residue-amended soils. *Applied Soil Ecology*, 37: 150-160.

Faria, C.A., Wäckers, F.L., Prichard, J., Barrett, D.A., Turlings, T.C.J., 2007. High susceptibility of *Bt* maize to aphids enhances the performance of parasitoids of lepidopteran pests. *PLoS ONE*, 2: 1-11 (e600).

Farinós, G.P., de la Poza, M., Hernández-Crespo, P., Ortego, F., Castañera, P., 2004. Resistance monitoring of field populations of the corn borers *Sesamia nonagrioides* and *Ostrinia nubilalis* after 5 years of Bt maize cultivation in Spain. *Entomologia Experimentalis et Applicata*, 110: 23-30.

Farinós, G.P., de la Poza, M., Hernández-Crespo, P., Ortego, F., Castañera, P., 2008. Diversity and seasonal phenology of aboveground arthropods in conventional and transgenic maize crops in Central Spain. *Biological Control*, 44: 362-371.

Felke, M., Lorenz, N., Langenbruch, G.-A., 2002. Laboratory studies on the effects of pollen from Bt-maize on larvae of some butterfly species. *Journal of Applied Entomology*, 126: 320-325.

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

Gatehouse, A.M.R., Ferry, N., Raemaekers, R.J.M., 2002. The case of the monarch butterfly: a verdict is returned. *Trends in Genetics*, 18: 249-251.

Gathmann, A., Wirooks, L., Eckert, J., Schuphan, I., 2006a. Spatial distribution of *Aglais urticae* (L.) and its host plant *Urtica dioica* (L.) in an agricultural landscape: implications for *Bt* maize risk assessment and post-market monitorng. *Environmental Biosafety Research*, 5: 27-36.

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

Griffiths, B.S., Caul, S., Thompson, J., Birch, A.N.E., Scrimgeour, C., Andersen, M.N., Cortet, J., Messean, A., Sausse, C., Lacroix, B., Krogh, P.H., 2005. A comparison of soil microbial community structure, protozoa, and nematodes in field plots of conventional and genetically modified maize expressing the *Bacillus thuringiensis* Cry1Ab toxin. *Plant and Soil*, 275: 135-146.

Griffiths, B.S., Caul, S., Thompson, J., Birch, A.N.E., Scrimgeour, C., Cortet, J., Foggo, A., Hackett, C.A., Krogh, P.H., 2006. Soil microbial and faunal community responses to Bt-maize and insecticide in two soils. *Journal of Environmental Quality*, 35: 734-741.

Griffiths, B.S., Caul, S., Thompson, J., Birch, A.N.E., Cortet, J., Andersen, M.N., Krogh, P.H., 2007a. Microbial and microfaunal community structure in cropping systems with genetically modified plants. *Pedobiologia*, 51: 195-206.

Griffiths, B.S., Lars-Henrik, H., Caul, S., Thompson, J., Scrimgeour, C., Krogh, P.H., 2007b. Varietal effects of eight paired lines of transgenic Bt maize and near-isogenic non- Bt maize on soil microbial and nematode community structure. *Plant Biotechnology Journal*, 5: 60-68.

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

Harwood, J.D., Wallin, W.G., Obrycki, J.J., 2005. Uptake of Bt endotoxins by non target herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem. *Molecular Ecology*, 14: 2815-2823.

Harwood, J.D., Samson, R.A., Obrycki, J.J., 2007. Temporal detection of Cry1Ab-endotoxins in coccinellid predators from fields of *Bacillus thuringiensis*. *Bulletin of Entomological Research*, 97: 643-648.

Hellmich, R.L., Siegfried, B., Sears, M.K., Stanley-Horn, D.E., Mattila, H.R., Spencer, T., Bidne, K.G., Lewis, L.C., 2001. Monarch larvae sensitivity to *Bacillus thuringiensis* purified proteins and pollen. *Proceedings of the National Academy of Sciences of the United States of America*, 98: 11925-11930.

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

Hopkins, D.W., Gregorich, E.G., 2003. Detection and decay of the Bt endotoxin in soil from a field trial with genetically modified maize. *European Journal of Soil Science*, 54: 793-800.

Hopkins, D.W., Webster, E.A., Chudek, J.A., Halpin, C., 2001. Decomposition in soil of tobacco plants with genetic modifications to lignin biosynthesis. *Soil Biology & Biochemistry*, 33: 1455-1462.

Höss, S., Arndt, M., Baumgarte, S., Tebbe, C.C., Nguyten, H.T., Jehle, J.A., 2008. Effects of transgenic corn and Cry1Ab protein on the nematode, *Caenorhabditis elegans*. *Ecotoxicology and Environmental Safety*, 70: 334-340.

Hubert, J., Kudlíková-Křížková, I., Stejskal, V., 2008. Effect of MON810 Bt transgenic maize diet on stored-product moths (Lepidoptera: Pyralidae). *Crop Protection*, 27: 489-496.

Huang, F., Buschman, L.L., Higgins, R.A., Li, H., 2002. Survival of Kansas Dipel-resistant European corn borer (Lepidoptera: Crambidae) on Bt and non Bt corn hybrids. *Journal of Economic Entomology*, 95: 614-621.

Huang, F., Rogers Leonard, B., Cook, D.R., Lee, D.R., Andow, D.A., Baldwin, J.L., Tindall, K.V., Wu, X., 2007. Frequency of alleles conferring resistance to *Bacillus thuringiensis* maize in Louisiana populations of the southwestern corn borer. *Entomologia Experimentalis et Applicata*, 122: 53-58.

Hunt, T.E., Higley, L.G., Witkowski, J.F., Young, L.J., Hellmich, R.L., 2001. Dispersal of adult European corn borer (Lepidoptera: Crambidae) within and proximal to irrigated and non-irrigated corn. *Journal of Economic Entomology*, 94: 1369-1377.

Icoz, I., Stotzky, G., 2008a. Cry3Bb1 protein from *Bacillus thuringiensis* in root exudates and biomass of transgenic corn does not persist in soil. *Transgenic Research*, DOI:10.1007/s11248-007-9133-8.

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

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

Jesse, L.C.H., Obrycki, J.J., 2000. Field deposition of Bt transgenic corn pollen: lethal effects on the monarch butterfly. *Oecologia*, 125: 241-248.

Jesse, L.C.H., Obrycki, J.J., 2003. Occurrence of *Danaus plexippus* L. (Lepidoptera: Danaidae) on milkweeds (*Asclepias syriaca*) in transgenic Bt corn agroecosystems. *Agriculture, Ecosystems & Environment*, 97: 225-233.

Johnson, K.L., Raybould, A.F., Hudson, M.D., Poppy, G.M., 2007. How does scientific risk assessment of GM crops fit within the wider risk analysis? *Trends in Plant Science*, 12: 1-5.

Krogh, P.H., Griffiths, B.S., 2007. ECOGEN - Soil ecological and economic evaluation of genetically modified crops. *Pedobiologia*, 51: 171-173.

Krogh, P.H., Griffiths, B.S., Demšar, D., Bohanec, M., Debeljak, M., Andersen, M.N., Sausse, C., Birch, A.N.E., Caul, S., Holmstrup, M., Heckmann, L.-H., Cortet, J., 2007. Responses by earthworms to reduced tillage in herbicide tolerant maize and *Bt* maize cropping systems. *Pedobiologia*, 51: 219-227.

Kroghsbo, S., Madsen, C., Poulsen, M., Schrøder, M., Kvist, P., Taylor, M.L., Gatehouse, A., Shu, Q.Y., Knudsen, I., 2008. Immunotoxicological studies of genetically modified rice expressing PHA-E lectin or Bt toxin in Wistar rats. *Toxicology*, 245: 24-34.

Lang, A., 2004. Monitoring the impact of *Bt* maize on butterflies in the field: estimation of required sample sizes. *Environmental Biosafety Research*, 3: 55-66.

Lang, A., Vojtech, E., 2006. The effects of pollen consumption on transgenic Bt maize on the common swallowtail, *Papilio machaon* L. (Lepidoptera, Papilionidae). *Based and Applied Ecology*, 7: 296-306.

Lang, A., Ludy, C., Vojtech, E., 2004. Dispersion and deposition of *Bt* maize pollen in field margins. *Zeitschrift Für Pflanzenkrankheiten Und Pflanzenschutz*, 111: 417-428.

Lehman, R.M., Osborne, S.L., Rosentrater, K.A., 2008. No differences in decomposition rates observed between *Bacillus thuringiensis* and non- *Bacillus thuringiensis* corn residue incubated soil in the field. *Agronomy Journal*, 100: 163-168.

Lilley, A.K., Bailey, M.J., Cartwright, C., Turner, S.L., Hirsch, P.R., 2006. Life in earth: the impact of GM plants on soil ecology? *Trends in Biotechnology*, 24: 9-14.

Losey, J.E., Rayor, L.S., Carter, M.E., 1999. Transgenic pollen harms monarch larvae. *Nature*, 399: 214.

Lövei, G.L., Arpaia, S., 2005. The impact of transgenic plants on natural enemies: a critical review of laboratory studies. *Entomologia Experimentalis et Applicata*, 114: 1-14.

Ludy, C., Lang, A., 2006a. A 3-year field-scale monitoring of foliage-dwelling spiders (Araneae) in transgenic *Bt* maize fields and adjacent field margins. *Biological Control*, 38: 314-324.

Ludy, C., Lang, A., 2006b. Bt maize pollen exposure and impact on the garden spired, *Araneus diadematus*. *Entomologia Experimentalis et Applicata*, 118: 145-156.

Lumbierres, B., Albajes, R., Pons, X., 2004. Transgenic Bt maize and *Rhopalosiphum padi* (Hom., Aphididae) performance. *Ecological Entomology*, 29: 309-317.

Malone, L.A., 2004. Potential effects of GM crops on honey bee health. *Bee World*, 85: 29-36.

Malone, L.A., Pham-Delègue, M.-H., 2001. Effects of transgene products on honey bees (*Apis mellifera*) and bumblebees (*Bombus* sp.). *Apidologie*, 32: 287-304.

Marchetti, E., Accinelli, C., Talamè, V., Epifani, R., 2007. Persistence of Cry toxins and *cry* genes from genetically modified plants in two agricultural soils. *Agronomy for Sustainable Development*, 27: 231-236.

Marvier, M., McCreedy, C., Regetz, J., Kareiva, P., 2007. A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. *Science*, 316: 1475-1477.

Meissle, M., Vojtech, E., Poppy, G.M., 2005. Effects of Bt maize-fed prey on the generalist predator *Poecilus cupreus* L. (Coleoptera: Carabidae). *Transgenic Research*, 14: 123-132.

Melé, E., Peñas, G., Palaudelmàs, M., Serra, J., Salvia, J., Pla, M., Nadal, A., Messeguer, J., 2007. Effect of volunteers on maize gene flow. In: Stein, A., Rodríguez-Cerezo, E. (Eds.), *Books of abstracts of the third International Conference on Coexistence between Genetically Modified (GM) and non-GM-based Agricultural Supply Chains*, European Commission, pp. 249-250.

Mendelsohn, M., Kough, J., Vaituzis, Z., Matthews, K., 2003. Are *Bt* crops safe? *Nature Biotechnology*, 21: 1003-1009.

Moar, W., Roush, R., Shelton, A., Ferré, J., MacIntosh, S., Leonard, B.R., Abel, C., 2008. Field-evolved resistance to *Bt* toxins. *Nature Biotechnology*, 26: 1072-1074.

Morales, C.L., Traveset, A., 2008. Interspecific pollen transfer: magnitude, prevalence and consequences for plant fitness. *Critical Reviews in Plant Science*, 27: 221-238.

Mulder, C., Wouterse, M., Raubuch, M., Roelofs, W., Rutgers, M., 2006. Can Transgenic Maize Affect Soil Microbial Communities? *PLoS Computational Biology*, 2: 1165-1172 (e128).

Oberhauser, K.S., Rivers, E.R.L., 2003. Monarch butterfly (*Danaus plexippus*) larvae and *Bt* maize pollen: a review of ecological risk assessment for non-target species. *AgBiotechNet* 5: 1-7.

Oberhauser, K.S., Prysby, M., Mattila, H.R. Stanley-Horn, D.E., Sears, M.K., Dively, G.P., Olson, E., Pleasants, J.M., Lam, W.K.F., Hellmich, R.L., 2001. Temporal and spatial overlap between monarch larvae and corn pollen. *Proceedings of the National Academy of Sciences of the United States of America*, 98: 11913-11918.

Obrist, L.B., Dutton, A., Albajes, R., Bigler, F., 2006a. Exposure of arthropod predators to Cry1Ab toxin in Bt maize fields. *Ecological Entomology*, 31: 143-154.

Obrist, L.B., Dutton, A., Romeis, J., Bigler, F., 2006b. Biological activity of Cry1Ab toxin expressed by Bt maize following ingestion by herbivorous arthropods and exposure of the predator *Chrysoperla carnea*. *Biocontrol*, 51: 31-48.

Parrot, W., 2008. Study of Bt impact on caddisflies overstates its conclusions: Response to Rosi-Marshall et al. *Proceedings of the National Academy of Sciences of the United States of America*, 105: E10.

Pleasants, J.M., Hellmich, R.L., Dively, G.P., Sears, M.K., Stanley-Horn, D.E., Mattila, H.R., Foster, J.E., Clark, T.L., Jones, G.D.., 2001. Corn pollen deposition on milkweeds in or near corn field. *Proceedings of the National Academy of Sciences of the United States of America*, 98: 11919-11924.

Pont, B., Nentwig, W., 2005. Quantification of Bt-protein digestion and excretion by the primary decomposer *Porcellio scaber*, fed with two Bt corn varieties. *Biocontrol Science and Technology*, 15: 341-352.

Pons, X., Lumbierres, B., Lopez, C., Albajes, R., 2005. Abundance of non-target pests in transgenic Bt-maize: A farm scale study. *European Journal of Entomology*, 102: 73-79.

Prasifka, P.L., Hellmich, R.L., Prasifka, J.R., Lewis, L.C., 2007. Effects of Cry1Ab-expressing corn anthers on the movement of monarch butterfly larvae. *Environmental Entomology*, 36: 228-233.

Qureshi, J.A., Buschman, L.L., Throne, J.E., Ramaswamy, S.B., 2005. Adult dispersal of *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) and its implications for resistance management in *Bt*-maize. *Journal of Applied Entomology*, 129: 281-292.

Ramirez-Romero, R., Chaufaux, J., Pham-Delègue, M.H., 2005. Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee *Apis mellifera*, a comparative approach. *Apidologie*, 36: 601-611.

Ramirez-Romero, R., Bernal, J.S., Chaufaux, J., Kaiser, L., 2007. Impact assessment of Bt-maize on a moth parasitoid, *Cotesia marginiventris* (Hymenoptera: Braconidae), via host exposure to purified Cry1Ab protein or Bt-plants. *Crop Protection*, 26: 953-962.

Ramirez-Romero, R., Desneux, N., Decourtye, A., Chaffiol, A., Pham-Delègue, M.H., 2008. Does Cry1Ab protein affect learning performances of the honey bee *Apis mellifera* L. (Hymenoptera, Apidae). *Ecotoxicology and Environmental Safety*, 70: 327-333.

Raubuch, M., Roose, K., Warnstorff, K., Wichern, F., Joergensen R.G., 2007. Respiration pattern and microbial use of field-grown transgenic *Bt*-maize residues. *Soil Biology & Biochemistry*, 39: 2380-2389.

Romeis, J., Meissle, M., Bigler, F., 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nature Biotechnology*, 24: 63-71.

Rose, R., Dively, G.P., Pettis, J., 2007. Effects of Bt corn pollen on honey bees: emphasis on protocol development. *Apidologie*, 38: 368-377.

Rosi-Marshall, E.J., Tank, J.L., Royer, T.V., Whiles, M.R., Evans-White, M., Chambers, C., Griffiths, N.A., Pokelsek, J., Stephen, M.L., 2007. Toxins in transgenic crop by products may affect headwater stream ecosystems. *Proceedings of the National Academy of Sciences of the United States of America*, 104: 16204-16208.

Saeglitz, C., Bartsch, D., Eber, S., Gathmann, A., Priesnitz, K.U., Schupman, I., 2006. Monitoring the Cry1Ab susceptibility of European corn borer in Germany. *Journal of Economic Entomology*, 99: 1768-1773.

Saxena, D., Stotzky, G., 2001a. *Bacillus thuringiensis* (Bt) toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil. *Soil Biology & Biochemisty*, 33: 1225-1230.

Saxena, D., Stotzky, G., 2001b. Bt corn has a higher lignin content than non-Bt corn. *American Journal of Botany*, 88: 1704-1706.

Saxena, D., Stotzky, G., 2002. Bt toxin is not taken up from soil or hydroponic culture by corn, carrot, radish, or turnip. *Plant and Soil*, 239: 165-172.

Saxena, D., Flores, S., Stotzky, G., 2002. Bt toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events. *Soil Biology & Biochemistry*, 34: 133-137.

Saxena, D., Stewart, C.N., Altosaar, I., Shu, Q., Stotzky, G., 2004. Larvicidal Cry proteins from *Bacillus thuringiensis* are released in root exudates of transgenic *B. thuringiensis* corn, potato, and rice but not of *B. thuringiensis* canola, cotton, and tobacco. *Plant Physiology and Biochemistry*, 42: 383-387.

Schmidt, K., Wilhelm, R., Schmidtke, J., Beißner, L., Mönkemeyer, W., Böttinger, P., Sweet, J., Schiemann, J., 2008. Farm questionnaires for monitoring genetically modified crops: a case study using GM maize. *Environmental Biosafety Research*, 7: 163-179.

Schmitz, G., Pretscher, P., Bartsch, D., 2003. Selection of relevant non-target herbivores for monitoring the environmental effects of Bt maize pollen. *Environmental Biosafety Research*, 2: 117-132.

Schorling, M., Freier, B., 2006. Six-year monitoring of non-target arthropods in Bt maize (Cry 1Ab) in the European corn borer (*Ostrinia nubilalis*) infestation area Oderbruch (Germany). *Journal of Consumer Protection and Food Safety*, 1(S1): 106-108.

Schrader, S., Münchenberg, T., Baumgarte, S., Tebbe, C.C., 2008. Earthworms of different functional groups affect the fate of the Bt-toxin Cry1Ab from transgenic maize in soil. *European Journal of Soil Biology,* 44: 283-289.

Schuphan, I., 2006. *Protecting the benefits of Bt-toxins from insect resistance development by monitoring and management*. RWTH Aachen, http://www.bio5.rwth-aachen.de/german/downloads/EU-Review.pdf

Sears, M.K., Hellmich, R.L., Siegfried, B.D., Pleasants, J.M., Stanly-Horn, D.E., Oberhauser, K.S., Dively, G.P., 2001. Impact of Bt corn pollen on monarch butterfly populations: a risk assessment. *Proceedings of the National Academy of Sciences of the United States of America*, 98: 11937-11942.

Séralini, G.-E., Cellier, D., Spiroux de Vendomois, J., 2007. New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. *Archives of Environmental Contamination and Toxicology*, 52: 596-602.

Sims, S.R., Holden, L.R., 1996. Insect bioassay for determining soil degradation of *Bacillus thuringiensis* subsp. *kurstaki* CryIA(b) protein in corn tissues. *Environmental Entomology*, 25: 659-664.

Stanley-Horn, D.E., Dively, G.P., Hellmich, R.L., Mattila, H.R., Sears, M.K., Rose, R., Jesse, L.C.H., Losey, J.E., Obrycki, J.J., Lewis, L.C. 2001. Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies *Proceedings of the National Academy of Sciences of the United States of America*, 98: 11931-11936.

StMUGV, 2006. *Monitoring of the environmental effects of the Bt gene*. Schriftenreihe Bayerische Landesanstalt Landwirtschaft 10/2006, http://www.lfl-neu.bayern.de/publikationen/daten/schriftenreihe_url_1_43.pdf

Stodola, T.J., Andow, D.A., Hyden, A.R., Hinton, J.L., Roark, J.J., Buschman, L.L., Porter, P., Cronholm, G.B., 2006. Frequency of resistance to *Bacillus thuringiensis* toxin Cry1Ab in southern United States corn belt population of European corn borer (Lepidoptera: Crambidae). *Journal of Economic Entomology*, 99: 502-507.

Stotzky, G., 2004. Persistence and biological activity in soil of the insecticidal proteins from *Bacillus thuringiensis*, especially from transgenic plants. *Plant and Soil*, 266: 77-89.

Tabashnik, B.E., Carrière, Y., Dennehy, T.J., Morin, S., Sisterson, M.S., Roush, R.T., Shelton, A.M., Zhao, J.Z., 2003. Insect resistance to transgenic *Bt* crops: lessons from the laboratory and field. *Journal of Economic Entomology*, 96: 1031-1038.

Tabashnik B.E., Gassmann, A.J., Crowder, D.W., Carrière Y., 2008a. Insect resistance to *Bt* crops: evidence versus theory? *Nature Biotechnology*, 26: 199-202.

Tabashnik B.E., Gassmann, A.J., Crowder, D.W., Carrière Y., 2008b. Reply to Field-evolved resistance to *Bt* toxins. *Nature Biotechnology*, 26: 1074-1076.

Tapp, H., Stotzky, G., 1995. Insecticidal activity of the toxins from *Bacillus thuringiensis* subspecies *kurstaki* and *tenebrionis* adsorbed and bound on pure and soil clay. *Applied and Environmental Microbiology*, 61: 1786-1790.

Tapp, H., Stotzky, G., 1998. Persistence of the insecticidal toxin from *Bacillus thuringiensis* subsp. *kurstaki* in soil. *Soil Biology & Biochemistry*, 30: 471-476.

Tapp, H., Calamai, L., Stotzky, G., 1994. Adsorption and binding of the insecticidal proteins from *Bacillus thuringiensis* subsp. *kurstaki* and subsp. *tenebrionis* on clay minerals. *Soil Biology & Biochemistry*, 26: 663-679.

Tarkalson, D.D., Kachman, S.D., Knops, J.M.N., Thies, J.E., Wortmann, C.S., 2008. Decomposition of Bt and non-Bt corn hybrid residues in the field. *Nutrient Cycling in Agroecosystems*, 80: 211-222.

Toschki, A., Hothorn, L.A., Roß-Nickoll, M., 2007. Effects of cultivation of genetically modified Bt maize on epigeic arthropods (Araneae; Carabidae). *Environmental Entomology*, 36: 967-981.

Turrini, A., Sbrana, C., Nuti, M.P., Pietrangeli, B.M., Giovanetti, M., 2004. Development of a model system to assess the impact of genetically modified corn and aubergine plants on arbuscular mycorrhizal fungi. *Plant Soil*, 266: 69-75.

Tyutyunov, Y., Zhadanovskaya, E., Bourguet, D., Arditi, R., 2008. Landscape refuges delay resistance of the European corn borer to Bt-maize: a demo-genetic dynamic model. *Theoretical Population Biology*, 74: 138-146.

Van Rensburg, J.B.J., 2007. First report of field resistance by the stem borer, *Busseola fusca* (Fuller) to Bt-transgenic maize. *South African Journal of Plant and Soil*, 24: 147-151.

Vercesi, M.L., Krogh, P.H., Holmstrup, M., 2006. Can *Bacillus thuringiensis* (Bt) corn residues and Bt-corn plants affect life-history traits in the earthworm *Aporrectodea caliginosa*? *Applied Soil Ecology*, 32: 180-187.

Vojtech, E., Meissle, M., Poppy, G.M., 2005. Effects of Bt maize on the herbivore *Spodoptera littoralis* (Lepidoptera: Noctuidae) and the parasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae). *Transgenic Research*, 14: 133-144.

Wandeler, H., Bahylova, J., Nentwig, W., 2002. Consumption of two Bt and six non-Bt corn varieties by the woodlouse *Porcellio scaber*. *Basic and Applied Ecology*, 3: 357-365.

Wang, H., Ye, Q., Wang, W., Wu, L., Wu, W., 2006. Cry1Ab protein from Bt transgenic rice does not residue in rhizosphere soil. *Environmental Pollution*, 143: 449-455.

Whalon, M.E., Mota-Sanchez, D., Hollingworth, R.M., Duynslager, L., 2008. *Arthropod pesticide resistance database*. Michigan State University, 2004-2008, http://www.pesticideresistance.org

Widmer, F., 2007. Assessing effects of transgenic crops on soil microbial communities. *Advances in Biochemical Engineering/Biotechnology*, 107: 207-234.

Wilkinson, M.J., Sweet, J., Poppy, G.M., 2003. Risk assessment of GM plants: avoiding gridlock? *Trends in Plants Science*, 8: 208-212.

Wolfenbarger, L.L., Naranjo, S.E., Lundgren, J.G., Bitzer, R.J., Watrud, L.S., 2008. Bt crop effecs on functional guilds of non-target arthropods: a meta-analysis. *PLoS ONE*, 3: 1-11 (e2118).

Wolt, J.D., Peterson, R.K.D., Bystrak, P., Maede, T., 2003. A screening level approach for nontarget insect risk assessment: transgenic Bt corn pollen and the monarch butterfly (Lepidoptera: Danaidae). *Environmental Entomology*, 32: 237-246.

Zangerl, A.R., McKenna, D., Wraight, C.L., Carroll, M., Ficarello, P., Warner, R., Berenbaum, M.R., 2001. Effects of exposure to event 176 Bacillus thuringiensis corn pollen on monarch and black swallowtail caterpillars under field conditions. *Proceedings of the National Academy of Sciences of the United States of America*, 98: 11908-11912.

Zwahlen, C., Hilbeck, A., Gugerli, P., Nentwig, W., 2003a. Degradation of the Cry1Ab protein within transgenic *Bacillus thuringiensis* corn tissue in the field. *Molecular Ecology*, 12: 765-775.

Zwahlen, C., Hilbeck, A., Howald, R., Nentwig, W., 2003b. Effects of transgenic Bt corn litter on earthworm *Lumbricus terrestris*. *Molecular Ecology*, 12: 1077-1086.

Zwahlen, C., Hilbeck, A., Nentwig, W., 2007. Field decomposition of transgenic Bt maize residue and the impact on non-target soil invertebrates. *Plant Soil*, 300: 245-257.