TESTBIOTECH Background 11- 12 - 2013

High-Level-Risk-Maize 1507

Shortcomings at the European Food Safety Authority (EFSA) and in EU Commission decision making should prompt reassessment of genetically engineered maize 1507[1]

Testbiotech e. V.
Institute for Independent Impact Assessment in Biotechnology

Christoph Then & Andreas Bauer-Panskus

This project was realised with support by Grassroots Foundation

Content

1 corrected version – table 1 from Mendelsohn was deleted because its content was not sufficiently clear

Summary

Maize 1507 is a genetically engineered plant that produces a Bt toxin (Cry1F) and is also resistant to the herbicide glufosinate. There is no doubt that maize 1507 poses a high level of risk to the environment:

- The content of insecticidal Bt proteins (Cy1F) in the plants is much higher compared to other genetically engineered plants such as MON 810.
- Risk assessment of maize 1507 implies a high level of uncertainty. There has been hardly any testing of the effects of the Bt toxin Cry1F on species such as protected butterflies.
- From current data, it is likely that the spectrum of organisms that are sensitive to the toxin Cry1F is broader than known from Cry1Ab (as produced in MON 810).

Although the European Food Safety Authority, EFSA, does not dispute any of these facts, in its risk assessment the authority has so far failed to request highly relevant data on:

- true variations and real content of the Bt toxin in all parts of the plants under various environmental conditions;
- real specificity and selectivity of Cry1F;
- sensitivity of protected European butterflies;
- effects on non-target organisms such as soil organisms and wild species;
- combinatorial effects between Cry1F and other environmental stressors.

Further, EFSA did not properly assess

- unintended effects in the genome caused by the process of genetic engineering;
- significant changes in the plants composition that were observed in maize 1507;
- the effects of spraying with the herbicide glufosinate.

Overall, EFSA ignored relevant facts and presented its opinion despite apparently not having enough data to reach conclusions. For example in 2011, EFSA identified a substantial lack of data on risks for protected European butterfly species, but failed to request more data. Instead, EFSA suggested collecting the relevant data after market authorisation (!).

The history of this application is nothing less than chaotic. The EU Commission made several unsound decisions and there were several errors and substantial changes in the EFSA opinions:

1. During the approval process, EFSA made contradictory statements on the Bt concentration in the pollen, which is most relevant for environmental risk assessment. According to EFSA in 2005, the Bt concentration is much higher in maize 1507than in other Bt plants. In 2008, EFSA stated there would be no difference compared with other Bt plants, but in 2011, it once again confirmed an extremely high concentration of Bt toxins. These substantial changes in the EFSA opinions were made on the basis of data consistently showing a high content of Bt toxins in the plants. One could assume that in 2008 EFSA deliberately tried to misinform the EU decision makers and the public ahead of a first vote taken on approval for cultivation in 2009.

2. The way in which the EU Commission dealt with the application was very confusing. In 2007, the EU Commission presented a draft decision not to allow the cultivation of maize 1507 because the level of uncertainty was too high. Serious questions were consequently raised about risk assessment. The Commission then withdrew its draft decision as soon as industry instigated legal proceedings at the EU Court of Justice (ECJ). Consequently, the serious concerns previously raised by the Commission were never addressed .
3. In 2009, instead of defending a high level of protection for the environment and consumers the EU Commission proposed approving maize 1507 for cultivation. However, after the Member States failed to come to a decision, it did not forward the proposal to the Council of Member States as foreseen by EU regulations. This failure on the part of the EU Commission enabled Pioneer to file a new, successful, challenge at the EU Court of Justice. In 2013, the court decided that this delay in the authorisation process was a violation of EU regulations.
4. Several EFSA opinions and statements were filed between 2005 and 2012. New publications were put forward alongside a highly questionable method for modeling the risks of maize 1507. At the same time, there was no formal procedure for Member States to comment on these EFSA opinions and the Commission did not start a process for public consultation, thus hindering participatory elements that are normally established before a decision on approval is made.
5. The EU Commission proposal to allow cultivation of maize 1507 is not underpinned by the EFSA opinion. EFSA recommends collecting further data through case specific monitoring and / or including precautionary measures for sensitive species in protected habitats. However, the draft decision of the EU Commission (EU Commission 2013) does not include these measures.

The planned vote on the approval of maize 1507 for cultivation gives the Member States a final chance to correct the series of flaws and errors made in recent years. The proposal of the EU Commission should be rejected.

Zusammenfassung

Hochrisiko-Mais 1507: Schwerwiegende Fehler in der Bewertung der EU-Lebensmittelbehörde EFSA und bei Entscheidungen der EU-Kommission müssen zu einer Neubewertung der Risiken von Mais 1507 führen.

Mais 1507 ist gentechnisch so verändert, dass er ein Bt-Insekengift (Cry1F) produziert. Zudem wurde er gegen das Unkrautvernichtungsmittel Glufosinat resistent gemacht. Es gibt keinen Zweifel daran, dass der kommerzielle Anbau von Mais 1507 ein hohes Risiko für die Umwelt bedeutet:

- Der Gehalt an Insektengift (Cry1F) im Mais 1507 ist wesentlich höher als in anderen gentechnisch veränderten Pflanzen wie z.B. Mon810.
- Die Risikobewertung von Mais 1507 birgt erhebliche Unsicherheiten: Die Auswirkungen von Cry1F auf Tierarten wie geschützte europäische Schmetterlinge wurden bisher kaum untersucht.
- Aus den vorliegenden Daten kann abgeleitet werden, dass Cry1F für eine größere Anzahl von Tierarten giftig ist, als dies vom Insektengift Cry1Ab bekannt ist (das im Mais Mon810 produziert wird).

Obwohl auch die europäische Lebensmittelbehörde EFSA diese Fakten nicht in Abrede stellt, versäumt es die Behörde dennoch, von der Industrie die entscheidenden Daten zu folgenden Bereichen zu verlangen:

- das tatsächliche Ausmaß der Schwankungen des Bt-Gehalts in den Pflanzen unter verschiedenen Umweltbedingungen;
- die Zielgenauigkeit der Giftwirkung von Cry1F;
- die Empfindlichkeit europäischer Schmetterlingsarten gegenüber Cry1F;
- Auswirkungen auf Nichtzielorganismen wie Bodenorganismen und Wildtiere;
- Wechselwirkungen zwischen dem Insektengift und anderen Stressfaktoren für die Umwelt.

Zudem hat die EFSA folgende Risiken nicht ausreichend bewertet:

- unbeabsichtigte Auswirkungen auf das Genom der Pflanzen, die durch die gentechnische Veränderung hervorgerufen wurden;
- signifikante Veränderungen in der Zusammensetzung der Inhaltsstoffe, die bei Mais 1507 beobachtet wurden;
- Auswirkungen der Anwendung von Glufosinat bei den Pflanzen.

Zusammenfassend lässt sich feststellen, dass die EFSA relevante Fakten ignoriert und ihre Stellungnahmen verabschiedet hat, obwohl ganz offensichtlich entscheidende Daten fehlen. So identifizierte die EFSA 2011 erhebliche Lücken bei den Daten zu Auswirkungen auf geschützte Schmetterlinge. Dennoch verlangte die Behörde keine weiteren Untersuchungen – stattdessen schlägt sie vor, entsprechende Daten nach der Zulassung (!) zu erheben.

Der Antrag zum Anbau von 1507 hat eine chaotische Geschichte. Es gibt eine Reihe offensichtlicher Fehler und Widersprüche in den Stellungnahmen der EFSA sowie mehrere falsche Entscheidungen der EU-Kommission:

- Während des Zulassungsprozesses bewertete die EFSA den Gehalt an Bt-Insektengift in den Pollen, die für die Risikobewertung wichtig sind, ganz unterschiedlich. 2005 bewertete die EFSA den Bt-Gehalt als sehr hoch, 2008 sah sie hingegen keinen Unterschied zu anderen gentechnisch veränderten Pflanzen, während die Behörde 2011 erneut einen sehr hohen Gehalt bestätigte. Diese unterschiedlichen Stellungnahmen erfolgten auf der Grundlage einer unveränderten Datenlage, die immer gezeigt hat, dass die Bt-Konzentration in den Pollen sehr hoch ist. Es drängt sich die Vermutung auf, dass die Behörde 2008 versucht haben könnte, die Entscheidungsträger und die Öffentlichkeit vor einer Abstimmung im Jahr 2009 in die Irre zu führen.
- Die Art und Weise, wie die Kommission mit dem Antrag verfuhr, war in höchstem Maße verwirrend: 2007 präsentierte die Kommission den Entwurf einer Entscheidung, nach der ein Anbau von Mais 1507 nicht zugelassen werden sollte, weil es zu viele Unsicherheiten gebe. Dabei wurden schwerwiegende Mängel der Risikobewertung herausgestellt. Diese Entscheidung wurde jedoch zurückgezogen, als die Industrie eine Klage vor dem EU-Gerichtshof einreichte. Im Ergebnis wurden die von der Kommission genannten Lücken in der Risikobewertung nie geschlossen.
- Anstatt den Schutz der Umwelt und Verbraucher vor Gericht zu verteidigen, schlug die EU-Kommission 2009 vor, den Anbau von Mais 1507 zuzulassen. Bei einer ersten Abstimmung unter den Mitgliedsstaaten wurde keine ausreichende Mehrheit erreicht. Die Kommission legte danach den Fall nicht zu einer zweiten Abstimmung vor, wie dies von den EU-Regeln festgelegt wird. Diese Verletzung formaler EU-Vorschriften erlaubte es der Industrie, eine neue, erfolgreiche Klage beim EU-Gerichtshof einzureichen. 2013 entschied der Gerichtshof, dass die Aussetzung des Zulassungsprozesses nicht zulässig war.
- Zwischen 2005 und 2012 wurden verschiedene Stellungnahmen der EFSA zum Mais 1507 veröffentlicht, bei denen auch neue Publikationen und insbesondere neue, umstrittene Methoden zur Modellierung der Umweltrisikien eingeführt wurden. Trotzdem gab es keinerlei – wie sonst üblich – offizielle Verfahren und Fristen zur Kommentierung dieser Stellungnahmen durch die Öffentlichkeit und durch die Experten der Mitgliedsländer. Damit entfiel in diesem Falle die sonst vorgesehene Möglichkeit zur Partizipation.
- Der Vorschlag der EU-Kommission, den Mais 1507 zum Anbau zuzulassen, steht nicht im Einklang mit den Stellungnahmen der EFSA. Diese schlägt vor, ein gezieltes Monitoring duchzuführen, um Risiken für die Umwelt nach einer Zulassung genauer zu untersuchen und/oder Sicherheitsabstände zu Naturschutzgebieten vorzuschreiben. Doch in dem Vorschlag der EU-Kommission findet sich keine dieser Maßnahmen (EU-Commission, 2013).

Die geplante Abstimmung über die Zulassung von Mais 1507 für den Anbau gibt den Mitgliedsländern der EU jetzt die Gelegenheit, diese Irrtümer und falschen Entscheidungen der Vergangenheit zu korrigieren. Der Vorschlag der EU-Kommission sollte daher zurückgewiesen werden.

1. Introduction

Maize 1507 (also known as DAS1507 or TC1507 and sold under the brand name Herculex I) is a variety of genetically engineered maize that is marketed jointly by Dow AgroSciences and Pioneer/DuPont. Maize 1507 produces a Bt toxin (Cry1F) active against lepidopteran target pests such as the European corn borer, *Ostrinia nubilalis*, and species belonging to the genus *Sesamia*, and is also resistant to the herbicide glufosinate. It was first approved for cultivation in the USA (2001) and Canada (2002). In the EU, an initial application for the market authorisation of maize 1507 was submitted in 2001. In 2006, it was approved for food and feed uses. A vote on market approval for cultivation was taken in 2009, however no qualified majority was reached and no decision has been made since then. In 2013, after a complaint from industry, the European Court of Justice ordered the Commission to end the deadlock in the approval process. In November 2013, the EU Commission presented a draft decision on the authorisation of maize 1507 for cultivation, and Member States will be invited to vote on this authorisation request.

2. Substantial weaknesses and flaws in EFSA risk assessment

2.1 Unintended effects in the plants

Maize 1507 was created by a process known as particle bombardment. This genetic transformation process has unintended effects and numerous additional gene fragments can be found in the genome. Open reading frames were identified that can give rise to unintended gene products in the plants. There are further indications that unintended tRNA is produced in the plants, while it is unclear if this is also the case with new proteins. Although some consideration was given to possible risks of tRNA and proteins, other biologically relevant substances such as double stranded RNA were not taken into account (see Zhang et al., 2011).
In comparison to its conventional counterparts, many significant differences were observed in compositional analysis but these were not investigated further. Instead historical data unrelated to the actual field trials, were used to conclude that these differences are not of biological relevance. Since it is not sufficiently clear under which specific conditions these additional historical data were generated, this kind of comparison inevitably leads to major uncertainties.

In agronomic parameters, several significant differences were identified in comparison to the control plants. However, the differences were not consistent over all field trials. The reason for this might be that these differences only emerge under particular environmental conditions, yet there has been no investigation under various defined environmental conditions to determine interactions between the genome and the environment. Since it is known that genetically engineered plants can exhibit unexpected reactions under stress conditions (see for example: Matthews et al., 2005), these kind of investigations would have been necessary in this case. Instead, the significant differences were dismissed without considering specific interactions between the genome and the environment.

2.2 Risk assessment of the Bt toxin

Maize 1507 produces an insecticide originally derived from *Bacillus thuringiensis* classified as Cry1F. The expression of the Bt toxin is much higher compared to other plants producing Bt toxins (such as MON 810 and Bt11). In particular, there is an extremely high concentration of the Bt toxin

in maize 1507 pollen which is highly relevant for the environmental assessment. EFSA (2011) confirmed this by stating:

> *„The 32 ng/mg dry weight of Cry1F protein in pollen of maize 1507 is about 350 times the Cry1Ab protein content expressed in maize MON 810 pollen.“* (EFSA 2011)

Due to the high content of Bt in the pollen, the environmental risks of cultivating maize 1507 are presumed to be much higher than the risks associated with MON 810. EFSA confirmed this view by stating:

> *„Indeed, in general, the potential risks without mitigation are considerably greater for maize 1507 than was the case for maize MON 810, due to the greater toxicity of the former and assuming the same exposure.“* (EFSA, 2011)

Despite these findings indicating a particularly high level of risk for cultivation of maize 1507 (compared to other Bt plants), EFSA failed to

- reliably determine the expression rate and maximum concentration of the Bt toxin in the plants;
- assess the true selectivity of the toxin;
- properly assess the risks to non-target organisms.

2.2.1 Failure to determine the expression rate of Bt toxins

Some basic prerequisites have to be met in order to carry out proper environmental risk assessment. If these data are not available, assessment of environmental risks cannot be designed, performed or interpreted in a meaningful way. One of these prerequisites is exact data on the expression of the newly expressed Bt proteins. These data are relevant for the emergence of resistance in pest insects and for the risk assessment to non-target organisms such as the larvae of protected butterflies. If it is already known that the rate of expression of Bt toxins is much higher (compared to other genetically engineered plants) then it is imperative to have exact data to show the true range of variations.

To make these data available the following have to be taken into account:

- The protocol used for measuring the Bt toxins is known to be highly influential on the outcome. Slight differences in the method/ protocol used in measuring can cause enormous differences in the results. There are, however, no standardised protocols to measure the content of Bt toxins reliably so that the results can be compared to results from different laboratories (Székács et al., 2011). Further, no reliably validated and reproducible method has been made available to independent laboratories, with the result that major uncertainties remain about the exact content of Bt toxin in maize 1507.

- The data provided by industry are known to be unreliable on the true range of variation of Bt toxins in the plants, as for example shown by Nguyen and Jehle (2007).

- The expression rate of the newly introduced proteins is known to be influenced by factors such as temperature, soil and stress conditions, and can vary substantially (Then & Lorch,

2008). Huge variations in Bt content have, for instance, been found in genetically engineered cotton plants (Adamczyk et al., 2008). Further, there has been no systematic investigation to determine the Bt content in maize 1507 under varying environmental conditions. As a result, the true range of variation of Bt content in the plants is unknown.

- Some relevant data are missing completely: It is known that Bt toxins can be exuded into the soil through the roots of genetically engineered plants (see for example Saxena, 2002). However, although such data are relevant for the risk assessment of soil organisms, none has been made available.

Since the Bt content in maize 1507 is much higher than in other genetically engineered plants, failure to determine the exact concentration of Bt toxins in all parts of the plants calls the whole of the EFSA risk assessment into question. No final conclusions can be drawn on the risks to the environment from exposure to Bt toxins and the actual risks for non-target organisms such as larvae of protected butterflies.

2.2.2 Failing to assess the real risks for butterflies

It is known and accepted by EFSA that the toxin Cry1F affects another spectrum of sensitive insects than is the case with Cry1Ab (which is produced in crops like MON 810). However, the statement made by EFSA on these differences is questionable:

> *"It is well-documented that larvae of a range of Lepidoptera can be affected by the Cry1F protein with a spectrum of sensitivity which is quantitatively different from the Cry1Ab protein." (page 15)*

EFSA is suggesting that the spectrum of sensitivity between Cry1Ab and Cry1F is only *quantitatively* different. However, this assumption is not sufficiently based on facts. The observed differences in the spectrum of sensitivity of Bt toxins are likely to be based on differences in the binding site between Cry1F and Cry1Ab described by Hua et al. (2001) as well as by Tan et al. (2013). It is not clear why these differences should be considered only as quantitative and not as *qualitative* differences.

The observed differences are highly relevant for assessing the selectivity of the toxin and the risks for non-target organisms. The most relevant publication in regard to risk for non-target organisms is Hanley et al. (2003), which shows a huge difference in toxicity and selectivity between Cry1Ab and Cry1F in larvae of the greater wax moth. The greater wax moth (*Galleria mellonella*) is an important and often used model organism for assessing toxic effects in insects. Hanley et al. (2003) show that 1507 maize Cry1F is much more toxic to the greater wax moth than Cry1Ab in MON 810. While pollen with Cry1Ab did not show any significant impact on the larvae of the greater wax moth, pollen containing Cry1F was nearly 100 percent toxic.

> *"We found that the mortality of larvae fed Cry1F corn pollen was significantly greater than the mortality of larvae fed Cry1A(b) corn pollen or non-transgenic corn pollen ($P < 0.05$). In each trial Cry1F fed larvae showed 100% mortality."*

These findings were overlooked by EFSA (2005) but mentioned in a Testbiotech report in 2010 (Bauer-Panskus & Then, 2010) which was forwarded to EFSA by the EU Commission. EFSA's response was astonishing:

> *„The sensitivity of the pest species Galleria mellonella (see Annexes to EFSA, 2005) could*

not be quantified by Hanley et al. (2003) because, although the Cry1F protein clearly caused mortality, the non-standard bioassay technique involved a diet comprising solely of pollen offered in no-choice feeding trials.“ (EFSA 2011)

This statement is also questionable for several other reasons:

- According to EFSA (2011), the Hanley et al (2003) investigation is the only one performed on honey bees with Cry1F. EFSA accepted this publication (EFSA 2005) insofar as it showed no effects on honey bees. If this publication had not been accepted, there would be no peer reviewes data available on the risks of maize 1507 for honey bees.
- The most significant outcome of the Hanley et al. (2003) investigation is that the greater wax moth is highly sensitive to Cry1F while it is not sensitive to Cry1Ab. Thus Hanley et al. show large qualitative differences in the toxicity of CRy1Ab and Cry1F that do not seem to be a matter of exact quantification. Thus, the statement made by EFSA seems to be a deliberate misinterpretation of the Hanley et al. (2003) findings and an example of EFSA applying double standards to an individual publication.

If the findings of Hanley et al, 2003, are taken into account, it is evident that large parts of the EFSA risk assessment carried out in 2005 (as well as in later opinions which are largely based on the 2005 EFSA opinion), are based on false assumptions. Most of the publications mentioned by EFSA (2005) concern Cry1Ab. But Hanley et al. (2003) show that there are huge differences in the toxicity of the two toxins, especially in the most relevant group of non-target organisms which are butterflies (Lepidoptera). As mentioned, the greater wax moth (which belongs to the group of Lepidoptera commonly known as butterflies) is an important model organism that is used by many biologists to assess toxicity. Finding that Cry1F is toxic to the greater wax moth but Cry1Ab is not must be seen as a strong indication that further specific investigations with Cry1F are needed. Based on the findings of Hanley et al, 2003 it has to be assumed that more lepidopteran species can be endangered by the cultivation of maize 1507 than by MON 810. This is particularly relevant for protected butterflies found in regions where 1507 might be grown in the EU. The fact that EFSA first overlooked the findings of Hanley et al (2003) on the greater wax moth and then did not interpret these findings correctly, should be a reason for EFSA to withdraw its opinions as the agency recently did with its opinion on environmental risk assessment of maize 59122[2].
These flaws in the opinions of EFSA and the questions they raise still cannot be rectified or answered since most data are missing. Even after many years in which this application has been pending, there is still no peer reviewed publication on the effects of Cry1F pollen on any protected European butterfly species and the sensitivity of the most relevant species. This lack of knowledge is highly problematic, as butterflies (Lepidoptera) are the species deemed most sensitive to Cry1F toxin.

In 2011, the applicants forwarded two studies to EFSA on the effects of pollen from maize 1507 (Long et al., 2011a) and a stacked event 1507 x NK603 (Long et al., 2011b) on the European butterfly Painted Lady, known in Germany as the Distelfalter (*Vanessa cardui*). These studies have not been published in any peer reviewed journal and are not available to the general public. Up until now, these two studies from industry are the only ones to investigate the effects of maize 1507 on European lepidopteran. Both studies show reduced larval weights after consumption of a diet containing 10% pollen from maize 1507. Both studies have serious flaws. For example, only very few larvae were tested per group (8 to 12 in Long et al., 2011a, up to 15 per group in Long et al., 2011b). In effect, the statistical power of these two studies is extremely limited. It should be

2 http://www.efsa.europa.eu/en/efsajournal/pub/3443.htm

acknowledged that even within species there can be great differences in the sensitivity to Bt toxins, especially if species have a large regional distribution (Van Frankenhuyzen, 2009). This is especially relevant in the case of the Painted Lady, since it is a migrating butterfly species, stemming from North Africa and found in regions such as North America, Australia, Asia and Europe. There is no doubt that a dozen individuals originating from one source are not enough to perform risk assessment for this protected species.

Figure 1: Painted Lady, Source: Wikipedia, Author: Quartl

In the light of the substantial data gaps, EFSA has conceded that there are not enough studies on European butterflies:

> *"However, the EFSA GMO Panel does not agree with the applicant's conclusion that this study provided adequate evidence that there is a negligible risk of maize 1507 to non-target Lepidoptera in the EU. Firstly, as stated above, there is no evidence that the average sensitivity found for the single species V. cardui will be typical of other species of Lepidoptera or even of other Nymphalids. Secondly, the exposure assessment used by the applicant assumes that the host-plant is exclusively the nettle Urtica dioica when it is known that some populations of V. cardui are known to prefer thistles (Cirsium spp. and Carduus spp.) (Janz, 2005). Finally, the use of the data of Gathmann et al. (2006) on densities of Urtica in maize fields in the exposure assessment was incorrect."* (EFSA 2011)

EFSA proposes collecting crucial data that should have been made available before risk assessment after allowing cultivation (!):

> *"the EFSA GMO Panel recommends to carry out further field studies on non-target Lepidoptera. The purpose of these studies should be to estimate whether non-target Lepidoptera larvae, with a high sensitivity to the Cry1F protein, are in reality feeding on host-plants in and adjacent to maize fields at the time of pollen deposition, and if so (a) to estimate the proportions of these populations likely to be affected; and (b) to determine the overall effect on maintaining a favourable status of these populations."* (EFSA 2011)

For this reason, a case specific monitoring should be established according to the 2011 EFSA opinion:

> *"The EFSA GMO Panel recommends case-specific monitoring (CSM) to assess the efficacy of risk management measures put in place to reduce levels of risk and scientific uncertainty for (….) (2) the risk to sensitive non-target Lepidoptera from maize 1507 pollen. The EFSA GMO Panel considers that risk managers should adapt monitoring methodologies to their local receiving environments and management systems."*

In 2012, precautionary measures were proposed for protected habitats:

> *"Spatial arrangements of non-Bt-maize should always be placed to maximise the average distance of non-target lepidopteran larvae from the nearest source of maize 1507 pollen."* (2012b)

2.2.3 Data gaps on non-target organisms besides butterflies

Although the application has been pending for so many years there are still hardly any publications available on the effects of maize 1507 on other relevant non-target organisms (besides butterflies) such as soil organisms, pollinators, and aquatic organisms. As aforementioned, there is only one peer reviewed publication on the risks for honey bees (Hanley et al., 2003).

Health risks for fish, birds and mammals living in the wild have not been assessed. In fish and birds, tests were carried out to investigate nutritional effects but there have been no detailed studies on health effects. The feeding trial to investigate health effects in mice fed with maize kernels produced significant findings that were not investigated further (Dona & Arvanitoyannis, 2009). No studies have been performed on wild mammal species, or investigations conducted with other parts of the plants than kernels. Large-scale cultivation will bring many wild species into contact with these plants, which have a high content of Bt toxins in all parts.

There have been no detailed empirical investigations of the organisms in the receiving environments, including several tiers of the food web. Previous studies with Cry1Ab on insect predators (Obrist et al., 2006) and slugs (Zurbrügg & Nentwig, 2009), have shown that Bt toxins can accumulate in the food web, reaching higher content than in the genetically engineered plants. Further, carabids (Peterson et al., 2009), thrips (Akthar et al., 2010) and earthworms (Zeilinger et al., 2010) can distribute Bt toxins in the environment. None of these routes of exposure have so far been investigated in relation to maize 1507. EFSA should have requested an assessment of the effects on insect predators, rodents, birds and other animal species.

2.2.4 True level of complexity ignored by EFSA

Risk assessment of Bt toxins as performed by EFSA and companies like Pioneer/Dupont is largely based on the assumption of selective toxicity of Bt toxins for certain groups (orders) of insects such as Lepidoptera (butterflies), Diptera (flies), Coleoptera (beetles). However, this classification is only partially based on facts. So far, it is not fully understood how Bt toxins work and the whole issue is controversial (Pigott & Ellar, 2007). A warning from Van Frankenhuyzen, (2009) that many Bt proteins can be toxic across the borders of specific groups of insects must be taken very seriously. According to Van Frankenhuyzen, (2009), there is evidence showing that this classification needs to be reconsidered:

> *"The first classification of crystal proteins reflected that order specificity, and included only one family with dual specificity. Since then, testing has revealed cross-order toxicities in 15 of the 87 families distinguished at the secondary rank that are known to be pesticidal."*

Further van Frankenhuyzen (2009) states that the investigation into toxin selectivity has so far

largely been biased towards previous assumptions, while only a small proportion of the toxins was really empirically tested:

> *„Of the crystal protein families reported to have toxicity that is restricted to one of the three major insect orders, few were actually tested outside that order (Diptera: 5 out of 21 families; Coleoptera: 9 out of 20; Lepidoptera: 6 out of 15), illustrating how the selection of tests species is biased towards the order in which activity was initially reported. Such bias obviously limits the evaluation of cross-order toxicities, and more cross-order activities are likely to be uncovered as testing is expanded."*

There are several peer reviewed publications showing that Bt toxins can indeed impact on a wider spectrum of species than previously thought. There are even indications that Bt toxins might cause adverse health effects in mammals (Thomas & Ellar, 1983; Shimada et al., 2003; Huffmann et al., 2004; Ito et al., 2004; Mesnage et al., 2012; Bondzio et al., 2013). For example, these publications also have to be taken into account when it comes to the risk assessment of mammalian species living in the wild.

The data gaps – which are especially relevant for Cry1F - concern the sensitivity of non-target species, mode of action, changes in the toxicity after transferring the toxins into plants, potential synergies and other combinatorial effects that might enhance toxicity (see below). EFSA did not elaborate on the uncertainties and partially contradictory findings on the impact of Bt toxins. It only made a very general statement:

> *"Whilst scientific uncertainty has been expressed about the mode of action and specificity of Cry proteins (see publications by Hilbeck and Schmidt , 2006; Then, 2009), the EFSA GMO Panel considers that these are sufficiently understood to inform the ERA of Cry-expressing plants. The general mode of action of Cry proteins is to bind selectively to specific receptors on the epithelial surface of the midgut of susceptible lepidopteran species, leading to death of larvae through pore formation, cell burst and subsequently septicemia (Broderick et al., 2006, 2009; OECD, 2007; Bravo and Soberón, 2008; Raymond et al., 2009; Soberón et al., 2009; Van Frankenhuyzen et al., 2010; Sanahuja et al., 2011). The lepidopteran-active Cry1F protein belongs to the group of three-domain Cry proteins."* (EFSA 2011)

All the references made by EFSA in this statement refer to investigations carried out on Cry1Ab, once more highlighting a lack of specific publications dealing with Cry1F. A further reason for concern is that EFSA only mentions some relevant publications, but does not really investigate uncertainties in the mode of action and specificity of Bt toxins. Besides the publication from van Frankenhuyzen, there are several other publications calling into question the role of specific receptors in general (Crickmore, 2005; Zhang et al., 2006; see also Pigeott & Ellar, 2007). The gaps in the current understanding of the mode of action of Bt toxins are very relevant for the risk assessment of non-target organisms pointed out by Lövei et al. (2009):

> *„Both the Cry1Ab and Cry1Ac toxins are members of the large family of three-domain Cry toxins, meaning that they share homologous aminoacid sequences in three regions, which are implicated in receptor-specific binding and toxin specificity. Four distinct classes of receptors have been identified: cadherin-like proteins, aminopeptidases, alkaline phosphatases and certain glycolipids, and it is clear that the understanding of receptor and toxin specificity is far from complete. Even well-studied Cry toxins have an incompletely determined range of toxicity. Although it is clear that Cry1Ab and Cry1Ac are toxic mainly to Lepidopteran species, it is not yet possible to infer toxin specificity from toxin structure, and thus toxin specificity of a Cry toxin is a scientific hypothesis, not a scientific fact.*

Moreover, truncation and mutagenesis of synthetic toxins might alter their range of toxicity compared with the native toxins.“

In conclusion, EFSA did not assess the true known level of uncertainty. Neither did EFSA consider potential synergies and accumulated effects as highlighted by some of the publications cited its statement. Synergistic effects can become highly problematic for non- target organisms, because they can cause higher toxicity and selectivity (see Broderick et al., 2006 and 2009; Kramarz et al., 2007 and 2009; Then, 2009). These effects may impact the ecosystems on various levels. For example, Duan et al. (2008) point out that co-stressors can impact the health of honey bees. In general, there needs to be systematic screening of synergistic or accumulated effects on a sufficiently broad range of organisms (Dolezel et al., 2011). According to Directive 2001/18, EFSA is obliged to take into account the accumulated effects of genetically engineered organisms. However, potential combinatorial effects of parallel cultivation of maize MON 810 and maize 1507 or combinatorial feeding were not considered.

It also has to be mentioned that most of the studies on Cry1F were performed with the toxin as produced by microorganisms. EFSA saw these tests as comparable because the structures of the insecticidal proteins are almost identical. However, as Saeglitz et al. (2006) show, Bt toxins with identical structures but derived from differing sources can vary extensively in their toxicity. Therefore, uncertainties remain about whether data derived from toxins derived from bacteria can be used in the risk assessment of maize 1507. The DNA inserted into the plants seems to be a synthetic variant of the naturally occurring toxin. It is well known that the insertion of the relevant DNA into plants and accompanying changes in the structure of the Bt toxins can cause a change in its toxicity (Hilbeck & Schmid, 2006). We are not aware of any investigation carried out with Cry1F to examine this question. All in all, there are further uncertainties on toxicity, selectivity and risks of maize 1507.

The way in which EFSA is dealing with gaps in knowledge regarding the toxicity of Cry1F shows the agency is deliberately following a ”do not look, do not find approach”, denying the actual risks and not taking into account the current limits of knowledge and reporting only a small proportion of the actual uncertainties regarding the safety of maize 1507.

2.2.5 Modelling without reliable data

EFSA (2011) admits at least some uncertainties regarding the risks for larvae of protected butterflies. The agency does not agree with the applicant that there is sufficient data to assess the actual risks for this group of non-target organisms. EFSA concludes that there are indeed risks for highly sensitive butterfly (Lepidoptera) species. This is in contradiction to their first opinion:

“The EFSA GMO Panel concludes that the cultivation of maize 1507 could have the following adverse effects on the environment in the context of its intended uses (…) reductions in populations of certain highly sensitive non-target lepidopteran species where high proportions of their populations are exposed over successive years to high levels of maize 1507 pollen deposited on their host-plants.” (EFSA, 2011).

However, the aforementioned uncertainties show that risks can hardly be limited to the highly sensitive lepidopteran, and are relevant for a much broader range of species. Further, the way in which the risks for highly sensitive lepidopteran are assessed is highly questionable. EFSA uses a modelling scheme developed together with of members of the GMO Panel (Perry et al., 2010; 2011;

2012). Basically, this modelling scheme tries to combine data on the sensitivity of protected Lepidoptera to Bt toxins with the Bt content in the pollen and the dispersal of maize pollen in the environment. Lang et al. (2011), Camastra et al. (2013) and Holst et al. (2012) have all criticised the model developed by Perry et al. and used by EFSA. Their publications show that it can generate major uncertainties, even in the case of Cry1Ab, where much more data are available (see Lang et al., 2011). Using modelling schemes is tantamount to educated guesswork since there are no reliable date available for the abundance of protected butterfly species around maize fields, the uptake of pollen by the larvae, the content of Bt in pollen, the sensitivity of the various butterfly species or interactions with other stressors. For example, Camastra et al. (2013), come to the conclusion that the EFSA data

> *"cannot be absolutely considered to fulfil the worst case scenario, recommended in the Directive 2001/18/EC of the European Community."*

There are still severe doubts about the deposition of pollen. While the modelling scheme used by EFSA is based on a single study from the US (Wraight et al., 2000), data from field trials in Germany point to higher pollen deposition values (Hofmann, 2010, 2011), so that the EFSA model is likely to underestimate the exposure of sensitive butterflies to pollen.

EFSA seems to be aware of major uncertainties associated with its method of modelling. That is why the agency is passing on the responsibility of requesting further data to the risk manager. EFSA now proposes to collect the necessary data that should have been made available before risk assessment during commercial cultivation:

> *"the EFSA GMO Panel recommends to carry out further field studies on non-target Lepidoptera. The purpose of these studies should be to estimate whether non-target Lepidoptera larvae, with a high sensitivity to the Cry1F protein, are in reality feeding on host-plants in and adjacent to maize fields at the time of pollen deposition, and if so (a) to estimate the proportions of these populations likely to be affected; and (b) to determine the overall effect on maintaining a favourable status of these populations."* (EFSA 2011)

2.3. Risks of spraying with glufosinate

Maize 1507 is engineered to be resistant to the herbicide glufosinate. Dow AgorSciences advertise the application of glufosinate (brand name Liberty) on maize 1507 (marketed as Herculex I) as follows:

> "In addition to insect protection, corn hybrids with the Herculex I trait contain LibertyLink® technology which provides tolerance to glufosinate-ammonium herbicides by the expression of a protein generally referred to as PAT. Corn plants possessing this tolerance can be directly sprayed after emergence with glufosinate-ammonium herbicides (e.g., LIBERTY® herbicide), allowing for broad spectrum weed control without herbicide damage to the corn plant. Benefits to the farmer are convenient and effective weed control that ultimately enhances yield potential for the corn." [3]

According to draft decision of the EU Commission (2013), spraying glufosinate on maize 1507 is not foreseen in the EU. At the same time, glufosinate herbicides are still on the market in the EU

3 http://www.dow.com/productsafety/finder/herc.htm

and could easily be applied on maize 1507 without anyone knowing. There are substantial health concerns associated with the use of this herbicide, which is marketed under brand names such as Basta and Liberty. It will not be removed from the EU market until 2017[4]. EFSA should therefore have conducted risk assessment in cases where maize 1507 has been sprayed with glufosinate. This issue has been almost completely left aside in the opinions of EFSA. In 2005, the agency made a dubious statement without real substance:

> *"The Panel considers that the presence of the pat gene and the use of glufosinate is not likely to give an increased impact on biodiversity in most situations."*

4. Failures of the Commission

The way in which the EU Commission dealt with this application is particularly confusing. Several erroneous decisions were made that are undermining the high level of protection for the environment and human health as required by EU regulations.

In 2007, the EU Commission presented a draft decision on maize 1507 (EU Commission, 2007). In this document, the EU Commission exposed several deficiencies in the opinion of EFSA (EFSA, 2005). Amongst others, the Commission stated:

> *"In view of the above there are still serious indications that the cultivation of 1507-maize could (i) adversely affect non-target organisms, such as particular species of butterflies, (ii) increase the presence of parasitoids in caterpillars and thus modify the food chains, (iii) generate an uneven concentration of the Bt-toxin on plants of the same locations,(iv) influence the composition of the microbial community and (v) lead to the persistence of Bt-toxin in aquatic environments. As the studies indicate that the spread of these potential effects in the environment would be wide, the concentration of Bt-toxin uneven, the affected organisms and eco-systems considerably diverse and the potential damage on the environment irreversible, it is not possible to establish appropriate management measures which would effectively mitigate the potential damage on the environment."* (Point 21)

According to the Commission, the degree of uncertainty in the EFSA opinion is too high:

> *"Taking into account (i) EFSA's opinions [...] and (ii) the on-going scientific debate on key issues regarding effects on non-target organisms, long term effects as well as the methodology to assess these effects, the degree of uncertainty attached to the results of the evaluation of the available scientific information as regards cultivation is considerably high. This uncertainty is such that it could compromise the high level of protection of the environment as foreseen by Article 174(2) of the EC Treaty and Directive 2001/18/EC."* (Point 24)

In conclusion, the EU Commission is of the opinion that maize 1507 cannot be approved for cultivation in the EU:

> *"In the light of the above, Zea mays L. line 1507 should not be approved for placing on the market for cultivation in the Community. This Decision is provisional and subject to review, depending on the development of scientific knowledge. The relevant scientific evidence may be made available to the Commission by any source, including the notifier."*

4 Regulation of the European Parliament and of the Council on the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC http://www.europarl.europa.eu/oeil/file.jsp?id=5372312

However, this draft decision was withdrawn even though the questions raised by the EU Commission had not been resolved. In 2007, Pioneer/ Dupont initiated legal action at the EU Court of Justice (Case T-139/07) to gain market authorisation for cultivation of 1507 despite the concerns of the Commission.

Instead of defending its substantiated position at the Court, the Commission sent a list of more or less arbitrary publications to EFSA for further assessment. EFSA presented its opinion in 2008 (EFSA, 2008) after superficially assessing the documents. Interestingly, in its statement, the agency denied the risks posed by the high content of Bt toxin in maize 1507. The Commission accepted this without criticism, even though it is in contradiction to the 2005 EFSA opinion. In 2009, the Commission then asked the Member States to take a vote on the application for cultivation of maize 1507 on the basis of the flawed EFSA opinions. Consequently, the pending court case was stopped, because the ECJ thought it had become unnecessary.

Experts from the Member States voted on the application in February 2009. No qualified majority was reached and a simple majority of the Member States voted against the approval. Maybe the Commission now remembered its previous doubts about the risk assessment. In any case, the application was not referred to EU Member States for a second vote as foreseen by EU regulations. Instead, the EU Commission again requested further information from EFSA. This failure of the Commission to proceed with the application as required by EU regulations enabled Pioneer/ Dupont to file a new case (T-164/10) at the EU Court of Justice, which led to a ruling in their favour in 2013.

As a result, the EU Commission has helped the company twice. Firstly, by not defending its substantiated scientific reasoning in court and secondly, by failing to fulfill the EU regulation requirements.

In its draft decision the Commission (EU Commission, 2013) is once again serving the interests of industry. EFSA recommended a case specific monitoring of the impact on non-target organisms, and in 2011 (EFSA, 2011 and 2012 it proposed precautionary measures to safeguard butterflies in protected areas (EFSA 2012). None of these measures were included in the draft decision of the EU Commission. The only measure suggested by the Commission is the general surveillance of risks for non-target organisms by using questionnaires for farmers or cooperation with existing networks of nature observation. These measures are not adequate to detect or prevent environmental damage and are not even meant for the systematic collection of missing data. In this regard, the draft decision of the EU Commission is not backed by the EFSA opinion.

5. Conclusions and recommendations

In the light of seriously erroneous decisions made by the EU Commission, substantial errors at EFSA and a level of uncertainty that is too high, the Member States should not adopt the draft decision of the EU Commission to allow maize 1507 for cultivation in the EU. Instead, the issues and questions raised by the EU Commission in 2007 should be upheld. EFSA should officially withdraw its previous opinions from 2005, 2006 and 2008 and also reconsider its opinions from 2011 and 2012. Risk analysis of maize 1507 should be performed in a sound and much more systematic manner before any further decisions are made.

References

Adamczyk, J.J. Jr., Perera, O., Meredith, W.R. (2008) Production of mRNA from the cry1Ac transgene differs among Bollgard lines which correlates to the level of subsequent protein. Transgenic research, 18(1), 143-149

Akhtar, Z.R., Tian, J.C., Chen, Y., Fang, Q., Hu,C., Chen, M., Peng, Y.F., Ye, G.Y. (2010) Impacts of six Bt rice lines on nontarget rice feeding thrips under laboratory and field conditions. Environmental Entomology, 39(2): 715-726.

Bauer-Panskus, A. & Then, C. (2010) Testbiotech opinion concerning the application for market approval of genetically modified maize 1507 (DAS-Ø15Ø7-1). http://www.testbiotech.org/node/369

Bondzio, A., Lodemann, U., Weise, C., & Einspanier, R. (2013). Cry1Ab Treatment Has No Effects on Viability of Cultured Porcine Intestinal Cells, but Triggers Hsp70 Expression. PloS one, 8(7): e67079.

Broderick, N.A., Raffa, K.F., Handelsman, J. (2006) Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. PNAS, 103(41): 15196-15199.

Broderick, N.A., Robinson, C.J., McMahon, M.D., Holt, J., Handelsman, J., Raffa, K.F. (2009) Contributions of gut bacteria to *Bacillus turingiensis*-induced mortality vary across a range of Lepidoptera. BMC Biology, 7: 11.

Camastra, F., Ciaramella, A., Staiano, A. (2013) A note on some mathematical models on the effects of Bt-maize exposure. Environmental and Ecological Statistics, 1-9. http://arxiv.org/pdf/1308.6243

Crickmore, N. (2005) Using worms to better understand how *Bacillus thuringiensis* kills insects. Trends in Microbiol, 13: 347-350.

Dona, A., Arvanitoyannis I.S. (2009) Health Risks of Genetically Modified Foods. Critical Reviews in Food Science and Nutrition, 49: 164-175.

Dolezel, M., Miklau, M., Hilbeck, A., Otto, M., Eckerstorfer, M., Heissenberger, A., Tappeser, B., Gaugitsch, H. (2011) Scrutinizing the current practice of the environmental risk assessment of GM maize applications for cultivation in the EU. Environmental Sciences Europe, 23:33.

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

EFSA (2005) Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/ES/01/01) for the placing on the market of insect-tolerant genetically modified maize 1507, for import, feed and industrial processing and cultivation, under Part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds, The EFSA Journal, 181: 1-33.

EFSA (2006) Clarifications of the Scientific Panel on Genetically Modified Organisms following a

request from the Commission related to the opinions on insect resistant genetically modified Bt11 (Reference C/F/96/05.10) and 1507 (Reference C/ES/01/01) maize. http://www.efsa.europa.eu/de/efsajournal/pub/1561.htm

EFSA (2008) Scientific Opinion of the Panel on Genetically Modified Organisms on a request from the European Commission to review scientific studies related to the impact on the environment of the cultivation of maize Bt11 and 1507. The EFSA Journal, 851: 1-27. http://www.efsa.europa.eu/en/efsajournal/pub/851.htm

EFSA (2010) Request from the European Commission to review scientific studies related to the Testbiotech report on the cultivation of maize 1507. Adopted part of the minutes of the 61st plenary meeting of the Scientific Panel on Genetically Modified Organisms held on 20-21 October 2010.http://www.efsa.europa.eu/en/events/event/gmo101020.htm.

EFSA (2011) Scientific Opinion updating the evaluation of the environmental risk assessment and risk management recommendations on insect resistant genetically modified maize 1507 for cultivation. EFSA Journal, 9(11): 2429.

EFSA (2012a) EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion updating the risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize 1507. EFSA Journal, 10(10):2933. doi:10.2903/j.efsa.2012.2933. Available online: www.efsa.europa.eu/efsajournal

EFSA (2012b) EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion supplementing the conclusions of the environmental risk assessment and risk management recommendations on the genetically modified insect resistant maize 1507 for cultivation. EFSA Journal, 10(11): 2934. Available online: www.efsa.europa.eu/efsajournal

EU Commission (2007) Draft Commission Decision of [...] concerning the placing on the market, in accordance with Directive 2001/18/EC of the European Parliament and of the Council, of a maize product (*Zea mays* L., line 1507) genetically modified for resistance to certain lepidopteran pests and for tolerance to the herbicide glufosinate-ammonium. ENV/07/, www.testbiotech.org/node/979

EU Commission (2013) Proposal for a COUNCIL DECISION concerning the placing on the market for cultivation, in accordance with Directive 2001/18/EC of the European Parliament and of the Council, of a maize product (Zea mays L., line 1507) genetically modified for resistance to certain lepidopteran pests, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=COM:2013:0758:FIN:en:PDF

Hanley, A.V., Huang, Z.Y., Pett, W.L. (2003) Effects of dietary transgenic Bt corn pollen on larvae of *Apis mellifera* and *Galleria mellonella*. Journal of Apicultural Research, 42(4):77-81.

Hilbeck, A. & Schmidt, J.E.U. (2006) Another view on Bt proteins – How specific are they and what else might they do? Biopesticides International, 2(1): 1-50.

Holst, N., Lang, A., Lövei, G. (2013) Increased mortality is predicted of *Inachis io* larvae caused by Bt-maize pollen in European farmland. Ecological Modelling, 250: 126-133.

Hofmann, F., Epp, R., Kruse, L., Kalchschmied, A., Maisch, B., Müller, E., Kuhn, U., Kratz, W., Ober, S., Radtke, J., Schlechtriemen, U., Schmidt, G., Schröder, W., v. d. Ohe, W., Vögel, R., Wedl, N., Wosniok, W. (2010). Monitoring of Bt-Maize pollen exposure in the vicinity of the nature reserve Ruhlsdorfer Bruch in northeast Germany 2007 to 2008. Umweltwissenschaften und Schadstoff-Forschung, 22(3), 229-251.

Hofmann, F., Otto, M., Kuhn, U., Ober, S., Schlechtriemen, U., Vögel, R. (2011) A new method for in situ measurement of Bt-maize pollen deposition on host-plant leaves. Insects, 2(1): 12-21.

Hua, G., Masson, L., Jurat-Fuentes, J. L., Schwab, G., Adang, M. J. (2001) Binding Analyses of *Bacillus thuringiensis* Cry -Endotoxins Using Brush Border Membrane Vesicles of Ostrinia nubilalis. Applied and Environmental Microbiology, 67(2):872-879.

Huffmann, D.L., Abrami, L., Sasik, R., Corbeil, J., van der Goot, G., Aroian R.V. (2004) Mitogen-activated protein kinase pathways defend against bacterial pore-forming toxins. Proceedings of the National Academy of Sciences of the United States of America , 101: 10995-11000.

Ito, A., Sasaguri, Y., Kitada, S., Kusaka, Y., Kuwano, K., Masutomi, K., Mizuki, E., Akao, T., Ohba, M. (2004) *Bacillus thuringiensis* crystal protein with selective cytocidal action on human cells. J Biol
Chem, 279: 21282-21286.

Kramarz, P. E., Vaufleury, A., Zygmunt, P. M. S., Verdun, C. (2007) Increased response to cadmium and bacillus thuringiensis maize toxicity in the snail Helix aspersa infected by the nematode *Phasmarhabditis hermaphrodita*. Environ Toxicol Chem, 26(1): 73-79.

Kramarz, P., de Vaufleury, A., Gimbert, F., Cortet, J., Tabone, E., Andersen, M., Krogh, P. (2009) Effects of Bt-Maize Material on the Life Cycle of the Land Snail *Cantareus aspersus*. Applied Soil Ecology, 42: 236-242.

Lang, A., Brunzel, S., Dolek, M., Otto, M., Theißen, B. (2011) Modelling in the light of uncertainty of key parameters: a call to exercise caution in field predictions of Bt-maize effects. Proceedings of the Royal Society B: Biological Sciences, 278(1708): 980-98.

Long, S., Ostrem, J., Kang, Q. (2011a) Laboratory Characterization of Painted Lady Butterfly (*Vanessa cardui*) Larval Response to DAS-Ø15Ø7-1 Maize Pollen in Artificial Diet. Unpublished.

Long, S., Ostrem, J., Kang, Q. (2011b) Laboratory Characterization of Painted Lady Butterfly (*Vanessa cardui*) Larval Response to DAS-Ø15Ø7-1xMON-ØØ6Ø3-6 Maize Pollen in Artificial Diet. Unpublished.

Lövei, G.L., Andow, D.A., Arpaia, S. (2009) Transgenic insecticidal crops and natural enemies: a detailed review of laboratory studies. Environmental Entomology, 38(2): 293-306.

Matthews, D., Jones, H., Gans, P., Coates, S., Smith, L.M.J. (2005) Toxic secondary metabolite production in genetically modified potatoes in response to stress. Journal of Agricultural and Food Chemistry, 53(20): 7766-7776.

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

Mesnage, R., Clair, E., Gress, S., Then, C., Székács, A., Séralini, G.-E. (2012) Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. Journal of Applied Toxicology, 33(7): 695-699.

Nguyen, H.T. & Jehle, J.A. (2007) Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize MON810: Journal of Plant Diseases and Protection, 114(2): 82-87.

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

Perry, J. N., Devos, Y., Arpaia, S., Bartsch, D., Gathmann, A., Hails, R.S., Kiss, J., Lheureux, K., Manachini, B., Mestdagh, S., Neemann, G., Ortego, F., Schiemann, J., Sweet, J.B. (2010) A mathematical model of exposure of non-target Lepidoptera to Bt-maize pollen expressing Cry1Ab within Europe. Proceedings of the Royal Society B: Biological Sciences, 277: 1417-1425.

Perry, J.N., Devos, Y., Arpaia, S., Bartsch, D., Gathmann, A., Hails, R.S., Kiss, J., Leureux, K., Manachini, B., Mestdagh, S., Neemann, G., Ortego, F., Schiemann, J., Sweet, J.B. (2011) The usefulness of a mathematical model of exposure for environmental risk assessment. Proceedings of the Royal Society B: Biological Sciences 278: 982-984.

Perry, J.N., Devos, Y., Arpaia, S., Bartsch, D., Ehlert, C., Gathmann, A., Hails, R.S., Hendriksen, N. B., Kiss, J., Messean, A., Mestdagh, S., Neemann, G., Nuti, M., Sweet, J. B., Tebbe, C.C. (2012) Estimating the effects of Cry1F Bt-maize pollen on non-target Lepidoptera using a mathematical model of exposure. Journal of Applied Ecology, 49: 29-37.

Peterson, J.A., Obrycki, J. J., Harwood, J. D. (2009) Quantification of Bt-endotoxin exposure pathways in carabid food webs across multiple transgenic events. Biocontrol Science and Technology, 19: 613-625.

Pigott, C. R. & Ellar, D. J. (2007) Role of Receptors in *Bacillus thuringiensis* Crystal Toxin Activity. Microbiol Mol Biol Rev, 71(2): 255-281.

Saeglitz, C., Bartsch, D., Eber, A., Gathmann, K., Priesnitz, K. U., Schuphan, I. (2006) Monitoring the Cry1Ab Susceptibility of European Corn Borer in Germany. J Econ Entomol, 99(5): 1768-1773.

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

Shimada, N., Kim, Y.S., Miyamoto, K., Yoshioka, M., Murata, H. (2003) Effects of *Bacillus thuringiensis* Cry1Ab toxin on mammalian cells. J Vet Med Sci, 65: 187-191.

Székács, A., Weiss G., Quist, D., Takács, E., Darvas, B., Meier, M., Swain T., Hilbeck A. (2011) Inter-laboratory comparison of Cry1Ab toxin quantification in MON 810 maize by ezyme-immunoassay. Food and Agricultural Immunology, 23(2): 99-121.

Tan, S. Y., Cayabyab, B. F., Alcantara, E. P., Huang, F., He, K., Nickerson, K. W., & Siegfried, B. D. (2013). Comparative binding of Cry1Ab and Cry1F *Bacillus thuringiensis* toxins to brush border membrane proteins from *Ostrinia nubilalis*,*Ostrinia furnacalis* and *Diatraea saccharalis*(Lepidoptera: Crambidae) midgut tissue. Journal of invertebrate pathology, 114(3): 234-240.

Then, C. & Lorch, A. (2008) A simple question in a complex environment: How much Bt toxin do genetically engineered MON810 maize plants actually produce? In: Breckling, B., Reuter, H. &Verhoeven, R. (eds), 2008, Implications of GM-Crop Cultivation at Large Spatial Scales, Theorie in der Ökologie 14. Frankfurt, Peter Lang.

Then, C. (2009) Risk assessment of toxins derived from *Bacillus thuringiensis* - synergism, efficacy, and selectivity. Environ Sci Pollut Res Int, 17(3): 791-797.

Thomas, W.E. & Ellar, D.J. (1983) *Bacillus thuringiensis* var israelensis crystal delta-endotoxin: effects on insect and mammalian cells in vitro and in vivo. Journal of Cell Science, 60(1): 181-197.

Van Frankenhuyzen, K. (2009) Insecticidal activity of Bacillus thuringiensis crystal proteins. Journal of Invertebrate Pathology, 101: 1-16.

Wraight, C.L., Zangerl, A.R., Carroll, M.J., Berenbaum, M.R. (2000) Absence of toxicity of Bacillus thuringiensis pollen to black swallowtails under field conditions. Proceedings of the National Academy of Sciences, 97(14): 7700-7703.

Zeilinger, A.R., Andow, D.A., Zwahlen, C., Stotzky, G. (2010) Earthworm populations in a northern US cornbelt soil are not affected by long-term cultivation of Bt maize expressing Cry1Ab and Cry3Bb1 proteins. Soil Biology & Biochemistry, 42: 1284-1292.

Zhang, X., Candas, M., Griko, N.B., Rose-Young, L., Bulla, Jr., L.A. (2006) A mechanism of cell death involving an adenylyl cyclase/PKA signaling pathway is induced by the Cry1Ab toxin of *Bacillus thuringiensis*. Proc.Nat. Acad Sci USA, 103: 9897-9902.

Zhang, L., Hou, D., Chen, X., Li, D., Zhu, L., Zhang, Y., Li, J., Bian, Z., Liang, X., Cai, X., Yin, Y., Wang, C., Zhang, T., Zhu, D., Zhang, D., Xu, J., Chen, Qu., Ba, Y., Liu, J., Wang, Q., Chen, J., Wang, J., Wang, M., Zhang, Q., Zhang, J., Zen, K., Zhang, C.Y. (2011) Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA, Cell Research 22: 107-126.

Zurbrügg, C. & Nentwig, W. (2009) Ingestion and excretion of two transgenic Bt corn varieties by slugs. Transgenic Research, 18 (2): 215-25.

Annex:

Chronologic overview of proceedings for the EU market application of maize 1507

- 2001: Pioneer/ Dupont applies for EU market authorisation of maize 1507
- 2004: EFSA delivers its first opinion on 1507 for food and feed usage (EFSA 2004)
- 2005: EFSA delivers its first opinion on cultivation of maize 1507. A statement is made about the high concentration of Bt toxins in the pollen (EFSA 2005)
- 2005: The Commission approves maize 1507 for feed.
- 2006: EU approves maize 1507 for food and feed (food/feed).
- 2006: EFSA publishes clarifications to its previous opinions (EFSA 2006).
- 2007: The EU Commission raises a series of questions on deficiencies in the risk assessment of maize 1507 and maize Bt 11 (EU Commission 2007). These questions are not answered. Instead, the EU Commission asks EFSA to review some publications.
- 2008: EFSA publishes a statement on some new publications. A high concentration of Bt toxins in the pollen is denied (EFSA 2008)
- 2009: EFSA publishes its opinion for renewal of market authorisation of maize 1507 for usage in food and feed.
- 2009: The expert committee of the EU Member States takes a vote on the application of 1507 for cultivation, however no majority is reached. The EU Commission fails to forward the proposal to the Council of the Member States as required by EU regulations.
- 2011: EU Commission renews market authorisation of maize 1507 for food feed.
- 2010: the EFSA GMO Panel confirmed that, considering recent studies and advances in methodology, there was a need to further analyse the potential adverse effects of maize 1507 pollen on non-target Lepidoptera, as well as to clarify its recommendations to risk managers.
- 2011: the EFSA GMO Panel concludes that risk management measures may be needed under specific conditions in order to reduce the exposure of sensitive protected butterfly species to pollen from maize 1507. A high concentration of Bt toxins in the pollen is confirmed again (EFSA, 2011). No official process for comments by experts of Member States or the public was started.
- 2012: the European Commission requests the EFSA GMO Panel to deliver a Scientific Opinion updating the risk assessment and/or management of maize 1507 in the light of recent scientific publications.
- 2012: EFSA publishes two opinions supplementing and updating risk assessment and risk management. EFSA proposes precautionary measures to protect non-target butterflies to increase the distance between pollen and protected species (EFSA 2012 a and 2012 b). No official process for comments by experts of Member States or the public was started.
- 2013: the EU Court of Justice decides that the EU Commission was not acting in line with EU regulations because after the vote of the expert committee in 2009, the proposal to allow cultivation of 1507 was not forwarded to the Council of Member States to vote again.
- 2013: the EU Commission again makes a proposal to allow cultivation of maize 1507.