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Testbiotech opinion concerning the application for market approval of genetically modified maize 1507 (DAS-Ø15Ø7-1)

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Content

Summary

In the present study Testbiotech assesses genetically modified maize line 1507 with respect to the pending approval process for cultivation in Europe. 1507 maize produces the insecticidal protein Cry1F as well as the PAT protein that makes the plant tolerant to pesticides containing glufosinate. In comparison with Cry1Ab toxin, which is expressed in MON 810 or Bt11 maize, the efficacy of Cry1F is different, although both toxins originate from a soil bacterium (*Bacillus thuringiensis*) and belong to the group of Bt toxins. These toxins are believed to affect only certain insects, although their mode of action is not fully understood.

The following conclusions have been drawn by Testbiotech from the opinions published by EFSA, some comments from Member States and several stakeholders, as well as from scientific literature:

- EFSA's risk assessment is largely based on analogies and conclusions are drawn from other Bt toxins (Cry1Ab) which differ in their mode of action as well as their effects. From a scientific point of view, this approach is flawed. EFSA took no notice of the obvious differences between the toxins. EFSA did not correctly assess scientific data that indicates high risks of 1507 maize to non-target organisms. There is a proof of adverse effects in a species (greater wax moth) being abundant in Europe. It is not a protected species, but used as a model organism in toxicology tests. Since no other non-target European butterfly species were investigated in the context of 1507, these results have to be taken very serious.

- 1507 maize produces high amounts of Bt toxin in pollen. For example, the toxin content is much higher than in the MON810 genetically engineered maize line. Because many non-target organisms are exposed to this part of the transgenic plant, a detailed investigation of the toxicity of 1507 maize would be absolutely necessary before any market approval. EFSA's conclusion that the risks of 1507 maize to non-target organisms are not higher than in the case of MON810 is pure speculation and is in contradiction to scientific facts.

- To date, very few peer reviewed studies on 1507 maize and Cry1F toxin have been published. A high proportion of these papers was published by the biotech industry. There is an almost complete lack of independent studies or long-term studies.

- The tests undertaken by the applicants almost exclusively used bacterially produced Bt toxin and not the transgenic plant. Specific risks of genetically modified plants therefore may not covered. Furthermore, the applicants' tests are not published and therefore lack public scrutiny.

- During the process of gene transfer, numerous fragments of the gene construct as well as other genetic material were transferred unintentionally along with the construct. Possible effects on plant components, human health and environment were not thoroughly investigated by EFSA.

- Studies on the effect of Cry1F on soil are lacking almost completely. Two out of three studies were published by the applicants, only one of them was undertaken under field conditions. From the evidence presented it can't be concluded that there are no detrimental effects on soil and soil microorganisms. The EFSA assessment concerning the effects of 1507 maize on soil is therefore highly speculative.
- The effects of the use of glufosinate have not been evaluated by the EFSA's GMO panel, whereas EFSA pesticide experts state that this pesticide has negative effects on reproduction. According to the German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV), glufosinate will even be banned throughout Europe by 2017.
- Scientific data only recently published shows that the cultivation of 1507 maize has led to field resistance of certain target organisms after only a short period of time. Resistance of plant pests is a major risk in Bt crops, as is pest replacement, that was also observed with Cry1F.
- Feeding studies that were not thoroughly assessed by EFSA are linked to detrimental health effects.

The EFSA opinions on 1507 maize are flawed, incomplete, speculative or contradictory in major parts and lack scientific scrutiny. They therefore do not provide a sufficient basis for the approval of 1507 maize for cultivation in the EU. Testbiotech accuses EFSA of negligence, because the authority did not even request or evaluate very basic scientific data that is absolutely essential for the risk assessment of 1507 maize.

1. Introduction

Two new transgenic maize lines could be approved for cultivation in the EU in the course of 2010. If there is no qualified majority against approving Bt11 and 1507 in the EU Council of Ministers, the application will be passed on to the EU Commission. Given the Commission's positive stance towards biotech crops, it is very likely that both applications will be granted.
Transgenic maize 1507 (also called TC1507, DAS Ø15Ø7-1, Herculex ® I Insect Protection or Cry1F maize) is an insect-resistant and herbicide-tolerant maize developed by the U.S. corporations Pioneer/DuPont and Dow AgroSciences (Mycogen Seeds). 1507 maize was first approved in the U.S. in 2001. It contains the gene cry1F, which makes the plant toxic to certain plant pests. It also contains the pat gene, which makes the plant tolerant to herbicides containing glufosinate as the active ingredient (tradenames Basta or Liberty). According to the applicants, 1507 maize is toxic to different corn pests including the European Corn Borer (*Ostrinia nubilalis*), Southwestern Corn Borer (*Diatraea grandiosella*), Fall Armyworm (*Spodoptera frugiperda*), Black Cutworm (*Agrotis ipsilon*), Pink Stem Borer (*Sesamia spp.*) and Western Bean Cutworm (*Striacosta albicosta*).

According to the applicants, MON810 (Cry1Ab) and 1507 (Cry1F) differ in their effects on some pests (such as *Striacosta albicosta*). Buntin (2007) found out that Cry1F shows a higher efficacy against Fall Armyworm than Cry1Ab. These are some of the reasons why data for the risk assessment of Cry1F cannot be derived from data about Cry1Ab.

The EU Commission received an application for the cultivation of 1507 maize in 2003. In 2005, the European Food Safety Authority (EFSA) published its opinion on 1507 maize. The agency comes to the conclusion that 1507 maize is as safe as conventional maize (EFSA, 2005). After criticism was voiced by several EU member states, EFSA published a clarification on Bt11 and 1507 in 2006. Again, both lines are cleared (EFSA, 2006). In 2007, the Commission prepared an analysis of EFSA's opinion and compiled an extensive list of deficiencies (Commission of the European Communities, 2007).

This list has never been assessed by EFSA. Instead, the Commission submitted to EFSA a list of various publications for evaluation. This list did not include new studies on 1507. In 2008, EFSA published an opinion concerning the studies proposed by the Commission and once again concluded that there are no safety risks (EFSA, 2008). An application for approval of 1507 maize based on this latest opinion was discussed by the Standing Committee on the Food Chain in February 2009. However, no qualified majority for or against the application could be reached.

2. Molecular genetics

Event 1507 was obtained by ballistic transformation. The gene construct contains the cry1F gene (more precisely, cry1Fa2) from soil bacterium *Bacillus thuringiensis* (*ssp. aizawai*) as well as the pat gene (for resistance to herbicides with glufosinate as the active ingredient). The process of genetic transformation obviously leads to numerous unexpected changes in the plant DNA. For example the process of genetic engineering led unintentional to superfluous DNA as well as fragments of the genetic construct. It is a fact that many transgenic plants already approved for cultivation or import in the EU also suffer from extensive restructuring, omissions or duplication of the insert (Collonier et al., 2003). According to EFSA, numerous additional fragments can be found in 1507 maize (EFSA, 2005):

- a truncated form of the Cry1F gene,
- fragments of the plasmid,
- fragments of the pat gene,
- fragments of the ubiquitin promotor,
- fragments of the termination sequence,
- fragments of maize chloroplast DNA,
- sequences resembling retrotransposons (maize DNA).

Unintended changes are also mentioned by La Paz et al., 2006:

„Sequences 5'-flanking TC-1507 full-length insert were characterized and showed multiple rearrangements involving insert and maize chloroplast fragments."

Unintended gene constructs in transgenic plants may lead to the production of new ingredients and are therefore of major importance for the assessment of possible effects on human health as well as environmental impact.

1507 maize contains at least two „open reading frames" (630 and 753 base pairs) that could lead to the production of new RNA and proteins. This possibility is confirmed by EFSA.

„Bioinformatics analysis of the insert sequence indicates the presence, in addition to the two intended transcripts detected in the transgenic plant, further ORF of one of more than 300 bp length (ORF4: 630 bp) fragment on PHI8999A and a number of other ORFs (including ORF3, which is 753 bp long) spanning the junctions between maize DNA and DNA originating from the transformation fragment. This raises the possibility that new putative fusion proteins could be produced. [...] Northern analysis revealed no expression of ORF4 but a weak signal was detected using RT-PCR, which thus

indicated that the detected mRNA originates from a read-through product of the gene Cry1F." (EFSA, 2005)

Recent studies of the genetic makeup of 1507 maize brought to light that 61 different new proteins with no known biological function are being produced (EFSA, 2009):

„The new molecular data included updated bioinformatics analysis of the border regions of the insert 1507 and of all putative reading frames (defined from STOP codon to stop codon) spanning the 5 ‚and 3' insert - genomic DNA junctions or resulting from rearrangement of the original construct intended for insertion (eg junctions between the complete and / or partial copies of the insert PHI8999A original). Homology searches with the flanking regions as query sequences identified high scores with maize genomic DNA, homologous to retrotransposable elements, which raises no safety concern. In silico analysis of all reading frames at all new junctions resulting from the insertion, using updated databases, identifed 61 putative peptides with no similarity to known allergens, toxins or other bioactive peptides."

Several scientific papers suggest that severe disturbances of plant DNA are common in genetically engineered plants produced by ballistic transformation (e. g. Latham et al., 2006; Makarevitch et al., 2003). Because of the many potential effects on the genome, the different gene activities in genetically engineered plants should be studied as in MON810 (Zolla et al., 2008). As the numerous technical flaws of 1507 maize may lead to a change in gene activity, EFSA should have asked for proteomic and metabolomic testing.

3. Changes in the plants components

In 1507 maize, there are many changes in the expression of plant compounds, also according to EFSA:

„In summary, the analysis of nutrient composition of maize kernels from line 1507 (glufosinate treated and non-treated) occasionally revealed statistically significant differences in some compounds. For example, kernels of maize in 1507 contained higher overall levels of potassium, linoleic acid, linolenic acid, and tocopherols, as well as lower levels of fat, manganese, stearic acid, oleic acid, cysteine, methionine, and vitamin B1, than control kernels in the season 1998-1999. The levels of protein, amino acids (Ala, Asp, Glu, Gly, His, Leu, Phe, Pro, Ser, Thr, Tyr, and Val), and potassium were increased, while the level of vitamin B2 was decreased in kernels of maize in 1507 (both sprayed and non-sprayed) compared with control kernels 1999. In the 2000 season, ash, amino acids (Ala, Phe, Tyr), and potassium were increased, while manganese was decreased in kernels of maize line 1507 (both sprayed and non-sprayed) compared with controls." (EFSA, 2005)

EFSA usually tries to interpret significant differences in plant constituents by claiming that differences fall within the range of natural variability. In 1507 maize however, differences seem to be beyond the ranges reported in literature.

„All analytical data were either very close to or within the ranges published in the literature." (EFSA, 2005)

These significant differences are a further indication of substantial disturbances in gene regulation in the genetically engineered plants. In addition to a more accurate analysis of gene activity, it would be absolutely necessary to assess the actual changes in plant metabolism under different environmental conditions in order to exclude, for example, harmful antinutritive effects. But instead of asking the applicant for such systematic studies, EFSA did not take into account the observed differences just because they vary from region to region:

„Statistically significant differences were occasionally observed in some GM plants, for example increased overall levels of carbohydrates and decreased levels of fat in forage of maize line 1507 (both sprayed and non-sprayed) in the 2000 season. However, there were no differences that were observed consistently over years and at each location." (EFSA, 2005)

From a scientific point of view, this conclusion is highly questionable, as unintended interactions between genome and environment might be the cause of these changes (see e. g. Then & Lorch, 2008). Also the Austrian authorities request more information regarding possible genome – environment interaction, also taking into account the genetic background of certain varieties and impact

of the treatment with glufosinate[1]:

"No assessment of expression of cry1F and pat in different genetic backgrounds is possible due to missing information on the origin of tested GM maize 1507 hybrids. We therefore request submission of data from the notifier from recent trials in the EU assessing the differences in expression between different varieties, years and locations and systematically assessing the effect of the Glufosinate-treatment on expression of transgenes."

Given the available data, the genetically modified plants can't be regarded as substantially equivalent, thus much more data is necessary for the risk assessment of these plants (EFSA, 2006 a).

1 Application EFSA-GMO-RX1507, Comments and opinions submitted by Member States during the three-month consultation period

4. Environmental risks

Very few studies have been published so far on the ecological effects of 1507 maize (Lövei et al., 2009). Thus, the EFSA based its opinion on only three published studies that deal specifically with Cry1F protein or the event 1507 and its potential environmental risks (EFSA, 2005): one study that determines the effects of 1507 maize on the monarch butterfly (Hellmich et al., 2001) and two laboratory studies testing the breakdown of Cry1F toxin in soil (Herman et al., 2001, Blackwood & Buyer, 2004). In addition, EFSA relies on company data that was not published in peer reviewed journals. The quality of these tests therefore can't be evaluated by independent experts.

In its opinion EFSA (2005) tries to make up for this lack of basic data by comparing Cry1F with other Bt toxins, particularly Cry1Ab (as produced by MON810 or Bt11). This comparison is highly questionable, as Cry1F stems from a different subspecies of *Bacillus thuringiensis*, has a different spectrum of activity and a different molecular weight (68 kD, compared to 63 kD in Mon810). Furthermore, the toxin binds to different parts of the insect gut as Cry1Ab (González-Cabrera et al., 2006). EFSA fails to take a systematic look at the differences between the efficacy of Cry1Ab and Cry1F, even though according to the applicants the toxins have different effects.

Even the EU Commission criticised EFSA for their environmental assessment. In a draft decision calling for a ban on 1507 maize (Commission of the European Communities, 2007), which was subsequently withdrawn without any scientifical reasons (Then & Lorch, 2008 b), the Commission states:

„Although the majority of the studies are mainly available from maize expressing Bt toxin another, CryIAb (instead of Cry1F for Zea mays L. line 1507), EFSA indicates in its opinion that ‚effects of Bt plants expressing different Cry proteins are considered to be comparable'."

The second EFSA opinion on maize 1507 (and Bt11) demonstrates that even then no further peer reviewed studies on 1507 maize were available (EFSA, 2008). A literature review confirms that even up to 2010 only very few further studies of any use for ecological risk assessment were published. In the light of this situation, EFSA should have been calling for further studies. Furthermore, the EFSA opinions are negligent because until now neither the mechanisms of action of Bt toxins in detail is sufficiently known, nor have interactions with other factors been adequately studied (Then, 2009). Given the lack of data concerning the toxicity of Cry1F, the EFSA environmental risk assessment as well as the agency's conclusions are unacceptable.

4.1 Content of Bt toxin in pollen

According to several publications the content of Bt toxin of maize 1507 is very high in pollen. The content is more than a hundred times higher in 1507 than in Bt 11 (EFSA, 2005):

"On the other hand, according to the data presented in the respective dossiers, Cry1F concentration in 1507 maize pollen is higher in comparison with CryIAb concentration in Bt11 pollen (1.3 ng Cry toxin mg-1 plant protein in Bt11 pollen compared with 160 ng Cry protein mg-1 plant protein in 1507 maize pollen)." (EFSA, 2005)

A higher content of Bt toxin in comparison with other Bt crops is also confirmed by further publications such as EPA (2001), Mendelsohn et al. (2003) and USDA (2004). But these facts did not result in any detailed risk assessment by EFSA. On the opposite EFSA concluded surprisingly that adverse effects in non target lepidoptera being very unlikely:

"Considering toxicity and exposure of Cry1F, the Panel agrees with the assessment of the applicant that risk of exposure of non-target lepidoptera to harmful toxin concentrations via 1507 maize pollen is negligible and that adverse impacts on populations are very unlikely." (EFSA, 2005)

Even more dubious, in later opinions as published by EFSA the authority takes a U-turn in assessing the Bt content in the plant (EFSA, 2006, 2008). Contrary to their first opinion, the GMO panel is now claiming the Bt content in maize 1507 is similar to that in Bt11 and MON810 and similar biological effects could be expected (EFSA, 2008):

"The amount of biologically active Cry protein in pollen of maize Bt11, 1507 and MON810 is relatively low resulting in similar toxicological effects on non-target lepidopteran populations exposed to pollen from these events (Mendelsohn et al., 2003), in contrast to maize Bt176 which contains higher levels of the Cry1Ab protein in pollen (Hellmich et al., 2001)."

This assumption by EFSA is simply wrong. The publication mentioned by EFSA (Mendelsohn et al., 2003) does not state that maize 1507 only produces low amounts of biologically active Cry protein. On the contrary, Mendelsohn (2003) presents a table to compare Bt toxins contents in genetically engineered plants that reveals significant higher levels of the Bt toxin in maize 1507 (see figure 1).

Fig. 1: Bt content in various genetically engineered crops (source: Mendelsohn et al., 2003)

Crop	Leaf (ng/mg)	Root (ng/mg)	Pollen	Seed (ng/mg)	Whole plant (ng/mg)
Cry1Ab corn Bt11	3.3	2.2-37.0 (a)	<90 ng/g b)	1.4	NS
Cry1Ab corn MON810	10.34	NS	<90 ng/g b)	0.19-0.39	4.65
Cry1F corn TC 1507	56.6-148.9	NS	113.4-168.2 a)	71.2-114.8 a)	830.2-157.2 a)
Cry3A potato	28.27	0.39 c)	NS	NS	3.3
Cry1Ac cotton	2.04	NS	11.5 ng/g	1.62	NS

All values ref fresh weight unless otherwise noted. NS, not submitted at the time of the assessment.
a) ng/mg total protein b) per dry weight c) Tuber

Maybe EFSA (2006, 2008) is relying on some speculative interpretation that Cry1F might be biologically less active compared to Cry1Ab, but this is just another false presumption. The investigations by Hanley et al. (2003) show the opposite is true (see 4.2). Hanley et al. (2003) found that plants producing Cry1F are much more toxic for some European Lepidoptera than those producing Cry1Ab.

In addition to the wrong interpretation by EFSA of the published scientific findings, it has to be emphasised that the overall data published so far are in no way sufficient for drawing final conclusions on risks caused by Cry1F. For example, no systematic testing to compare the toxicity of Cr1F to Cry1Ab in European Lepidoptera has been published.

Further, an indispensable prerequisite for assessing the Bt crops are standardised protocols (by ring testing) for the measurement of the Bt protein in the plant. Further systemic testing of Bt content under changing environmental conditions has to be requested since the Bt content shows a high variability (Nguyen & Jehle, 2007; Then & Lorch, 2008 a). These basic necessary basic data do not even exist for MON810, which has been cultivated for more than ten years. The fact that these basic data were not requested in the case of 1507 can only be interpreted as some kind of systematic negligence.

4.2 Effects on non target organisms

In the opinion of EFSA (2005) there is only one published study which explicitly concerns the effects of maize 1507 on non-target organisms. In this study (Hellmich et al., 2001) a lower toxicity was found for the monarch butterfly. These data (concerning a butterfly not abundant in Europe) seem to be the reason for EFSA's conclusion that there are no risks to non-target organisms in Europe.

But Hanley et al. (2003) show that 1507 maize Cry1F is much more toxic to the greater wax moth (*Galleria mellonella*) than Cry1Ab in MON810. While pollen with Cry1Ab did not show any significant impact, pollen containing Cry1F was nearly 100 percent toxic to the wax moth.

"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."

This study clearly shows the need for more in-depth investigations concerning the specific risks of maize Cry1F for non-target organisms. The study by Hanley et al. (2003) was already published when EFSA published its first opinion (2005) but it is not mentioned. Taking into account this study, the toxicity of Cry1F has to be judged as being much higher for certain European Lepidoptera than can be concluded from Hellmich's publication (2001).

A survey of recent publications showed an extremely low number of studies concerning the effects of 1507 on target organisms. Wolt et al. (2005) investigated effects on butterflies from Asia. Pioneer and Dow scientists did not find any impact on the abundance of insects and other arthropods in fields with maize 1507 (Higgins et al., 2009). Mason et al. (2010) studied the effects of maize 1507 on a lacewing species from North and Latin America. Dolezel et al. (2009) mention that sensitivity data (LD50) were conducted with 15 lepidopteran species, most of them being potential pests. These data were not published.

It can be concluded that so far only one single study has been published on effects on butterflies being abundant in Europe (Hanley et al., 2003). This study showed significant effects on the greater wax moth, leaving it open if these effects are caused by higher susceptibility or by higher concentration of the Cry1F in pollen of maize 1507:

„In the greater wax moth study, we found that mortality in larvae fed Cry1F corn pollen was significantly higher than those fed Cry1A(b) and non-transgenic corn pollen. Even though both Cry1F and Cry1A(b) are toxins targeting lepidopteran insects, only Cry1F corn pollen showed a significant effect. This might be due to the fact that Event TC1507 produces more protein in pollen (31–33 ng Cry1F protein per mg pollen, US Environmental Protection Agency, 2001) than the Cry1A(b) protein produced by Event Bt11 (1.1–7.1 ng/mg pollen,

Sears et al., 2001). However, we can not rule out the possibility that wax moth larvae are more sensitive to Cry1F protein, because Bt proteins are known to be highly species-specific."

In the light of these findings, further studies for risk assessment in maize 1507 are absolutely necessary.

The applicants have provided further data for the market authorisation of maize 1507. But these data have not been published in scientific (peer reviewed) journals. According to Hilbeck et al. (2008) these studies in most cases (24 of 32 cases) were not performed with genetically engineered plants but solely with Bt proteins produced by bacteria. The origin of the Bt protein can cause substantial differences in the observation of its effects. Several publications show that Bt proteins from bacteria can be different in their toxicity compared to those in transgenic plants (see Hilbeck et al., 2006). For example the Bt toxin as produced in MON810 can still be effective, even if pest insects acquired resistance against the protein produced by bacteria (Li et al., 2007). Thus studies using bacterial proteins cannot be seen as being sufficient for assessing the safety of Bt crops.

4.3 Effects in soil

Even less published scientific data is available on the effects of maize 1507 in soil. Only two studies on Cry1F and maize 1507 were available at the date when EFSA published its opnions (EFSA, 2005, 2008). Blackwood & Buyer (2004) show significant effects occurring after a short period of time in one type of soil and conclude long term investigations are necessary. In the other publication cited by EFSA (Herman et al., 2001) only bacterial derived Cry1F proteins were used. After a short period of only three days the Dow company experts could find no more Cry1F proteins. In a recently published Dow study (Shan et al., 2008) no toxin was found after three years of cultivating maize 1507. The authors were using a specific test protocol for their measurements. The results differ from those being performed on Cry1Ab which show their presence in soil over a longer period of time (see for example Stotzky 2004).

In general, the recovery rate of Bt in soil is relatively low (see Baumgarten & Tebbe, 2005). In the light of this problem, the method as developed by Dow (Shan et al., 2008) needs validation in ring testing before conclusions can be drawn from its result. So far EFSA (2005) has not even requested data on maize 1507 and Bt content in roots. It has also not been published if the toxin is directly transmitted into the soil via exudate from the roots as is known with MON810 (Saxena et al., 1999).

Altogether only three studies concerning the effects of Cry1F in soil have been published. Two of them were performed by the industry, and only one was

conducted under environmental conditions. Nevertheless EFSA (2005) assumed these studies to be sufficient to show no effects on soil and soil organism can be expected.

4.4 Resistance in pest insects

First reports about the emerging resistance of pest insects in fields growing maize 1507 were published some years ago. In the magazine Nature Biotechnology Moar et al. (2008) report:

„In this case, there was a change in field performance resulting in field failures, and subsequently it was demonstrated that S.frugiperda showed no mortality at the highest concentration of Cry1F tested in laboratory bioassays. As a result, there was an immediate voluntary discontinuation of commercial cultivation of Cry1F Bt corn in Puerto Rico."

This report was confirmed by Tabashnik et al. (2009), who present the most comprehensive overview so far on the resistance of pest insects to Bt crops. According to Tabashnik et al. (2009) the fall armyworm (*Spodoptera frugiperda*) established resistance against Cry1F after the Bt maize had been grown over a period of only four years. Never before was such resistance observed to have emerged so fast in the fields:

„Field-evolved resistance of S. frugiperda to Bt corn producing Cry1F occurred in 4 year in the United States territory of Puerto Rico (Matten et al. 2008), making this the fastest documented case of field-evolved resistance to a Bt crop. This is also the first case of resistance leading to withdrawal of a Bt crop from the marketplace."

Further, the usage of Cry1F for controlling pest insects has to be put into question because it is known it is only up to 80 percent effective in killing insects such as the western bean cutworm (Eichenseer et al, 2008). There is a substantial risk that the cultivation of these plants will foster the quick spread of resistant populations and speed up pest replacement (Then, 2010).

The recent publications on resistance and low efficacy of Cry1F indicate that maize 1507 has a high potential to enhance pest resistance in the fields. *Spodoptera frugiperda* and *Striacosta albicosta* are not abundant in Europe. Further investigations in European pest insects have to be conducted here. For example Dolezel et al. (2009) mention that basic data showing the efficacy of Cry1F against Sesamia species was not determined which is a relevant pest in Spain.

The risk of ‚pest replacement' is also relevant in this context but has not been investigated by EFSA (Then, 2010). Recent investigations showing that maize 1507 can cause pest replacement were published by Virla et al. (2010): by killing the target organism *S. frugiperda*, another pest insect *Dalbulus maidis* showed a higher incidence in the fields.

4.5 Effects from combined use with herbicides

The risks of combined use of maize 1507 with the herbicide glufosinate was not assessed by EFSA. Apparently this is due to a systemic deficiency also prevailing in other opinions of EFSA on herbicideresistant plants. The GMO panel declares itself not responsible for assessing the toxicity of complementary herbicides used in combination with transgenic plants. In the case of glufosinate which can be applied in maize 1507 this problem is extremely relevant.

According to the German Ministry of Agriculture, glufosinate showing toxicity to reproductive organs will be banned from the market at the latest in 2017 as the EU Directive on pesticides will be redrafted (BMELV, 2009). EFSA's pesticide experts have also concluded glufosinate is toxic for humans, animals and the environment (EFSA, 2005a). In the light of this evidence the possible risks of glufosinate application in maize 1507 needs to be integrated in risk assessment with the GMO application. Further, the EU Commission has left no doubt that in the case of herbicide-tolerant crops (GMHT) the EU Directive 2001/18 also requires the complementary herbicide be assessed. As DG Sanco explains in a letter to EFSA (European Commission, 2008):

"Under Directive 2001/18/EC it is necessary to cover under the GMO risk assessment the possible effects on biodiversity and non-target organisms which any individual GMHT crop may cause due to the change in agricultural practices (including those due to different herbicide uses)".

"In sum, the consequences of the change in agricultural practices due to the herbicide use in GMHT plants have to be duly considered within the environmental risk assessment under Directive 2001/18/EC". cantly decreased in female rats fed 33% 1507 maize compared with those fed 33% near isogenic control and reference maize." (EFSA, 2005)

5. Health effects

Pioneer presented the results of a ninety day feeding trial with rats. This study was accepted by EFSA but shows some major deficiencies and cannot be seen as proof that maize 1507 does not pose a risk to human health. A prerequisite for performing proper feeding trials is the use of isogenic lines as the comparator. But as EFSA (2005) states the company only used a variety with a similar genetic background:

„A 90-day oral toxicity study has been performed on rats in five groups (12 animals/sex/group) fed diets containing 1507 maize (11 and 33%), a non transgenic control line with comparable genetic background (11 and 33%), and another non transgenic maize line as reference (33%).“

Feeding trials with no direct possibility of comparing the genetically engineered plants with isogenic lines can be used to mask undesired effects (Lorch & Cotter, 2005). The Dutch authority also criticises the study because it was conducted with too small a number of animals. Thus the outcome is not valid from a statistical point of view. (BAC, 2009). It is difficult to conceive why EFSA accepted the study without any critical comment.

Further, the study showed a significant drop in the number of certain white blood cells, an effect that was not assessed properly by EFSA (2005):

„In addition, serum counts of eosinophil leukocytes were statistically significantly decreased in female rats fed 33% 1507 maize compared with those fed 33% near isogenic control and reference maize.“ (EFSA, 2005)

EFSA (2005) assumes that these effects are not of biological relevance, arguing the findings as only being significant in female animals. But Seralini et al. (2009) explain that differences between sexes are typical for many adverse health effects and should not be a reason to dismiss significant findings.

EFSA (2008) mentions a second 90 day feeding study commissioned by Pioneer (MacKenzie et al., 2007). The outcome of this study is interpreted by EFSA as valid proof of the safety of maize 1507, EFSA thus accepting Pioneer‘s assumptions. But Dona & Arvanitoyannis (2009), who did further analysis of the data, present different results in a peer reviewed paper:

1. effects in hepatic enzymes:

“Alterations have also been observed in hepatic enzymes after consumption of raw rice expressing GNA lectin (Poulsen et al., 2007), GM Bt with vegetative insecticidal protein gene (Peng et al., 2007) and in DuPont’s subchronic feeding study in rats fed diets containing GM corn 1507 (MacKenzie et al., 2007). These alterations in hepatocyte cells and enzymes may be indicative of hepatocellular damage.“

2. smaller kidneys:

“Smaller kidneys were developed in DuPont’s study in rats fed diets containing GM corn 1507 (MacKenzie et al., 2007).“

3. a decreased number of red blood cells and a changed hematocrit:
"DuPont's study in rats fed diets containing GM corn 1507 showed a decrease in red blood cell count and hematocrit of females (MacKenzie et al., 2007)."

4. a decreased number of certain white blood cells:
"DuPont's subchronic feeding study in rats fed diets containing GM corn 1507 showed that eosinophils concentration in females was decreased (MacKenzie et al., 2007)."

The Austrian authorities also put in question the interpretation of the results of MacKenzie et al., 20071:

"The results showed significantly higher feed consumption in males of the high-dose group. Furthermore haematology analyses revealed lower mean red cell count, hemoglobin and number of eosinophils only in females of the high-dose group. The clinical chemistry evaluation showed a lower level of alkaline phosphatase in males of the high dose group. Additionally the kidney weight was lower in these male rats. Mean body weight gain in male and female rats fed diets containing 33% 1507 was higher on most test days than that of rats fed the control diet, but mean body weight gains were similar over individual test day intervals. Such transient effects should not be underrated, since they do not mean that the test substance is safe in the long run. Aberrant feeding behaviour only found on a daily or weekly basis thus not presenting a consistent trend, could be triggered by an aversion to or preference of the new feed or any numbers of physiological short-term needs of the animals. Short-term feeding tests with adult animals are not sufficient to prove safety beyond doubt. Feed effects are more likely to become apparent in times of high performance, e.g. reproduction. Therefore more generation tests should be conducted, especially when transient significant differences are discovered even in a 90day study with rodents."

These comments raise substantial doubts regarding the food safety of maize 1507 and should engender a reassessment of the risks of maize 1507. But to date EFSA has not assessed Dona & Arvanitoyannis's publication (2009), nor taken into account the comments by the Austrian authorities.

6. Recommendations

EFSA's opinions should be rejected for several reasons. The opinions are wrong in several details. They are not sufficient because of a substantial lack of data. They are no longer valid because of more recent publications. Any market authorisation on the basis of the existing opinions would be acting negligently and irresponsibly from a scientific point of view.

Since EFSA recently presented a draft for new guidelines for the risk assessment of genetically engineered plants (EFSA, 2010), the final version of these should be drawn up before maize 1507 undergoes further assessments in depth. Any further discussion about risk assessment should take particular account of the deficiencies and open questions as presented by the European Commission in 2007 (Commission of the European Communities, 2007). Up to now none of the points raised by the Commission has been answered.

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