Genetic Engineering

Toxin in GM maize

New research reveals risks of Bt maize grown in Europe

Preliminary version*

GREENPEACE

editor: Greenpeace e.V., Große Elbstraße 39, 22767 Hamburg, phone 0049-(0)40/30618-0, fax 0049-(0)40/30618-100; responsible according to press law: Christoph Then; Politische Vertretung Berlin, Marienstraße 19-20, 10117 Berlin; Email: mail@greenpeace.de, internet: www.greenpeace.de; authors: Antje Lorch, Christoph Then; graphic design title: Ulrike Hemme, Hamburg; photos: Bernhard Nimtsch/Greenpeace, Martin Langer/Greenpeace, Thomas Stephan/BLE, Bonn

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Introduction

The GM maize MON810 that is grown in Europe is a problematic plant.

- Genetic modification causes unplanned and unwanted changes to the genotype and to plant metabolism.
- Pollination and admixture contaminates harvests and food products.
- The maize produces a toxin against insects that normally only exists in bacteria but now is becoming a permanent part of food and feed and can persist in fields.

Bt maize was developed in the United States primarily to control the European corn borer (ECB, *Ostrinia nubilalis*). This moth lays its eggs on maize leaves, damaging the plant. The larvae then tunnel into the leaves and the stalk. Into the autumn they migrate down the stalk and then spend the winter in the lowest part of the stalk or the top part of the roots. The stalks of the infected plants often break off.

The European corn borer was introduced to North America between 1910 and 1920 and then spread rapidly as a pest. In Europe it is found naturally on a number of different plants. Only one of the two corn borer strains in Europe actually attacks maize. This strain is native only to some parts of Europe; for example it is not found in northern Germany or Great Britain. However, the ECB strain that attacks maize is slowly spreading northward and in Germany has been found as far as Brandenburg. In conventional agriculture ECB is usually controlled simply by ploughing the fields.

In autumn 2006, the approval to grow the GM maize MON810 in the EU will expire, which means the authorities will need to re-appraise this maize. Therefore Greenpeace has compiled the latest research from Germany and other countries, drawn up a list of open questions and clarified possible risks.

For Germany, the authors mostly used an analysis of research findings from a project of the German Ministry of Education and Research (BMBF 2006), "Safety research and monitoring for Bt maize cultivation 2001-2004", the results of which have only been published in part. The studies investigated MON810 and another type of GM maize (Bt 176) that is no longer being cultivated.

A report published in April 2006 by the European Commission shows that safety problems with GM crops have become more and more obvious over the last years (European Communities 2005). The new data confirm this alarming finding. The wealth of indications that are available now show that the problems with GM maize are even more complex than originally assumed. The risks apply to the smallest soil organisms, to protected species such as butterflies and to beneficial insects such as bees and even extend to health risks for humans and animals.

The latest findings and the list of open questions clearly show that approval for commercial cultivation of the GM maize was granted prematurely and contradicts the precautionary principle that is part of EU legislation. The EU’s approval of the GM maize therefore must be withdrawn.

1. The cycle of the toxin in the environment

Normally the *Bacillus thuringiensis* toxin only exists in soil bacteria. This toxin has been used for many years to control agricultural pests. It is considered so harmless that it is even allowed in organic agriculture. But by genetically engineering the toxin into maize plants its characteristics have been changed fundamentally.

1. In nature, the toxin only exists in very low concentrations. If it is sprayed for pest infestations, then it is used selectively and for a very short period of time.
2. The toxin in its natural form only kills certain insects. It comes in a non-active form (protoxin) and it is not turned into the active form until it is in the insect’s gut.

Genetic engineering however changes the characteristics of the toxin:

1. It is produced in high concentration during the whole vegetation period of the plant and it is released through the roots, parts of the plant and pollen into the environment.
2. The toxin binds to soil particles and can survive in the soil for months. It can be passed on in the food chain and can even be passed through the gut of farm animals.
3. The toxin is not present in the inactive form, but in an active version. This changes the range of possibly sensitive organisms.
4. Although the different toxic proteins are all called Cry1Ab, they are fundamentally different from the natural protein, and they are different from each other.

Cultivating Bt maize creates a completely new cycle of distribution and concentration of Bt toxin in the environment and in the food chain. This has been confirmed by the latest research.

Effects of Bt plants on the soil have only been investigated since the mid/late 1990s, i.e. only after Bt maize had already been cultivated in the USA, and only after Bt176 and MON810 had been approved for cultivation in the EU.

Many of the studies that have been published since the end of the 1990s on the topic of "Bt crops and soil" reveal unexpected effects, particularly negative environmental effects. These results also show that most areas have not been studied at all – and that nearly everywhere where research is done, indications of negative effects can be found.

How does the toxin get into the environment?

Bt toxins can get into the soil via different routes: as a living plant material (roots, Jehle 2005), as dead fine roots and root exudates during the growth period (Saxena et al. 1999, Saxena & Stotzky 2000), pollen (Losey 1999) that is washed into the soil, harvest residues (roots, stalks, leaves) after harvest (Tapp & Stotzky 1998, Stotzky 2000, Zwahlen et al. 2003b, Baumgarte & Tebbe 2005), and in animal excrement (Einspanier et al. 2004).

In recent years a series of studies with varying approaches was conducted to study the persistence of Bt toxins in the soil, but there are only very few studies that investigate the amount and form of Bt toxin during and after the growth period. In 2005 it was still unknown how much toxin is actually exudated by the roots.

“To our knowledge, it is not known how much Cry1Ab protein is produced in the rhizosphere of Bt-maize under agricultural practice and how much of that protein remains in the soil after harvesting” (Baumgarte & Tebbe 2005).

Apparently rather high toxin levels can be found in the soil close to the roots. Some of the toxin is found in the soil even months after the harvest, even though higher levels are found in the remaining plant residues:

“The amount of Cry1Ab protein in bulk soil of MON810 field plots was always lower than in the rhizosphere, the latter ranging from 0.1 to 10 ng/g soil. Immunoreactive Cry1Ab protein was also detected at 0.21 ng/g bulk soil 7 months after harvesting, i.e. in April of the following year. At this time, however, higher values were found in residues of leaves (21 ng/g) and of roots (183 ng/g), the latter corresponding to 12% of the Cry1Ab protein present in intact roots” (Baumgarte & Tebbe 2005).

Even though it is known that roots contain Bt toxin and can exudate it into the soil, this issue is not considered a factor at all in some risk assessments of Bt maize. For example, in the approval application for Bt maize 1507 that is currently pending at the EU, the Bt concentrations for different parts of the plant are given – but not that of the roots. Nevertheless, the EU authority EFSA gave a positive opinion for the commercial cultivation of this Bt maize.

The path of the toxin through roots, pollen and plant material is not the only path through which Bt toxin is released into the environment. Initial research into the degradation of Bt protein in the gut of cows shows that “remarkable” amounts of Bt toxin are found in the gastrointestinal tract, and that the animals’ faeces contains the toxin (Einspanier et al. 2004).

How long does the toxin stay in the soil?

A number of studies are investigating the persistence, activity and degradation of Bt toxin in the soil, but because different issues are being studied (persistence of complete or partly degraded toxins, activity of the target organism etc.) and because of different methods used (lab studies, use of isolated bacterial Cry1Ab, dried and pulverized leaves, plant residues from the field) the results cannot be compared readily.

Field studies and monitoring show in any case that the toxin can still be detected several months after the harvest in plant residues and in soil, and it is active. Earlier studies, which extrapolated the results of unrealistic laboratory studies, are therefore refuted.

Different soil types influence the persistence of Bt toxins in the soil. The toxin can persist in clay soils for a particularly long time.

According to Dolezel et al. (2005), “persistence of Bt toxins released into the soil is a function of

- the amount of the toxin present,
- the rate of consumption and inactivation by insect larvae,
- the rate of degradation by microorganisms , and
- the rate of abiotic inactivation (Stotzky 2004)."

Saxena et al. (2002a) were able to detect Bt toxin in Bt maize exudates and in degrading Bt maize plant material in the soil after 350 days (the longest period studied). In other studies, isolated Bt toxin could still be found after 234 days (when the study was stopped;Tapp & Stotzky 1995, Palm et al. 1996, Koskella & Stotzky 1997, Tapp & Stotzky 1998). This also refutes earlier studies.

Sims & Holden (1996) had calculated from lab studies that 90% of the toxin would be degraded after 41 days and concluded that the Cry1Ab toxin in Bt maize would be unstable under field conditions and would be degraded fast under growing conditions. Applicants and authorities still frequently refer to this study (Sims & Holden 1996), even though it clearly does not reflect reality. As explained below, this is an example of how unrealistic studies are often used for risk assessments of Bt maize even today.

Sims & Holden, for example, assumed for their lab study a constant soil temperature of 24-27ºC. This is completely unrealistic for soil temperatures in European maize cultivation areas. Zwahlen et al. (2003a) recorded in field trials soil temperatures of 8.5ºC. As Zwahlen et al. (2003b) explain, the degradation of Bt toxins depends largely on microbial activities (Palm et al. 1996, Koskella & Stotzky 1997) which are reduced at lower temperatures.

Therefore, it must be expected that the degradation in temperate regions differs substantially from that observed in laboratory trials with high constant temperatures (Zwahlen et al. 2003b). The authors (Zwahlen et al. 2003a) also showed in a comparative study that Bt plant material under (comparable) field and lab conditions is degraded slower in the fields and stays toxic longer.

This trend is also confirmed by the most recent studies on this topic in a project of the German Ministry of Education and Research (BMBF). Baumgarte & Tebbe (2005) observed that surface roots of MON810 maize still contained 12% of the toxin levels of intact roots seven months after the harvest, that is, shortly before the next sowing. This level droppeed then in the following two months.

Does the toxin accumulate in the soil?

Bt toxins bind to surface-active soil particles and are therefore protected from biological degradation (Saxena & Stotzky 2001a, Saxena & Stotzky 2000). The complete binding is completed within 30 minutes (Schröder 2005). Once bound, the Bt toxins do not detach easily (Lee et al. 2003).

Saxena & Stotzky (2002) observed that soil with Bt plant material showed different toxicity after 120 to 180 days depending on the composition of soil minerals. Bt toxins bind better to soil particles with higher cation exchange capacity and with a more distinct surface structure.

Lee et al. (2003) confirm this results. They showed that the majority of the Bt toxins (88-98%) bind firmly to clay particles, and that even with great amounts of Cry1Ab protein no saturation effect occurred. They did not observe any structural changes for the bound Bt toxins, but there was persistent toxic activity. After 45 days the toxicity of bound Bt proteins was even higher than of free toxins.

It is therefore clear that Bt toxin accumulates to different degrees in different soil types, depending on climatic conditions, and that it can then can show different levels of activity. However, these effects have not yet been sufficiently investigated, something that is confirmed by the latest studies from Germany.

In a three-year study at three sites (Halle and in the Rhineland) all soils had similar mineralogical composition with high clay content. But the bedrock and the climate were different so that according to the scientists "important soil characteristics differed" (Schröder 2005). They observed that the mobility of the Bt toxin Cry1Ab at the different locations varied greatly (Schröder 2005).

"In regard to soil chemistry parameters the following summary statements apply: The higher the content of organic substances is, especially in the topsoil, the less the binding of Bt toxin occurs. [...] The bigger the surface of the soil particles, the more Bt toxin is bound to the soil particles" (Schröder 2005). Schröder (2005) concludes that "this information has to be taken into account when evaluating our results in terms of a monitoring method for the release of genetically modified plants."

In general one has to assume that the toxin concentrates in the soil, and can accumulate for years (Hopkins & Gregorich 2003, Lang & Arnd 2005). There is a great need to conduct research about toxin accumulation in soil: "In the second year of Bt maize cultivation at both locations, the observed Bt levels [in the soil] were clearly higher than those from 2002. The increase in toxin levels on the different locations was five to seven times higher than in the previous year, depending on the location. The toxin could even be detected in soil samples that were taken in April 2003, i.e. before the next sowing" (Arndt 2005).

Bt toxin in the food chain

When the Bt toxin is ingested by insects and other animals it has not disappeared from the food chain. In some cases the Bt toxin was found in animals that had fed on the Bt maize plants without being acutely harmed by it.

When these animals were eaten by others then their predators also took up the toxin. In spider mites the Bt toxin became so concentrated that they contained more Bt toxin then the Bt maize plants themselves. Studies of the spider mite *Tetranychus urticae* found the Bt concentrations of the animals ranged from Bt levels similar to those of Bt maize leaves (Dutton et al. 2002) to levels that were three times higher than those of the leaves (Obrist et al. 2005).

Compared with other herbivorous insects (thrips, aphids and grasshoppers) the Bt levels in spider mites were the highest (Dutton et al. 2004b). These levels were up to 33 times higher then those of cicadas that feed on the same parts of the plant (Dolezel et al. 2005).

Therefore, we should not only assume that the toxin is passed on by the (unaffected) spider mites, but also that the Bt toxin concentrates in spider mites, whereby predators are subjected to higher Bt concentrations then those found in the Bt plants themselves.

In other animals, the Bt toxin was found both in the gut and in the faeces (e.g. in earthworms, aphids, spiders and woodlice; Saxena & Stotzky 2001a, Raps et al. 2001, Harwood et al. 2005, Wandeler et al. 2002). These faeces are part of the soil so that the Bt toxin can be ingested by further organisms.

Bt toxin from maize plants even survives the stomachs of ruminants and is excreted with the faeces (Einspanier et al. 2004). In this way the Bt toxin can be returned to the fields through animal dung.

2. Effects on soil organisms

In principle, Bt toxins can affect all parts of the soil ecosystem. One has to take into account that Bt toxins get into the soil in different ways (living and dead roots, root exudate, dead stalks and leaves, pollen, faeces and liquid manure) and that it is present at different times in different forms and different concentrations, but so far little data exists.

Effects of Bt plants on non-target organisms in the soil were not studied at all before the end of the 1990s. Apparently there was no clear idea that Bt toxin would be produced in the roots, that there are non-target organisms in the soil of agricultural fields, and that in agricultural practice (in contrast to scientific studies) Bt plant material resides in the soil. The lack of such studies is glaring.

Effects on micro-organisms

The importance of micro-organisms is beyond doubt as they are responsible for 90% of the carbon turnover in the soil. More than 10^9 micro-organisms live in 1 gram of field soil. For a depth of 10 cm, this amounts to 10^{17} micro-organisms per hectare. Micro-organisms are also directly associated with specific insect groups. Insects such as the larvae of fungus gnats decompose decaying plant material that has already been partly degraded by micro-organisms.

Several studies describe the effects of Bt maize on soil micro-organisms, which vary depending on soil type. For several years there has been "some indication of anti-bacterial effects of Bt toxins" (Escher et al. 2000, Zalunin et al. 2003; cited in Lang 2005)

In greenhouse experiments, Castaldini et al. (2005) found differences in the bacteria communities of the rhizosphere (root area) of three maize varieties (Bt176, Bt11 and a non-Bt maize). For up to four months, plant residues affected bacterial communities, soil respiration, and mycorrhizal symbiosis (Castaldini et al. 2005).

Baumgarte & Tebbe (2005) also observed that plant age and field heterogeneity had a strong influence on the bacterial communities of MON810 cultivation. In two of the three years they found structual changes in the bacteria communities of the rhizosphere of Bt maize (Dolezel et al. 2005). Baumgarte & Tebbe (2005) concluded that "there is persumably an effect of the presence of the Cry1Ab protein on the structure of the bacterial community but this effect was masked by more selective factors", and they "emphasize the importance of considering post-harvest effects on non target organisms."

Effects on mycorrhiza

Mycorrhiza is a symbiosis between fungi and plants in which the fungus is in contact with the system of fine roots of a plant. The mycorrhiza fungi help the plant to take up nutrients and water more easily from the soil. In addition the symbiosis gives the plant some protection against diseases and enables the plant to grow better during drought. Mycorrhiza of crop plants is an important ecological parameter and should be part of any risk assessment. However, the current approval applications for Bt maize do not take mycorrhiza colonization into account.

Two studies show that the roots of Bt maize plants are less colonized with mycorrhiza. If this happens, the Bt maize not only loses a symbiotic partner and its contribution to plant nutrition, but the plants might even be more susceptible to pest insects because without mycorrhiza colonization maize attracts fewer natural enemies of the pests.

Turrini et al. (2004) were the first to study mycorrhiza colonization of Bt plants. They found that the fungi were not able to develop viable structures on the roots of Bt176.

Castaldini et al. (2005) conducted a second study on this issue and also found a significantly lower mycorrhiza colonization of Bt maize roots. In laboratory experiments a significant decrease of mycorrhiza colonization was found at Bt176 roots, but not for Bt11 maize. A healthy mycorrhiza makes crop plants more attractive to natural enemies of aphids which are a maize pest (Guerrieri et al. 2004 cited in Dolezel et al. 2005). Lower mycorrhiza colonization as described by Turrini et al. (2004) and Castaldini et al. (2005) would make Bt maize more susceptible to pest insects because fewer natural enemies of the pests come to the maize plant (Dolezel et al. 2005 p.37). However, so far no studies have investigated the issue of a higher susceptibility to pest insects.

Bt maize harms fungus gnats

Fungus gnats (Sciaridae) are 2 to 3 mm long gnats that, just like their 1mm long larvae, feed on dead plant material. They live in the upper layers of soils where they hatch in rates up to 6,000 individuals per m^2. They play an important role in soil ecology and for soil fertility as decomposers of plant material in the soil.

According to a new study in Germany (Büchs 2005), Bt maize MON810 harms fungus gnats. Their mortality rate is higher and their pupation rate is lower. In addition, the Bt toxin can harm the beetles that feed on the larvae (Langenbruch et al. 2006).

Larvae of the fungus gnat *Lycoriella castanescens* feeding on MON810 maize needed significantly longer to pupate than larvae feeding on non-Bt maize (Büchs et al. 2004, Büchs 2005). The period until pupation is a very important factor for the larvae because they do not have a hard-shelled chitin cover and have a limited ability to move. The longer it takes till pupation, the higher the chance that the larvae can be attacked by parasites or diseases. Larvae that only fed on MON810 material were eaten more often by other insects because the larval period lasted longer (Langebruch et al. 2006). Therefore the time until pupation is a key parameter for assessing negative effects for these insects that are particularly important for degradation and soil fertility. Particularly if Bt maize is grown over years on the same field, the decomposers' community can be altered, affecting the formation of compost and soil development (Langebruch et al. 2006).

The effects on the population of fungus gnat larvae only become visible after some time. Fungus gnat larvae feed by degrading plant residues in the soil. Therefore negative effects in the field are not necessarily obvious in the first year of Bt maize cultivation, and may only appear when the Bt plant material has actually been incorporated into the soil. This was observed in a three-year study. During the first year on MON810 fields, there were even increased number of species, hatching and decomposition activity, but this changed in the second year. In the third year the degradation rate on MON810 fields was significantly lower than on the control field. This decrease coincided with an increase in the Bt toxin levels in the plant material by a factor of more than 2.5 (Langenbruch et al. 2006).

How difficult it is to study effects of the Bt maize on organisms such as fungus gnat larvae is shown by the finding that the amount of Bt toxin alone apparently is not the only contributing factor. The Bt toxin Cry1Ab in the Bt maize variants Bt176 and MON810 is claimed to be the same, but the toxins might have different biological effects.

When fungus gnat larvae *Lycoriella castanescens* were fed maize pollen, a slower development was observed with MON810 but not with Bt176 even though the Bt176 variety Valmont used in this study contains 2962 ng/g Pollen – 30 times more then the Bt toxin content of the MON810 variety Novelis (97 ng/g; Büchs 2005).

The negative effects of Bt maize on fungus gnat larvae can influence the next levels of the food web in two ways.

Firstly, the changes in the life span of the larvae also has an effect on the predators that feed on these larvae. Initially the predators can find more prey when the larvae need longer till pupation. Over the long term however, this effect could reverse, so that soil fertility could be fundamentally negatively affected (Langenbruch et al. 2006). However, there are no studies on such long-term effects on the food web in the soil of repeated Bt maize cultivation.

Secondly, the negative effect is reproduced directly in the food chain. When the fungus gnat larvae raised on MON810 plant material were given as prey to the larvae of two beetle varieties (*Atheta coriaria* and *Poecilius cupreus*), which naturally feed on these larvae, the beetle larvae also experienced developmental delays (Büchs 2005).

Nematodes neglected

Nematodes (roundworms) are the most numerous organism group in the soil besides bacteria and fungi. They only have limited mobility, are relatively susceptible to stress and include groups with very diverse feeding types. They can be affected directly and indirectly by Bt toxins, because there are herbivorous and decomposing nematodes, and also parasites and nematodes that feed on insects (Manachini et al. 2004).

Several studies have described the adverse effects of the Bt toxin on nematodes (references in Land & Arndt 2005 p. 62). Nevertheless there is little interest in the interaction of Bt plants and nematodes.

In the early 1990s, different studies showed that the toxins of several *Bacillus thuringiensis* strains negatively affect the eggs and larvae of nematodes (Meadows et al. 1990, Bottjer et al. 1985; cited in Manachini et al. 2004). When Bt toxins persists in the soil for longer or when Bt toxins from harvest residues or root exudates accumulate (Tapp & Stotzky 1998, Saxena et al. 1999), a risk for the nematode fauna cannot be excluded (Lang & Arndt 2005).

Negative effects on nematode under field conditions are difficult to study because, due to the high diversity of feeding forms, little can be concluded from the total number of nematodes. The numbers of nematodes of the different feeding types need to be measured.

Only a few studies investigated individual nematode species, but these lab studies showed negative effects. Soil from the rhizosphere of MON810 and Bt176 negatively affected the growth and reproduction rate of the nematode *Caenorhabditis elegans* (Lang & Arndt 2005). *C. elegans* also showed a possible sensitivity to the Bt toxin in the field, especially in the soil from the rhizosphere of the MON810 variety Novelis (Mananchini & Lozzia 2003).

In the field, differences in the composition of nematode populations were observable. In a field with Bt176 maize plants - while there was no difference in the abundance of the major nematode genera - some nematode species that feed on bacteria were not found, but some nematode species that feed on fungi were found here and not in the control field (Manachini & Lozzia 2002). The scientists concluded that “the decreased number of bacteria-eating nematodes in the Bt maize field could have been caused by a direct effect of the Bt toxin on the nematodes or by an indirect effect on another level of the food web (bacteria, fungi, predator).”

Earthworms not factored in

Earthworms are important and beneficial organisms in agricultural fields, which makes it all the more astonishing that they get almost no attention in the risk assessment of Bt maize. Earthworms degrade plant material, their tunnels contribute greatly to soil movement, the walls of their tunnel form niches that are rich in oxygen in otherwise oxygen-poor soils, and their faeces add to soil fertility.

Nevertheless there are only a few studies (Ahl Goy et al. 1995, Saxena & Stotzky 2001a, Zwahlen et al. 2003a, Lang & Arndt 2005, Vercesi et al. 2006) that also only focus on three different species of earthworms (*Eisenia fetida, Lumbricus terrestris, Aporrectodea caliginosa*). Only one of these species – the one studied in the most recent study from June 2006 (Vercesi et al. 2006) – is of relevance for agricultural land. In three of the studies (Ahl Goy et al. 1995, Saxena & Stotzky 2001a, Lang & Arndt 2005) the main focus was on the absolute mortality rate or the abundance of the animals.

Even in studies (Ahl Goy et al. 1995) that could not detect an acute effect of Bt maize, the Cry1Ab toxin could be detected in the gut and faeces of the earthworms. Nevertheless, there are no further studies about the question of how the Bt toxin is spread via the faeces of earthworms and through the soil movement that they cause.

Zwahlen et al. (2003a) report that the mortality rate and the growth of juvenile and adult *L. terrestris* was largely unaffected over 160 days of feeding on Bt maize. However, for the final measurement after 200 days, the adult earthworms that were fed Bt maize weighed significantly less. This study gives an important indication of what the long-term or chronic effects of Bt maize on earthworms might be, even though *L. terrestris* is an earthworm species that is not very common in agricultural soils (Vercesi et al. 2006).

Vercesi et al. (2006) studied for the first time different life history parameters (such as survival rate, hatching rate, reproduction, growth). They studied a species (*A. caliginosa*) that is probably the most abundant species in agricultural soil in the temperate climate zone, and the MON810 variety Monumental (Vercesi et al. 2006).

The majority of the parameters studied were not negatively affected by the Bt maize, but there was a significant decrease in the number of earthworms hatching from their cocoons. This is a negative effect that could greatly reduce the population numbers of this earthworms in a Bt maize field and this could also influence other soil organisms that depend on the manifold interactions of earthworms.

3. Effects on bees, butterflies and other organisms

Not enough lab, field and monitoring studies have been done to rule out effects on non-target organisms. Especially indirect and long-term effects have only been studied rarely. Nevertheless a majority of these studies show individual negative effects, that in general have not been investigated in follow-up studies, let alone refuted. This points to a "remarkable number of cases" (Lövei & Arpaia 2005) with negative effects.

Only in the rarest cases did studies bother to first assess which species are present in the (European) agricultural landscape and then chose their test organisms from them. The few studies that did such a survey show how necessary this is: A survey of the butterfly fauna around maize fields, for example, produced a list of 79 species with diverse abundance and different potential for endangerment (Lang 2005). A survey of bees and wasps on a MON810 field listed 200 species of which 39 species are on the German Red List of endangered species (Gathmann 2005).

Of the numerous groups of non-target organisms none or only few species have been studied, and most of these are species from North America. Only in the last years – years after MON810 and Bt176 where approved for cultivation in the EU – were studies conducted for the first time on

European species; a lot of these in Germany.

The US agriculture and agricultural landscape are fundamentally different from those in Europe – but nevertheless the ecological conditions in the US are used as basis for the risk assessment of GM crops in Europe.

Lang (2005, pp.49-50) gives a good overview of the published peer-reviewed scientific field studies. In the course of 2005, this situation basically did not change even though a few more studies have been published, most of which show negative effects.

"These studies are rather diverse with regard to the animal groups studied, the research period, the maize varieties, the field sizes, the sample size, the detection method used, the geographic location, and more, which makes a direct comparison of these studies difficult (Orr & Landis 1997, Pilcher et al. 1997a, Lozzia 1999, Manachini 2000, Wold et al. 2001, Bourguet et al. 2002, Hassel & Shepard 2002, Jansinski & Eisley 2003, Kiss et al. 2003, Musser & Shelton 2003, Dively & Rose 2003, Mayne et al. 1997, Rathinasabapathi 2000, Volkmar & Freier 2003). The majority of the studies are from the USA (47%), 13% (i.e. 2 publications each) from France, Italy and Spain, and one each from Hungary and Germany (Volkmar & Freier 2003). Six studies (40%) were done over a period of only one year, eight studies over two years (53%) and only one study from Spain was done over a period of three years. Mainly predator arthropods that feed on aphids, such as lady bugs, lace wings, parasitic wasps and predatory bugs were studied." (Lang 2005)

However, it is now known that the phloem that aphids eat does not contain Bt toxin so no direct effect on aphids and those animals that feed on aphids can be expected. The whole design of these studies appears questionable, in addition to the fact that so few studies are undertaken in Europe or with European species, and that no real long-term studies were undertaken.

Butterflies

The majority of studies on the effects of the Bt toxin on butterflies and butterfly larvae are studies with the Monarch in the United States. First studies in Europe of European butterflies have identified butterflies that are present in or around maize fields, among them a number of endangered species. These studies show that species such as Peacock and Swallowtail can be damaged lethally or sublethally by even small amounts of Bt toxin (Felke & Langenbruch 2003, 2004, 2005).

Important longitudinal studies such as the monitoring of butterfly larvae in and around Bt maize fields have so far not (or not sufficiently) been conducted – due to a lack of money for such studies.

"Based on what we know now, it is impossible to predict whether specific butterfly species could be endangered on the species level by the cultivation of transgenic Bt maize. At least on the population level negative effects cannot be ruled out. Indigenous butterflies are endangered by a number of anthropogenic influences. The biggest danger stems from loss of habitat. The cultivation of Bt maize is an additional threat whose impacts on numerous species has not been determined. [...] A negative effect of pollen of Bt maize on butterfly larvae should [...] be expected especially where a bigger maize field borders on a much smaller butterfly habitat such as a hedge or the edge of a field. [...] Particularly populations of those species whose larval habitats are mainly grassland or other areas in the agricultural landscape and that are regarded as regionally endangered have to be considered as potentially endangered. Especially for species that are distributed in patches, damaging a single population can impact the total population of a specific region." (Felke & Langenbruch 2005)

Studies in the United States have primarily been of the Monarch butterfly (*Danaus plexippus*). At the end of the 1990s it was accidentally observed that the Monarch could be affected by Bt maize (Losey 1999). Since then, it has been shown repeatedly that Monarch caterpillars can be affected by cultivation of Bt maize and its pollen. "Interestingly the effect was found using the Bt11 maize variety N4640. Bt11 is known to have less Bt toxin then the pollen of Bt176." (Felke & Langebruch 2005).

In the meantime, studies have shown that Bt pollen is not necessarily always acutely toxic for monarch butterflies. However, longitudinal studies have shown clear negative effects on monarch caterpillars (Dively et al. 2004). In this study, too, MON810 and Bt11, whose pollen contains much less Bt toxin than Bt176, were used. Before Dively et al. published their study it was assumed that MON810 would have next to no effects on butterfly larvae.

Studies with European butterflies

When German scientists identified butterflies in the immediate surroundings of a Bt maize field, they found 26 day- and 53 night-active butterfly species (Felke & Langenbruch 2005). According to their data, the risk to 33 of the listed species cannot currently be assessed because it is unknown how sensitive they are to the Cry1Ab toxin. All of these species are owlets, belonging to the moth family.

For 16 species a minimal risk is assumed because they are common, widespread species. Twenty-three species are classified as slightly threatened, because they are not present all over the country; their population densities are lower then those of the 16 species mentioned earlier. Five butterfly species are only present sporadically in a lot of areas and are therefore considered to be highly threatened. These species already have a decline in population or are already considered endangered species – at least in some parts of Germany (Felke & Langenbruch 2005).

A lab study with seven butterfly species native to Germany showed that the caterpillars of six species were sensitive to the Bt toxin in Bt176 maize. When this pollen was on their feeding plants, the caterpillars fed less and their weight increased more slowly; there also was a higher mortality rate (Felke & Langenbruch 2005).

In a second part of the study the scientists determined more exactly how sensitively the different butterfly species reacted and they found there were major differences. They determined the so-called LD_{50} values, i.e. the amount of Bt toxin that will kill half of the caterpillars when they consume it once. Three of the species (Peacock, Small tortoiseshell and Small white) were as sensitive as the European corn borer that is supposed to be killed by the Bt maize. The Diamondback moths reacted even more sensitively (Felke & Langenbruch 2005). Another study showed a similarly high sensitivity for the Common swallowtail (Lang & Voijtech 2006)

Species		**LD_{50}** **[number of pollen grains]**
Diamondback moth	*Plutella xylostella*	8
Common swallowtail	*Papilio machaon*	14
European corn borer	*Ostrinia nubilalis*	32
Small tortoiseshell	*Aglais urticae*	32
Peacock	*Inachis io*	37
Small white	*Pieris rapae*	39

Table 1: LD_{50} value of Bt176 pollen for butterflies native to Germany. The LD_{50} value describes the amount that if consumed once causes the death of half of the test animals (source: Felke & Langenbruch 2005; Lang & Vojtech 2006)

Even below the LD_{50} threshold, delays in development have been measured for the Peacock and the Diamondback moth among others problems (Felke et al. 2002). Feeding on Bt pollen made caterpillars lethargic so they stayed on the top of the leaf instead of feeding on it from below, which made it harder to hide from predators (Felke et al. 2002). Even small, non-lethal effects of the Bt pollen can cause the butterfly pupae or hatched butterflies to weigh less, so they lay fewer eggs and die earlier (Dolezel et al. 2005 p.16).

One has to take into account that butterfly larvae in the lab are usually kept under optimal conditions. Here, the caterpillars are not subjected to any other stress factor likely to be encountered in the wild (agrochemicals, parasites, weather conditions, suboptimal feeding due to a lack of specific feeding plants, etc). In the field, Bt maize is an added stress factor for butterfly species that are already endangered.

Apparently some of the findings in the lab are valid in the field as well: "Besides their lab experiments, M. Felke and G. A. Langenbruch also conducted field experiments with Peacock larvae and Bt176 maize. This unpublished results show that under field conditions the flight of Bt176 pollen also has negative effects on Peacock larvae." (Lang & Arndt 2005).

Based on their LD_{50} values, Felke & Langenbruch (2005) estimated the distance from the edge of the field at which the butterfly larvae could be damaged: "If one counts in a safety margin of the factor 100, then the Diamondback moth (*Plutella xylostella*) should only be exposed to 0.08 pollen grains. At a distance of 32 metres from the edge of a flowering maize field an average of 3 to 5 pollen were counted per square centimetre. The maximum number at that distance was 34. This means that negative effects on species that react as sensitively as the Diamondback can not be ruled out at a distance of 32 metres from a Bt176 maize field. This is also true for the neonate larvae of Peacock and Small tortoiseshell butterflies." (Felke & Langenbruch 2005).

There is no EU regulation that requires a safety buffer between Bt maize and the habitat of butterflies or other protected animal species.

Aphids

The effect of Bt maize on aphids has been studied several times. No special effects could be observed (Manachini et al. 1999, Vidal 2005). The lack of such effects is often used as a proof that in general there are no negative effects on non-target organisms. A subsequent study (Raps et al. 2001), however, showed that the phloem, on which aphids feed, does not contain any Bt toxin.

Aphids were also used to study effects on predators of maize pests such as *Chrysoperla carnae* (lacewing; Manachini et al. 1999). Neither negative effects on the development, nor a higher mortality of *C. carnae* could be observed (Vidal 2005). This is not surprising since there is no Bt toxin in the phloem, the aphids' food source.

Bees illustrate research difficulties

For approval applications feeding studies with bee larvae that have been conducted under unrealistic conditions failing to meet scientific criteria are often used. For the approval application for 1507 maize, for example, bee larvae were fed with Bt maize pollen only once before their acute mortality was measured. The few scientific studies that were conducted with bees and Bt maize did not show negative effects. This is explained in large part by the way that bees feed.

In a field study Kaatz et al. (2005) show a more differentiated result. He and his colleagues could not show a chronic toxic effect for Bt maize (Bt176 and MON810) on healthy honey bee colonies, but they found a significant negative effect on bees that were weakened by other factors.

"The first year the bee colonies happened to be infested with parasites (microsporida). This infection led to a decrease in the number of bees and to reduced breeding activity in the hives that were fed with Bt pollen, as well as in those fed with non-Bt pollen. As a result, the experiments were stopped prematurely. The effect was much stronger on the bee colonies fed with Bt maize pollen. (The significant differences indicate an interaction of toxin and pathogen on the epithelial cells in the gut of the honey bee. The underlying mechanism of action is unknown.)" (Kaatz 2005)

This observation reveals two important problems for research: On the one hand, studies, especially lab studies, are conducted with healthy test organisms isolated from further external influences. This may be a valid scientific approach and study design, but it disregards the fact the once a GM crop is cultivated the non-target organisms are subject to these, possibly cumulative factors.

But even if additional factors were to be studied, this could not happen unless the organisms and their pathogens could be bred and kept in the lab. For example, in the bee study described (Kaatz 2005), it was not possible to repeat and study the parasite infection under controlled conditions because the parasites could not be bred in the lab.

Spiders: Neglected persistently

Only a few studies have investigated the possible effects of Bt maize on spiders. (Details for these studies can be found in Lang 2005). Bt maize appears to threaten orb-weaving spiders as a result of several different factors. They ingest the Bt toxin either directly as pollen (e.g. through the recycling of their web) or indirectly through prey (Lang 2005). A longitudinal study shows negative effects of Bt pollen on orb-weaving spiders (Lang 2005, Ludy & Lang 2006). According to the scientists these could be indirect effects caused by a reduced number of prey or by a lower feed quality of the prey. Similar indirect effects have already been described for lacewings (Hilbeck et al. 1998, Dutton et al. 2003a).

Spiders are more abundant in maize fields then expected. At the outset of a field study, Lang (2005) counted 50 species in the field and on the field margins, two of which were endangered species.

According to Ludy & Lang (2006) the exposure of orb-weaving spiders to maize pollen can be very high, but also very variable. In the maize field the exposure, with up to 6900 pollen in a web, is much higher then on the field margins.

In a lab study, 65% of the orb-weaving spiders ingested pollen from the web (Volkmar & Freier 2003), even if only in low amounts. A monitoring study (Lang 2005) showed that more than 7% of all spiders collected in the Bt maize field had Cry1Ab in their systems, which indicates long-term exposure. Ludy & Lang (2006) concluded that in their experiments the Bt toxin did not seem to have any relevant negative effects, but they point out "that the sample size sometimes was rather low and therefore possible effects of the Bt toxin could not be statistically validated" (Ludy & Lang 2006).

Under certain conditions the Bt maize even had a positive effect on the spiders. In 2003, there was a greater abundance of spiders in the Bt176 maize field (Lang 2005). The reason for this seems to lie in unplanned change to metabolism of the GM maize (Saxena & Stotzky 2001b, Magg et al. 2001, Hassel & Shepard 2002). For unknown reasons the Bt maize stays green longer during drought. This effect raises questions about whether the metabolism of GM plants is altered to a much greater extent than intended. Other indicators of this phenomenon are higher concentrations of lignin (see below).

Beneficial insects: Victim of Bt maize

The Ichneumon wasp *Trichogramma brassicae* is a natural enemy of the European corn borer (ECB). It lays its eggs in the ECB's larvae and is therefore used in organic farming for targeted pest control.

Naturally occurring Ichneumon wasps are endangered by Bt maize cultivation. If they lay their eggs in ECB larvae, substantially fewer wasps hatch than in non-Bt fields (Manachini & Lozzia 2004b). In addition, *Trichogramma* finds less prey in the Bt fields.

In regions with intensive Bt maize cultivation, the ECB's natural enemies are at risk of becoming (locally) extinct. Specialized natural enemies can even be more threatened than the pest itself because they can only reproduce in those fields where the pest is present. The regional loss of specialized natural enemies could lead to increased pest infestation in other maize fields (Sisterson & Tabashnik 2005).

Trichogramma is doubly threatened because they also feed on maize pollen. The Bt toxin could be especially dangerous for them because of their small body size. This has not been proven, though. In lab studies no negative effects on the life expectancy of female wasps or on the total number of eggs laid could be determined (Langenbruch et al. 2006).

Another natural enemy of the ECB is the lacewing (*Chrysoperla carnae*). Their development is substantially delayed if the larvae feed on ECB larvae raised on Bt maize (Hilbck et al. 1998a,b). This is another example of how the Bt maize toxin can affect several levels of the food web.

4. Poor quality

The Bt concentration can be different in different parts of the plant, in one part at different moments during the growing season, and in different varieties with the same genetic modification. Even different parts of one leaf can contain different amounts of Bt toxin. There are no comprehensive studies on this issue.

Quite often Bt plant material is used in experiments without a determination of how much Bt it contains, which makes it impossible to compare these studies and it remains to be seen whether or not they describe real conditions.

The Bt concentrations of different plant parts and at different times were (and are) often not listed in sufficient detail in EU approval applications, even though it is known that the Bt content in Bt maize varies a lot (Felke & Langenbruch 2005).

Data on Bt exudates from roots or the Bt content in the soil in the rhizosphere are not required for approval applications, even though these data are important for estimating the risk to soil organisms.

Possible differences between different varieties into which the Bt gene is being incorporated are not being studied either, even though concentrations of the toxin can fluctuate depending on the variety.

Toxin levels depend on the variety, growth, environment and climate

The production of Bt varies by season and by plant parts and can be influenced by environmental factors. These variations in different parts of the plant occur to different degrees. Data from other climate zones or from other varieties can therefore not be used for an environmental risk assessment.

The variations in Bt production have been known for more than 10 years, but so far there has been no serious research in the reasons. We have initial indications that higher temperatures reduce or silence Bt production and/or that Bt production is correlated with photosynthesis activity of the plant parts.

Several studies show that young and old Bt maize plants produce different amounts of Bt toxin (Fearing et al. 1997, Dutton et al. 2004b). A new study from Germany confirms this fluctuation. According to Jehle (2005), "the toxin content varies seasonally and among plant parts". The toxin concentrations measured "differed to some extent considerably from those known from corresponding studies in the U.S., but tendencies could be confirmed. This result underlines the importance of studies under local climatic conditions with local varieties." (Jehle 2005).

In general, younger leaves seem to produce more Bt toxin than older ones. Within one leaf there can be different Bt concentrations (Abel & Adamczyk 2004). In MON810 the Bt toxin production is more pronounced in early stages of plant development (Jehle 2005). When Dutton et al. (2005) studied the Bt content of different leaves of Bt11 plants, they found that the youngest leaves showed the highest variation – with the highest concentration at the tip of the leaf and lower values in the growth region of the leaf, close to the stalk. In contrast, the Bt levels in older leaves were much more consistent.

Dutton et al. (2004a) also found similar results in MON810 plants grown under different conditions in the greenhouse and in the field. Young plants had nearly twice the Bt content of the older plants.

The Bt content within one plant differs depending on the plant part. In MON810 the Bt content is highest in the leaves and lowest in the cobs (Jehle 2005). In MON810 leaves the Bt content is four to seven times higher than in the roots (Mendelsohn 2003, Nguyen 2004, Baumgarte & Tebbe 2005). In Bt176 plants, however, the highest concentrations can be found in the pollen and leaves (Fearing et al. 1997).

In both Bt176 and MON810 the cobs have very low Bt content (Jehle 2005). Maize kernels or cobs are usually used for feeding studies on possible negative effects with mammals and birds, but since the Bt content in cobs is considerably lower than in leaves, these studies can not be used to assess the risk for animals that feed on other parts of the maize plant.

Burns & Abel (2003) discovered that lower nitrogen levels coincide with reduced Bt levels in leaf tissue. Dutton et al. (2004a) also found that higher Bt levels in young plants coincide with higher nitrogen values. They assume that differences in temperature reduce Bt production or prevent it altogether. 'Gene silencing' under extreme (weather) conditions and especially when the plants are under heat stress has been discussed for other GM plants for quite a while.

Abel & Adamczyk (2004) studied the Bt content of different parts of the maize leaf. They found significantly lower concentrations of Bt toxin in the white-yellow parts of the leaf than in the green ones. Their results show that plant parts with little chlorophyll and less photosynthesis activity produce less Bt toxin (Abel & Adamczyk 2004).

Photosynthesis activity of plants is influenced by several environmental factors such as temperature, water and light, therefore it stands to reason that these factors could also influence

Bt production. This is why Bt values measured in one place cannot be generalized for cultivation under diverse environmental and climatic conditions.

Indications of the effects of environmental factors and climatic conditions on Bt production were also found when the Bt concentration were measured in different years and at different locations. Even at locations in Germany, Bt concentrations can vary by nearly 50% (Jehle 2005). Samples of Bt176 and MON810 maize for several sub-projects of the 'Safety research and monitoring for Bt maize cultivation 2001-2004' project of the German Ministry of Education and Research (BMBF) were tested. At the two locations in Germany (Bonn and Halle) clear differences were found. "The toxin levels at one location were about 6-49% higher than at the other location during nearly all development stages during the three years of the trial." (Jehle 2005).

In two successive years, the differences can even be greater, as two examples from this project show. Büchs (2005) registered Bt toxin in MON810 litter that was 2.5 times higher than in the previous year. Such differences could have a considerable impact on the studies of non-target organisms or Bt persistence in the soil.

Some Bt plants do not produce any Bt toxin at all. According to information from Monsanto, this affects 2% of the maize plants in a field (N. Mülleder; Monsanto Agrar Deutschland GmbH; pers. communication cited in Magg et al. 2001). Jehle (2005) was not able to detect Bt toxin in 9% of the plants tested in the German study. No Bt toxin could be detected in 32% of Bt176 roots (Jehle 2005). No studies have looked into why some Bt plants do not produce Bt toxin.

Pollen: More toxin than expected

In general it is assumed that the Bt content in the pollen of Bt176 plants is considerably higher than in the pollen of Bt11 or MON810 plants (Felke & Langenbruch 2005). The different varieties can produce very different levels (Nguyen 2004).

The Bt content for MON810 pollen is usually specified as very low (e.g. 0.09 µg/g pollen; Stanley-Horn et al. 2001). Results from German fields were much higher. Nguyen et al. (2001) found Bt levels in pollen as high as 0.32-6.6 µg/g – nearly as high as the Bt content in Bt176 pollen (7.1 µg/g; Stanley-Horn et al. 2002). The high variation in toxin production in MON810 maize could be caused by abiotic factors as well as by differences among different varieties.

Felke & Langenbruch (2005) conclude: "It is therefore essential that the pollen of MON810 maize be biotested further to clarify whether the different MON810 varieties have different toxin concentrations in the pollen and whether there are individual differences in toxin expression among plants of the same variety."

Toxins differ

The effects caused by one Bt maize cannot directly be used to assess another Bt maize, because the Bt production is regulated differently. In addition, the Bt toxins (CryAb) are different in the different Bt plants. Lower (absolute) Bt levels can therefore not necessarily be equated with less impact.

Different Bt maize plants (MON810, Bt176, Bt11 etc.) use different promoters to produce the Bt protein in the plant cell. It is known that different promoters activate the Bt production in different plant tissues (e.g. pollen, roots or phloem) differently (Dotton et al. 2003b), but there is no safety research on this issue.

The Bt toxins in different Bt maize plants are not identical even though they are all called Cry1Ab.

First of all they are all fundamentally different from the natural Bt toxin, produced in the bacteria *Bacillus thuringiensis*. The natural Bt protein is much bigger and develops its toxic properties only when it is partly degraded in the insect gut by specific enzymes. However, not all insects produce the right enzyme.

Therefore the primary characteristic of the natural protein, as it is used in organic agriculture, is that it is non-toxic. It only becomes a toxin when it is ingested by specific insects. In the gut of the insect the protein is turned into a toxin. However, the Bt toxin in Bt plants is present in a shortened form and is already toxic, which is why the Bt sprays used in organic agriculture cannot be compared with genetically modified Bt plants.

In addition the Bt toxins of different Bt plants are not identical. The Bt protein in MON810 is slightly larger than that of Bt176 (92 kDa and 65kDa, respectively, Nguyen 2004). In general it is assumed that the Bt toxins in Bt crops are identical and that only the amount of Bt toxin is relevant to possible effects on other insects. However there are indications that these different Bt proteins can cause different effects.

Studies on Monarch butterflies revealed long-term negative effects of MON810 and Bt11 even though they have lower Bt levels in the pollen than Bt176 (Dively et al. 2004). A newer study makes this even more clear. Compared to MON810 and Bt176, pollen from MON810 harmed the fungus gnat *Lycoriella castanescens.* This negative effect could not be observed with Bt176 pollen even though it contains 30 times as much Bt toxin in the pollen (Büchs 2005). The scientists concluded that "apparently there is no connection between the observed effect and the absolute Bt content for different Bt maize varieties."

Disturbed metabolism in Bt maize

Bt maize has a substantially higher lignin (wood) content than unmodified maize. This is presumably an unintended consequence of the genetic modification that is now known and has been measured (Saxena & Stotzky 2001b), but it has not been resolved why this is the case. Without doubt higher lignin levels have effects on the environment and influence whether or not Bt maize is suitable as food or animal feed (Poerschmann et al. 2005).

For MON810 and Bt176 the lignin content in stalks is considerably higher. In contrast, the levels in the leaves are not much different from the control plants (Poerschmann et al. 2005). This unexpected and unintended effect of the genetic modification could be a reason why the stems of Bt maize are harder and why harvest residues of Bt maize decompose more slowly in the soil (Poerschmann et al. 2005).

Higher lignin content also changes the soil ecology. The Bt plant material decomposes more slowly and the Bt toxin is likewise protected from degradation because it is bound to lignin in the plant material (Poerschmann and Kopinke 2001, Stotzky 2000, Saxena er al. 200b). The higher lignin content could also be a reason why the Bt plants have a lower nutritional value for organisms such as the larvae of fungus gnats. Poerschmann et al. (2005) conclude that studies of the lignin content of genetically modified maize are indispensable.

The higher lignin content is not the only unwanted difference compared to normal maize. Other studies show that the leaves of Bt maize stayed green longer then those of control plants (Lang 2005). Other pleiotropic effects of Bt maize have also been described by other scientists (Saxena & Stotzky 2001b, Magg et al. 2001, Hassel & Shepard 2002, Lumbierres et al. 2004; cited in Lang 2005). Nitrogen levels of leaves also seem to be higher (Escher et al. 2000).

During EU approval procedures Bt maize is considered 'substantially equivalent' to normal maize. This implies that the approval authorities assume that – besides the additional Bt toxin – there is no difference between Bt and non-Bt maize. It is because Bt maize is assessed in this way that further (health) risk assessment steps are dispensed with. However the examples of changes to metabolism presented here show that Bt maize is not substantially equivalent to normal maize.

MON810 was developed using a so-called gene gun. Cells are bombarded with metal particles to get the additional gene construct (transgene) into the plant. The insertion of the transgene is completely random and cannot be controlled. Apparently not only does this method disturb the normal maize genome, it can also change the transgene itself. The transgene's DNA sequence that is actually present in MON810 plants is different from the sequence described in the EU approval application (Hernandez et al. 2003).

5. GM maize is ecological and economic nonsense

In recent years more and more of the mechanisms that plants use to naturally protect themselves against pests have been discovered. Maize plants have an indirect mechanism to protect themselves against Lepidoptera such as the European corn borer (ECB). If the plant is damaged by caterpillars feeding on them, the plant produces a scent that attracts natural enemies of the ECB such as the ichneumon fly *Trichogramma* (Degenhardt 2005).

"Every maize variety has a typical basic scent pattern and every plant has an individual form of this pattern that is acquired during its lifetime while undergoing constant changes. A young maize plant does not release any scent. Not until the intensive growth phase does the plant produce its scent pattern, depending on local conditions and on pest and pathogen infestations. There are also differences in the release of volatile substance throughout the day. Plants that appear practically identical to the naked eye, that have similar weights and that are infested with similar numbers of Lepidoptera larvae, can still release very different levels of scent. Maybe there is also a fungal infection, root pests or other factors that influence the scent pattern." (Degenhardt 2005).

Different maize varieties therefore have differences in their natural susceptibility to pests such as the ECB. Evidently the (North American) varieties used for genetic modifications are more susceptible to pests.

MON810 has been shown to produce fewer natural substances to protect itself from pests. For MON810 and unmodified control varieties significant differences were found in the amount of protective substances produced. It was shown that the difference was not caused by the genetic modification, but was due to a varietal effect (Degenhardt 2005). This means that the Bt maize MON810 starts out at a natural disadvantage compared to other maize varieties in regard to the problem that it is supposed to have solved. Genetic engineering repeats the problems of the Green Revolution when it tries to replace multiple better-adapted varieties with only a few varieties.

Lower mycorrhiza levels can make the plants more susceptible to pests. Mycorrhiza symbiosis also makes crops more attractive to the natural enemies of aphids (Guerrieri et al. 2004, cited in Dolezel et al. 2005). Less mycorrhiza colonization (Turrini et al. 2004, Castaldini et al. 2005, see above) makes Bt maize plants more susceptible to pests since they cannot attract as many natural enemies of the pests (Dolezel et al. 2005 p.37).

In EU approval procedures the question is not being raised as to whether gene manipulation has an impact on these natural defence mechanisms or whether the source plant material used for the genetic modification might be more susceptible to certain pests from the outset.

Who needs Bt maize?

In an issue of the ForschungsReport (1/2006), a publication of the German Ministry for Food, Agriculture and Consumer Protection (BMEVL) on the topic of genetic engineering and safety research, ECB infestation in Germany is described as follows:

"In Germany maize is cultivated on 1.7 million hectares, of which 350,000 hectares are in the infestation area of the ECB (*Ostrinia nubilalis*), the only maize pest that is controlled year round on larger fields in Germany. ECB is mainly controlled through preventive measures as chaffing the stubbles and clean ploughing after the maize harvest. This kills the larvae in stubbles and robs them of material for pupation on the soil surface. The application of insecticides (on about 35,000 hectares per year) is laborious, because they have to be applied with special machinery due to the height of the plants. On about 14,000 hectares per year *Trichogramma* larvae are applied as an organic method of controlling the eggs of the pests." (Langenbruch et al. 2006).

This means that only a fifth (20%) of the German maize acreage is infested with the European corn borer. Insecticides are applied to only 10% of this acreage, which means in turn that only 2% of the German maize acreage is treated with insecticides to control ECB.

The main argument for Bt maize cultivation is usually that farmers would not need to spray pesticides to control ECB any more, thus reducing overall applications of pesticides. For Germany this argument does not hold true because in general little pesticides are used to control ECB to begin with. Even worse is that on every field that would not have been treated with pesticides to control ECB, Bt maize cultivation releases additional Bt toxins on a large scale into the environment. So the overall amount of poison that gets into the soil and damages other organisms beside ECB increases.

Preventive practices to control ECB using mechanical field work such as chaffing the maize straw and clean, deep ploughing can decimate 80-90% of the ECB populations (Hurle et al. 1996, Langenbruch 2003; cited in Schorling 2006 p.14).

During acute ECB infestation there is also the possibility of applying *Trichogramma* or of using Bt sprays. "With Bt sprays only members of specific insect groups in a specific development stage over a short period of time (of about a week) are harmed after the spray has been applied." (Langenbruch et al. 2006).

The amount of Bt toxin released on the field by Bt plants is considerably higher than that from Bt sprays (Szekacs et al. 2005).

European corn borer infestation: Not a problem in Europe

Bt maize was developed in the United States. The situation in the United States is completely different from the situation in Germany and Europe in several respects, especially when it comes to the issue of ECB infestation. In the assessment of whether Bt maize is necessary and/or safe to grow in Germany, these issues are not usually considered sufficiently.

Ostrinia nubilalis (ECB) is a member of the butterfly family (Lepidoptera). It is native to Europe. Here, it has two different strains and not only colonizes maize (Liebe 2004; cited in Schorling 2006). ECB was introduced to the United States between 1910 and 1920 and spread fast as a maize pest; hence its name in English: European corn borer. In hot regions, ECB can produce two or three generations per year. In Germany (and in most of the EU), *O. nublilalis* only has one generation per year. The larvae spend the winter in the stems and roots left on the field and can usually be controlled by chaffing the plant residues and ploughing them under.

Furthermore, ECB infestations are cyclical, i.e. in some years there is high infestation while in other years there will be only few or no incidents. In Germany pesticides are used very infrequently to control the European corn borer. *O. nubilalis* has natural enemies in Europe. For example, ichneumon flies (*Trichogramma*, see above*)* lay their eggs in ECB larvae and thereby reduce the ECB populations.

Maize plants actively contribute to controlling ECB when they are affected by this pest by releasing volatile substances that attract *Trichogramma* (Degenhardt 2005). *Trichogramma* is also used as an organic pest control – though on only a small part of the limited acreage.

In North American varieties the ability to produce these volatile substances was lost in the course of plant breeding, while teosinte (the predecessor of maize) and European varieties use it to attract natural enemies, e.g. when the corn is infested with the corn rootworm (Rasmann et al. 2005).

With regard to MON810 this means that probably a conventional maize variety was genetically modified which has a poor interaction with beneficial insects that occur naturally or that are used intentionally as beneficial insects. Therefore MON810 is structurally more susceptible to exactly the pest that it is intended to control.

European agriculture differs significantly from North American agriculture. German agriculture is much more small scale. Field margins and hedges are an important part of the agricultural landscape. Similar structures are often missing from large-scale North American maize cultivation.

Effects on non-target organisms have to be assessed under conditions that represent the structures (and organisms) of the respective croplands. For instance, this is key to the question of whether Bt pollen negatively affects butterflies and butterfly populations. The amount of pollen deposited at field margins varies greatly and depends on a range of environmental factors (Dolezel et al. 2005 p.16). It must accordingly be assumed that the effects on the environment will be more serious in Europe than in the United States.

Summary

The overview presented here shows the many ways Bt maize impacts the environment. And there are also the possible adverse effects on the health of humans and animals. The number and complexity of already known effects show that it will be impossible to assess all relevant risks in approval procedures and field trials.

The list of open questions and uncertainties is long:

- Neither the number of gene sequences nor the location where the new genes are inserted can be controlled.
- The interactions with other genes and the metabolism of the plants cannot be predicted.
- The ecosystemic effects are complex and can only be partially assessed scientifically.
- Health impacts cannot be ruled out.
- Pollen migration and contaminants in the harvest contaminate food products and seed.

European framework legislation (Directive 2001/18 and Regulation 1829/2003) gives high priority to the precautionary principle. Directive 2001/18, Article 4(1) states:

"Member States shall, in accordance with the precautionary principle, ensure that all appropriate measures are taken to avoid adverse effects on human health and the environment which might arise from the deliberate release or the placing on the market of GMOs."

In the light of the many known repercussions and because it is factually impossible to thoroughly study and assess all relevant risks, the requirements for EU marketing approval are not in place. While the cultivation of these plants and their use for animal feed serves the financial interests of a few companies, the potential long-term effects make such cultivation untenable.

References

Abel, C.A. & Adamczyk, J.J. 2004. Relative concentration of Cry1A in maize leaves and cotton bolls with diverse chlorophyll content and corresponding larval development of fall armyworm (Lepidoptera: Noctuidae) and Southwestern corn borer (Lepidoptera: Crambidae) on maize whorl leaf profiles. Journal of Economic Entomology 97(5): 1737-1744.

Ahl Goy, P., Warren, G., White, J., Pivalle, L., Fearing, P.L. & Vlachos, D. 1995. Interaction of insect tolerant maize with organisms in the ecosystem. Mitteilungen des Biologischen Bundesamts für Forst- und Landwirschaft 309: 50-53.

Arndt, N. 2005. Abbau von Bt-Mais und Auswirkungen auf die mikrobielle Aktivität. Sicherheitsforschung und Monitoring zum Anbau von Bt-Mais 2001-2004, und http://www.biosicherheit.de/de/sicherheitsforschung/2 1.doku.html

Baumgarte, S. & Tebbe, C.C. 2005. Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. Molecular Ecology 14(8): 2539-2551.

BMBF 2005. Ölosystem Maisfeld. Ergebnisse des Projektverbund Sicherheitsforschung und Monitoring zum Anbau von Bt-Mais 2001-2004. http://www.biosicherheit.de/de/mais/zuensler/317.doku.html

Bootjer, K.P., Bone, L.W. & Gills, S.S. 1985. Nematoda: suspectibility of the eggs to Bacilus thuringiensis toxins. Experimental Parasitology 60: 239-244.

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

Büchs, W. 2005. Auswirkungen von Bt-Mais auf Trauermückenlarven als Zersetzer. Sicherheitsforschung und Monitoring zum Anbau von Bt-Mais 2001-2004. http://www.biosicherheit.de/de/sicherheitsforschung/14.doku.html

Büchs, W., Prescher, S., Müller, A. & Larink, O. 2004. Effects of Bt-maize on decomposition capacity, reproduction success and survival of saprophagous Diptera larvae and their predators. Präsentationsposter, Statusseminar 16.6.2004, Berlin. Sicherheitsforschung und Monitoring zum Anbau von Bt-Mais 2001-2004. http://www.biosicherheit.de/pdf/statusseminar2004/poster14.pdf

Burns, H.A. & Abel, C.A. 2003. Nitrogen fertility effects on Bt d-endotoxin and nitrogen concentrations of maize during early growth. Agronomy Journal 95: 207-211.

Castaldini, M., Turrini, A., Sbrana, C., Benedetti, A., Marchionni, M., Mocali, S., Fabiani, A., Landi, S., Santomassimo, F., Pietrangeli, B. et al. 2005. Impact of Bt corn on rhizospheric and soil eubacterial communities and on beneficial mycorrhizal symbiosis in experimental microcosms.

Applied and Environmental Microbiology 71(11): 6719-6729.

Degenhardt, J. 2005. Auswirkungen von Bt-Mais auf Schmetterlinge und deren Gegenspieler. Sicherheitsforschung und Monitoring von Bt-Mais 2001-2004. http://www.biosicherheit.de/de/sicherheitsforschung/23.doku.html

Dively, G.P. & Rose, R. 2003. Effects of Bt transgenic and conventional inscticide control on the non-target natural enemy community in sweet corn. In: Van Driesche, R.G. Proceedings of the First International Symposium on Biological Control of Arthropods, Honolulu, USA, January 14-18, 2002. USDA Forest Service, Morgantown, WVA, USA.

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 continous exposure to Cry1Ab-expressing corn during anthesis. Environmental Entomology 33(4): 1116- 1125.

Dolezel, M., Heissenberger, A. & Gaugitsch, H. 2005. Ecological effects of genetically modified maize with insect resistance and/or herbicide tolerance. Forschungsberichte der Sektion IV. Band 6/2005. Bundesministerium für Gesundheit und Frauen, Sektion IV, Vienna, Austria.

Dutton, A., Romeis, J. & Bigler, F. 2005. Effects of Bt maize expressing Cry1Ab and Bt spray on Spodoptera littoralis. Entomologia Experimentalis et Applicata 114(3): 161-169.

Dutton, A., D'Alessandro, M., Romeis, J. & Bigler, F. 2004a. Assessing expression of Bt-toxin (Cry1Ab) in transgenic maize under different environmental conditions. IOCB/WPRS Working Group "GMOs in Intergrated Production" 27(3): 49-55.

Dutton, A., Obrist, L.B., D'Alessandro, M., Diener, L., Müller, M., Romeis, J. & Bigler, F. 2004b. Tracking Bt-toxin in transgenic maize to assess the risk on non-target athropods. IOCB/WPRS Working Group "GMOs in Intergrated Production" 27(3): 57-63.

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

Dutton, A., Romeis, J. & Bigler, F. 2003b. Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: Bt-maize expressing Cry1Ab as a case study. BioControl 48: 611-636.

Dutton, A., Klein, H. & Romeis, J. 2002. Uptake of Bttoxin by herbivores feeding on transgenic maize and consequences for the predator Chrysoperla carnea. Environmental Entomology 27: 441-447.

Einspanier, R., Lutz, B., Rief, S., Berezina, O., Zverlov, V., Schwarz, W. & 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(3): 269-173.

Escher, N., Käch, B. & Nentwig, W. 2000. Decomposition of transgenic Bacillus thuringiensis maize by microorganisms and woodlice Porcelli scaber (Crustacea, Isopoda). Basic and Applied Ecology 1: 161-169.

European Communities - Measures affecting the approval and marketing of Biotech products (DS291, DS292, DS293). Comments by the European Communities on the scientific and technical advice to the panel. 28 January 2005.

Fearing, P.L., Brown, D., Vlachos, D., Meghji, M. & Privalle, L.S. 1997. Quantitative analysis of CryIA(b) expression in Bt maize plants, tissues, and silage and stability of expression over

successive generations. Molecular Breeding 3:169-176.
Felke, M. & Langenbruch, G.-A. 2005. Auswirkungen des Pollen von transgenem Bt-Mais auf ausgewählte Schmetterlingslarven. BfN-Skripten. No. 157. Bundesamt für Naturschutz.

Felke, M. & Langenbruch, G.-A. 2004. Untersuchungen zu subletalen Effekten geringer Pollenmenge der transgenen Maislinie Bt176 auf Raupen des Tagpfauenauges (Inachis io) und der Kohlmotte (Plutella xylostella). Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft 396.

Felke, M. & Langenbruch, G.-A. 2003. Wirkung von Bt- Mais-Pollen auf Raupen des Tagpfauenauges im Laborversuch. Gesunde Pflanze 55(1): 1-4.

Felke, M., Lorenz, N. & Langenbruch, G.-A. 2002. Laboratory studies on the effects of pollen from Btmaize on larvae of some butterfly species. Journal of Applied Entomology 126(6): 320-325.

Gathmann, A. 2005. Effekte des Anbaus von Bt-mais aufdie epigäische und Krautschichtfauna verschiedener trophischer Bezüge. BMBF-Verbundprojekt: Sicherheitsforschung und Monitoring zum Anbau von Bt-Mais 2001-2004. http://www.biosicherheit.de/de/sicherheitsforschung/3 2.doku.html

Harwood, J.D., Wallin, W.G. & Obrycki, J.J. 2005. Uptake of Bt endotoxins by nontarget herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem. Molecular Ecology 14(9): 2815-2823.

Hassel, R.J. & Shepard, B.M. 2002. Insect populations on Bacillus thuringiensis transgenic sweet corn. Journal of Economic Entomology 37: 285-292.

Hernandez, M., Pla, M., Esteve, T., Prat, S., Puigdomènech, P. & Ferrando, A. 2003. A Specific Real-Time Quantitative PCR Detection System for Event MON810 in Maize YieldGard Based on the 3'-Transgene Integration Sequence. Transgenic Research 12(2): 179-189.

Hilbeck A, Moar WJ, Pusztai CM, 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.

Hilbeck A, Baumgartner M, Fried PM, Bigler F (1998a): Effects of transgenic Bacillus thuringiensis corn-fed prey on mortality and development time of immature Chrysoperla carnes (Neuroptera: Chrysopidae). Environmental Entomology 27(2), 480-487.

Hilbeck A, Moar WJ, Pusztai CM, Filippini A, Bigler F (1998b): Toxicity of Bacillus thuringiensis CrylAbtoxin to the predator Chryosperla canae (Neuroptera: Chrysopidae). Environmental Entomology 27(4), 1255-1263.

Hopkins, D.W. & Gregorich, E.G. 2003. Detection and decay of the Bt endotoxin in soil from a field trial with genetically modified maize. European Journal of Soil Science 54(4): 793-800.

Jansinski, J. & Eisley, J. 2003. Select nontarget arthropod abundance in transgenic and non transgenic fieldcrops in Ohio. Environmental Entomology 32: 407-411.

Jehle, J.A. 2005. Toxinproduktion und Qualitätskontrolle von rekombinanten Cry1Ab in heterologen Expressionssystemen (Abschlußbericht) BMBFVerbundprojekt: Sicherheitsforschung und Monitoringmethoden zum Anbau von Bt-Mais, und http://www.biosicherheit.de/de/sicherheitsforschung/3 1.doku.html

Kaatz, H.-H. 2005. Auswirkungen von Bt-Maispollen auf die Honigbiene. BMBF-Verbundprojekt: Sicherheitsforschung und Monitoring zum Anabu von Bt-Mais.

http://www.biosicherheit.de/de/sicherheitsforschung/68.doku.html

Kiss, J., Szentkiralyi, F., Toth, F., Szenasi, A., Kadar, F., Arpas, K., Szekeres, D. & Edwards, C.R. 2003. Btcorn: impact on non-targets and adjusting to local IPM systems. In: Lelley, T., Balázs, E. & Tepfer, M. Ecological impact of GMO dissemination in agroecosystems. OECD Workshop, September 27-28, 2002, Grossrussbach, Austria. 157-172.
Koskella, J. & Stotzky, G. 1997. Microbial utilization of free and clay-bound insecticidal toxins from Bacillus thuringiensis and their retention of insecticidal activity after incubation with microbes. Applied and Environmental Microbiology 63(9): 3561-3568.

Lang, A. 2005. Effekte von Mais auf flugfähige Blütenbesucher und Prädatoren höherer Straten (Schlussbericht). Sicherheitsforschung und Monitoring-Methoden zum Anbau von Bt-Mais 2001-2004, und http://www.biosicherheit.de/de/sicherheitsforschung/ 68.doku.html

Lang, A. & Arndt, N. 2005. Monitoring der Umweltwirkungen des Bt-Gens. Schriftenreihe. No. 2005/7. Bayerischen Staatsministeriums für Umwelt, Gesundheit und Verbraucherschutz, München.

Lang, A. & Voijtech, E. 2006. The effects of pollen consumption of transgenic Bt maize on the common swallowtail, Papilio machanon L. (Lepidoptera, Papilionidae). Basic and Applied Ecology 7(4): 296-306.

Langenbruch, G.-A., Hassan, S.A., Büchs, W., Bürgermeister, W., Freier, B. & Hommel, B. 2006. Biologische Sicherheitsforschung mit Bt-Mais. ForschungsReport 1/2006: 8-12.

Lee, L., Saxena, D. & Stotzky, G. 2003. Activity of free and clay-bound insecticidal proteins from Bacillus thuringiensis subsp. israelensis against the mosquito Culex pipiens. Applied and Environmental Microbiology 69(7): 4111-4115.

Liebe, D. 2004. Molekulargenetische Untersuchungen zur Abgrenzung von Populationen des Maiszünslers Ostrinia nubilalis Hübner als eine Voraussetzung für das Insektenresistenzmanagement (IRM) von Bacillus thuringiensis-Mais (Bt-Mais) (Dissertation). Justus-Liebig-Universität Giessen.

Losey, J.E. 1999. Transgenic pollen harms monarch larvae. Nature 399: 214-214.

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

Lozzia, G.C. 1999. Biodiversity and structure of ground beelte assemblages (Coleoptera, Carabidae) in Bt corn and its effects on non-target insects. Bollettino di Zoologia Agraria e di Bachicoltura Ser II, 3: 37- 58.

Ludy, C. & Lang, A. 2006. Bt maize pollen exposure and impact on the garden spider, Araneus diadematus. Entomologia Experimentalis et Applicata 118(2): 145-156.

Lumbierres, B., Albajes, R. & Pons, X. 2004. Transgenic Bt maize and Rhopalosiphum padi (Hom., Aphididae) performance. Ecological Entomology 29 (3): 309-317.

Magg, T., Melchinger, A.E., Klein, D. & Bohn, M. 2001. Comparison of Bt maize hybrids with their nontransgenic
counterparts and commercial varieties for resistance to European corn borer and for agronomic traits. Plant Breeding 120: 397-403.

Manachini, B., Landi, S., Fiore, M.C., Festa, M. & Arpaia, S. 2004. First investigations on the effects of Bttransgenic Brassica napus L. on the trophic structure of the nematofauna.

IOCB/WPRS Bulletin 27(3): 103- 108.

Manachini, B. & Lozzia, G.C. 2003. Biodiversity and structure of nematofauna in Bt corn (Präsentation). Biodiversity Implications of Genetically Modified Plants 7-13 September 2003, Ascona, Switzerland.

Manachini, B. & Lozzia, G.C. 2002. First investigations into the effects of Bt corn crop on Nematofauna. Bollettino di Zoologia Agraria e di Bachicoltura 34(1): 85-96.

Manachini,B. 2000. Ground beetle assemblages (Coleoptera, Carabidae) and plant dwelling nontarget athropods in isogenic and transgenic corn crops. Bollettino di Zoologia Agraria e di Bachicoltura 32(2): 181-198.

Manachini, B., Agosti, M. & Rigamonti, I.E. 1999. Environmental impact of Bt-corn on non target entomofauna: Synthesis of field and laboratory studies. Proceedings of the XI Symposium for Pesticide Chemistry: 873-882.

Mayne,M.B., Coleman,J.R. & Blumwald,E. 1997. Differential response to drought and abscisic acid of two cDNAs corresponding to genes expressed during drought conditioning in jackpine seedlings. New Forests 13: 165-176.

Mendelsohn, M. 2003. Are Bt crops safe? Nature Biotechnology 21: 1003-1009. Musser, F.R. & Shelton, A.M. 2003. Bt sweet corn and selective insecticides: Impacts on pests and predators. Journal of Economic Entomology 96(1): 71-80.

Nguyen, H.T. 2004. Sicherheitsforschung und Monitoringmethoden zum Anbau von Bt-Mais: Expression, Nachweis und Wirkung von rekombinantem Cry1Ab in heterologen Expressionssystemen (Dissertation). Georg-August- Universität Göttingen.

Nguyen, H.T., Berlinghof, M. & Jehle, J.A. 2002. Expressionsmonitoring von Cry1Ab verschiedener Maislinien an zwei Freisetzungstandorten in Deutschland. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft 390: 542-543.

Obrist, L.B., Dutton, A., Albajes, R. & Bigler, F. 2005. Exposure of arthropd predators to Cry1Ab toxin in Bt maize fields on Spain. Meeting on Ecological Impact of GMOs 1-3 June 2005.

Orr, D.B. & Landis, D.A. 1997. Oviposition of European corn borer (Lepidoptera: Pyralidae) and impact of natural enemy populations in transgenic versus isogenic corn. Journal of Economic Entomology 90 (4): 905-909.

Palm, C.J., Donegan, K.K., Harris, D. & Seidler, R.J. 1994. Quantification in soil of Bacillus thuringiensis var. kurstaki delta-endotoxin from transgenic plants. Molecular Ecology 3(2): 145-151.

Pilcher, C.D., Obrycki, J.J., Rice, M.E. & Lewis, L.C. 1997a. Preimaginal development, survival, field abundance of insect predators on transgenic Bacillus thuringiensis corn. Environmental Entomology 26(2): 446-454.

Poerschmann, J., Gathmann, A., Augustin, J., Langer, U. & Gorecki, 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.

Poerschmann, J. & Kopinke, F. 2001. Sorption of very hydrophobic organic compounds (VHOCs) on dissolved humic organic matter (DOM). 2. Measurement of sorption and application of a Flory-Huggins concept to interpret the data. Environmental Science & Technology 35: 1142- 1148.
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: Aphididae) for the presence of Cry1Ab. Molecular Ecology 10(2): 525-533.

Rasmann,S., Köllner,T.G., Degenhardt,J., Hiltpold,I., Toepfer,S., Kuhlmann,U., Gershenzon,J. & Turlings,T.C.J. 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature 434: 732-737.

Rathinasabapathi, B. 2000. Metabolic engineering for stress tolerance: Installing osmoprotectant synthesis pathways. Annals of Botany 86: 709-716.

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

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

Saxena, D., Flores, S. & Stotzky, G. 2002a. Vertical movement in soil of insecticidal Cry1Ab protein from Bacillus thuringiensis. Soil Biology and Biochemistry 34: 111-120.

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

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

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

Saxena, D. & Stotzky, G. 2000. Insecticidal toxin from Bacillus thuringiensis is released from roots of transgenic Bt corn in vitro and in situ. FEMS Microbiology Ecology 33: 35-39.

Saxena,D., Flowers,S.A. & Stotzky,G. 1999. Transgenic Plants: Insecticidal toxin in root exudates from Bt corn. Nature 402: 480.

Schorling, M. 2006. Ökologische und phytomedizinische Untersuchungen zum Anbau von Bt-Mais im Maiszünsler-Befallsgebiet Oderbruch (Dissertation). Mathematisch-Naturwissenschaftliche Fakultät der Universität Potsdam.

Schröder, D. 2005. Quantifizierung der Beweglichkeit von Bt-Toxin in Böden (Schlussbericht) Sicherheitsforschung und Monitoringmethoden zum Anbau von Bt-Mais 2001-2004, und http://www.biosicherheit.de/de/sicherheitsforschung/95.doku.html

Sims, S.R. & Holden, L.R. 1996. Insect bioassay for determining soil degradation of Bacillus thuringiensis subsp. kurstaki CryIA(b) protein in corn tissue. Environmental Entomology 25(3): 659-664.

Sisterson, M.S. & Tabashnik, B.E. 2005. Simulated effects of transgenic bt crops on specialist parasitoids of target pests. Environmental Entomology 34(4): 733-742.

Stanley-Horn, D.E., Dively, G.P., Hellmich, R.L., Mattila, H.R., Sears, M.K., Rose, R., Hansen Jesse, L.C., Losey, J.E., Obrycki, J.J. & Lewis, L.C. 2001. Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. Proceedings of the National

Academy of Sciences of the United States of America 98(21): 11931-11936.

Stotzky, G. 2000. Persistence and biological activity in soil of insecticidal proteins from Bacillus thuringiensis and bacterial DNA bound on clays and humic acids. Journal of Environmental Quality 29: 691-705.

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

Tebbe, C. 2005. Untersuchungen zum abbau von Bt-Mais in Böden und Auswirkungen auf die mikrobielle Bodenökologie. Schlussbericht zum Forschugnsvorhaben 0312631E, Verbundprojekt: sicherheitsforschung und Monitoring-Methoden zum anbau von Bt-Mais.

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

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

Szekacs, A., Juracsek, J., Polgar, L.A. & Darvas, B. 2005. Levels of expressed Cry1Ab toxin in genetically modified corn DK-440-BTY (YIELDGARD) and stubble . FEBS 272 (s1) L3-005.

Vidal, S. 2005. Untersuchungen zu Kaskadeneffekten einr Bt-Expression von Maispflanzen auf Pflanze- Herbivor-Parasitoid-Systeme amd Beispiel von Blattläusen und ihren Parasitoidkomplexen (endbericht). Sicherheitsforschung und Monitoring zum Anbau von Bt-Mais 2001-2004, und http://www.biosicherheit.de/de/sicherheitsforschung/17.doku.html

Volkmar, C. & Freier, B. 2003. Spider communities in Bt maizeand not genetically modified maize fields. Journal of Plant Diseases and Protection - Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 110(6): 572-582.

Wandeler, H., Bahylova, J. & Nentwig, W. 2002. Consumption of two Bt and six non-Bt corn varieties by the woodlouse Porcellio scaber. Basic and Applied Ecology 3(4): 357-365.

Wold,S.J., Burkness,E.C., Hutchison,W.D. & Venette,R.C. 2001. In-field monitoring of beneficial insect populations in transgenice corn expressing a Bacillus thuringiensis toxin. Journal of Entomological Science 36(765): 775.

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

Zwahlen, C., Hilbeck, A. & Nentwig, W. 2003b. Degradation of the Cry1Ab protein within transgenic Bacillus thuringiensis corn tissue in the field. Molecular Ecology 12(3): 765-775.