

EFSA fails again: insect resistant GM *Bt* maize 1507 (C/ES/01/01) should not be grown in Europe

Lorch, A. & Cotter, J.

Greenpeace Research Laboratories, Department of Biological Sciences, University of Exeter, Exeter EX4 4PS, UK

December 2005

Technical Note: 13/2005

EFSA fails again: insect resistant GM *Bt* maize 1507 (C/ES/01/01) should not be grown in Europe

A. Lorch & J. Cotter

Greenpeace Research Laboratories Technical Note 13/2005

December 2005

Summary

Pioneer's genetically modified (GM) maize 1507 is both insect resistant and herbicide tolerant. 1507 produces the *Bt* toxin Cry1F. There are very few peer-reviewed studies on the environmental risks and human and animal health risks of Cry1F. Therefore, the evaluation of GM maize 1507 requires considerable care. EFSA has evaluated 1507 and given a positive opinion. However, EFSA's assessment is woefully inadequate. Each time there is uncertainty in the data submitted, EFSA makes a considerable effort to negate the possible consequences of these uncertainties with phrases such as "unlikely" or "not of biological relevance". The assessment of 1507 is yet another example of EFSA disregarding scientific evidence to give a positive opinion of a GM crop even when there remain serious scientific uncertainties and unknowns regarding the environmental and food/feed safety.

There remain many scientific unknowns regarding the safety of GM maize 1507 for the environment, human and animal health. Below, the major outstanding scientific questions are described. Together, they are grounds for rejection of GM insect resistant maize 1507 to be grown or imported into the EU.

Greenpeace urges EU Member States and the European Commission to:

- **reject EFSA's report on 1507 maize because there is a serious lack of scientific scrutiny in the manner that it has been performed,**
- **apply the Precautionary Principle and reject the cultivation of 1507 maize,**
- **re-organise EFSA's to ensure that experts are independent and perform their work with the necessary scrutiny.**

Introduction

Pioneer's 1507 maize¹ is a genetically modified (GM) maize (corn) containing two types of inserted genetic constructs. One insert produces a modified form of the *Bacillus thuringiensis* (*Bt*) Cry1F protein, which is toxic to lepidoptera (butterflies and moths), in order to make the GM maize resistant to larvae of the European corn borer (*Ostrinia nubilalis*) and of the Mediterranean corn borer (*Sesamia nonagrioides*). The second GM trait is tolerance to the herbicide, glufosinate ammonium, sold under brand names including Basta or Liberty.

Cry1F is a different *Bt* toxin than that produced by current GM insect resistant maize, such as Bt176, Bt11 and MON810, which produce Cry1Ab. There are very few peer-reviewed studies on the environmental risks and human and animal health risks of Cry1F. Therefore, the evaluation of GM maize 1507 requires considerable care. EFSA gave a positive opinion of GM maize in January 2005. This is the first time that EFSA has assessed a GM crop, not only for the import and processing, but also for cultivation. EFSA's assessment is woefully inadequate and there remain

¹ 1507 maize is also known as TC1507 or as unique identifier DAS-01507-1

many scientific uncertainties and unknowns regarding the safety of GM maize 1507 for the environment, human and animal health. Below, the major outstanding scientific questions are described. Together, they are grounds for rejection of GM insect resistant maize 1507 to be grown or imported into the EU.

1) Unknown effects of unintended gene fragments

Genetic engineering is known to cause additional gene fragments to be inserted and rearrangements of portions of the plant's own DNA. Indeed, there are several unintended additional fragments in 1507 (EFSA, 2005) including:

- **Fragment of the Cry1F gene** located at the beginning (5' end) of the intended insert.
- **Fragments of other parts of the inserted genes** including fragments of the plasmid, the *pat* gene, the maize ubiquitin promoter and the terminator sequence.
- **Fragments of chloroplast DNA.**

It is unknown where these additional unintended gene fragments are located within the plant genome, or whether they interrupt the plant's own genes or regulatory elements. These unintended fragments result in two substantial open reading frames (ORFs, genes without a "stop" codon), which could produce unintended RNA or novel or altered protein. The possibility that these fragments are expressed has not been discounted as a faint signal indicating the presence of unintended RNA:

"Northern analysis revealed no expression of ORF4 but a weak signal was detected using RT-PCR, which also indicated that the detected mRNA originates from a read-through product of the cry1F gene." (EFSA, 2005)

EFSA consider it *"very unlikely"* that any protein could be expressed from this open reading frame. However, similar statements regarding expression of the fragments in Roundup Ready soya were made in 2000 (Monsanto, 2000) but were later found to be transcribed (Monsanto, 2002a,b). The consequences of additional copies and fragments of the inserted gene, of these fragments being expressed as far as RNA, or being expressed in conjunction with other genes is extremely difficult to predict. Potentially, any unintended RNA or novel or altered protein is a serious consequence of the molecular irregularities in 1507.

EFSA (2005) consider that any expressed protein from this open reading frame would not have adverse effects because it would bear no resemblance to *"known allergenic, toxic or gluten sensitive enteropathy-related proteins"*. However, it has recently been shown that only minor structural changes to protein structure are necessary to cause significant effects on toxicity (Prescott et al., 2005). Hence, any unintended protein (or altered plant protein) can be allergenic even if bears no resemblance to known allergens.

There are many irregularities in the genome of 1507 caused by the genetic engineering process itself. These by themselves, on the basis of the Precautionary Principle, should be ground for rejection of 1507 as they can give rise to unintended and unexpected effects. However, combined with the significant differences seen in composition between GM 1507 and its non-GM counterpart and the significant differences in the toxicological assessment, this gives serious cause for concern. The implications of the genome irregularities in 1507 to the environmental and food/feed safety of this GM maize remain unknown.

The failure of EFSA to recognise these warning signs and failure to investigate the possibility that irregularities in the genome of 1507 could have caused these significant differences, signifies that EFSA itself is a failure.

2) Differences in composition between GM 1507 and non-GM control maize

Statistically significant differences were found from the analysis of plant composition. However, EFSA (2005) disregard the differences between GM 1507 and the non-GM control line because a lack of consistency, instead relying on comparisons with “*ranges published in the literature*”. This is not scientifically valid. In addition, such composition differences between the GM and non-GM maize must be taken very seriously, given the excessive number of unintended additional DNA fragments in the GM plant genome.

There are many statistically significant differences in plant composition:

“In summary, the analysis of nutrient composition of kernels from maize line 1507 (glufosinate treated and non-treated) occasionally revealed statistically significant differences in some compounds. For example, kernels of 1507 maize contained higher overall levels of potassium, linoleic acid, linolenic acid, and tocopherols, as well as lower levels of fat, manganese, stearic acid, oleic acid, cysteine, methionine, and vitamin B1, than control kernels in the 1998-1999 season. The levels of protein, amino acids (Ala, Asp, Glu, Gly, His, Leu, Phe, Pro, Ser, Thr, Tyr, and Val), and potassium were increased, while the level of vitamin B2 was decreased, in kernels of 1507 maize (both sprayed and non-sprayed) compared with control kernels in 1999. In the 2000 season, ash, amino acids (Ala, Phe, Tyr), and potassium were increased, while manganese was decreased in kernels of maize line 1507 (both sprayed and non-sprayed) compared with controls. Across locations and between years, however, there were no consistent statistically significant differences. All analytical data were either very close to or within the ranges published in the literature.” (EFSA, 2005)

The comparison to literature ranges is not valid as it is the experimental control that has to be used for comparison. Clearly, GM 1507 is not compositionally equivalent to that of the non-GM control maize.

Indeed, many statistically significant differences are seen in the compositional analysis of GM maize 1507. Despite this, referring to possible DNA deletions caused by the genetic engineering process, EFSA (2005) states that there is “*No indication that such a [DNA] deletion produces any phenotypic effect in the transformed maize line.*” There may not be any indication in the agronomic indicators of any unintended changes, but there are several statistically significant differences in plant composition and in animal toxicity. Therefore, GM maize 1507 is not equivalent to its non-GM counterpart. These differences could cause adverse effects in the long term on human and animal health or the environment.

3) Differences found in the toxicological assessment.

Statistically significant differences between rats fed (unsprayed) 1507 maize kernels and the non-GM control group in the subchronic oral toxicological assessment were found but EFSA rejects these differences either by considering them not “*biologically relevant*” or relying on comparisons with “*another non-transgenic line as reference*” (EFSA, 2005). Such differences are grounds for rejection of 1507, especially in conjunction with the high number of molecular irregularities in this GM maize.

- Statistically significant **increase of feed consumption** was found in male rats. EFSA (2005), however, argues that no significant difference was found between 1507 and the reference group, and that the difference between 1507 maize and the control therefore pose no “*concerns over the safety and nutritional value of 1507 maize*”.

- Statistically significant **decrease of serum counts of eosinophil leukocytes** (blood cells) were found in female rats. EFSA (2005, p. 12) does not consider these findings “*biologically relevant*” because they were considered to be an “*isolated finding*” of a parameter with ‘inherent variability’ and because “*it was observed in one sex only*”.

For the risk assessment of a GM crop, the pertinent question is whether there is a difference between the modified and the unmodified variety, the inclusion of another GM maize line is not valid and only serves to mask any differences that have been observed. It must be concluded that differences with feeding studies were observed. Therefore, it remains unknown whether GM maize 1507 is safe, especially in the long-term, as human food or animal feed. EFSA has failed because it should, at the very least, have required the reasons for these differences to be further investigated.

4) Possible effects on non-target organisms

The *Bt* levels in GM 1507 maize pollen are extremely high and relevant studies of the potential effects on non-target organisms (long-term exposure and multi-trophic levels) do not appear to have been performed. It is unknown whether *Bt* Cry1F is exuded through the roots of 1507.

“The data are presented on a ng Cry1F protein/mg tissue dry weight basis and show that the expression values fall within the same order of magnitude for cultivation in different years and at different locations. Maximum expression (on a tissue dry weight basis) was found in pollen (average 20.0 and maximum 29.3 ng Cry1F protein/mg tissue dry weight). The values for whole plant extracts ranged between 1.0 and 6.9 ng Cry1F protein/mg tissue dry weight and for kernels between 1.2 to 3.1 ng Cry1F protein/mg tissue dry weight.” (EFSA, 2005)

This level of *Bt* expression in pollen is much higher than the concentrations of Cry1Ab in Bt176 (up to 7.1 ng/mg of pollen, Stanley-Horn et al., 2001). Bt176 was found to cause adverse effects on larvae of the monarch butterfly (Stanley-Horn et al., 2001). Although Hellmich et al. (2001) did not find acute toxicity to monarch butterfly larvae, chronic (long-term) toxicity has not been examined, nor excluded. Indeed, it has been found with MON810 and Bt11 that whilst there was no acute toxicity to monarch butterfly larvae, there is long-term toxicity (Dively et al., 2004). This was despite the fact that MON810 and Bt11 contain approximately 30 times less *Bt* in pollen than Bt176. Therefore, long-term experiments are necessary to evaluate potential toxicity to non-target lepidoptera.

Whilst the acute toxicity of Cry1F may be lower to monarch butterfly larvae, it is unknown if this is the case for all non-target lepidoptera. Recent research (Dutton et al., 2005) shows that assumptions about sensitivity and non-sensitivity of lepidoptera to *Bt* toxins do not necessarily hold true. It has been reported that *Spodoptera littoralis*, a secondary lepidopteran pest, was not sensitive to Cry1Ab (Raps et al., 2001). However, more recent studies found that *S. littoralis* larvae was sensitive on the long term, showing a higher mortality and longer development time (Dutton et al., 2005).

The statement by EFSA “*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)*” is highly misleading. Dively et al. (2004) found that the larvae of monarch butterfly ingested maize pollen that had fallen on milkweed plants within and surrounding maize fields, rather than maize itself as a food source and this was the cause of the toxic effects. Over 20 % fewer larvae reached the adult stage. Secondly, the conclusion that that toxic effects affected 0.6 % of the monarch population was only made after an extensive study of the calculations of overlap between the range of monarch butterflies in North America and the area of GM *Bt* maize. No such study has been performed in Europe where in addition the structure of the agricultural landscape is different.

Insect populations differ in the different geographic and climate zones in Europe. It has not been considered which lepidoptera are associated with maize fields (including hedgerows and field margins) in the different Member States, nor has experimental data been provided to support the assumption of the applicant that these insects would not be adversely affected. Several studies have been conducted on the potential impact of insect-resistant Cry1Ab *Bt* maize varieties on a diversity of non-target organisms besides the monarch butterfly, such as the peacock butterfly (*Inachis io*) and black swallowtail (*Papilio polyxenes*). However, even though this information has been published in peer-reviewed scientific journals (Felke & Langenbruch, 2003; Zangler et al., 2001), it was not included in the notification. Many species of butterflies and other insects are already under threat (Thomas et al., 2004) from factors such as climate change and loss of habitat. Increased stress from exposure to *Bt* pollen could further threaten certain species.

Therefore, the toxicity of 1507 to non-target lepidoptera is unknown. The long-term toxicity has not been studied, and there have been no studies of the toxicity of this maize to European lepidoptera. This issue is critical to the biodiversity considerations of insect resistant GM crops and demonstrates that EFSA is not capable of conducting an environmental assessment for the cultivation of a GM crop.

5) Unknown plant Cry1F degradation in soil and exudation by roots

The notifier found a remarkably short (and, frankly, difficult to believe) effective half-life of approximately three days for Cry1F in the soil. While Cry1F is a different protein to Cry1Ab, this result is in direct contradiction to studies that have found Cry1Ab to persist in the soil and remain active for up to over 200 days (Koskella & Stotzky, 1997; Tapp & Stotzky, 1998; Stotzky, 2000; Zwahlen et al., 2003a).

One possible explanation for the discrepancy is that purified Cry1F was added to soil, rather than maize tissue containing Cry1F. The purified protein is more liable to breakdown than *Bt* in maize tissue. As the *Bt* toxin would be incorporated into the soil as maize tissue post harvesting, if ever grown in EU, then the appropriate experiment would be to analyse the degradation rates of Cry1F in maize tissue.

Furthermore, it is unknown whether Cry1F is exuded through the roots of 1507. *Bt* crops containing Cry1Ab have been found to exude *Bt* through the roots (Saxena et al., 2004). This is important to determine as Cry1F could accumulate in the soil, affecting non-target soil organisms, affecting soil health.

EFSA (2005) states that “*no conclusive evidence has yet been presented that currently released transgenic Bt-resistant crops are causing significant direct effects on the soil environment*”. However, for the only *Bt* maize variety approved for cultivation in the EU, such effects were not monitored. Regulatory practices in other countries, such as the USA, do not require monitoring. Therefore, the fact that no evidence has been presented is because such evidence has not been looked for. Absence of evidence is not evidence of absence.

The long term, cumulative effects of continued growth over several years of GM plants expressing toxins are not well known but recent studies (Zwahlen et al., 2003b) have shown that there may be detrimental effects to soil fauna (earthworms) of long term exposure to *Bt*. No relevant long term studies, either of persistence, accumulation or toxicity of Cry1F have been undertaken. Therefore, it is unknown whether Cry1F would be exuded by roots of 1507 and whether it could accumulate in the soil.

6) Possible altered lignin content

The investigation of lignin levels of 1507 maize compared to its non-GM counterpart is an essential component of determining the possibility of unexpected and unintentional effects on digestibility, effects on herbivores, plant growth architecture, soil organic matter stabilization and/or decomposition processes. In particular, the persistence and biological activity of the *Bt* toxin would be enhanced by increased lignin, as this causes plants to decompose more slowly (Poerschmann et al., 2005).

Higher lignin levels have been observed in other GM crops as an unintended effect. Therefore, special attention should be given to the lignin levels of 1507. Levels of '*ADF and NDF, which comprise lignin*' in forage are described as '*comparable to those in control maize and within the background range*' (EFSA, 2005). However, recent studies show that ADF and NDF levels in forage are not sufficient to answer concerns about increased lignin levels in stems and roots. Poerschmann et al. (2005) found significant differences in lignin levels in stems but not in leaves. They also point out that lignin levels change during plant development. It is, therefore, not sufficient to measure only the two plant components in forage. Instead, lignin itself needs to be measured in the different tissues, especially in those that remain on the field after harvest.

Hence, possible increases in the lignin content of parts of 1507 cannot be ruled out. This may be important in terms of degradation of 1507 plant material, subsequent accumulation of *Bt* in the soil and possible adverse effects on non-target soil organisms.

7) Contamination and co-existence

Although there are plans for the surveillance of adverse effects of 1507 maize, these do not seem to include any consideration of the co-existence of GM and non-GM crops. GM maize is described as presenting a '*medium to high risk*' for cross-pollination with conventional or organic maize (Treu & Emberlin, 2000). Thus, there is a high probability that conventional and organic maize could become contaminated with 1507 maize. Although GM maize is not expected to become a persistent or invasive weed, should any volunteer GM maize plants inadvertently grow near a conventional maize crop, the resulting pollen could cross-pollinate with maize in fields, producing genetic contamination. Maize plants have been shown to survive over a single growing season, even in the UK, a comparatively cold part of Europe (Crawley et al., 2001). Survival might be expected to be higher in warmer (Mediterranean) parts of Europe.

The notifier cites freezing temperatures as the main factor limiting germination of seeds, but many maize growing areas in Europe do not always experience freezing temperatures in winter. Maize volunteers have been noted occasionally growing from spilled seed in uncultivated fields and by roadsides in the year following maize production (Eastham & Sweet, 2002). Who will control these volunteers and how? The spraying of roadsides, which are valuable wildlife habitats, is simply not acceptable. There do not appear to be any plans to monitor or control such volunteers in the proposed monitoring plan, nor contingency plans for contamination of non-GM maize.

Genetic contamination of neighbouring maize crops is a known risk and has not been included in the risk assessment. This is a serious omission by EFSA.

8) Monitoring

The proposed case-specific monitoring plan fails to monitor the critical issue of possible effects on non-target organisms, such as lepidoptera.

“The Panel agrees with the risk assessment that no adverse effects on other non-target organisms are anticipated and thus this should not be included in the case-specific monitoring plan.” (EFSA, 2005)

The function of the monitoring programme is to test the assumptions made in the risk assessment. The EU Council Guidance Notes makes this quite clear:

“Case-specific monitoring serves to confirm that scientifically sound assumptions, in the environmental risk assessment, regarding potential adverse effects arising from a GMO and its use are correct.” (EU Council, 2002/811/EC).

EFSA’s assertion that no adverse effects on non-target organisms are anticipated is derived from a series of questionable assumptions. There is much scientific concern regarding effects on non-target organisms from insect resistant *Bt* crops, and especially from 1507 as there are hardly any studies examining effects on non-target organisms from Cry1F.

In addition, there are many science-based concerns with 1507 that have not been adequately examined by EFSA, including the exudation of *Bt* toxins into the soil, the accumulation and persistence of *Bt* toxins in the soil, and possible effects on non-target soil organisms (both short-term and long-term). Yet no case-specific monitoring is proposed.

In the EU Council Guidance Notes, the objectives of monitoring are made clear:

“The environmental risk assessment aims, on a case by case basis, to identify and evaluate potential adverse effects of the GMO, either direct or indirect, immediate or delayed, on human health and the environment arising from its placing on the market. This assessment may also need to take account of potential long-term effects associated with the interaction with other organisms and the environment. [...]” (EU Council, 2002/811/EC).

In direct contradiction to this, EFSA justifies the exclusion of lepidoptera from the case-specific monitoring because they consider it too expensive. This is in clear contradiction to the understanding that the monitoring should be based on scientific reasoning.

The general surveillance plan is also wholly inadequate:

- There is no monitoring outside the users’ own fields and premises.
- Only users with an interest in growing and processing 1507 maize are involved in the general surveillance. There are no scientists involved to monitor general effects on the fields, besides those who undertake an additional study. Relying on the existing infrastructure of users, seed distributors and company representatives is not sufficient for environmental monitoring.
- The monitoring is restricted to a subset of growers in representative regions. Unanticipated effects are even more likely to become visible under extreme conditions. This restriction to only some users, representative regions and model organisms could be read as an attempt to ensure that adverse effects will go unrecorded.

The inadequacy of the general surveillance plan and the lack of a recommendation to test for adverse effects on lepidoptera or the wider environmental effects of the *Bt* toxin from 1507. are serious failures of the EFSA assessment for 1507. This casts severe doubt on the ability of EFSA to evaluate the environmental risks associated with GM crops.

Conclusions

There are many scientific unknowns and uncertainties regarding the environmental, regarding the safety of GM maize 1507 for the environment, human and animal health. These include:

1. several unintended additional fragments of the inserted genes. The implications of these genome irregularities in 1507 to the environmental and food/feed safety of this GM maize remain

unknown.

2. many statistically significant differences are seen in the compositional analysis of 1507. These differences could cause adverse effects on human and animal health or the environment.
3. statistical differences were seen in the toxicological assessment of 1507. Therefore, it remains unknown whether GM maize 1507 is safe, especially in the long-term, as human food or animal feed.
4. unknown toxicity of 1507 to non-target European lepidoptera.
5. unknown exudation of Cry1F through the roots of 1507 and unknown accumulation in the soil.
6. A possible increase in the lignin content of parts of 1507 cannot be ruled out. This may be important in terms of degradation of 1507 plant material, subsequent accumulation of *Bt* in the soil and possible adverse effects on non-target soil organisms.
7. Genetic contamination of neighbouring maize crops is a known risk but has not been included in the assessment.
8. The monitoring plan lacks a recommendation to test for adverse effects on lepidoptera or the wider environmental effects of the *Bt* toxin from 1507. The general surveillance plan is inadequate.

The assessment of GM maize 1507 is woefully inadequate. Each time there is uncertainty in the data submitted, e.g. open reading frames, significant differences in feeding trials, EFSA makes a considerable effort to negate the possible consequences of these uncertainties with phrases such as “unlikely” or “not of biological relevance”.

What is striking about this application is the number of uncertainties. For example, the presence of so many unintended DNA fragments in 1507 resulting in open reading frames coupled with the significant differences in the feeding trials should cause concern to any regulatory body and be grounds for rejection of 1507. Similarly, the lack of studies on relevant non target lepidoptera, such as European butterflies, is a shocking omission where EFSA should have demanded more studies.

This is the first time that EFSA has assessed a GM crop, not only for the import and processing, but also for cultivation. The misquoting by EFSA from studies such as Dively et al (2004) on adverse effects on non-target organisms raises serious questions regarding the effectiveness of EFSA as a body to give a adequate scientific advice on the food/feed and environmental safety of GM crops. EFSA have not yet been critical of a single GM crop, even though many scientists have raised concerns in the past (e.g. in the assessment of MON863). This assessment of 1507 is yet another example of EFSA disregarding scientific evidence to give a positive opinion of a GM crop even when there remain serious scientific uncertainties and unknowns regarding the environmental and food/feed safety.

Greenpeace urges EU Member States and the European Commission to:

- **reject EFSA’s report on 1507 maize because there is a serious lack of scientific scrutiny in the manner that it has been performed,**
- **apply the Precautionary Principle and reject the cultivation of 1507 maize,**
- **re-organise EFSA’s to ensure that experts are independent and perform their work with the necessary scrutiny.**

References

- Birch, A.N.E., Geoghegan, I.E., Majerus, M.E.N., McNicol, J.W., Hackett, C.A., Gatehouse, A.M.R. & Gatehouse J.A. 1999. Tri-trophic interactions involving pest aphids, predatory 2-spot ladybirds and transgenic potatoes expressing snowdrop lectin for aphid resistance. *Molecular Breeding* 5: 75-83.
- Bourguet, D., Chaufaux, J., Micoud, A., 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.
- Brooks, G. 2000. Weed control rookies. *Farm Industry News*. 29 February 2000.
- Crawley, M.J., Brown, S.L., Hails, R.S., Kohn, D.D. & Rees, M. 2001. Transgenic crops in natural habitats. *Nature* 409: 682-683.
- 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.
- Dutton, A., Romeis, J. & Bigler, F. 2005. Effects of *Bt* maize expressing Cry1Ab and *Bt* spray on *Spodoptera littoralis*. *Entomologica Experimentalis et Applicata* 114: 161.
- Eastham, K. & Sweet, J. 2002. Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer. Expert's Corner Series, European Environment Agency, Copenhagen.
- EFSA (2005): Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/ES/01/01) for the placing on the market of insect-tolerant genetically modified maize 1507, for import, feed and industrial processing and cultivation, under part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds. *The EFSA Journal* 181: 1-33.
http://www.efsa.eu.int/science/gmo/gmo_opinions/827_en.html
- Felke, M. & Langenbruch, G.-A. 2003. Wirkung von *Bt*-Mais-Pollen auf Raupen des Tagpfauenauges im Laborversuch (Effect of *Bt*-maize-pollen on caterpillars of *Inachis io* in a laboratory assay). *Gesunde Pflanze* 55: 1-4.
- 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.
- Hellmich, R.L., Siegfried, B.D., Sears, M.K., Stanley-Horn, D.E., Daniels, M.J., 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* 98: 11925-11930.
- Jank, B. & Haslberger, A. 2000. Recombinant DNA insertion into plant retrotransposons. *Trends in Biotechnology*, 18: 326-327.
- Koskella, J. & Stotzky, G. 1997. Microbial utilization of free and clay-bound insecticidal toxins from *Bacillus thuringiensis* and their retention of insecticidal activity after incubation with microbes. *Applied and Environmental Microbiology* 63: 3561-3568
- Monsanto (2000) Dossier containing molecular analysis of Roundup Ready soya:
http://www.foodstandards.gov.uk/pdf_files/acnfp/dossier.pdf.
- Monsanto (2002a) Transcript analysis of the sequence flanking the 3' end of the functional insert in Roundup Ready Soybean event 40-3-2. Available at: <http://www.food.gov.uk/science/ouradvisors/novelfood/assess/assess-uk/60500/>
- Monsanto (2002b) Additional characterisation and safety assessment of the DNA sequence flanking the 3' end of the functional insert of Roundup Ready Soybean event 40-3-2.
Available at: <http://www.food.gov.uk/science/ouradvisors/novelfood/assess/assess-uk/60500/>
- Poerschmann, J., Gathmann, A., Augustin, J., Langer, U. & Górecki, T. 2005. Molecular composition of leaves and stems of genetically modified *Bt* and near-isogenic non-*Bt* maize – Characterization of lignin patterns. *Journal of Environmental Quality* 34: 1508-1518.
- Prescott, V.E., Campbell, P.M., Moore, A., Mattes, J., Rothenberg, M.E., Foster, P.S., Higgins, T.J.V. & Hogan, S.P. 2005. Transgenic expression of bean alpha-amylase inhibitor in peas results in altered structure and immunogenicity. *Journal of Agricultural And Food Chemistry* 53: 9023 – 9030.
- Raps A., Kejr, J., Gugerli, P., Moar, W.J., Bigler, F., Hilbeck, A. 2001. Immunological analysis of phloem sap of *Bacillus thuringiensis* corn and the nontarget herbivore *Rhopalosiphum padi* (Homoptera: Aphidiae) for the presence of Cry1Ab. *Molecular Ecology* 10: 525-533.
- 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.
- 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., Obyrcki, J.J. & Lewis, L. 2001. Assessing the impact of Cry1Ab-expressing corn pollen on Monarch butterfly larvae in field studies. *Proceedings of the National Academy of Sciences* 98: 11931-11936.
- Stotzky, G. 2000. Persistence and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis* and of bacterial DNA bound on clays and humic acids. *Journal of Environmental Quality* 29: 691-705.
- Tapp, H. & Stotzky, G. 1998. Persistence of the insecticidal toxin from *Bacillus thuringiensis* subsp. *kurstaki* in soil. *Soil Biology & Biochemistry* 30: 471-476.
- Thomas, J.A., M.G. Telfer, D.B. Roy, C.D. Preston, J.J.D. Greenwood, J. Asher, R. Fox, R.T. Clarke & J.H. Lawton. 2004. Comparative losses of British butterflies, birds and plants and the global extinction crisis. *Science* 303: 1879-1881.
- Treu, R. & Emberlin, J. 2000. Pollen dispersal in the crops maize (*Zea mays*), oil seed rape (*Brassica napus* ssp *oleifera*), potatoes (*Solanum tuberosum*), sugar beet (*Beta vulgaris* ssp *vulgaris*) and wheat (*Triticum aestivum*). A report for the Soil Association from the National Pollen Research Unit. Available at <http://www.soilassociation.org>
- Zanger, A.R., McKenna, D., Wraight, C.L., Carrol, 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. *PAMA* 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 the earthworm *Lumbricus terrestris*. *Molecular Ecology* 12: 1077 –1086.