Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safeguard clause invoked by Hungary according to Article 23 of Directive 2001/18/EC[1]

(Question No EFSA-Q-2005-055)

Opinion adopted on 8 June 2005

SUMMARY

On 20 January 2005, Hungary invoked Article 23 of Directive 2001/18/EC (safeguard clause) to provisionally prohibit the cultivation of the authorised genetically modified maize line MON 810 on its territory. On 27 January 2005, the Commission received from Hungary a detailed list of reasons with documents supporting Hungary's measures.

As a consequence, the European Commission requested in a letter dated 8 April 2005, a scientific opinion from the European Food Safety Authority (EFSA) as to whether the statement and documents submitted by the Hungarian authorities are in accordance with Article 23 of Directive 2001/18/EC. Further EFSA was requested to consider whether new information affects the environmental risk assessment in the light of existing information and on the basis of new scientific knowledge.

Following investigation of the evidence presented in the Hungarian submission, EFSA's Scientific Panel on Genetically Modified Organisms (GMO Panel) concludes there is no new information affecting scientific evidence, in terms of risk to human health and the environment, that would invalidate the risk assessment of genetically modified maize line MON 810 established under Directive 90/220/EEC (repealed by Directive 2001/18/EC from 17 October 2002) and that would justify a prohibition to cultivation of these genetically modified crops in Hungary. The GMO Panel strongly recommends that in order to facilitate a thorough assessment of the identified risk, Member States should support any claims to invoke the safeguard clause by supplying an appropriate risk assessment accompanied by the supporting new scientific data of a quality which can be subjected to detailed scientific scrutiny.

Key words: GMOs, maize (*Zea mays*), MON 810, Hungary, safeguard clause, human health, environment, Directive 2001/18/EC.

[1] For citation purposes: Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safeguard clause invoked by Hungary according to Article 23 of Directive 2001/18/EC, The EFSA Journal (2005) 228, 1-14.

TABLE OF CONTENTS

BACKGROUND

On 13 April 2005, EFSA has received a request from the Commission to provide a scientific opinion on the statement and documents submitted by Hungary in the context of the safeguard clause invoked under Article 23 of Directive 2001/18/EC (EC, 2001). The mandate for the request was adopted at the plenary meeting of the GMO Panel on 20-21 April 2005.

On 21 January 2005, Hungary invoked Article 23 (safeguard clause) of Directive 2001/18/EC to provisionally prohibit the production, use and distribution of seeds derived from the authorised genetically modified maize line MON 810 (Reference C/F/95/12-02), along with the importation on its territory. The prohibition does not apply to the uses as food and feed of the genetically modified maize line MON810.

MON 810 maize (C/F/95/12-02) was authorised in the European Union for all uses with the exception of food by Commission Decision 98/294/EC on 22 April 1998 (EC, 1998) and final consent was granted by the French competent authority on 3 August 1998. Food use of maize derivatives was notified according to Regulation (EC) 258/97 – Art. 5 on 6 February 1998 (EC, 2004).

Hungary is not the only country that invoked the safeguard clauses on MON 810. On 1 June 1999 Austria invoked Article 16 of Directive 90/220/EEC (EC, 1990). The Scientific Committee on Plants (on 24 September 1999) delivered an opinion providing that the justification and information submitted by the Austrian authorities did not impact on the original assessment in terms of risks to human health or the environment (SCP, 1999). In January 2003 Austria provided the Commission with additional information which had been submitted to the European Food Safety Authority (EFSA) for an opinion. On 8 July 2004 EFSA concluded that there was no new scientific evidence, in terms of risk to human health and the environment, that would invalidate the risk assessments of genetically modified maize line MON 810 established under Directive 90/220/EEC or Directive 2001/18/EC and that would justify a prohibition of these genetically modified crops authorised under Directive 90/220/EEC or Directive 2001/18/EC in Austria (EFSA, 2004b).

TERMS OF REFERENCE

EFSA was requested, under Article 29(1) and in accordance with Article 22(5) of Regulation (EC) No 178/2002, to provide a scientific opinion, within 60 days, as to whether, in accordance with Article 23 of Directive 2001/18/EC, the statements and documents submitted by the Hungarian authorities comprise new or additional information affecting the environmental risk assessment or re-assessment of existing information on the basis of new or additional scientific knowledge such that detailed grounds exist to consider that the above authorized GMOs, for the uses laid down in the corresponding consents, constitute a risk to human health or the environment.

EFSA was not requested to give an opinion on political, economic and legal arguments put forward by Hungary in the context of the application of legislation or requests for further legislative/implementing measures.

ASSESSMENT

1. Introduction

Several authorisations for the placing on the market of GMOs were granted under the previous Directive 90/220/EEC, which was repealed by Directive 2001/18/EC on 17 October 2002. Of these products, seeds from GM maize line MON 810 have been authorised for the placing on the market to include cultivation as a use. Pending the renewal of consents, updates to notifications for these products will have to be submitted under Directive 2001/18/EC before 17 October 2006 according to Article 17 of Directive 2001/18/EC.

Article 23 of the Directive states that

- Where a Member State, as a result of new or additional information made available since the date of the consent and affecting the environmental risk assessment or reassessment of existing information on the basis of new or additional scientific knowledge, has detailed grounds for considering that a GMO as or in a product which has been properly notified and has received written consent under this Directive constitutes a risk to human health or the environment, that Member State may provisionally restrict or prohibit the use and/or sale of that GMO as or in a product on its territory. The Member State shall ensure that in the event of a severe risk, emergency measures, such as suspension or termination of the placing on the market, shall be applied, including information to the public. The Member State shall immediately inform the Commission and the other Member States of actions taken under this Article and give reasons for its decision, supplying its review of the environmental risk assessment, indicating whether and how the conditions of the consent should be amended or the consent should be terminated, and, where appropriate, the new or additional information on which its decision is based.

- A decision shall be taken on the matter within 60 days in accordance with the procedure laid down in Article 30(2). For the purpose of calculating the 60 day period, any period of time during which the Commission is awaiting further information which it may have requested from the notifier or is seeking the opinion of the Scientific Committee(s) which has/have been consulted shall not be taken into account. The period of time during which the Commission is awaiting the opinion of the Scientific Committee(s) consulted shall not exceed 60 days. Likewise, the period of time the Council takes to act in accordance with the procedure laid down in Article 30(2) shall not be taken into account.

The genetically modified maize line MON 810 (Reference C/F/95/12-02) has been evaluated at the national and EU level prior to their market approval and thereafter. MON 810 maize was assessed by the Scientific Committee for Plants (SCP, 1998; 1999b).

2. Evaluation of documents delivered by Hungary

The GMO Panel has examined the submission and supporting documents [docs #3-11; see below: Documents provided to EFSA] from Hungary. The 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, 2004a).

Two main aspects were considered:

- whether new scientific evidence had been presented by Hungary which would change the risk assessment conducted on the MON 810, to which is currently given marketing consent in the EU.
- whether there was scientific evidence supplied which would indicate that the environment or ecology of Hungary was different from other regions of the EU and merited separate risk assessments from those conducted for other regions of neighbouring states.

Risk assessment and approval of GMOs according to Directive 90/220/EEC (repealed by Directive 2001/18/EC) is done on a case by case basis. The Directive provides the possibility for Member States to raise objections against marketing of specific GMOs. If necessary, the risk assessment may include features specific to certain geographical regions or sub-regions. Furthermore, the Directive provides safeguards in the case where new or additional information would affect the risk assessment of an authorised GMO. The provisions foreseen by Hungary seek to provisionally prohibit MON 810 seeds from cultivation.

The Hungarian submission stated that research was carried out in Hungary under the direction of the Plant Protection Institute of the Hungarian Academy of Sciences. This research focused on genetically modified maize DK 440 BTY containing the genetic construction YieldGardTM MON 810. The appellants state that laboratory and small-scale parcel field experiments showed that the long-term presence of the plant in the ecosystem may have adverse effects, such as:

I. *"The Bt maize produces 1500-2000 times as much Bt-toxin as is released through a single treatment in conventional crop protection, with the chemical called DIPEL, which contains Bt toxin."*

II. *"Other experiments have found that the residues of Bt plants are slower to decompose than their isogenic lines. Some 8% of the toxin produced by the plant remained in the field after harvesting. Indeed, a substantial share of this active toxin quantity could be identified in the soil 11 months later."*

III. *"In the soil of the field under the transgenic plant, the entire biological activity was lower than in the control field."*

IV. *"The caterpillars thriving on herbs in and on the edges of maize fields, hatching during the pollination period, are the most substantially affected by the Bt toxin produced by MON 810. 16 % of the 187 protected butterfly genera in Hungary may be developing on herbaceous weeds along field edges as well. According to the research findings, Bt containing pollen is most dangerous to Inachis io L. and Vanessa atalanta L."*

Hungary provided a total number of nine documents (#4-12) supporting the statements I. to IV.. Eight of these nine documents are abstracts of reports presented at scientific conferences (supporting documents #4-11) which summarise results of research studies but do not contain any data that can be evaluated on a scientific basis. These summaries are presented without sufficient information on experimental design and how data have been collected and statistically analysed. The GMO Panel strongly recommends that in order to facilitate a thorough assessment of the identified risk, Member States should support any claims to invoke the safeguard clause by supplying an appropriate risk assessment accompanied by the supporting new scientific data of a quality which can be subjected to detailed scientific scrutiny.

Document #12 summarizes some survey data of Lepidoptera *potentially* occurring in maize field margins in Hungary, data on maize pollen shed and estimated pollen densities on host plant leaves. These data are relevant but not sufficient for a full risk assessment. For the following reasons i) there are no scientific data from laboratory and field studies demonstrating acute toxic effects of pollen at the densities that would be encountered in the field (Cry 1Ab expression in MON 810 pollen is generally low);
ii) pollen densities were measured with "sticky plates" in different distances from the maize fields. Based on this data potential pollen densities on leaves were calculated without considering leaf surface or orientation characteristics; iii) in addition, other environmental factors (e.g. rain, wind) and agronomic factors (e.g. other pesticide usage, refuges cultivation) influencing exposure of larvae were not quantified.
The GMO Panel recommends that a full risk assessment should be based on reliable data on toxicity, environmental exposure and statistical analysis of the impact on the populations of the Lepidoptera species. Additionally a full quantitative risk assessment would include:

- a scientifically sound estimate of the coincidence of maize pollen shed and larval activity periods,
- the proportion of a particular Lepidopteran population utilizing weed stands in and near maize fields (on landscape level),
- the mode of adoption and exploitation of Bt maize in Hungary (for example in Spain the refuges areas of non-Bt maize are grown around the outside of the Bt crop so that pollen dispersal is minimised) and
- the potential adoption rate of MON 810 in Maize growing areas of Hungary.

An example of such studies is given by the studies conducted in the USA on the impact of Bt maize (corn) on Monarch butterflies in recent years (see 3b below and Dively *et al* 2004). Scientific evidence presented within document # 12 does not contain scientific information that would alter the risk assessment of the MON 810 maize event.

3. Evaluation of other relevant documents

As stated in document # 3, Hungary invoked Article 23 of Directive 2001/18/EC (EC, 2001) with concerns on the impact of MON 810 on

- biological soil activity through accumulation of Bt toxin (see concern I-III above),
- effects on non-target and endangered caterpillars (see concern IV above).

The GMO Panel has considered the relevance of these concerns again in the light of other scientific data.

3.1. Biological soil activity and Bt toxin accumulation in soil

The cultivation of Bt maize will result in the respective Bt toxins being incorporated into the soil from root exudates, pollen deposits, decomposing roots, stems and leaves after harvest. Some scientific publications indicate that the Bt toxin may persist in soil during cultivation of Bt maize and may accumulate in sequential crops and that this might affect soil organisms. Therefore, both direct and indirect impacts of the toxin or the Bt maize (*e.g.* potential increase of lignins content in combination with a possible delay in decomposition) on non-target organisms and soil function should be considered in risk assessment (Saxena *et al.* 2002, Zwahlen *et al.* 2003a). Data on potential effects of Bt plants are available from several maize events expressing Cry1Ab such as Bt11, MON 810, and Bt176. As effects of Bt plants expressing similar Cry proteins are considered to be comparable, the GMO Panel has taken published data on other Bt maize cultivars into account. Saxena & Stotzky (2001) reported that Cry1Ab had no apparent effect on earthworms and nematodes in a 45-days study. Zwahlen *et al.* (2003b) reported a 200-day study investigating the impact of transgenic Bt maize event Bt11 (expressing Cry1Ab) on immature and adult *Lumbricus terrestris* in a single worst-case laboratory study and in a single small scale field test. At the end of the laboratory test the earthworms showed a significant weight loss of 18% (compared with their initial weight) when fed (Bt+) maize litter whereas a weight gain of 4% occurred with non-GM control maize. No difference was found in the higher tier small scale field test. The experimental design did not allow the authors to exclude the possibility that the weight loss of earthworms fed with Bt maize in the laboratory test was due to other factors.

The effects of Bt11 maize on soil microbial community structure were assessed in growth chamber experiments using three soil types with different textures (Blackwood & Buyer, 2004). Very few significant effects on soil microbial communities due to the presence of the Bt toxins were found, whereas the soil type significantly influenced the composition of the soil microflora. Similarly, other studies on transgenic plants expressing Cry toxins did not reveal any negative, long-lasting impact on the soil or plant-associated microorganisms (Devare *et al.*, 2004; Donegan *et al.*, 1995). Koskella & Stotzky (2002) reported that Bt proteins showed no toxicity to bacteria, fungi and algae. Field studies were done in Germany to assess how much of the transgenic, insecticidal protein Cry1Ab, was released from Bt-maize MON 810 into soil and whether bacterial communities inhabiting the rhizosphere of MON 810 maize were different from those of the rhizosphere of non-transgenic maize cultivars (Baumgarte & Tebbe 2005). The concentrations of Cry1Ab protein in the rhizosphere soil did not accumulate during the growing season, despite the affinity of the Cry1Ab protein for soil particles. The concentrations of the Cry1Ab protein in soil from Bt-maize fields are in the range between 0.1 and 10 ng/g in bulk soils and rhizospheres. Baumgarte & Tebbe (2005) are not aware of any non target or target organism that would directly respond to such low concentrations as a bioindicator. In addition, the bacterial community structure was less affected by the Cry1Ab protein than by other environmental factors, i.e. the age of the plants or field heterogeneities.

A four year study on the decay of transgenic maize Bt toxin was published by Hopkins and Gregorich (2003). This followed the rate at which the toxin in Bt-maize leaves decomposed in soil from a field in which Bt-maize had been cultivated for four years. In addition, Hopkins and Gregorich (2005) determined the concentrations of the Cry1Ab protein in organic residues from MON 810 maize plants at increasing stages of ageing and decay, and the subsequent decomposition in soil of these residues and the Cry1Ab protein in them. The Cry1Ab protein decomposes faster than the bulk organic carbon in residues and it is likely to fall below the detection limit by ELISA within months of entering the soil. The results suggested that much of the Cry1Ab protein in crop residues is highly labile and quickly decomposes in soil, but that a small fraction may be protected from decay in relatively recalcitrant residues. It is known from experience with conventional Bt sprays, that Bt toxins (including Cry1Ab) can persist in soils, e.g. for at least 28 months as reported by Vettori *et al.* (2003).

Saxena *et al.* (2002) found that the release of Cry1Ab proteins by roots is a common phenomenon with transgenic maize. Although the release of Bt toxin from roots and decaying plant material has theoretical implications for the activity and survival of root-feeding invertebrates and organisms involved in decomposition processes, there is currently little evidence for any significant adverse effects of the Bt toxin on non-target soil organisms, either from transgenic plant material expressing the toxin or from extensive studies with *B. thuringiensis* preparations used historically as a control agent. Saxena, Stotzky (2001) and Stotzky (2004) did not report any deleterious effects on soil microorganisms, earthworms or nematodes with Bt-maize. In addition, tests with *B. thuringiensis* preparations showed no deleterious effects on a variety of invertebrates (Glare and O'Callaghan, 2000).

Recently, the decomposition of different plant species expressing Bt toxins was analysed in laboratory experiments and results were discussed in relation to lignins contents and potential environmental consequences (Flores *et al*., 2005). Generally, Bt plants showed lower decomposition rates than non-Bt plants. However, this effect was not clearly related to lignification or reduced microbial activity in soil. The authors concluded that lower decomposition rates may be beneficial as organic matter derived from plants would persist for a longer period improving soil structure and reducing erosion. In addition, Flores *et al*. (2005) discussed potential effects on target and non-target insects due to the longer persistence of Bt toxins in soil. In relation to soil organic content, it has been shown that even distinct increases in decomposition resistant compounds such as lignins result in only modest increases in organic carbon in the topsoil. Changes in soil management have a much more pronounced effect (Sessitsch *et al*., 2004). Considering the available information on potential effects of Bt plants on the soil environment and in particular on soil non-target organisms, adverse effects due to slightly altered decomposition rates are unlikely.

In general, there are no published data on the impact of Bt maize on biological soil functions which indicate the need for a change in the original environmental risk assessment.

3.2 Effects on non-target and endangered caterpillars

The temporal occurrence, spatial range and dispersion of maize pollen, and thus potentially Bt maize pollen densities in field margins is an important part of the risk assessment of Bt maize as it characterises the potential environmental exposure of non-target organisms to Bt pollen. Knowledge of naturally occurring maize pollen densities on food plants is indispensable for assessing the expected effects of Bt maize on butterfly larvae along field edges together with the toxin amount of the Bt maize pollen, and its toxic effect on butterfly larvae (Lang *et al.* 2004).

It is well documented that a range of lepidopteran species may be affected by Bt toxins and some may be present in maize fields (Schmitz *et al*., 2003; for a review see Evans 2002). However, exposure of any populations of lepidoptera to the toxin is restricted to those consuming the Bt plant or its products. In the vicinity of the Bt maize field larvae may be most exposed to the toxin when Bt maize pollen is deposited on plants on which they are feeding. Maize, a recently introduced species into Europe, is not a significant food source for endemic lepidoptera and impacts due to pollen dispersal are likely to be transient and minor as demonstrated by studies on monarch butterflies in the USA (Dively *et al*., 2004). Published studies investigating potential effects of GMOs due to the expression of Bt toxins have been mainly performed with maize Bt11 and Bt176, both producing Cry1Ab. Generally similar effects on the environment due to the presence of different *cry* genes can be expected, however, the severity of potential effects will depend on the expression of the relevant gene and the toxicity of the resulting toxin. Considering toxicity and exposure of Cry1Ab, the risk of exposure of non-target lepidoptera to harmful toxin concentrations via Mon 810 maize pollen is negligible and that adverse impacts on populations are very unlikely.

The abundance of non-target predators preying upon the target organisms *Ostrinia nubilalis* or *Sesamia nonagrioides* will vary with the abundance of their prey. Thus, a reduction in prey either by cultivation of Bt maize or by insecticides may negatively effect the food source of predators like *Chrysoperla carnea* (Hilbeck *et al*. 1998a,b). However, current knowledge on toxicity and exposure give sufficient scientific evidence that Bt maize poses no risk to this predator (Dutton *et al*. 2003a,b; Romeis *et al.* 2004). Most field studies confirm that predator and parasitoid abundances and biocontrol functions are very similar in Bt and non-Bt fields (Candolfi *et al*. 2004, Pons & Stary, 2003, Musser & Shelton, 2003). As part of a Spanish specific monitoring program for Bt maize (Bt176), a farm-scale study was initiated in 2000 to assess the potential impacts of Bt maize on predatory arthropods. The trials were conducted at two maize growing areas over 3 years. The data suggest that Bt maize has no adverse effect on naturally occurring predators (De La Poza *et al.* 2005) and on aphids, leafhoppers, cutworms and wireworms (Pons *et al.* 2005).

Reductions of population densities of specialist *Ostrinia* predators and parasitoids are expected as this pest is the target to be controlled in Bt maize fields. Bourget *et al.* (2002) and Siegfried *et al.* (2001) have found that populations of specific natural enemies of *Ostrinia* are less abundant in Bt maize fields than in non-Bt maize fields. This is not thought to be due to the direct effects of the Cry toxin consumed while predating or parasitizing *Ostrinia* but is due to decreased availability of specific prey. Results of field studies comparing the effects of Bt maize with insecticide treatments against the target pest, show that broad-spectrum insecticides, like pyrethroids, reduce abundances of a range of predator and parasitoid species not specific to *Ostrinia* (Dively & Rose 2003, Candolfi *et al.* 2004). Such effects have not been reported in Bt maize.

Previous worst-case scenario tests on Bt maize reporting potential adverse effects on non-target organisms have been proven irrelevant in laboratory and environmental field tests. Bt toxin (Cry1Ab) has no direct effect on larvae of the green lacewing (Romeis *et al.*, 2004). A substantial number of other entomophagous arthropods are not sensitive to Cry1Ab (Dutton *et al.*, 2003a). Ecological field tests in France (Bourguet *et al.*, 2002) have also shown no effects on non-lepidopteran species. A study by Sears *et al.* (2001) suggests that the impact of Bt maize pollen from current commercial hybrids on monarch butterfly populations is negligible.

In 2001, the US Environmental Protection Agency assessed data collected during the process of renewing the registration of Bt crop whose registration expired. These crops had been cultivated in the US since 1996. The study concluded that Bt crops, including Bt maize MON 810, posed no significant risk to the environment or to human health (Mendelson *et al.*, 2003).

Finally, O'Callaghan *et al.* (2005) concluded in a review, that the extensive testing on non-target plant-feeding insects and beneficial species that has accompanied the long-term and wide-scale use of Bt plants has not detected significant adverse effects. In addition, Bt plants appear to have little impact on soil biota such as earthworms, collembolans, and general soil microflora.

No conclusive evidence has yet been presented that currently released transgenic Bt crops are causing significant direct effects on the environment. The effects of transgenic Bt maize in these experiments were small, if they existed at all. In addition, the available data do not indicate a chain of events that might result in long-term effects. Therefore, it seems likely that in commercial cropping conditions, where crop rotations are used, the consequences of effects on soil functions and soil organisms are negligible. Overall, the evidence presented by Hungary contains no new scientific information on the environmental or human health impacts of the specified GM maize event. No scientific evidence is presented which shows that Hungary has unusual or unique ecosystems that require separate risk assessments compared with other similar regions of Europe. No specific data were presented to show that transgenic Bt maize crops have an adverse effect on biodiversity, either directly or indirectly through changes in agricultural practices.

CONCLUSIONS

The Scientific Panel on Genetically Modified Organisms, having considered the scientific information submitted by Hungary, is of the opinion that

- there is no new data that would invalidate the provisions for the environmental risk assessment established under Directive 90/220/EEC or Directive 2001/18/EC.

- there is no specific scientific evidence, in terms of risk to human health and the environment, that would justify a prohibition of the genetically modified crops authorised under Directive 90/220/EEC or Directive 2001/18/EC in Hungary.

In conclusion, the Panel finds that the scientific evidence currently available does not sustain the arguments provided by Hungary. The GMO Panel strongly recommends that in order to facilitate a thorough assessment of the identified risk, Member States should support any claims to invoke the safeguard clause by supplying an appropriate risk assessment accompanied by the supporting new scientific data of a quality which can be subjected to detailed scientific scrutiny.

DOCUMENTATION PROVIDED TO EFSA

1. Letter to Mr. Geoffrey Podger, dated 8 April 2005 with ref. DG Environment B4/BV/D(05) 6231, from Mrs. Catherine Day from Environment Directorate-General requesting a consultation of the Scientific Panel on Genetically Modified Organisms with supporting documents:
2. Letter from Dr. Persanyi to Stavros Dimas, dated 21 January 2005, to Environment Directorate-General
3. Document No. 104130/2005: Announcement of Dr. Németh Imre, Minister of Agriculture and Rural Development, Hungary, dated 20 January 2005
4. Darvas Béla, Kincses Judit, Vajdics Gyöngyi, Polgár A. Lászlo, Juracsek Judit, Ernst András and Székács András (2003). Effect of pollen of Dk-440-BTY (yieldgard) Bt maize on the larvae of *Inachis Io.* L. (nymphalidae). Abstract *49th Plant Protection Days (25-26 February 2003)*
5. Ilona Villanyi, Zoltan Naar, Istvan Kiss, Gabor Bakonyi and Borbala Biro (2003). Comparative assessment of the decomposition of material line maize producing Bt toxin and its C:N ratio. Abstract *49th Plant Protection Days (25-26 February 2003)*
6. Gabor Bakonyi, Istvan Kiss, Fruzsina Szira, Borbala Biro, Ilona Villanyi, Judit Juradecsek and Andras Szekacs (2003). Effects of Maize producing Bt toxin (DK-440-BTY) on the biological activity of the soil and the territory and food selection of collembolan. Abstract *49th Plant Protection Days (25-26 February 2003)*
7. Csóti Attila, Peregovits László, Ronkay László and Darvas Béla (2003). Data for the risk analysis of Bt maize pollen sensitive butterfly larvae. Abstract *49th Plant Protection Days (25-26 February 2003)*
8. Laszlo A. Polgar, Gyöngyi Vajdics, Judit Juracsek, Andrai Szekacs, Gabor Fekete and Bela Darvas (2003). Effects of transgenic maize (DK-440-BTY) in host-parasitoid (*plodia interpunctella/venturia canescens*) system. Abstract *49th Plant Protection Days (25-26 February 2003)*
9. Tombacz and Emöke Magyar (2003) The place and role of GMO environmental assessment in the licensing procedure. Abstract *49th Plant Protection Days (25-26 February 2003)*
10. Borbala Biro, Ilona Villanyi, Zoltan Naar, Gabor Bakonyi (2003). Changes in certain soil microbiological characteristics in the root zone of the genetically modified Bt maize. Abstract *49th Plant Protection Days (25-26 February 2003)*
11. Bela Darvas, Adel Gharib, Attila Csoti, Andras Szekacs, Gyongyi Vajdics, Laszlo Peregovits, Laszlo Ronkay and Laszlo Polgar (2002). On the pollen of *yieldgard* genetically modified maize. Abstract *48th Plant Protection Days (6-7 March 2002)*
12. Béla Darvas, Attila Csóti, Adel Gharib, László Peregovits, László Ronkay, Éva Lauber and László A. Polgár (2004). Data for the risk analysis in Hungary of Bt maize pollen and larvae of protected butterfly species. Növényvédelem (Plant Protection) 40 (9) 441-447, with English translation

REFERENCES

Baumgarte, S. & Tebbe, 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.

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.

Bourguet D., Chaufaux J., Micoud, A., Delos, M., Naibo, B., Bombardes 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). Environ. Biosafety Res., 1, 49–60.

Candolfi, M.P., Brown, K., Grimm, C., Reber, B. & Schmidli, H., 2004. A faunistic approach to assess potential side-effects of genetically modified Bt-corn on non-target arthropods under field conditions. Biocontrol Science and Technology, 14, 129-170.

De La Poza, M., Pons, X., Farinósa, 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.

Devare, M.H., Jones, C.M. & Thies, J.E., 2004. Effect of Cry3Bb transgenic corn and tefluthrin on the soil microbial community: biomass, activity and diversity. J. Environ. Qual., 33, 837- 843.

Dively G.P. & Rose R., 2003. Effects of Bt transgenic and conventional insecticide control on the non-target natural enemy community in sweet corn. Proceedings of the 1st International Symposium on Biological Control of Arthropods, 265-274.

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. Environ.Entomol., 33(4), 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 expressiong the Bacillus thuringiensis var. kurstaki endotoxin. Appl. Soil Ecol., 2, 111-124.

Dutton, A., Romeis, J. & Bigler, F., 2003a. Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: Bt-Maize expressing Cr1Ab as a case study. BioControl, 48, 611-636.

Dutton A., Klein H., Romeis J.& Bigler F., 2003b. Prey-mediated effects of *Bacillus thuringiensis* spray on the predator Chrysoperla carnea in maize. Biological Control, 26, 209-215.

EC, 1990. Council Directive 90/220/EEC of 23 April 1990 on the deliberate release into the environment of genetically modified organisms. Official Journal of the European Communities, L117, 15-27. http://europa.eu.int/smartapi/cgi/sga_doc?smartapi!celexapi!prod!CELEXnumdoc&lg=EN&numdoc=31990L0220&model=guichett

EC, 1998. Commission Decision 98/294/EC of 22 April 1998 concerning the placing on the market of genetically modified maize (*Zea mays* L. line MON 810), pursuant to Council Directive 90/220/EEC. Official Journal of the European Communities L131, 32-33. http://europa.eu.int/eur-lex/pri/en/oj/dat/1998/l_131/l_13119980505en00320033.pdf

EC, 2001. Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal of the European Communities, L106, 1-39.

http://europa.eu.int/eur-lex/pri/en/oj/dat/2001/l_106/l_10620010417en00010038.pdf

EC, 2004. Notifications pursuant to Article 5 of Regulation (EC) N° 258/97 of the European Parliament and the Council. http://europa.eu.int/comm/food/food/biotechnology/novelfood/notif_list_en.pdf

EFSA, 2004a. Guidance document for the risk assessment of genetically modified plants and derived food and feed, prepared by the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority, The EFSA Journal (2004) 99, 1-94. http://www.efsa.eu.int/science/gmo/gmo_guidance/660/guidance_docfinal1.pdf

EFSA, 2004b. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Austrian invoke of Article 23 of Directive 2001/18/EC, The EFSA Journal (2004) 78, 1-13. http://www.efsa.eu.int/science/gmo/gmo_opinions/507/opinion_gmo_safeguard_clauses_austria_en1.pdf

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

Glare, T.R. & O´Callaghan, M., 2000. Bacillus thuringiensis: Biology, Ecology and Safety. Wiley and Sons, Chichester. 350 p.

Hilbeck, A., W.J. Moar, M. Pusztai-Carey, A. Filippini & F. Bigler, 1998a. Toxicity of *Bacillus thuringiensis* Cry1Ab toxin to the predator *Chrysoperla carnea* (Neuroptera: *Chrysopidae*). Environ. Entomol., 27, 1255–1263.

Hilbeck, A., M. Baumgartner, P.M. Fried & F. Bigler, 1998b. Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: *Chrysopidae*). Environ. Entomol., 27, 480–487.

Hopkins, D.W. and 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. & Gregorich, E.G., 2005. Decomposition of residues and loss of the δ-endotoxin from transgenic (Bt) corn (Zea mays L.) in soil. Canadian Journal of Soil Science, 85, 19 -26.

Koskella, J. & Stotzky, G. 2002. Larvicidal toxins from Bacillus thuringiensis subsp. kurstaki, morrisoni (strain tenebrionis), and israelensis have no microbicidal or microbiostatic activity against selected bacteria, fungi, and algae in vitro. Can. J. Microbiol., 48, 262- 267.

Lang, A., Ludy C. & Vojtech, E., 2004. Dispersion and deposition of Bt maize pollen in field margins. J. Plant Diseases and Protection, 111, 417-428.

Mendelson, M., Kough, J., Vaituzuis, Z. & Matthews, K., 2003. Are Bt crops safe? Nature Biotechnology, 21, 1003-1009.

Musser, F.R. & Sehlton, A.M., 2003. Bt sweet corn and selective insecticides: Impacts on pests and predators. J. Econ. Entomol., 96, 71-80.

O'Callaghan, M., Glare, T.R., Burgess, E.P.J., & Malone, L..A., 2005. Effects on plants genetically modified for insect resistance on nontarget organisms. Annu. Rev. Entomol., 50, 271-292.

Pons, X. & Stary, P., 2003. Spring aphid-parasitoid (Hom.Aphididae, Hym. Braconidae) association and intractions in a Mediterranean arable crop ecosystem, including Bt maize. J. Pest Sci., 76, 133-138.

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.

Romeis, J., Dutton, A. & Bigler, F., 2004. Bacillus thuringiensis toxin (Cry1Ab) has no direct effect on larvae of the green lacewing Chrysoperla carnea. Journal of Insect Physiology, 50, 175-183.

Saxena, D. & Stotzky, G., 2001. 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 and Biochemistry, 33, 1225–1230.

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 and Biochemistry, 34, 133–137.

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.

Sessitsch, A., Smalla, K., Kandeler, E. & Gerzabek, M.H., 2004. Effects of transgenic plants on soil microorganisms and nutrient dynamics. In Plant Microbiology (Eds. M. Gillings & A. Holmes), BIOS Scientific Publishers, London and New York.

SCP, 1998. Opinion of the Scientific Committee on Plants regarding the genetically modified, insect resistant maize lines notified by the Monsanto Company. http://europa.eu.int/comm/food/fs/sc/scp/out02_en.html

SCP, 1999. Opinion of the Scientific Committee on Plants on the Invocation by Austria of Article 16 ('safeguard' clause) of Council Directive 90/220/EEC with respect to the placing on the market of the Monsanto genetically modified maize (MON 810) expressing the Bt cryia(b) gene, notification C/F/95/12-02. http://europa.eu.int/comm/food/fs/sc/scp/out49_en.html

Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Oberhauser, K.S., Pleasants, J.M., Mattila, H.R., Siegfried, B.D. & Dively, G.P., 2001. Impact of Bt corn pollen on monarch butterfly populations: A risk assessment. Proc. Natl. Acad. Sci. USA, 98, 11937-11942.

Siegfried, B.D., Zoerb, A.C. & Spencer, T., 2001. Development of European corn borer larvae on Event 176 Bt corn: influence on survival and fitness. Entomol. Exp. et Appl., 100, 15-20.

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.

Vettori C., Paffetti D., Saxena D., Stotzky G. & Giannini R., 2003. Persistence of toxins and cells of Bacillus thuringiensis subsp. kurstaki introduced in sprays to Sardinia soils. Soil Biology and Biochemistry, 35, 1635-1642.

Zwahlen, C., Hilbeck, A., Howald, R. & Nentwig, W., 2003a. Effects of transgenic Bt corn litter on the earthworm Lumbricus terrestris. Molecular Ecology, 12, 1077–1086.

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

SCIENTIFIC PANEL MEMBERS

Hans Christer Andersson, Detlef Bartsch, Hans-Joerg Buhk, Howard Davies, Marc De Loose, Michael Gasson, Niels Hendriksen, John Heritage, Sirpa Kärenlampi, Ilona Kryspin-Sørensen, Harry Kuiper, Marco Nuti, Fergal O'Gara, Pere Puigdomenech, George Sakellaris, Joachim Schiemann, Willem Seinen, Angela Sessitsch, Jeremy Sweet, Jan Dirk van Elsas and Jean-Michel Wal.

ACKNOWLEDGEMENT

The GMO Panel wishes to thank Achim Gathmann for his contribution to the draft opinion.