maize

BT 11 MAIZE - C/F/96.05.10 NOTIFICATION FOR CULTIVATION

REPORT PREPARED ON BEHALF OF GREENPEACE INTERNATIONAL BY ANTJE LORCH

REPORT 2005

bt 11 maize - C/F/96.05.10 notification for cultivation

GREENPEACE

report | europe | **issue** genetic engineering

maize

BT 11 MAIZE - C/F/96.05.10
NOTIFICATION FOR CULTIVATION
PUBLISHED SEPTEMBER 2005

© JENS CHRISTIANSEN

GREENPEACE

prepared by Antje Lorch on behalf of Greenpeace International
date September 2005
author Antje Lorch, *lorch@ifrik.org*
editor Christoph Then, *Greenpeace Germany*
design & layout Tania Dunster, kÏ design, Sweden
contact persons Christoph.Then@greenpeace.de and judit.kalovits@int.greenpeace.org

Summary

On 20 May 2005 European Food Safety Authority (EFSA) delivered a positive opinion on Syngenta's application for insect-resistant genetically modified (GM) maize Bt11.

The notifier (now Syngenta) applied for the approval to cultivate Bt11 maize in the European Union. Bt11 produces the Bt toxin Cry1Ab against Lepidoptera (moths and butterflies) to protect maize against the two insect pests European stem borer (ECB, Ostrinia nubilalis) and Mediterranean stem borer (MCB, Sesamia nonagrioides). In addition, Bt11 is herbicide-tolerant against the glufosinate-ammonium (BASTA, Liberty). The notifier claims that Bt11 will not be marketed for its herbicide tolerance, but in other countries, such as the USA and Canada, Bt11 is marketed for both GM traits.

The application contains almost no original data about risk assessment studies, and in several cases the summaries make clear that the few studies that were undertaken lack scientific relevance for growing Bt11 in the field. They lack any study of medium or long-term effects, effects on European species (especially European butterflies) and studies on impacts on the soil biota. Even Member States stated that it would not be possible to draw positive recommendations from this limited data. Information about the actual insert, about the insertion site and possible additional, unintended inserts are classified as confidential business information, thereby making it impossible for third parties to undertake an independent assessment.

Bt11 should not be grown in the EU because:

* The notification lacks original data that would enable an independent assessment to be made of the studies undertaken and their results.
* The non-target studies are insufficient to enable a risk assessment to be undertaken, while the scientific literature gives enough indications of adverse effects of Bt11 on non-target organisms, including multitrophic interactions between plants, herbivores and pests. Effects on soil organisms have not been studied at all.
* There are unexplained irregularities in the molecular data discovered by independent scientists, including rearrangements and possible contamination with Bt176. A summary of a (otherwise as CBI classified) sequencing acknowledges the integration of several pieces of vector backbone DNA, but does not clarify the other irregularities found earlier in an independent study. The site of the insertion is a region where interruptions are likely to interfere with the basic metabolism of the GM plants.
* There are no sufficient data from feeding and toxicity studies.
* Bt11 is also tolerant to the herbicide glufosinate (brand name Liberty, Basta). This transgenic trait has not undergone any risk assessment, yet can have environmental effects; either directly or through changes in agricultural practices.
* The monitoring plan is inadequate. As even the EFSA points out, not enough attention is given to resistance development and adverse effects on non-target organisms. As well as farmers who are supposed to fill in questionnaires, but who might have conflicting interests, only a vague list of existing networks (including seed producers, and actors in the food and feed industry) is given without information on how such a monitoring exercise would work and whether these organisations are interested in participating. It has been acknowledged by the EFSA that some of these organisations lack the necessary scientific expertise.

maize

BT 11 MAIZE - C/F/96.05.10
NOTIFICATION FOR CULTIVATION

PUBLISHED SEPTEMBER 2005

General

Bt11 is a genetically modified (GM) maize that produces the Bacillus thuringiensis (Bt) toxin Cry1Ab against the European corn borer (ECB, Ostrinia nubilalis) and the Mediterranean corn borer (MCB, Sesamia nonagrioides). It is tolerant to the broad spectrum herbicide glufosinate-ammonium . The notifier claims that Bt11 will not be marketed for its herbicide tolerance, but in other countries, such as the USA and Canada, Bt11 is marketed for both GM traits.[1]

In North America, ECB has been an important maize pest since the 1910s. In Europe, however, this pest is only an agronomic problem in some parts of Europe, although the area of ECB is currently spreading north by about 10-12 km per year. For Germany, the northern limit of ECB infestation currently lies on a line of Cologne – Westerwald – Oderbruch (Saeglitz, 2004). There are no known ECB infestations in the UK (UK Competent Authority, 2004), and climatic conditions are considered to be a natural barrier (Kluge, 1999, quoted in Saeglitz, 2004).

ECB larvae spend the winter in the lowest part of the maize stalk and in the roots, and common agricultural practices such as ploughing can destroy them in autumn. ECB infestations are cyclic, and usually do not pose problems for several years in a row (Benbrook, 2001), a pattern which can also change from region to region. In warmer regions, ECB can have two or three infestations in hot regions such as in the south of the USA, with as many as four generations emerging per year. Even during infestations farmers often do not treat an infested field. In the notification dossier itself, Syngenta states that “currently only 5 to 10 percent of the total maize acreage is treated with insecticides for ECB control” (C/F/96.05.10, Appendix 10).

The notification dossier C/F/96.05.10 contains almost no original data from studies, but presents summaries instead. The non-target studies quoted are part of the approval procedure at the US Environmental Protection Agency (EPA) and are not part of the notification dossier. It is therefore impossible to assess design and results and to draw valid conclusions from them for an environmental risk assessment (e.r.a.). Experience shows that many studies lack realistic study designs and/or are not even set up to determine whether possible differences are statistically significant (Lövei & Arpaia, 2005), so the missing information in the non-target studies raise serious concerns. Relevant information about the molecular characterisation of Bt11 (including for example information on the insertion site) are classified as confidential business information (CBI).

During its assessment, the European Food Safety Authority (EFSA) requested additional information five times. While the replies and the provided material from Syngenta are available to third parties, the questions asked by the EFSA have not been disclosed.

© JENS CHRISTIANSEN

1 | Most GM varieties in the USA and Canada are sold under brand names and lines, but without the explicit mention of the actual transformation event. Different seed distributors use different brand names, so that it becomes difficult to know which genetic modification is sold under which name. Bt11 hybrids are listed with "Characteristics: Cry1Ab Corn borer protection, Glufosinate herbicide tolerance" by the National Corn Growers Association (USA, http://lepton.marz.com/ncga/search_hybrids/traitChart1.asp?Event_ID=1). Several seed producers sell their sweetcorn explicitly for protection against ECB and as tolerant to the Liberty herbicide, as well as hybrids that are also tolerant to glyphoste (Roundup). (See for an example: http://www.garstseed.com/GarstClient/Technology/agrisure.aspx and http://www.garstseed.com/GarstClient/Technology/agrisure.aspx)

I MOLECULAR CHARACTERISATION

The event Bt11 was obtained by protoplast transformation (De Scrijver & Moens, 2003) and then regenerated in tissue culture. In contrast to the information given by the notifier, rearrangements were found when Belgian scientists compared the insert with the original data, and that several parts of the insert were truncated or unexpectedly inserted, e.g. t35S sequences which can act as a stop codon (DE Schrijver & Moens, 2003; for details see below).

The sequence of the Bt11 insert and the flanking DNA is stated to be confidential business information (CBI) in Appendix 8 of the dossier and in the additional information provided by the notifier in 2005. The notifier states in the single paragraph available to third parties that the insert contains the two intended inserts as well as three parts of vector backbone, but no partial fragments. No information is given about truncations or rearrangements. Since the original data are not available to third parties it has become impossible to follow this up or to assess whether such rearrangements can have adverse effects for human and animal health or the environment.

The actual act of transforming a genome as well as tissue culture technologies are known to cause mutations that go beyond the effect of the expression of the transgenic insert (Wilson et al., 2004).

Concerns were raised by Member States, but the EFSA (2005) does not refer to these.

i Sequence data

Research on the genetic map of Bt11 shows irregularities and rearrangements of the genetic insert that do not match the molecular data of the notification dossier (De Schrijver & Moens, 2003). On 27 January 2005, Syngenta provided EFSA with "Further experimental evidence that there are no secondary events in the Bt11 event." Only one summary paragraph is available to third parties. The detailed information is classified as CBI. Except for one point, the summary of this new information does not answer the concerns raised by De Schrijver & Moens (2003), nor does EFSA's opinion refer to this report.

De Schrijver & Moens (2003) were able to determine the insert location, which was not given in the dossier C/F/96.05.10. The insertion is in a region known as "knob DNA". This needs to be taken up in the risk assessment, because interruptions in such DNA might only become obvious under stress situations (see page 6). The research also found additional DNA from the vector, and additional sequences that were not described as being part of the insert.

Vector backbone DNA has been confirmed by Syngenta (2005a) as being present before, after and between the two parts of the insert. Syngenta states that they used parts of the whole vector as a probe and could not find partial DNA fragments or additional sequences of the intentionally inserted DNA.

However, no answer appears to be given on De Schrijver & Moens' (2003) findings of unexpectedly inserted DNA, including a sequence that does not come from the unmodified plant, nor from the vector as described.

Earlier, the whole sequence of the insert of Bt11 was determined by TEPRAL (France) and described in a report by the Belgian Service of Biosafety and Biotechnology. "The insert consists of a single copy of the vector fragment carrying both the Cry1Ab and pat gene. It was found that rearrangements have taken place in the insert compared to the original insert and that several parts of the plasmid have been truncated or unexpectedly inserted, e.g. t35S sequences." The presence of t35S fragments was confirmed by another French institute, INRA (De Schrijver & Moens, 2003).

t35S is a 'stop' codon. It is present in the primary insert, even though t35S is not the stop codon used in Bt11. "Since it has been shown that unexpected t35S fragments are present in the primary insert, it should be clarified where these sequences come from. It should be determined whether they originate from the vector used for transformation or any other source." (De Schrijver & Moens, 2003). t35S are used in Bt176 and contamination with Bt176 has been suggested as a source of contamination by cross-pollination (De Schrijver & Moens, 2003). "Preliminary data of INRA (France) show that a set of primers designed on the edge fragment of Bt176 amplifies sequences from both Bt176 and Bt11. These data were obtained from six different Bt11 plant seeds received from Syngenta. They suggest that the presence of a fragment of Bt176 DNA might be the result of an initial contamination of Bt11 by Bt176" (De Schrijver & Moens, 2003).

According to De Schrijver & Moens (2003), in the dossier a 134 bp deletion is observed at p35S regulating pat gene expression. However, this is not mentioned in the dossier, nor in the summary of further studies.

In 2003, De Schrijver & Moens conclude: "There are still uncertainties concerning the molecular data in the dossier C/F/96.05.10: rearrangements in the insert and truncations of parts of the insert might have occurred. Therefore, the sequence of the insert should be further checked together with the number of inserts. [...] It should be determined whether [the unexpected t35S fragments in the primary insert] originate from the vector used for transformation or any other source. The molecular data presented in the dossier C/F/96/05-10 do not fulfil the Belgian requirements concerning molecular data. The sequence of the insert, together with the sequence of the flanking regions should be provided. In addition, the flanking regions of the insert should be analysed for the presence of chimaeric reading frames. It must be noted that the same Bt11 event as in notification C/F/96.05.10 [...] has been submitted for approval under Regulation (EC) 258/97, implying that the molecular data for both dossiers are similar. However one cannot entirely exclude [the possibility that] that backcrosses of the original event with a maize line for feed purposes or sweet maize might give rise to rearrangements at molecular level."

Without access to the information provided to the EFSA in January 2005, it is impossible for third parties to independently assess whether these uncertainties have now been clarified. Neither Syngenta nor the EFSA refer to De Schrijver & Moens (2003) in general, or to their finding of the t35S sequences, even though this report was written for the Belgian competent authority. It therefore remains unclear whether this uncertainties were actually studied. The earlier concerns about irregularities and rearrangements of the Bt11 genome can therefore not be put aside.

ii Insertion site

The insert has integrated on a 180 kb long knob-specific tandem repeat sequence. A 180 kb knob-specific repeat sequence is present at the 5' 35S promoter border of the Cry1Ab (Zimmermann et al., 2000, in De Schrijver & Moens, 2003). Between the maize plant DNA and the insert is a 1099 bp sequence homologous to the pUC (vector) backbone sequence containing part of the lacZ coding sequence. This sequence has now been confirmed by Syngenta (2005a).

The junction region at the 3'NOS terminator border showed a sequence that is also similar to the maize 180 bp knob associated tandem repeat (De Schrijver & Moens, 2003, Ronning, 2003). There is an additional DNA sequence homologous with pUC backbone sequence at the 3' NOS junction. "These data provide evidence that the Bt11 insert is integrated in the Zea mays 180 bp knob-associated tandem repeat locus" (De Schrijver & Moens, 2003). The insert location does not seem to be given in the dossier. (This part of the dossier is not available to third parties as CBI.)

Knob DNA is thought to be an important part of the maize chromosome and has been shown to have multiple functions and to influence several genetic effects, such as flowering time (Ananiev et al., 1998). In addition, knob DNA sequences are

©GREENPEACE/CARRASCO

complex and controlled by several elements including mobile elements such as retrotransposons. The implications of inserting the genetic construct into this region have not been studied. The disruption of a knob sequence might interfere with the function of either the knob sequence itself or retrotransposons. The mobility and functionality of retro-transposons are dependent on several factors, including environmental factors. Therefore, any adverse effects relating to the interruption of a knob sequence may only become apparent under specific environmental conditions, e.g. drought. The interruption of a knob sequence could give rise to unexpected effects, genetic instability in future generations, and possibly alter important plant functions such as flowering time (Jank & Hasselberger, 2000)

The notifier states that "knobs are a component of the maize chromatin, a class of chromatin known to be not transcribed" (Alberts et al., 1994 quoted in "Response to questions raised by Member States"). This formulation is repeated word by word by the EFSA (2005:6) in their opinion.

The reference "Alberts et al. 1994" is a university textbook, not a scientific article. Research in the last 10 years however has discovered much more about DNA function, including several functions of DNA that is not transcribed. One recent article in this context discusses, for example, the vital role played by 'junk DNA' (Bejerano et al., 2004). Therefore, although knobs may not be transcribed, they still may have some vital function.

It is alarming to see that not only Syngenta, but also the EFSA, rely on a textbook over ten years out of date in an area that has seen significant new discoveries in recent years, in order to discard the possibility of insertion effects in Bt11.

Conclusion

It is unacceptable that the information about the insertion site and the insert are classified as CBI, and that independent assessments have therefore been made impossible. The publicly available summary does not clarify earlier concerns about DNA sequences from unknown sources. It remains unclear whether the GM insert interrupts functions of un-transcribed DNA.

© JENS CHRISTIANSEN

II PLANT COMPOSITION

In the response from November 2003, the notifier replied to the request by the Scientific Committee on Plants (January 2000) to "provide information on the composition of plant tissues, including green tissues used for animal feeding. Data should be provided from different field trial locations and growing seasons."

* The notifier presents a mix of studies in which different tissues are tested for different contents. These levels cannot necessarily be compared to one another. In the summary in the "Responses to Member States" (2003) the notifier discards significant differences found in individual studies:
"The purpose of these compositional analyses is to determine whether unintentional effects have occurred as a result of the genetic modification. As various protocols were used, results cannot be directly compared between the different studies, and data for Bt11 hybrids should therefore primarily be compared to data obtained for the non-modified control hybrids within the same study. Nevertheless, the fact that no difference was observed, which is statistically significant for a specific compound over the whole range of studies3 supports the absences of undesired pleiotropic effects." (original Footnote: "3 Some sporadic statistically significant difference were observed in some studies, see study reports for details.")
* Significant differences were found between Bt and non-Bt varieties at several instances, but even then the notifier stated that they still all fell in the range for maize varieties. In one of the studies the authors (Edwards & Pilancinski, C/F/96.05.10, Appendix 13) came to the conclusion that "given that the hybrids are near-isogenic [near-identical] and that the range of variation among hybrids is great compared to Cry1Ab-associated effects, one is left with the conclusion that Cry1Ab hybrids are not materially different from normal hybrids in amino acid and fatty acid content." This claims that because there is variation in maize anyway, additional variation does not matter. However it does matter if the variation is due to the GM process. No efforts seem to have been taken to find out why significant differences occur.

This is in contradiction with the recent FAO/WHO Codex Alimentarius (2003) guidelines, which state:

"The concept of substantial equivalence is a key step in the safety assessment process. However, it is not a safety assessment in itself; rather it represents the starting point which is used to structure the safety assessment of a new food relative to its conventional counterpart. This concept is used to identify similarities and differences between the new food and its conventional counterpart. It aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy to date for safety assessment of foods derived from recombinant-DNA plants."

All in all, the evidence regarding compositional data is a patchwork of studies with different objectives, which cannot be compared to one another. The conclusion of the notifier that – if all studies are put together – there is no significant difference, cannot be drawn from such diverse studies.

A more comprehensive study comparing different varieties on different locations would be necessary to judge the significant differences that have been observed in several studies.

i Lignin content

Several small increases were observed for lignin content of Bt11 plants (EFSA 2005, p. 8). However, the EFSA does not consider the increases to be relevant. Instead the EFSA discards the whole issue of a possible lignin increase in Bt maize as raised by Saxena and Stotzky (2001a) and Flores et al. (2005), by referring to a third study by Jung & Sheaffer (2004).

Lignin is of importance for Bt maize production, especially for Bt maize production in the kind of no-till farming systems as advocated for herbicide tolerant crops. The investigation of lignin levels of Bt11 maize compared to its isogenic lines is mandatory to study pleiotropic 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 Bt

toxins are expected to be enhanced by the protection of slower decomposing plant material (Poerschmann et al., 2005).

Since higher lignin levels have been observed in Bt11 and other Bt maize (Bt176 and MON810) before (Saxena & Stotzky, 2001a), the increased lignin contents recorded in the application C/F/96.05.10 cannot be discarded as easily. A newer study (Poerschmann et al. 2005), confirmed Saxena & Stoztky's results for Bt176 and MON810, but unfortunately the study did not include Bt11. The authors, however, raise several issues that can explain differences between the findings of Saxena & Stotzky (2001a) and Jung & Sheaffer (2004). They found significant differences for lignin content in the stems but not in the leaves, and they point out that lignin levels change with the development of the plant. Most importantly they point out that different methods produce different results.

A newer study (Poerschmann et al., 2005) confirms Saxena & Stotzky's results, but also raises concerns about the validity of Jung & Sheaffer's results. Until there is no explanation of why different methods used in the literature come to contradicting results, it unacceptable to discard the documented increase of lignin levels in Bt11 solely on the basis of one article. Instead, issues such as decomposition and effects on the bioavailability of the Cry1Ab toxin of Bt11 in the soil need to be studied further.

III BT EXPRESSION

Bt concentrations were determined on a small number of plants (C/F/96.05.10, Appendix 5). Different plant tissues of five greenhouse plants were sampled during the growth of the plants. In the field, four hybrids were sampled at two different locations, resulting in a total of 16 plants, compared to "similar" but not "isogenic" controls. Field plants were only sampled once from mature maize plants, and Bt toxin levels were determined for leaf, stalk, husk and kernel. The sampling was done on four plants of four hybrids (on two different locations), resulting in 16 plants. Bt levels of pollen and roots of Bt11 plants in the field or root exudates were not determined.

i Varying levels of Bt toxins

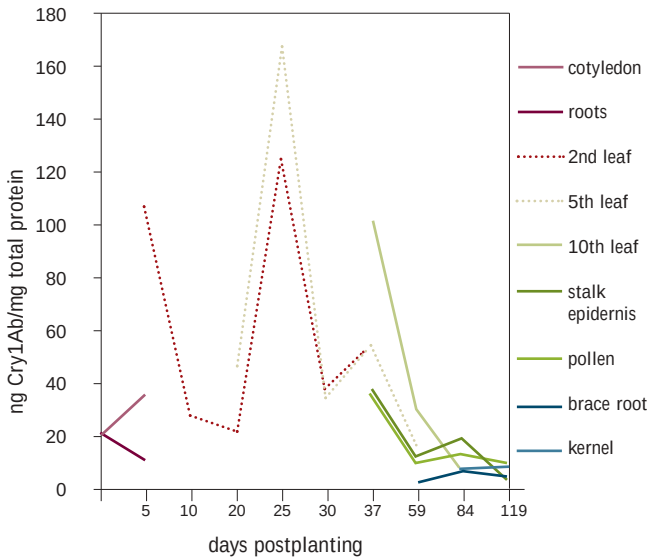
Comparison with other data is difficult because concentrations can be given in reference to fresh tissue, total protein, or extracted protein. Data from the greenhouse trial and from the plants from the field in Appendix 5 cannot be compared because they are given in different forms of concentration.

From the greenhouse plants it is obvious that the Cry1Ab concentrations in the plants vary extremely. For example, the fifth leave on day 25 after planting contains 168 ng Cry1Ab/mg plant protein (the maximum measured), but only 34 ng Cry1Ab/mg plant protein on day 30 (see Figure 1). Other tissues produce as little as less then 5 ng Cry1Ab.mg plant protein, and kernels on day 119 only contain 0.4 ng Cry1Ab/mg plant protein. No explanation is given for these extremely varying levels.

maize

BT 11 MAIZE - C/F/96.05.10
NOTIFICATION FOR CULTIVATION
PUBLISHED SEPTEMBER 2005

FIGURE 1: CRY1AB LEVELS IN BT11 TISSUES
CRY1AB CONCENTRATION IN BT11 GREENHOUSE PLANTS. NINE OUT OF 16 TISSUES SAMPLED, SEE P 27 FOR COMPLETE DATA. SOURCE C/F/96.05.10, APPENDIX 5.



* GM Bt plants in general are known to have varying levels of Bt toxin production, and the notifier even refers to several studies throughout the dossier, which mention varying concentrations (for example C/F96.05.10, Appendix 10: "survivors observed in the field arise from rare plants not expressing the Cry1Ab protein"). However varying concentrations of Bt are not mentioned in C/F/96.05.10 Appendix 5 titled "Levels of Btk [Cry1Ab] protein in transgenic plant tissues during plant life cycle (Bt11 maize). Neither is it mentioned that individual plants were recorded in the field which did not produce Bt toxin. The notifier gives data for different plant tissues throughout the growing season (from greenhouse plants) without any explanation of the variation in the different tissues and levels. For field plants only the average at the end of the growing season is given.

Variations in Bt levels are known in other Bt maize varieties. For Bt176, a producer confirmed that 1 to 2 % of the plants do not produce the Bt toxin (BMBF, 2003).

* Variations in Bt levels are of relevance for the risk assessment and for the agricultural application of Bt crops.

1. Bt plants that produce little Bt toxin compromise the high-dosage strategy in delaying resistance development in the target organism. This strategy relies on high Bt levels that will kill all pest insects even if they inherited Bt resistance from one of their parents. Plant with low Bt levels might even increase Bt resistance development (Chicutt & Tabashnik, 2004; Farinos, 2004)

2. Studies of the effect of Bt toxins on non-target organisms in general do not specify how much Bt toxin is present in the plant material used or whether the material was collected at the period during the growing season when the non-target organism would be subjected to it. However, if Bt levels vary over time and in different tissues, or if they can be completely absent, then it can be possible that non-target studies have been undertaken with too little Bt toxin.

3. Determination of the actual Bt levels in plant material at different times is also necessary for studies where purified Bt toxin from GM bacteria is used. The majority of these studies undertaken for approval procedures such as for C/F/96.05.10 use high levels of Bt toxin, but chronic, long-term effects from the constant feeding at low levels have different effects than acute toxic effects. It is therefore necessary to know on which Bt toxin levels target and non-target organisms will be feeding.

* Cry1Ab levels are noted as highest in the leaves, especially during the early stages of development. The protein is detectable in all parts of the plant. The Evaluation Report does not specify whether this is the average and what the maximum might be. The evaluation report states "It is estimated that 90% of the Cry1Ab protein is located in the leaves of the plant." No data is given about Cry1Ab levels in pollen or in the stems even though stemborer larvae will be found in the stems. "Generally, higher levels were detected at the younger stages of tissue

development. The level of Cry1Ab protein decreased as the plant reached full maturity and the tissues became senescent" (SNIF). "The Cry1A(b) protein levels in husks and kernels were found to vary, showing no consistent trend." (EC Scientific Committee on Food, 2002, p. 7). No further explanation is given, but it can be assumed that it will increase the rate of insect resistance build-up (see for example Knight, 2003)

ii Equivalence of plant and microbial Bt toxin

Microbial Bt sprays (as used by organic farmers) are fundamentally different to Bt crops. Microbial Bt sprays have very few effects on non-target organisms because the "pro-toxin" is produced. This pro-toxin is inactive and only becomes toxic when processed in the gut of certain (targeted) species of insect larvae. In contrast, GM Bt insect-resistant plants contain an artificial, truncated Bt gene that is active. It is therefore less selective, and may harm non-target insects that do not have the enzymes to process the pro-toxin, as well as the pests for which it is intended (Hilbeck, 2001; Hilbeck et al., 2000).

The notifier shows a molecular equivalence of Bt toxin produced by Bt plants or by GM E. coli bacteria in order to use GM bacterial Bt toxin for testing, e.g. to test the effects on non-target organisms. However, structural changes may occur between the Bt protein produced by the plant and by the bacteria. Therefore, bacterially-produced Bt toxin cannot be regarded as equivalent to Bt11 plant material.

No equivalence is shown between Bt11 plant material (or GM E. coli Bt toxin) and the Bt toxin used in traditional microbial Bt sprays. Nevertheless the long-term use of Bt (without considering the totally different form of application) is used as reference to claim that there are no adverse effects of Bt. This information is misleading because of the differences in production and the application of traditional Bt sprays.

iii Cry1Ab toxicity studies

No actual toxicology studies were performed with Bt11 on animals.

The notifier conducted two studies each on the toxicity of isolated Cry1Ab and PAT proteins: "one digestibility study; one acute oral toxicity study on mice" (C/F/96.05.10, Appendix 13, p.15). Very little information is given about these studies, let alone any information about the study design or original data, but it is clear that these studies are not sufficient to assess adverse impacts of Bt11. The digestibility study was apparently not performed with animals in a "simulated mammalian gastric environment". The acute toxicity studies were done with purified protein but not with the actual plant material. In the case of the Bt toxin, the tested Cry1Ab toxin was produced by GM E. coli bacteria instead of by Bt11.

The notifier used these studies to argue that no study of any chronic effect, and thereby no actual feeding trial, would be necessary to assess the food and feed safety of Bt11.

The notifier also refers to two 14-day studies which are not part of the application C/F/96.05.10: a 14-day poultry study and a 14 day dietary cow study. As Syngenta admitted in 2003, these studies are not toxicology studies.

Questions raised by Member States

" ***Italy:*** The feeding studies on toxicity and allergenicity on poultry and cows are conducted in a very short time; the data provided are only on the detection of Cry1Ab and PAT proteins.

Response: The studies referred to were not designed to detect toxic or allergenic effects but rather to assess the transfer of the Cry1Ab and PAT proteins to animal products. Further studies to assess the nutritional value of Bt11 maize to cattle have been performed and are described in Section 3.5: Animal and Human Safety (Response to the UK)." (Syngneta 2003, p. 31)

However, these studies also are no actual toxicology studies, and very little information is given. There is no information on the study design such as how many animals etc, nor is any original data available.

In a 14-day study lactating cows were fed whole-plant Bt11 maize to determine whether their milk contained Cry1Ab or PAT proteins. 12 cows were fed on either Bt11, Bt176 or a control maize. This control maize was not isogenic to Bt11 but to Bt176. The aim of this study was the transfer of the transgenic proteins, and the amount of "information on animal performance" (EFSA 2005, p. 13) was insufficient to count as a toxicity study. Milk yield, feed intake, composition of milk and udder health were studied and described as "similar for all study diet groups".

Another feeding study with 16 lactating cows over 21 days and a beef study over 101 days concentrated on comparing Bt11's feed qualities with a control group, studying feed intake, body weight, milk production and composition, ruminal ph and volatile acids. No other health parameters such as clinical chemistry and pathology were studied. Toxicology studies with other GM maize (such as 1507 maize or MON863) have shown statistically significant differences for example for blood parameters or organ weights.

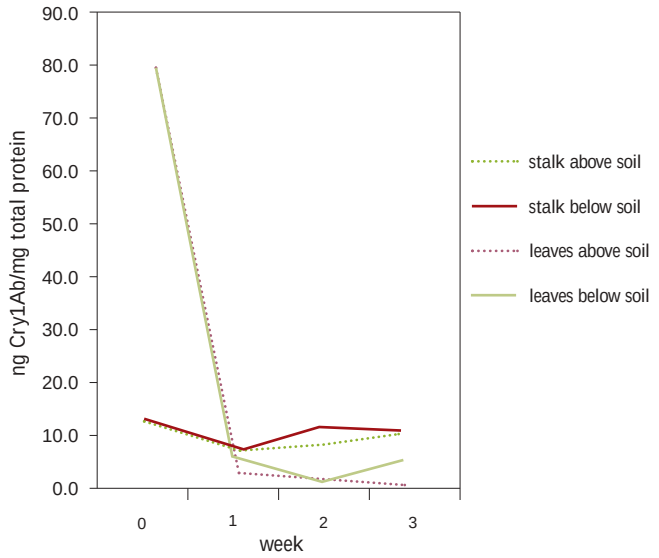
In fact no feeding study has been conducted as toxicology study. In those studies that were conducted for other aims as few as four animals received Bt11 in their feed. The few clinical parameters derived from these studies are not adequate as toxicity studies.

The EFSA (2005, p. 14), however, comes to the conclusion that "given the background of experience and knowledge already accumulated on the newly expressed proteins in Bt11 maize, there is no evidence of particular concerns for their safety" and "no additional subchronic toxicity studies are necessary". It is unacceptable that the need to conduct any toxicity feeding study is discarded on so little evidence, while feeding studies with other Bt maize events show statistically significant differences for a range of parameters (such as clinical chemistry, blood cell counts, organ weights) and raise serious concerns about the safety of GM Bt maize.

iv Cry1Ab degradation

The notifier conducted a study with Bt11 stalk and leaves above and below soil under greenhouse conditions. The notifier comes to the conclusion that "these results clearly show that if Cry1Ab protein was extracted from transgenic plant material during plant degradation, it would rapidly degrade in the soil." (C/F/96.05.10, Appendix 12).

FIGURE 2: DEGRADATION OF CRY1AB IN BT STALKS AND LEAVES.
3-WEEK GREENHOUSE STUDY WITH SOIL TEMPERATURE OF 20 AND 25ºC (NIGHT/DAY). SEE TABLE 2, P. 27 FOR COMPLETE DATA. SOURCE: C/F/96.05.10 APPENDIX 12.




© JENS CHRISTIANSEN

However the actual data provided by the notifier show a different result. After three weeks, about 70% of the Cry1Ab protein from stalks was still extractable from the soil (62% for stalks above soil, 74% for stalks laying in the soil). Leaves had a much higher Bt toxin concentration in the beginning, and Cry1Ab from the leaves degraded much faster in the first week. However in the following weeks, Cry1Ab levels in decomposing leaves in and above the soil stayed on a similar level, and even increased again for leaves that were incorporated in the soil (see Figure 2).

Scientific studies by Stotzky and co-workers also found much slower degradation. Cry1Ab persisted in the soil and remained active for over 200 days (Koskella & Stotzky, 1997; Tapp & Stotzky, 1998; Stotzky, 2000; Zwahlen et al., 2003).

Conclusion

Cry1Ab levels vary considerably over time and between different tissue types of the Bt11 maize. There has been no research into why this happens, and neither is there information on whether the (already extremely limited) toxicity studies used relevant Cry1Ab concentrations. Furthermore, no equivalence has been shown between the Cry1Ab protein produced in GM E. coli bacteria and Cry1Ab toxin incorporated in Bt11 plant material. No feeding studies have been conducted to study the toxicity of Bt11.

IV ENVIRONMENTAL RISK ASSESSMENT (E.R.A.)

The field trials referred to by the notifier in C/F/96.05.10 Appendix 14 (Earlier Releases) are described as tests about performance and for seed production. The environmental risk assessment (e.r.a.) was not the goal of these studies. The statement that no adverse effects were found in these releases is not valid, because the studies were not designed to find any adverse effects (COGEM, 2004).

Most studies put forward by the notifier are from North America. However, the European corn borer (ECB) has been an invasive (introduced) species in North America since the 1910s and therefore e.r.a.s done in North America only have limited representation for the situation of ECB in Europe. As an indigenous species in Europe, ECB can have more and different natural predators in Europe, and different feeding habitats.

For the notification C/F/96.05.10, no e.r.a. was undertaken for the herbicide resistance trait of Bt11. Such an e.r.a was requested in 2004 by EFSA and supplied in January 2005. This e.r.a. does not contain any studies or original data, but is a argument as to why the herbicide trait shows only negligible risks. The arguments and sources provided cannot replace a risk assessment.

i Target organisms

According to the Competent Authority in the UK (ACRE, 2003), the e.r.a. does not consider the potential wider biodiversity impact of the insect resistance trait resulting from the effect on target insects. This consideration should not only include consideration of the primary targets Ostrinia nubilalis and Sesamia nonagrioides but should also consider other maize and grass feeding Lepidoptera (moth and butterfly) larvae. The notifier only answers this concern for the UK, and only based on the understanding that ECB and other maize pests are rare in the UK. This does not say anything about wider biodiversity impacts in the whole EU.

A decreased number of target organisms impacts on the number of natural predators. The notifier compares impacts of Bt crops with those of insecticides, but they state themselves that only 5 to 10% of maize fields are treated with insecticides. The baseline for comparison should therefore be the untreated fields.

ii Non-target Lepidoptera

The notifier considers all non-target Lepidoptera larvae feeding on maize as pests and therefore as target organisms, independent of their actual agronomical impact. In addition, the coincidence of butterfly/moth larvae and maize pollen is simply considered as "unlikely" without any further source for this assumption. However, it is known from the numerous studies in North America on the monarch butterfly that these so coincide.

* The populations of non-target Lepidoptera, especially those in close proximity to maize fields, are different in the EU (and in the different European regions) than in North America, not least due to fundamental differences in the agricultural landscapes and in the ratio between agricultural land and wilderness. Species in Europe have adapted to the agricultural use of landscapes and have found niches in agricultural regions. Tests on non-target Lepidoptera need to be done with European species under European conditions.
* Feeding experiments with the larvae of the peacock butterfly (Inachis io) showed that these non-target Lepidoptera are susceptible to Bt toxin Cry1Ab. In a no-choice test with Bt176 (another Cry1Ab-producing GM maize), larvae showed lethal and sublethal damage from when their fodder plant was treated with Bt pollen. Although levels of Bt in pollen from Bt11 are less than that in Bt176, the study shows the general susceptibility of peacock butterflies to Cry1Ab in maize pollen. Felke & Langenbruch (2003) found sublethal effects such as smaller growth and delayed development. For peacock butterflies, Felke & Langenbruch (2003) do not expect a threat to the whole species, because peacock butterfly larvae and maize flowering does not always appear at the same time and place, but they state that there is a threat to butterfly and moth populations in agricultural landscapes. Sublethal effects could have long-term effects such as less activity, higher susceptibility to diseases, being easier prey to predators and parasitoides, producing fewer eggs or having a shorter life span (Felke & Langenbruch, 2003).
* To date, there have been no studies of the possible toxicity of Bt11 to the peacock butterfly. This is despite the conclusion from recent long-term studies on the monarch butterfly that showed adverse effects. Although no short-term effects (4-5 days) were noted (Stanley-Horn et al., 2001), longer-term studies (two years) found that over 20% fewer monarch larvae reached the adult butterfly stage when exposed to naturally deposited Bt pollen (Dively et al., 2004). 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.
* Of all butterfly species on the British Isles, only two have life cycles with larvae feeding outside the vulnerable period. Most species have herbaceous food plants, many of which are commonly found in the near vicinity of fields used for arable crops. Rarer species with limited distribution, e.g. the Chalk Hill Blue (Lysandra coridon) may be highly vulnerable if Bt11 maize is planted close to their restricted habitats. More then 250 species of moths on the British Isles have larvae stages feeding in July and include a substantial proportion which feed on herbaceous plants associated with agricultural land. Many of these must have the same potentially high vulnerability of the butterfly species. In comparison with the British Isles, the butterflies of continental Europe show a much greater diversity of species in all major orders (Stradling, 1999).

iii Non-target organisms

Several Member States raised concerns about the lack of data provided for impacts on non-target organisms. Even the few existing reports have been declared as confidential by the French authorities so that third parties cannot assess them. From the titles of these unpublished studies, it is clear that they were originally conducted in the early 1990s for the US EPA and that they comprise an apparently acute toxicity test at one trophic level, but not under realistic (feeding) conditions.

Competent authorities such as the Dutch authority have rejected the information provided by the notifier as inadequate to even conduct an e.r.a., let alone to draw a positive advice from it. The notifier answered concerns of the Member States in very general terms. It also acknowledged that the species tested may not be native to the EU, and did not see any reason to conduct studies with European non-target organisms

The EFSA however appears not to have asked for any additional studies. Instead it quotes a study carried out with aphids (Rhopalosiphum padi) on Bt176 maize to prove the lack of adverse effects (Lumbierres et al., 2004). As the EFSA itself states, the phloem on which the aphids feed does not contain any Bt toxin (Raps et al., 2001). On the other hand, the EFSA leaves out all other scientific studies on the effect of Bt maize on non-target organisms, for example the studies done by Hilbeck et al. (1999, 2000, 2001) that show adverse effects on the predator green lacewing (Chrysoperla carnea), or the review by Lövei & Arpaia (2005) that points out that relevant groups of non-target predators and parasitoids have not been not studied at all, and that most studies do not consider realistic conditions and/or worst case scenarios. In addition, there are no studies that take the stacked GM traits of Bt toxin production and herbicide resistance into account, even though herbicide application could have adverse effects on the non-target organism, or could increase adverse effects of Bt toxins.

It is alarming that the EFSA does not consider proper non-target organism studies with Bt11 itself to be necessary and refers only to the existing scientific literature. Instead the EFSA (2005, p. 17) comes to the conclusion that "the panel has no reason to consider that Bt11 maize will cause changes to non-target species that differ significantly from those caused by conventional farming."

Where are the studies on non-target organisms? Syngenta listed five unpublished studies by Monsanto as their supporting evidence:[2] but have not been able to supply these to Greenpeace, despite numerous requests. The French Ministry of Agriculture (to whom the original Bt11 application was made) eventually explained in a letter to Greenpeace that Monsanto considered the studies confidential and therefore could not disclose them. This is not in keeping with the spirit of transparency that is supposed to exist within the EU's regulation of GM crops. All unpublished studies should be made available to the public upon request. It is not clear whether any Member States have examined these studies, as they are not included in the dossier.

From the titles of these unpublished studies, it is clear that they were originally conducted in the early 1990s for the US EPA. Therefore, their application to an EU application is highly questionable. It is apparent that Syngenta have not conducted or commissioned any tests for potential toxicity to non-target organisms for the EU environment. The submitted titles of unpublished studies are apparently for simple, acute toxicity tests at one trophic level, e.g. direct feeding to green lacewing larvae when it has clearly been shown that this organism is affected by indirect toxicity, i.e. by ingesting Bt toxin by feeding on ECB larvae that have taken this up Bt (Hilbeck et al., 1999). This is despite pleas that several trophic levels should be studied in the assessment of Bt crops (Knols & Dicke, 2003).

Agricultural landscapes in Europe are different from those in North America, for example in terms of scale, the ratio between cultivated and uncultivated land, and specific habitats like field margins and hedges that are influenced by agricultural use. Species that regularly live and/or feed on agricultural land, such as the rapidly declining skylark, could be affected by changes in species composition at lower trophic levels (i.e. their food sources). Tests with non-target organisms need to be based on European species and include multiple trophic levels. It is inadequate and alarmingly complacent to rely on over-simplified, unpublished studies that are ten years old and for completely different ecosystems.

The competent authority, COGEM (Commissie Genetische Modificatie, Netherlands, 2004), considers the research material supplied by the notifier to be insufficient for a risk assessment. In 1999 COGEM asked for additional information about potential effects of Bt11 maize on non-target organisms. On 28 August 2003,

2 | Hoxter & Lynn (1992a, b, c)

COGEM concluded that "the report is again missing such information, so that it is not possible to conduct a sound risk-analysis. [...] The notifier handed in two tables (1a and 1b), in which a list of organisms is given that are tested on their sensitivity towards Cry1Ab. However, the tables do not give specific information about the Bt11 maize, but only about Bt maize in general. Also, the tests are only focused on short-term effects. Conclusions over long-term effects cannot be drawn on the basis of this research. [According to COGEM,] table 1b does not explain whether the organisms are fed with the GM Bt plants or with bacterial crystals. The website mentioned under the table as source of the data does not exist." Therefore COGEM considers the question of the effects of Bt11 maize on non-target organisms to be "unanswered." "In general, COGEM criticizes the additional information that it received on demand. In the goal of the field tests, the effects on non-target organisms are not mentioned, but in the conclusions it is stated that no negative impact on the environment was observed. COGEM thinks that such a conclusion cannot be drawn, if effects on non-target organisms are not considered."

Missing studies The notifier uses a tiered system for the environmental risk assessment with, for example, laboratory assays to test for toxicity as part of the first tier. Most studies performed or referred to by the notifier, however, were performed under conditions that are likely to differ from those which occur under real conditions, with small sample sizes and few replications, so it is questionable whether these are relevant. Any sublethal effects that can weaken individuals and populations, such as those found to be important for monarch butterflies (Dively et al., 2004), would not be identified in such studies.

The notifier considers that the testing was extensive, and only chose 'representative' non-target organisms. However, in some cases sample sizes were so small that statistic evaluation was not possible. The question of which non-target organisms were representative is still under discussion. The notifier did not present its criteria of how the non-target organisms tested were selected.

As Lövei, & Arpaia (2005) explained: peer-reviewed studies on non-target organisms have been done on a few species but whole other groups of predators and parasitoids (such as predatory flies, spiders, wasps or ants) have so far not been studied at all. The notifier did not conduct any such studies, and there is no scientific literature that could be used as an alternate reference.

Maize is dominantly wind-pollinated, but bees still need to be included in any e.r.a of GM maize, because bees feed regularly on maize pollen, especially when fed to larvae.

Only 5 to 10% of ECB infestations are treated with insecticides, as the notifier itself states. However, the effects of Bt11 maize on the target organisms and on the natural predators of the target organism are measured against the effects of insecticide use. Untreated ECB infestations do not impact the natural predators, but Bt11 plants can have an impact if large proportions of the predators' food source disappear.

"COGEM considers the dossier on placing Bt11on the market and its cultivation to be incomplete, because data concerning the effects of the Cry1Ab protein on non-target organisms under field conditions are missing. Additionally, data about the persistence of the Bt protein in the soil are missing. To be able to conduct a sound risk analysis it is necessary to have access to such data. COGEM considers that there is not enough support to be able to give positive advice at the moment." (COGEM, 2004, translated by A.Lorch)

iv Effects in and on soil

The release of Cry1Ab toxin by roots is a common phenomenom with transgenic Bt maize. Cry1Ab root exudates were found for all five GM Bt11 hybrids tested by Saxena et al. (2002) as well as for Bt176 and MON 810 varieties. Areas of soil where toxin had been released retained insecticidal activity for at least 180 days, the longest time studied. Larvae of the tobacco hornworm (Manduca sexta) in plant growth rooms showed mortalities of about 81%, while no mortality occurred in soil with the isogenic control lines. The weight of the surviving larvae was less then 10% of that of the control group. The effects of the five Bt11 varieties on the non-target larvae in the soil were stronger than that of Bt176 (one variety) and MON 810 (six varieties)

© JENS CHRISTIANSEN

Mortality in field trials was lower, while the decrease in larvae weight was even higher.

COGEM raised the issue of the persistence of Bt toxins in the soil and the consequent effects on non-target organisms. In its advice from 28 August 2003, COGEM pointed out that Bt proteins can bind to clay particles in the soil and so persist in the soil for several months. Research showed that in bioassays such soil can lead to mortality in insects (Groot & Dicke, 2002). The notifier replied that the persistence of Bt protein in the soil after cultivation of Bt11 maize was low, referring to the research report 'Assessment of the potential for persistence and accumulation for Cry1Ab protein in soil as a result of sustained Bt corn use' (Dupelman, 2003), which described field trials with Bt plants in three successive planting seasons and the persistence and accumulation of Bt proteins in the soil. However this report is not part of the dossier and COGEM considers it necessary to have access to this report in order to come to a sound judgement." (COGEM, 2004).

The notifier does not provide any monitoring regarding the persistence, activity and impact of Bt toxins from Bt11 in the soil (C/F/96.05.10, Appendix 12). The data provided by the notifier refer only to a simulated degradation cycle of three weeks.

The insecticidal activity of Bt plant material was measured in a simulated degradation cycle of three weeks. The Bt toxin was extracted from the soil and its insecticidal activity tested on ECB larvae. Such a study design however does not use realistic conditions.

* Soil organisms will be subject to Bt toxin that has accumulated from root exudates and degrading plant material. Even under the conditions of the study performed by the notifier, the results are questionable. In their study, it was not possible to give data on the amount of Bt toxin in the soil because standard extraction buffers did not work: "Quantitative extraction of Cry1Ab protein directly from the spiked soil was not possible. Apparently the Cry1Ab protein bound to the soil particles and was not released by the standard buffers" (C/F/96.05.10, Appendix 12). The Bt toxins bind to the surfaces of soil particles and retain insecticidal activity for at least 180 days (Saxena et al., 2002).
* The temperature will be much lower then the 20ºC and 25ºC used in the simulation. Therefore microbial degradation of the Bt toxin is bound to be slower. Preliminary results from other studies, at temperatures as they occur in Europe in autumn, show a much longer persistence and biological activity in the soil (Saxena et al., 2002; BMBF, 2003; Zwahlen et al., 2003).
* Other organisms than ECB will be subjected to Bt in the soil. ECB larvae, however, are not soil-dwelling organisms.

The notifier comes to the conclusions that "these results clearly show that if Cry1Ab protein was extracted from transgenic plant material during plant degradation, it would rapidly degrade in the soil. Additionally, the tight binding of Cry1Ab protein to the soil significantly reduced the biological activity of the Cry1Ab protein and could also [be] expected to reduce the mobility of Cry1Ab protein in soils." (C/F/96.05.10, Appendix 12). In contrast, other studies find a much longer persistence and higher biological activity, in which the binding to soil particles in particular plays an important role (Saxena et al., 2002; BMBF, 2003; Zwahlen et al., 2003).

Interaction between Bt toxin and glufosinate ammonium (such as increased persistence) was not studied by the notifier. Accinelli et al. (2004) showed in a preliminary study that in the presence of bacterial Cry1Ab protein glufosinate ammonium persistence in the soil was significantly increased.

Hilbeck et al. (2000) concluded that insufficient studies and insufficient studies of a relevant design (long term, multi-trophic studies) have been performed to meaningfully assess the ecological impact of transgenic Bt plants in soil ecosystems. This situation has not changed since early 2004 (Andow & Hilbeck, 2004). In their opinion(Andow & Hilbeck, 2004, it cannot be excluded that Bt toxins may accumulate in the soil under the large-scale production of transgenic Bt plants.

v Environmental risk assessment of herbicide resistance

The notifier had argued that no risk assessment would be necessary for GM herbicide resistance because Bt11 would not be marketed as herbicide resistant. However, after protests from Member States the EFSA requested in 2004 that the e.r.a. and post-market environmental monitoring should also consider the direct and indirect impacts of the herbicide tolerance trait. In January 2005, Syngenta supplied a 40-page paper on the "environmental risk assessment of the pat gene and PAT protein in connection with the potential non-legitimate use of glufosinate ammonium herbicide on Bt11 maize."

This paper did not contain any further studies that might have been undertaken as a risk assessment, but only arguments as to why there should be no or only negligible effects expected from the herbicide trait. It argues that volunteers on fields and roadsides will either die off in winter or can be treated with other herbicides. So-called 'volunteer crop plants' can grow from seeds that didn't germinate in the season they were sowed or from seed lost during harvest or transport.

Regarding the effects of herbicide tolerance on non-target organisms, Syngenta (2005a, p. 24) simply states that "direct feeding studies with pollen from Bt11 maize have shown no effects on honeybee development, lady beetles, insidious flower bug and green lacewing. Results from feeding studies of young quail fed with modified maize meal in their diet showed no adverse effects." There are no references or sources given for these studies. Syngenta does not even give any information which species they used in the study, and only refers to them by their (apparently American) common names so that it remains impossible to assess such information. These studies appear to be different from those (unpublished) studies about the non-target effects of Bt toxin (see Section 5.3.1) because those studies were undertaken with purified Cry1Ab toxin. There is no indication whether any of these studies was done with pollen from Bt11 plants with and without herbicide application.

The lack of access to this information, and the conclusion that these studies do not represent realistic conditions for studying the possible negative effects of the Bt toxin have been described earlier (see page 15).

These studies with Bt11 pollen are inadequate for studying the possible effects of the pat gene and the PAT protein because "the levels of PAT protein found in the pollen [...] were less than the level of detection" (C/F/96.05.10, Appendix 6). It is impossible to test the effect of a protein, if it is not expressed in a significantly higher amount then for the control.

In addition, since no details were available on the study protocol, no information is available on whether the Bt plant tissue comes from plants sprayed with the herbicide glufosinate-ammonium. However, a full risk assessment needs to include a study of the effects with and without a herbicide application.

It is unacceptable for Syngenta to claim that there would be no adverse effects on non-target organisms from herbicide tolerance by quoting studies without references, and which apparently use plant material (pollen) that does not contain the PAT protein and that probably also has not been sprayed with glufosinate.

It has to be concluded that, in effect, no e.r.a. was done for the adverse effects of herbicide tolerance on non-target organisms.

Conclusions

The original notification and the material submitted later are insufficient to undertake an environmental risk assessment. They lack relevant studies, and for those few studies that were undertaken they lack the original data by which third parties could assess the results themselves. From the submitted summaries, it appears that these studies are insufficient to assess effects on non-target organisms and effects on the soil. Competent authorities of the EU Member States have therefore rejected the e.r.a.. The EFSA however did it not find necessary to ask for more information, instead quoting some scientific articles, but leaving others out which do describe the effects of Cry1Ab toxin on non-target organisms.

The e.r.a. for the herbicide resistance trait does not contain any relevant studies in this context, and uses inadequate studies on plant material not even containing the PAT protein.

With this information it is impossible to draw a positive recommendation. It is alarming that the EFSA has not requested additional information on the effects on non-target organisms and soil biota, and it is alarming that the EFSA would consider the material on herbicide resistance as "a full environmental risk assessment of the pat gene in connection with the possible use of the complementary herbicide."

V MONITORING

The EFSA requested several improvements and clarifications from the notifier before it finished its evaluation. However, the post-market environmental monitoring plan, as presented now (Syngenta, 2005b), is insufficient. Even after three rounds of modification, the EFSA itself thinks that further modifications are necessary to improve the farmers' questionnaire which forms the main monitoring tool. The EFSA (2005, p. 23) also thinks that "management options for the cultivation of Bt11 maize should include measures to reduce exposure of non-target Lepidoptera and to delay the development of resistance to the Cry1Ab protein in target organisms." It remains unclear how the EFSA (2005, p. 24) can at the same time accept the monitoring plan while recommending "that cultivation should be accompanied by appropriate risk management strategies to minimise exposure of non-target organisms." Even based on the EFSA's own evaluation and recommendations, the monitoring plan is insufficient.

There is no case-specific monitoring, even though the EFSA points out that the management of Bt11 should include measures to delay resistance development of ECB. In its opinion on another Bt maize (1507 maize) the EFSA considered case-specific monitoring for this issue to be necessary. Why not for Bt11?

Some examples for effects which must be part of a monitoring exercise:

* **Long-term effects on butterflies:** A scientific study, conducted over a period of two years under field conditions and published in August 2004 (Dively et al., 2004), in which monarch butterfly larvae were exposed to pollen from Cry1Ab Bt plants, showed that over 20% fewer larvae reached the adult butterfly stage than in the control group. Before this long-term research was done, the Bt levels in pollen of most Bt plants were considered to be too low to cause adverse effects on non-target insects. Earlier studies had only focused on short-term effects.

  In a study with a common Eurasian butterfly, the larvae of the peacock butterfly showed susceptibility to a Cry1Ab Bt toxin (Felke and Langenbruch, 2003), but no study on the effects of Bt11 on European Lepidoptera was undertaken in the e.r.a. While this already is an unacceptable neglect in the e.r.a., such anticipated adverse effects have not even been considered in the monitoring plan.

  Instead the EFSA (2005, page 23), comes to the conclusion that "the recording of statistically significant Lepidoptera would demand a high input of personnel and costs, especially if larvae, as the most susceptible and immobile development stage, are to be monitored."

  It is simply unacceptable that the monitoring of European Lepidoptera is to be put aside because it would be too expensive. Lepidoptera are known to be effected by Bt pollen, and there has been no study on the effect on European Lepidoptera in European agricultural landscapes, which are different from those in North America. Money must not be the cause of neglecting risks in the e.r.a and to fail to monitor anticipated effects.

* **Effects on non-target organisms:** Research further suggested that transgenic Bt plants could also be harmful to organisms that feed upon pests exposed to the toxins. Swiss laboratory studies, for example, have demonstrated that the mortality of green lacewing (Chrysoperla carnea) larvae almost doubled after ingesting ESB which had fed on GM maize (Hilbeck et

al., 1999, Andow & Hilbeck, 2004). ECB larvae survives the Bt toxin for a while, so beneficial insects such as C. carnea, will be able to feed on them and suffer from the indirect effects of Bt maize cultivation. Insects such as C. carnea are also used as a beneficial insect for pest control in organic agriculture.

* **Changes in plant composition:** A study conducted by the Max Planck Institute, Jena, Germany, in 2001 to 2004 (Biosicherheit, 2005) compared specific plant defence mechanisms of Bt plants with a comparable (isogenic) line. The spectrum of volatile compounds used by the plants to defend themselves against pest insects showed significant differences which need to be studied further. (The results of this study have not yet been published as a scientific article.) Volatile compounds are important components of the secondary metabolic pathway in plants. They are used as a communication tool and alarm system against pest insects. For example, if a maize plant is attacked by ECB, it produces a specific profile of volatile substances that attract the natural enemies of ECB. If this composition is changed in such a way that it is not recognised by the beneficial insects any more, then the Bt crops becomes more susceptible to pest insects. This might include pests targeted by Bt production, but also other pests. Further, it might be a signal that other unintended compounds are being produced by the plant or that metabolic pathways are being disrupted (see also Firn and Jones, 1999).

* **Bt toxins in the soil:** Bt residues in the soil are another established aspect of Bt plant growth that are relevant for assessing the impact of Bt plants to the environment. Bt toxins containing Cry1Ab can be exuded by the roots of Bt crops (Saxena et al., 2002, Saxena et al., 2004) or enter the soil through degrading plant material. These toxins do not degrade quickly but persist in the soil, being absorbed in soil particles while remaining physiologically active for up to several months (Zwahlen et al., 2003). The long-term, cumulative effects of continued growth over several years of GM plants expressing toxins are important and should be considered as part of the environmental risk assessment (C/F/96.05.10, e.r.a.; Marvier, 2002; Andow & Hilbeck, 2004).

* **Spreading Bt toxins through manure:** In studies in Germany, the Bt toxin was unexpectedly found in cows' stomachs, intestines and dung, after feeding of Bt176 maize containing Cry1Ab toxin. Bt toxin in the plant material appears to degrade more slowly than had been assumed. Besides the possible harmful effects on the animals, this also shows an additional route for Bt to get into the environment. "Therefore, a potential distribution of Bt protein fragments on fields may be feasible considering the routine spreading of manure in e.g. dairy farms, and should be addressed in further studies" (Einspanier et al., 2004). Chowdhury et al. (2003) found similar results with pigs fed with Bt11.

Since it is known from the scientific literature that Bt crops can have these mentioned effects, it is a clear requirement that these issues are covered in case-specific monitoring.

i General surveillance

On other issues, both the notifiers and the EFSA (2005, p. 21) came to the conclusion that "the environmental risk assessment has not identified any risks specifically linked to Bt maize fields". The e.r.a. has been shown to be so incomplete that it is most improbable that any adverse effects would have been observed at all. Member states have criticized the non-target studies as inadequate and to be unuseable to produce a positive recommendation. Based on such a poor collection of data, it is obvious that no risk could be identified because most issues are either not or insufficiently studied.

The EFSA considers monitoring of Lepidoptera to be unnecessary mainly because of cost reasons .

"In addition it will be difficult to compare populations of Lepidoptera in conventional maize fields (sometimes treated with insecticides) with populations in Bt maize fields. [...] Furthermore, the recording of statistically sufficient data on the abundance of Lepidoptera would demand a high input of personnel and costs, especially if larvae as the most susceptible and immobile development stage, are to be monitored."


© JENS CHRISTIANSEN

It is unacceptable to deliberately not perform necessary monitoring for cost reasons.

The EFSA's GMO panel (EFSA, 2005, p. 23) is "not aware of any existing surveillance networks that would substantially fulfil the scientific requirements for the detection of any unforeseen environmental effect in relation to Bt11 maize cultivation. Thus the Panel agrees with the proposal of the [notifiers] to describe the generic approaches for using other existing surveillance networks". A number of networks are listed in very general terms, such as "seed producers' associations, university (agronomy), national environmental research institutes, nature conservation agencies, animal nutrition networks, food and feed industry associations". However, there is no indication given of whether these networks are actually interested or equipped to take part in the monitoring, and who will pay for it. It remains unclear whether any of the persons or organisations in the listed networks are committed to taking part in the monitoring.

There are no details given as to what should be monitored in the general surveillance. As described, the general surveillance is no more then an alert to the 'existing networks' to report anything they might see or hear to Syngenta. The general surveillance cannot be left to such a vague network, where it even remains unclear who will actually take part.

In addition, general surveillance of the fields is to be done by farmers completing and returning questionnaires to the company. The monitoring of the development of Cry1Ab resistance and of unanticipated adverse effects requires scientific knowledge. As Syngenta states: "It implies the collection, scientific evaluation and reporting of reliable scientific evidence". Non-scientific actors must not be asked to complete a scientific task they are unequipped to understand, especially when farmers in particular are likely to have conflicting interests between wanting to grow Bt11 maize, and having to look for adverse effects that might stop their Bt11 maize production.

Users (especially growers) might have conflicting interests in the cultivation of GM maize. For the case-specific monitoring of insect resistance, they may not appreciate the importance of reporting a few insects if these are not a danger to their own harvest. It is possible that they might consider safeguarding against insect resistance as a product protection.

All other effects on non-target organisms, including birds and other animals higher up the food chain, soil biota, and other plants are supposed to be monitored in the General Surveillance. In some cases growers might consider effects as positive or intended. This might be the case for decreased numbers of weeds, decreased numbers of non-pest insects or species and quantities of birds feeding on the field.

Growers might not consider certain effects unusual, or they might not recognise them as changes if they had not paid close attention to those issues before they cultivated Bt11 maize. No baselines are established against which growers and other users can compare possible effects. All responsibility for monitoring is given to growers and feed producers with an unknown level of relevant knowledge and training for such a task.

ii Insect resistance management (IRM)

Monitoring of insect resistance is also necessary because concerns have been raised about whether the proposed high dose/refuge strategy actually works.

It is unclear how the "Harmonized insect resistance management (IRM) plan for cultivation of Bt maize in the EU" ties in with the monitoring of Bt11. The IRM plan was developed by a corporate working group of Monsanto Europe, Pioneer Hi-Bred International and Syngenta Seed, called "EU Working Group on Insect Resistance Management" and covers the transformation events Bt11, Bt176, MON 810 and 1507. It is included in Section 3, Dossier C/F.96.05.10, Monitoring. The notifiers propose an IRM plan, but the implementation and its legal bindings remain unspecified.

Germany proposed discussing with other Member States an obligation for the notifier to bind customers contractually to comply with the requirements of the IRM implementation. However, the notifiers say only that they are "committed" to

"ensuring" the monitoring, without any information whether this will be legally binding, or how grower compliance can be ensured (C/F/96.05.10, Response to questions raised by Member States).

The IRM is based on non-Bt maize refuges but there is no scientific basis for such refuges, their size or form. The notifier acknowledges that "it is not clear what size refuge is needed, the ratio of refuge to ECB protected maize plot, nor its spatial arrangement" (C/F/96.05.10, Appendix 10).

The effectiveness of refuges has been brought into question. The hypothesis is based on pest insects moving from Bt plants to non-Bt plants for mating, and thus diluting possible resistance development. However, according to Farinos et al. (2004), several features in Spain and other Mediterranean countries may reduce the effectiveness of the high-dose/refuge strategy:

"(i) Bt maize varieties based on the 176 event, such as Compa grown in Spain are more likely to allow the survival of second and third generations of heterozygous resistant larvae (Onstad & Gould, 1998; Walker et al., 2000)

(ii) it has been shown that MCB females mate before they move for oviposition (Lopez et al.,1999), so that females emerging from refuge would rarely mate with potential resistant moths emerging from Bt maize fields and vice versa

(iii) ECB mobility is also reduced before oviposition in irrigated maize fields (Hunt et al., 2001), which corresponds to the agronomic practices of most maize growing areas in Spain."

Research even shows that refuges in close proximity to the Bt crops could even accelerate resistance development. This might be especially true in cases where refuges are not established as distinct parts of the field, but by mixing Bt and non-Bt seeds (Chilcutt & Tabashnik, 2004). In addition, Bt11 plants have also been reported to have decreasing Bt toxin levels in maturity, which will allow survival of semi-tolerant pests.

Conclusion

The proposed monitoring plan is insufficient to fulfil its task. Even the EFSA recommends that more monitoring is necessary for resistance development and non-target organisms. The monitoring plan is developed on the basis of the e.r.a., but the e.r.a. lacks so many relevant studies that the conclusion that "no risks were identified" cannot be drawn.

It remains unclear who besides farmers will actually participate in the general surveillance. The notifier lists a number of existing networks, but it is unclear whether these networks are actually interested or equipped to participate, who will pay for it, and what will actually be monitored.

© JENS CHRISTIANSEN

Conclusion

This report written on behalf of Greenpeace shows significant failures in current EU risk assessment by the EFSA.

According to Directive 2001/18/EC, which deals with the environmental considerations of GMOs (genetically modified organisms), any risk assessment must be based on the precautionary principle:

2001/18/EU Article 1

"***Objective*** In accordance with the precautionary principle, the objective of this Directive is to approximate the laws, regulations and administrative provisions of the Member States and to protect human health and the environment when:

* carrying out the deliberate release into the environment of genetically modified organisms for any other purposes than placing on the market within the Community,
* placing on the market genetically modified organisms as or in products within the Community."

The EFSA's published opinion clearly violates this requirement. Its assessment appears to be more oriented towards the interests of the notifier to get Bt11 approved then in considering the potential dangers for human health and the environment. The missing information and the way in which evidence for adverse effects are discarded document this.

The EFSA is very clear about considering the costs for the notifier (and the biotech industry as such) as of more relevance then the risks for human health and the environment. One of the EFSA's main reasons for rejecting the systematic monitoring of Bt11 on protected species such as butterflies and moths (Lepidoptera) are the potential costs:

"Furthermore, the recording of statistically sufficient data on the abundance of Lepidoptera would demand a high input of personnel and costs, especially if larvae, as the most susceptible and immobile development stage, are to be monitored."

Instead of scientific research and monitoring on and around the fields to study insects which are known to be susceptible to the Bt toxin, Syngenta proposes to send out questionnaires to farmers, and the EFSA have accepted this.

According to Directive 2001/18, every case needs specific monitoring and general surveillance because adverse effects might occur during the growth of GM crops that were not or could not be anticipated during risk assessment (Directive 2001/18/EC Annex VII). In October 2002, the Council decided upon relevant Guidance Notes (EU Council, 2002/811/EC). In the "Objectives" of these notes the following benchmarks are given:

"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. [...]"

In subsequent paragraphs, the guidance notes explain two basic concepts of the monitoring concept, case-specific monitoring (1.3.1) and general surveillance (1.3.2):

"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.

The approach should:

* focus on all the potential effects on human health and the environment identified in the risk assessment, taking into account i.e. different locations, soil types, climatic conditions, and
* define a specified time period in which to obtain results.

In contrast to case-specific monitoring, general surveillance should:

* Seek to identify and record any indirect, delayed and/or cumulative adverse effects that have not been anticipated in the risk assessment,
* Be carried out over a longer time period and possibly a wider area."

This is not how the EFSA interprets the monitoring requirements.

The EFSA's published opinion (2005) on the GM maize Bt11 is unacceptable. It interprets the requirements for risk assessment and monitoring of GMO in a lax way and privileges the interests of the notifier, which suggests establishing a precedent for future support of the biotech industry as a whole. The EFSA thereby undermines the EU legislation on the evaluation of GMOs. It supports a policy of Syngenta which is based on insufficient research, lack of transparency, and withholding data and studies from independent evaluation. This violates the EU regulation requiring full publication of all data that concern environmental risk assessments.

In the light of these findings, no approval for the cultivation of Bt11 can be given. Instead the EU authorisation process for GMOs should be halted, and a re-organisation of the EFSA and the EU regulation procedures begun. In addition, co-existence problems such as contamination of seeds, a legal framing of GMO-free zones and liability questions, all of which have been left out of this notification for Bt11, must be addressed as a high priority.

Abbreviations and Glossary

Bt	Bacillus thuringiensis
Cry1Ab	used synonymously for Btk, Bacillus thuringiensis subsp. kurstaki
CBI	Confidential Business Information.
ECB	European Corn Borer, Ostrinia nubilalis
EFSA	European Food Safety Authority
EPA	US Environmental Protection Agency
e.r.a.	environmental risk assessment
GM	genetically modified, genetically engineered
IR	insect resistant
IRM	Insect Resistance Management Plan
MCB	Mediterranean corn borer, Sesamia nonagrioides

Sources

Notification, additional material and Member State comments

ACRE (Advisory Committee on Releases to the Environment) 2004. Advice on a notification for marketing of insect resistant and herbicide tolerant GM maize. http://www.defra.gov.uk/environment/acre/advice/pdf/acre_advice46.pdf

ACRE (Advisory Committee on Releases to the Environment) 2003. Advice on a notification for marketing of insect resistant and herbicide tolerant GM maize. http://www.defra.gov.uk/environment/acre/advice/pdf/acre_advice35.pdf

C/F/96.05.10: Updated information for Dossier C/F/96.05.10, Syngenta Seeds SAS (Bt11 update 15 January 2003); and Response to questions raised by Member States, 28th November 2003.

C/F/96.05.10 Summary Information Format (SNIF) 'Deliberate Releases and Placing on the EU Market of Genetically Modified Organisms (GMOs)', as available in the dossier and on http://gmoinfo.jrc.it/gmc_browse.asp

Codex Alimentarius 2003. Joint FAO/WHO Food Standard Programme Codex Alimentarius Commission, Report of the twenty-fifth session, Rome, Italy 30 June - 5 July 2003 ALINORM 03/34. ftp://ftp.fao.org/codex/alinorm03/Al03_34e.pdf

COGEM (Commissie Genetische Modificatie) 2004. Aanvullende informatie marktdossier C/F/96/05-10 'InsectenreistenteBt11 maize' (Additional information market dossier C/F/96/05/10). http://vrom-ggo.bluedome.satama.nl/docs/im_markt/FR960510.ZIP

EC Scientific Committee on Food 2002. Opinion of the Scientific Committee on Food on a request to place genetically modified sweet maize line Bt11 on the market. SCF/CS/NF.DOS.14_ADD2 Final, 6 May 2002. http://europa.eu.int/comm/food/fs/sc/scf/out129_en.pdf

EFSA (European Food Safety Authority) 2005. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/F/96/05.10) for the placing on the market of insect resistant genetically modified maize Bt11 for cultivation, feed and industrial processing, under Part C of Directive 2001/18/EC from Syngenta Seeds. (Question No EFSA-Q-2004-012). The EFSA Journal 213: 1-33.

Scientific Committee on Plants 2000. Opinion of the Scientific Committee on Plants on the submission for placing on the market of genetically modified insect resistant and glufosinate ammonium tolerant (Bt-11) maize for cultivation. http://europa.eu.int/comm/food/fs/sc/scp/out86_gmo_en.html

Syngenta 2005a: Additional information submitted by Syngenta on 27 January 2005 in response to the EFSA's request for further information. includes Annex I: DNA analysis in Bt11 maize, and Annex II: Environmental risk assessment of the pat gene and PAT protein in connection with the potential non legitimate use of glufosinate ammonium herbicide n Bt11 maize.

Syngenta 2005b: Additional information submitted by Syngenta on 16 March 2005 in response to the EFSA's request for further information: General surveillance plan for Bt11 under the Directive 2001/18/EC.

Syngenta 2005c: Additional information submitted by Syngenta on 15 April 2005 in response to the EFSA's request for further information: Confirmation that the material used in the safety studies conducted with Bt11 maize is Bt11 maize.

UK Competent Authority, Feb 2004. Comments or objections to the placing on the market of a GMO under part C of Directive 2001/18/EC (Article 15), Response of United Kingdom Competent Authority, Notification number: C/F/96/05/10, Insect resistant and herbicide tolerant maize (Bt11). http://www.defra.gov.uk/environment/gm/regulation/pdf/f-96-05-10-2nd.pdf

UK Competent Authority (Sep 2003): Comments or objections to the placing on the market of a GMO under part C of Directive 2001/18/EC (Article 15), Response of United Kingdom Competent Authority, Notification number: C/F/96/05/10. http://www.defra.gov.uk/environment/gm/regulation/pdf/f-96-05-10.pdf

Scientific literature

Accinelli, C., Serepanti, C., Vicari, A. & Catizone, P. 2004. Influence of insecticidal toxins from Bacillus thuringiensis subsp. kurstaki on the degredation of glyphosate and glufosinate-ammonium in soil samples. Agriculture, Ecosystems and Environment, 103: 497-507

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. & Watson, J.D. 1994. Molecular Biology of the Cell. Garland Publishing Inc.

Ananiev, E.V., Philips, R.L. & Rhines, H.W. 1998. Complex structure of knob DNA on maize chromosome 9: retrotransposon invasion onto heterochromatin. Genetics 149, 2025-2037.

Andow, D.A. & Hilbeck, A. 2004. Science-based risk assessment for non-target effects of transgenic crops. Bioscience, 54: 637-649.

Bejerano, G., Pheasant, M., Makunin, I., Stephen, S., Kent, W.J., Mattick, J.S. & Haussler, D. 2004. Ultraconserved elements in the human genome. Science 304: 1321-1325

Benbrook, Ch. 2001. When Does It Pay To Plant Bt Corn? Farm-Level Economic Impacts of Bt Corn, 1996-2001. http://www.gefoodalert.org/library/admin/uploadedfiles/When_Does_It_Pay_To_Plant_Bt_Corn.pdf

Biosicherheit 2005. Auswirkungen von Bt-Mais auf Schmetterlinge und deren Gegenspieler. Max-Planck-Institut (MPI) für Chemische Ökologie, Abt. Biochemie der Pflanzen und Molekularbiologie, Jena. http://www.biosicherheit.de/projekte/23.proj.html

BMBF (Bundesministerium für Bildung und Forschung) 2003. Sicherheitsforschung und Monitoringmethoden zum Anbau von Bt-Mais. (Safety research and monitoring methods for the agro-production of Bt-maize). Overview over 12 projects and preliminary results at http://www.biosicherheit.de/mais/102.doku.html; final report yet unpublished.

Chilcutt, C.F. & Tabashinik, B.E., 2004. Contamination of refuges by Bacillus thuringiensis toxin genes from transgenic maize. PNAS 101(20), 7526-7529.

Chowdhury, E.H., Kuribara, H., Hino, A., Sultana, P., Mikami, O., Shimada, N., Guruge, K.S., Saito, M. & Nakajima, Y., 2003. Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11. Journal of Animal Science 81: 2546-2551.

DeSchrijver, A. & Moens, W. 2003. Report on the molecular characterisation of the genetic map of event Bt11. Service of Biosafety and Biotechnology, Belgium. IPH/1520/SBB/03-0325. http://www.biosafety.be/TP/MGC_reports/Report_Bt11.pdf

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.

Dupelman, 2003. quoted in Syngenta (2003): Response to Member States. – No further reference given.

Einspanier, R., Lutz, B., Rief, S., Berezina,O., Zverlov, V., Schwarz, W. and Mayer, J., 2004. Tracing residual recombinant feed molecules during digestion and rumen bacterial diversity in cattle fed transgene maize. European Food Research and Technology 218: 269-273.

Farinos, G.M., De la Poza, M., Hernandez-Crespo, P., Ortego, F. & Castanera, 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(1), 23.

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), 1-7.

Firn, A.D. & Jones, C.G. 1999. Secondary metabolism and the risk of GMOs. Nature 400, 13-14.

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

Groot, A.T. & Dicke, M. (2002). Insect-resistant transgenic plants in a multi-trophic context. Plant Journal, 31, 387-406.

Hillbeck, A. 2001. Transgenic host plant resistance and non-target effects . In Genetically engineered organisms: assessing environmental and human health effects. Letourneau, D.K. and B.E. Burrows [eds.] Boca Raton, FL: CRC Press.

Hilbeck, A., Meier, M.S. &, Raps, A. 2000. Bt proteins in soils: Is enough known to assess the impact of Bt plants on soil ecosystems. EcoStrat report to Greenpeace International. EcoStrat, Zürich.

Hilbeck, A., Moar, W.J., Pusztai-Carey, M., Filippini, A., Bigler, F. 1999. Prey-mediated effects of Cry1Ab toxin and protoxin and Cry2A protoxin on the predator Chrysoperla carnea. Entomologia Experimentalis et Applicata 91 305-316.

Hoxter K & Lynn S (1992a, b, c): Activated Cry1Ab HD-1 Protein: A Dietary Toxicity Study With [Green Lacewing Larvea | Parasitic Hymenoptera (Brachymeria intermedia) | Ladybird Beetles] Lab Project Numbers [WL-192-155, WL-192-157, WL-192-156]. Unpublished studies prepared by Monsanto Co. and Wildlife Int'l Ltd. MRID # 434680-03, -04, -05 in EPA (2001)

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.

Jank, B. & Hasselberger, A. 2000. Recombinant DNA insertion into plant retrotransposons. Trends in Biotechnology 18, 326-327.

Jung, H.G. & Sheaffer, C.C. 2004. Influence of Bt transgenes on cell wall lignification and digestibility of maize stover for sillage. Crop Science 44: 1781-1789.

Knight, J. 2003. Agency "ignoring its advisers" over Bt maize. Science 422, 5.

Kluge, E. 1999. Befallsatlas – Atlas der potentiellen Befallsgefährdung durch wichtige Schadorganismen im Ackerbau Deutschlands. Biologische Bundesanstalt, Berlin. (quoted in Saeglitz 2004)

Knols, B.G.J. & Dicke, M. 2003. Bt crop assessment in the Netherlands. Nature Biotechnology, 21 973-974.

Koskella, J. & Stotzky, G. 1997. Microbiological utilization of free and clay-bound insecticidal toxins from Bacillus thuringiensis and their retention of insceticidal activity after incubation with microbes. Applied and Environmental Microbiology 9: 3561-3568.

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.

Lopez, C., Sans, A., Asin, L. & Eizaguirre, M. 2001. Phenological model for Sesamia nonagrioides (Lepidoptera: Noctuidae). Environmental Entomology 30: 23-30.

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

Marvier, M. 2002. Improving risk assessment for nontarget safety of transgenic crops. Ecological Applications 12, 1119-1124.

Onstad, D.W. & Gould, F. 1998. Do dynamics of crop maturation and herbivorous insect life cycle influence the risk of adaptation to toxins in transgenic host plants? Environmental Entomology 27: 517-522.

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.

Raps, A., Kehr, J., Gugerli, P., Moar, W.J., Bigler, F. & Hilbeck, A. 2001. Immunological analysis of phloem sap of Bacillus thuringiensis corn and of the nontarget herbivore Rhopalosiphum padi (Homoptera: Aphidae) for the presence of Cry1Ab. Molecular Ecology 10: 525-533.

Ronning, S.B., Vaitilingom, M., Berdal, K.G. & Host-Jensen, A. 2003. Event specific real-time quantitative PCR for genetically modified Bt11 maize (Zea mays). European Food Research and Technology 216, 347-354.

Saeglitz, Ch. 2004. Untersuchungen der genetischen Diversität von Maiszünsler-Populationen (Ostrinia nubilalis, Hbn.) und ihrer Suszeptibilität gegenüber dem Bacillus thuringiensis (Bt)–Toxin als Grundlage für ein Resistenzmanagement in Bt-Maiskulturen. Dissertation, University Aachen. http://sylvester.bth.rwth-aachen.de/dissertationen/2004/110/04_110.pdf (2 Aug 2004)

Saxena, D., Stewart, 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.

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 & Stotzky G (2001a): Bt corn has a higher lignin content than non-Bt corn. American Journal of Botany 88(9), 1704-1706.

Saxena D & Stotzky G (2001b): 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 the soil. Soil Biology & Biochemistry 33, 1225-1230.

Schenkelaars Biotechnology Consultancy (2000): Use of microbial Bacillus thuringiensis formulations in Dutch agri- and horticulture. Report by order of Dutch Committee on Genetic Modification – COGEM. http://www.sbcbiotech.nl/publi_1.pdf (2 Aug 2004)

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. 2001. Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. PNAS. 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.

Stradling, J. 1999. Report on the potential for exposure if British Lepidoptera ot toxic GM maize pollen. Greenpeace

Tapp, H. & Stotzky, G. 1998. Persistence of the insecticidal toxin from Bacillus thuringiensis subsp. kurstaki in soil. Soil Biology and Biochemistry 30: 471-478.

Thomas, J.A, Telfer, M.G., Roy, D.B., Preston, C.D., Greenwood, J.J.D., Asher, J., Fox, R., Clarke, R.T. & Lawton, J.H. 2004. Comparative losses of British butterflies, birds and plants and the global extinction crisis. Science 303, 1879-1881.

Walker, K.A., Hellmich, R.L. & Lewis, L.C. 2000. Late-instar European corn borer (Lepidoptera: Crambidae) tunneling and survival in transgenic corn hybrids. Journal of Economic Entomology 93: 1276-1285.

Wilson, A., Latham, J. & Steinbrecher, R. 2004. Genome scrambling – myth or reality? Transformation-induced mutations in transgenic crop plants. EcoNexus Technical Report, October 2004.

Zimmermann, A., Lüthy, J. & Pauli, U. 2000. Event specific transgene detection in Bt11 corn by quantitative PCR at integration site. Lebensmittel Wissenschaft und Technologie 33 210-216.

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

Appendix

TABLE 1: BT CONCENTRATION IN DIFFERENT TISSUES
BT11 PLANTS GROWN IN GREENHOUSES. SOURCE: C/F/96.05.10, APPENDIX 5.

DAYS POST PLANTING	5	10	15	20	25	30	37	59	84	119
cotyledon	20.5	36.0								
roots	22.1	11.7					37.0	12.0	18.2	2.2
2nd leaf		106.0	27.9	22.4	125.0	38.0	55.6			
5th leaf				45.7	168.0	34.0	54.0	16.7		
10th leaf							102.0	30.0	9.4	
15th leaf								37.9	10.2	
stalk epidermis							36.0	10.4	12.6	9.0
stalk pith							27.0	19.2	18.0	8.8
tassel								8.0	8.8	6.8
pollen								1.3		
silk								2.4	6.6	5.2
ear shank								13.6	27.2	5.2
husk								24.8	15.4	2.6
cob								13.0	26.6	16.2
brace root								3.2	7.0	4.8
kernel									8.2	0.4

TABLE 2: DEGRADATION OF CRY1AB IN BT STALKS AND LEAVES.
THREE-WEEK GREENHOUSE STUDY WITH SOIL TEMPERATURE OF 20 AND 25ºC (NIGHT/DAY). SOURCE C/F/96.05.10 APPENDIX 12.

	STALK		LEAF	
WEEK	ABOVE SOIL	BELOW SOIL	ABOVE SOIL	BELOW SOIL
	[NG CRY1AB/G TISSUE]			
0	15.3	15.3	80.4	80.4
1	9.0	8.2	3.9	6.9
2	7.7	12.6	1.7	1.7
3	9.6	11.4	0.0	5.7
	[µG CRY1AB/G TISSUE]			
0	0.030	0.030	1.100	1.100
1	0.003	0.002	0.014	0.009
2	0.002	0.002	0.003	0.003
3	0.001	0.003	0.000	0.002
	[MG TOTAL PROTEIN/G TISSUE]			
0	1.69	1.69	13.40	13.40
1	0.35	0.23	3.17	1.09
2	0.22	0.48	2.02	1.30
3	0.13	0.29	1.19	0.53

maize

BT 11 MAIZE - C/F/96.05.10 NOTIFICATION FOR CULTIVATION
REPORT PREPARED ON BEHALF OF GREENPEACE INTERNATIONAL BY ANTJE LORCH

GREENPEACE

greenpeace international
Ottho Heldringstraat 5, 1066 AZ Amsterdam, Netherlands
t +31 20 718 2000 f +31 20 514 8151
www.greenpeace.org