A critique of the European Food Safety Authority's opinion on genetically modified maize MON810

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SUMMARY

Environmental safety

The European Food Safety Authority's (EFSA's) opinion on the environmental aspects of the cultivation of the genetically modified (GM) maize variety MON810 is woefully inadequate[1].

Failure to admit scientific uncertainty

There is much scientific evidence suggesting serious threats to biodiversity, yet EFSA admits no uncertainty on the environmental safety of MON810.

For example:

– Evidence suggests that non-target organisms such as butterflies and moths could be harmed by the cultivation of Bt maize. However, key laboratory studies on European species are, so far, absent. This critical issue has been raised by Member States. But instead of admitting that this is an area of uncertainty, EFSA produced its own model (which has not been peer-reviewed) and recommends that monitoring specifically for such effects is not needed, despite the fact that this is one of the main environmental concerns of MON810.

– It is accepted that MON810 exudes Bt proteins through roots into the soil. However, the fate of these proteins is not well understood. The accumulation of Bt proteins, and exposure of soil organisms to Bt cannot be excluded, nor can effects on soil microorganisms. However, scientific findings are dismissed and the uncertainty not admitted.

The uncertainty around the impacts of MON810 on the environment should be enough grounds for EFSA to at least declare that this maize has the potential to cause adverse effects and recommend that it should not be cultivated in the EU. But EFSA fails to admit the uncertainty of it's findings – and fails to safeguard the European environment.

Failure to consider Europe's diverse biogeographical regions

The environmental risk assessment data submitted by Monsanto does not adequately encompass European biogeographical regions. This is important because Europe is so diverse and the specific conditions of the European biogeographical regions, in which MON810 maize potentially could be grown, need to be considered.

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[1] Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMORX-MON810) for the renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON810; (2) feed consisting of and/or containing maize MON810, including the use of seed for cultivation; and of (3) food and feed additives, and feed materials produced from maize MON810, all under Regulation (EC) No 1829/2003 from Monsanto. *The EFSA Journal* (2009) 1149, 1-84.

Human safety

Several failures, shortcomings and omissions have been identified in the human safety assessment of the scientific opinion of EFSA on MON810. Under such circumstances its safety cannot be guaranteed and it poses a potential risk to human and animal health. These risks have been inadequately investigated by EFSA.

1) **The toxicological assessment of MON810 is not valid.** Based on the scientific references provided by EFSA, the assessment of the human toxicology of MON810 was either referenced wrongly or undertaken on a completely different GMO, namely MON863. Either way, the data provided by EFSA for the toxicological assessment of MON810 is not valid.

2) **EFSA has developed a new criterion for GMOs: "Not known = safe".** New unknown RNA fragments have been identified which are derived partly from the insert of the synthetic transgene from MON810 and the maize genome. These have the potential to produce new "putative recombinant" proteins as computer modelling suggests. EFSA agrees that these new proteins do not show similarity (or homology) with any known protein. However, instead of asking Monsanto to assess the toxicology properties of these unknown proteins, EFSA simply regarded them as safe without any further scientific studies or reference to peer-reviewed literature. The way EFSA comes to the conclusion on the safety of unknown novel proteins is a long way from any recognised scientific standard.

3) **EFSA is silent on unknown genetic fragments in their assessment of MON810.** In their earlier assessment of NK603 maize, EFSA looked at the potential risk from unknown RNA and DNA fragments developed as an unintended side product of the transgenic insert. However, in the assessment of MON810, EFSA is silent on this topic. In the scientific literature, the the role of DNA/RNA instimulating an immune, or allergenic response (immunostimulatory) in mammals, is getting more and more attention. Thus, these unknown RNA/DNA fragments may be important in determining the potential of MON810 to cause changes to the immune system or allergies in humans and animals. The silence of EFSA on the unknown DNA and RNA fragments of MON810 is not justified and of poor scientific standard.

4) **EFSA has made conflicting statements in the opinion on MON810.** Despite acknowledging the presence of new proteins EFSA then states that there are no new constituents and therefore a toxicological assessment is not needed.

5) **EFSA hides it sources of scientific information.** EFSA vaguely refers to scientific literature or data without citing the source of this information. For the reader it is impossible to check whether the information provided by EFSA is based on scientific data or not. Without correct scientific citation this opinion is not valid and again shows a low scientific standard of reporting.

6) **The detailed structure of the genetic insert in MON810 remains unknown.** EFSA accepted Monsanto's argument not to update its information on molecular characterization and flanks sequencing although there are questions arising about RNA and DNA fragments around the insert in MON810 maize. This is a serious issue since fragments of synthetic transgene from MON810 have been detected in the blood of animals.

7) **EFSA is not balanced when examining the peer-reviewed scientific literature.** EFSA sees shortcomings in scientific articles which show a risk of GM plants. In contrast, those articles which suggest that there is little risk were tolerated by EFSA although member states have identified shortcomings in those studies. This is a great imbalance in the way EFSA looks at scientific studies.

8-10) **EFSA has omitted studies on MON810 that indicate a risk or ask for further evaluation.** Why EFSA has failed to reference such studies, although they can be easily identified in scientific databases, is not clear. This goes in line with the impression that EFSA is shy to provide critical data on the safety of MON810.

The EFSA opinion on MON810 is inadequate to guarantee the safety of MON810. Important studies are ignored and safety concerns dismissed. MON810 contains unknown fragments of RNA and DNA, and unknown new proteins. Both could be important in determining the allergenicity and toxicity of MON810 in humans and animals. However, from the references EFSA provides and from the data it considers, it is clear that a thorough toxicological examination has not been made. The evaluation of MON810 has to be at the highest scientific standard – which EFSA has not able to provide.

Contamination of non-GM crops

There are other concerns regarding MON810 that fall outside of EFSA's remit. For example, co-existence is highly problematic. Non-GM maize (i.e. conventional and organic) is highly likely to become contaminated in Europe. There is no liability legislation in place that would award compensation for farmers whose crops are contaminated and therefore devalued by GM maize in Europe. This crucial aspect must be considered in terms of the cultivation of MON810.

ENVIRONMENTAL SAFETY

EFSA fails to admit uncertainty in the environmental risk assessment

In order to enable a decision-maker to take an informed decision, s/he has to be able to understand the underlying certainties and uncertainties and where in particular important gaps in our knowledge exist.

Although the European Food Safety Authortiy (EFSA) has conducted an extensive literature review and detailed many studies, it is the interpretations made from these studies where EFSA fails to protect the environment. Repeatedly, effects are noted but considered "unlikely", without any clear criteria on which this was based.

Interactions between the GM plant and non-target organisms (Section 6.1.4)

MON810 has been genetically modified to be toxic to certain species of moths and butterflies (Lepidoptera), e.g. the European corn borer (*Ostrinia nubilalis*), which are pests of maize. However, larvae of non-target moths and butterflies, for example the European peacock butterfly (*Inachis io*) may inadvertently ingest the Bt toxin whilst feeding on plants growing near Bt maize field. The effects of pollen from Bt maize on larvae of the monarch butterfly in North America is the most well known example of this phenomenon (Losey et al. 2001, Sears et al. 2001). Long-term exposure to Bt pollen from MON810 caused reduced survival of monarch butterfly larvae to adulthood (Dively et al. 2004). Many species of butterflies in Europe are already facing multiple threats, such as climate change and loss of habitat (Thomas et al. 2004), additional stress from exposure to Bt pollen could further threaten certain species of butterflies and moths. **Thus, there is a very real possibility that non-target organisms, such as butterflies, will be harmed by cultivation of Bt maize.**

In Section 6.1.4.1, EFSA lists publications that find the Bt protein moving up trophic levels that might affect predators, and conclude that "*the exposure to Cry1Ab protein differs between predatory taxa due to variability in phenology and feeding habits*". EFSA then list publications that consider the hazard, including those that found adverse effects (such as Naranjo 2009 and Meissle et al. 2005). But also points to studies that have found no effect. The science here is equivocal and EFSA should have admitted uncertainty.

On lacewings, EFSA lists the studies that have found adverse effects but considers "*lepidopteran larvae are not considered an important prey, especially after their first moult*". But this ignores the possibility that feeding preferences may change if the Lepidoptera become easy prey because they are affected by the Bt toxin. EFSA admits that "*chronic effects cannot be excluded completely*".

For the critical ladybird study (Schmidt et al. 2009), EFSA considers it as *"an outcome that needs to be confirmed based on more quantitative data (both on food intake and actual protein concentration). The EFSA GMO Panel is of the opinion that these data*

are not sufficient to identify a hazard or indicate a new mode of action of Cry proteins on the coccinellid species tested".

For invertebrate parasitoids, EFSA concluded that the *"results* [from studies] *suggest an effect on the parasitoid when delivered via the host feeding on plant tissue",* but this effect is not referred to again.

For this section, EFSA concludes that *"Rearrangements of species assemblages at different trophic levels are commonly associated with any pest management practice. The EFSA GMO Panel is of the opinion that maize MON810 will not cause reductions to natural enemies that are significantly greater from those caused by conventional farming where pesticides are used to control corn borers."* On the contrary, these studies give early indications that MON810 could affect populations of species at these low tropic levels, with unknown implications. Again, it is the admission of uncertainty that is lacking.

Non-target Lepidoptera (Section 6.1.4.2)

The section of the EFSA opinion on non-target Lepidoptera is a critical part of the environmental risk assessment. The Spanish competent authority's environmental risk assessment (Spanish Biosafety Commission 2009) reported that information on the potential adverse effects on relevant European Lepidoptera was lacking. This is critical as one of the principal concerns regarding MON810 is its potential to affect non-target Lepidoptera, some of which are protected in Europe, e.g. peacock butterfly (*Inachis io*).

EFSA lists all the studies that have noted adverse effects but considers that *"data on some aspects of exposure, such as phenology, are rare within Europe."*

Instead of admitting that this is an area of uncertainty, EFSA surprisingly built its own simulation model. *"In order to explore possible scenarios for the exposure of European species of butterflies to maize MON810 pollen, the EFSA GMO Panel built a simulation model to help quantify the risk assessment."* This is simply unacceptable. EFSA prides itself on only taking peer-reviewed studies into account. Yet this simulation has not been subject to peer-review, or indeed, any type of review. It is simply concocted by members of the panel. This is no way to conduct an environmental risk assessment and should be inadmissible. The possibility of adverse effects on non-target organisms should be enough grounds for EFSA to declare that this maize has the potential to cause adverse effects on non-target organism and recommend that it should not be cultivated in the EU.

From the modelling, EFSA concluded that *"a full exposure assessment is possible for several lepidopteran species, but it requires many factors to be taken into account, some of which had to be modelled with little available data. However, these predictions are relatively robust, as the difference between the best and most conservative (worst-case scenario) estimates led to no more than a 2.5 to 5-fold increase in the predicted mortality and sublethality."* This model has not been evaluated so the robustness of this finding cannot be evaluated.

Without the modelling, EFSA would have to admit that there **is** a risk to non-target Lepidoptera, and this should be grounds for refusal of cultivation of MON810 in the EU. However, although the GMO Panel does admit uncertainty over the model, *"EFSA GMO Panel is aware that all modelling exercises are subject to uncertainties; as with any ecological model, further data would refine the estimates reported here*.",

they simply recommend unspecified management measures, *"The EFSA GMO Panel considers it advisable that, especially in areas of abundance of non-target Lepidoptera populations, the adoption of the cultivation of maize MON810 be accompanied by management measures in order to mitigate the possible exposure of these species to MON810 pollen."* This exposes a major weakness in EFSA's approach to risk assessment. A risk has been identified and, instead of protecting the European environment and giving a negative opinion concerning MON810, as EFSA should, it simply passes the responsibility to others to deal with that risk.

Shockingly, although the Spanish Biosafety Commission suggested the potential effects of MON810 maize on non-target Lepidoptera should be considered more deeply in the post-market environmental monitoring plan, EFSA decided it was not practical to so this. "*An analysis of an existing dataset on butterfly communities in Switzerland (Aviron et al., 2009) have shown that case-specific monitoring would at best detect large effects in ubiquitous butterfly populations. … These authors and Lang (2004) also indicated that monitoring butterfly populations, particularly of infrequent species, is unlikely to achieve the level of sensitivity commensurate with the effects that are anticipated by the EFSA GMO Panel, unless thousands of samples are taken. Thus the EFSA GMO Panel is of the opinion that case-specific monitoring would not detect minor shifts in non-target Lepidoptera and is therefore not appropriate."*

No case-specific monitoring for non-target Lepidoptera is recommended by EFSA. This is despite the fact that this is one of the main environmental concerns of MON810. It is clear that the cultivation of MON810 has a high risk of adverse effects on biodiversity. Yet, this risk is largely dismissed, when EFSA should, at the very least, have said it was uncertain whether MON810 was safe for the European environment.

How can one check if the suggested management measures are working if there is no case-specific monitoring? EFSA admits that monitoring will not pick up any impacts on less abundant and rare butterflies. In such a case the precautionary principle should be applied and MON810 should be rejected.

Fate of Bt proteins in soil (Section 6.1.6.1)

As EFSA states, it is accepted that MON810 exudes Bt proteins through roots into the soil. However, the fate of these proteins is not well understood. Several studies have found long residence times and residual toxicity, as EFSA states. However, soil is complex and the residence time, and activity of Bt proteins in the soil is likely to be highly variable. Therefore, the accumulation of Bt proteins, and exposure of soil organisms to Bt cannot be excluded. EFSA does discuss the studies that find effects on soil microorganisms, but dismisses them as being temporal. *"Potential effects on soil microorganisms and microbial communities due to maize MON810 if they occur, will be transient, minor and localised in different field settings".* This is yet another area of uncertainty, but no uncertainty is expressed by EFSA and no robust scientific reasoning is offered to back up EFSA's opinion.

EFSA neglects scientific advice

The authors of several papers cited by EFSA as evidence for the absence of negative effects not only accentuate the remaining uncertainty of their results but also make other recommendations than EFSA. For example, regarding the potential impacts of MON810 EFSA cites Vercesi et al. (2006). But Vercesi et al. (2006) write that *"a sensible way to follow up on the results of this and previous studies, and to bolster a sound risk assessment of Bt-corn, would probably be to assess the effects of Bt-corn on earthworm populations in carefully designed field experiments*".

A further example of EFSA neglecting the advise of independent scientists concerns the data from experiments about the potential impact of MON810 on parasitoids. The results of several studies indicate a possible hazard of MON810 maize for parasitoids, and therefore they point out the need for more research. For example, Ramirez-Romero et al. (2007) write that, that *"the occurrence of direct effects of Cry1Ab protein on a hymenopteran parasitoid, such as C. marginiventris, merits further research because of the importance of these parasitoids as natural enemies in agroecosystems"*.

CONCLUSION:

EFSA has failed to follow European law and one of the basic principles of science – clearly identifying uncertainties. This in sharp contrast to other scientific bodies, such as the Intergovernmental Panel on Climate Change (IPCC), who clearly indicate the level of uncertainty and agreement within the panel and have developed a methodology for doing so (Risbey & Kandlikar 2007).

Biogeographical regions not considered

The extent and seriousness of the potential effects of GM insect-resistant crops on non-target organisms will depend on geographical factors as the same Bt maize plant could generate different ecological consequences in different biogeographical regions (Snow et al. 2005). The environmental risk assessment therefore should be region specific.

Given the diversity of agricultural practices in Europe and the regional variation in species composition and abundance, environmental risk assessment of MON810 maize in Europe requires a regional approach. For example, in regions with small-scale farming the interactions between MON810 maize and the surrounding ecosystems will be of orders of magnitude greater than in regions with large-scale MON810 cultivation (Knols & Dicke 2003).

Monsanto acknowledges biogeographic-specific differences where the potential development of resistance in the main target species is concerned. However, regarding the potential impacts of MON810 maize on non-target organisms, Monsanto takes an economic view and treats Europe as one single ecological area.

CONCLUSION:

As a consequence, the environmental risk assessment data submitted by Monsanto do not adequately encompass European biogeographical regions. Member States' competent authorities should ensure that the applicant provides adequate data that allow a risk assessment covering the specific conditions of the European biogeographical regions, in which MON810 maize potentially could be grown.

HUMAN SAFETY

A. Failures, conflicting silence, omissions, imbalances

1. EFSA human safety assessment is not valid

EFSA makes us believe that it has assessed a 90 days feeding study for MON810 as the following citation shows (EFSA 2009, page 19, Section 5.1.3.3. Toxicological assessment of the whole GM food/feed):

> *"The applicant provided a 90-day feeding study in Sprague-Dawley rats with grains of maize MON810 as a component of the diet. This study is available in the scientific literature (Hammond et al., 2006)"*

In the reference list "Hammond et al., 2006" is cited as: Hammond, B.G., Lemen, J., Dudek, R., Ward, D., Jiang, C., Nemeth, M., Burns, J., 2006. Results of a 90-day safety assurance study with rats fed grain from corn rootworm protected corn. Food and Chemical Toxicology, 44: 147-160."

This study deals with MON863 maize and does not cover 90 days feeding test with MON810.

CONCLUSION:

EFSA has either cited, or worse, analyzed a study on MON863 instead of MON810. Based on this data provided by EFSA we have to conclude that the safety evaluation of MON810 is not valid.

2. “Not known = safe”: EFSA’s new formula for safety assessment

EFSA (2009) states on page 12, paragraph 3:

> *“In silico translation of these transcripts identified 2 and 18 putative additional amino acids in different variants, all derived from the adjacent host genomic sequences, added to the truncated Cry1Ab protein. These putative* ***recombinant proteins did not show homology with any known protein and do not raise any new safety concerns.****”* [emphasis added].

The first part of this statement was taken word by word from Rosati et al. (2008) who state in their abstract: *“In silico translation of these transcripts identified 2 and 18 putative additional amino acids in different variants, all derived from the adjacent host genomic sequences, added to the truncated CRY1A protein. These putative recombinant proteins did not show homology with any known protein domains”.*

Because the authors have not analyzed the potential human health or environmental risk of these proteins they give no interpretation of their data in respect on safety issues.

In contrast EFSA (2009) added “***and do not raise any new safety concerns”*** but did not provide any data on how the safety of these recombinant proteins was tested, proven or analyzed.

CONCLUSION:

EFSA (2009) concludes without any scientific reference, that unknown “putative recombinant proteins” are safe. EFSA appears to have now developed a previously unknown scientific formula: “not known = safe”. This is in quite sharp contrast to the “concept of familiarity” where “not known = might be harmful and must be tested case by case.”

The way EFSA comes to conclusion on the safety of unknown novel proteins is far from any scientific standard. Without analysing the toxicological properties of any of the newly identified putative recombinant proteins the safety of MON810 cannot be assured.

3. EFSA does not follow its own practices

a) In 2003 EFSA analysed the consequences of RNA fragments

In its assessment of NK603 maize (EFSA 2003), the Authority is aware of the risks associated with RNA fragments of unknown origin as the following citation shows:

> *"… the RNA fragment observed in the product of the RT PCR amplification is not expected to have a regulatory function as described for micro RNAs which are short RNAs between 21 and 23 bp long derived from the processing of longer RNAs of around 70 bp (Jones, 2002). This is much shorter than the RNA fragments amplified from NK603."* (EFSA 2003, page 6, paragraph 3)

In other words the extra fragment is too long to have any regulatory function, but shorter fragments may pose a risk or give rise for concerns. Although EFSA's artificial separation between long and short RNA fragments is no longer valid (and was never valid)[2] it shows clearly that in 2003 EFSA saw a potential risk of RNA fragments.

b) In 2009 EFSA ignores the consequences of RNA fragments

Although several synthetic RNA fragments have been detected in MON810 (Rosati et al. 2008) EFSA (2009) is completely silent on the potential risks of the identified RNA fragments in MON810 which may – in EFSA terms - have a *"regulatory function"*. This is in contrast to its previous opinions such as NK603 (EFSA 2003).

Immunostimulatory DNA or RNA fragments

Proteins and nucleic acid can act as pathogen-associated molecular patterns (PAMP). Why nucleic acid is identified by the human (mammalian) immune system is still not fully clear, but some argue that nucleic acids represent a uniform conserved molecular pattern, allowing recognition independently of continuous evolutional changes to the outer membrane or capsid components of pathogens (Pawar et al. 2006).

Several receptors in the human immune system like Toll-like receptors (TLR) such as TLR3, TLR7, TLR8, TLR9 and retinoic acid-inducible protein1 (RIG-1) as well as MDA-5 are able to bind non-self nucleic acids i.e. DNA and RNA (Schlee et al. 2007). Toll-like receptors are evolutionary conserved among species (Pawar et al. 2006). Some nucleic acid sequences seem also to be "evolutionary conserved" and represent a universal code which is identified as a sequence from a pathogen by the innate immune system (Akira et al. 2006). New insights are gained on which sequences are recognized by the immune system (Schlee et al. 2007). The following figure gives an overview on some of these receptors and pathways.

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[2] The EFSA argumentation in 2003 that only short RNAs between 21 and 23 bp have a regulatory function is wrong. Even in 2003 several RNA databases showed that also long fragments of RNA show regulatory function. Kenzelmann et al. (2006) describes the current situation as following: Non coding RNAs range from 21-25 (siRNA and miRNA) to 100 – 200 nucleotides for small RNAs up to 10.000 nucleotides for RNAs involved in gene silencing. So any RNA regardless of its length is able to have regulatory function.

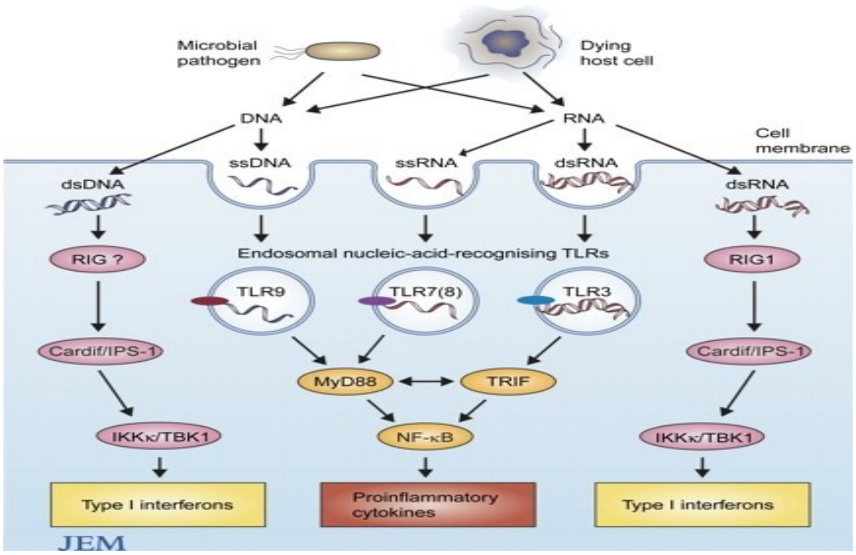

Figure 1: DNA/RNA recognition pathways in innate immune cells (Wagner and Bauer 2006)

Immunostimulatory DNA

Already in 1997, one year before MON810 was initially approved, David Pisetsky published his review on "DNA and the immune system" (Pisetsky 1997). Since then more and more publications on immunostimulatory DNA (e.g. Higgins et al. 2007, Kozy et al. 2009) or immunostimulatory RNA sequences (Bourquin et al. 2007, Hamm et al. 2007, Berger et al. 2009) have been published. Also in vivo studies show that that DNA from food (orally administered nucleic acid) interacts with the mammalian immune system (Rachmilewitz et al. 2004, Takahashi et al. 2006). Mazza et al. (2005) have traced fragments of synthetic transgenes into the blood of piglets fed with MON810 without providing data on how these fragments may interact with the immune system.

CONCLUSION:

It is of concern that EFSA (2009) fails to analyse the potential risks of synthetic DNA and RNA sequences in MON810, especially since immunostimulatory DNA/RNA has been getting more and more attention in the field of immune biology[3] .

As it seems that the recognition of RNA/DNA fragments by the immune system is based on evolutionary recognition patterns within the sequence of DNA/RNA fragments, the unknown unintended DNA and RNA (see Rosati et al. 2008) fragments in MON810, might bring some unexpected turbulences. It is therefore essential to investigate whether the synthetic RNA/DNA fragments of MON810 interact directly or indirectly with the human immune system. For example, by suppressing the capability of these receptors to correctly identify viral or other non-self DNA/RNA sequences or by affecting the ability of the immune system to distinguish correctly between self and non-self DNA/RNA fragments. Such potential interactions have to be assessed case by case to guarantee the safety of MON810.

[3] Akira et al. 2006, Pawar et al. 2006, Wagner und Bauer 2006, Schlee et al. 2006, Schlee et al. 2007, Bourquin et al. 2007, Hamm et al. 2007, Kozy et al. 2009, Chu et al. 2009, Berger et al. 2009

4. Conflicting statements in the same document

On page 19 in Section 5.1.3.2. *Toxicological assessment of new constituents other than proteins*, EFSA writes:

> *"Since no new constituents other than the above mentioned Cry1Ab protein are expressed in maize MON810 and because there is no indication of alteration in levels of endogenous compounds, a toxicological assessment for new constituents is not applicable."* (EFSA 2009)

Whereas EFSA states on page 12 in paragraph 3 that:

> *"In silico translation of these transcripts identified 2 and 18 putative additional amino acids in different variants, all derived from the adjacent host genomic sequences, added to the truncated Cry1Ab protein. These putative recombinant proteins did not show homology with any known protein…"* (EFSA 2009)

CONCLUSION:

These two statements are contradictory and the sentence on page 19 is misleading as EFSA clearly recognises that there are new "putative recombinant proteins" as well as fusion RNAs in MON810 maize. The new constituents have to undergo a toxicological risk assessment to fully address all risk of MON810.

5. Hide and seek - EFSA hides its source of information

In Section 3.1.1. *Transformation process and vector constructs* (EFSA 2009), EFSA refers many times to scientific literature or data without citing the source of this information. The following examples show how EFSA fails to provide clear information on the source of the data:

- *"In a previous molecular characterisation of maize MON810, it has been reported…"* (page 11, 3rd paragraph, line 1)
- *"Additional information provided in 2007"* (page 12, 2nd paragraph, line 1)
- *"Bioinformatic analyses were performed"* (page 12, 1st paragraph, line 3)

CONCLUSION:

Important statements are cited without scientific reference. For the reader it is impossible to check, if the information provided by EFSA is based on scientific data or not. We think it is neither the job of competent authorities nor of consumers to follow the "hide and seek" game performed by EFSA. Without correct scientific citation this opinion is not valid.

6. The mystery

Monsanto sees no need to update the information on molecular characterization and flank sequencing although the crop is already 10 years on the market:

> *"... evidence from a body of independent peer-reviewed literature on MON 810 that does not raise any safety issues (see Annex 3.1 of the "Specific Information" in this renewal application), do not indicate the need to update the information on molecular characterization and flanks sequencing"*

As pointed out above, EFSA cites some new information but hides most of its sources of information on the MON810 insert and appears to accept Monsanto's position not to provide more information.

CONCLUSION:

It is unclear why EFSA and Monsanto fail to provide full information and do not want to provide a clear picture on RNA and DNA fragments around the insert in MON810 maize. The fact that fragments of the synthetic transgene from MON810 has been detected in blood (Mazza et al. 2005) makes this "silence" a big concern.

7. The imbalance

EFSA is historically critical of studies which show potential risks of transgenic plants. For example, in a review on animal feeding trials in 2008 EFSA states:

> *"In some cases adverse effects were noted, which were difficult to interpret due to shortcomings in the studies."* (EFSA 2008a, page S4).

In contrast EFSA did not identify shortcomings in any of the studies on human health aspects of the renewal application of MON810 which do not show adverse effects. (EFSA 2009).

This is despite the fact that competent authorities from France (EFSA 2008c) and Austria (EFSA 2008b) do see shortcomings in studies provided by the applicant on MON810. See statement of France on MON810 (EFSA 2008c, page 30, 2nd paragraph):

> *"In fact, the protocol of the initial study by the enterprise has not been established in a way that could prove such a dose-effect as it limits itself to two dose levels only. What is more, for metabolic and hormonal disturbances, the response need not being linear. In each case, again, it is needed more than ever before that toxicological tests are performed with a longer duration and not only on rats. It should be reminded that the tragic history of thalidomide and its impact on the fetus was linked to the fact that only two animal models were utilized."*

CONCLUSION:

It is clear that EFSA is applying double standards when reviewing scientific studies. To declare shortcomings as the culprit not to consider a scientific publication is a very easy way to ignore adverse effects and to prove the safety. There is a clear imbalance in how the MON810 opinion was compiled as the scientific shortcomings identified by e.g. Austria and France still persist.

B. Important studies not considered by EFSA

8. The wrong track

α) EFSA has taken a very narrow view of the risks associated with transgenic fragments or genes as a result of the genetic modification. EFSA (2009) states on page 24 (last paragraph) that:

> *"the EFSA GMO Panel concludes that is very unlikely that the cry1Ab gene from maize MON810 would become transferred and established in the genome of microorganisms in the environment or in the hsuman* [correct citation] *and animal digestive tract."*

β) EFSA (2009) states on page 18 (last paragraph) that:

> *"A small fragment of the cry1Ab transgene was, together with endogenous maize genes, detected in blood, liver, spleen and kidney of animals fed the test diet.* ***However, no integration of the transgenic DNA in the host genome has been detected. Thus, transgenic DNA does not seem to behave differently from non-transgenic DNA with respect to transfer to animal tissue****."* [emphasis added]

EFSA (2009) does not state anything about other types of interference of DNA/RNA fragments with the immune system and limits its analysis to only risks that may arise from an integration of these fragments or the full gene into the host genome, which they state is unlikely.

However the integration of fragments into the genome is not the only potential risk from synthetic fragments. There is a substantial amount of scientific literature that deals with the detection of RNA and DNA in mammalian immune systems. A simple search of scientific databases reveals over 1000 scientific publications on the matter. [4]

CONCLUSION:

Research shows that DNA/RNA fragments orally administered are able to interact with the immune system (see e.g. Rachmilewitz et al. 2004, amongst others). EFSA themselves point into this direction when they analysed NK603 maize (EFSA 2003). The way EFSA handles this issue in their opinion on MON810 is far from satisfactory and far from the legal requirement of a comprehensive risk assessment as required by Regulation 1829/2003. The safety of MON810 for humans or animals cannot be guaranteed whilst the consequences of synthetic genetic material floating around blood streams are unknown. Why EFSA does not even mention that such synthetic DNAs detected in the blood might trigger immunostimmulatory effects is not comprehensible.

[4] Retrieved with scientific databases pubmed (http://www.ncbi.nlm.nih.gov/pubmed/) and highwire (http://highwire.org/) with keywords "immunostimulatory DNA/RNA, TLR3, TLR/, TLR7, TLR8, TLR9". For example: Akira et al. 2006, Pawar et al. 2006, Wagner und Bauer 2006, Schlee et al. 2006, Schlee et al. 2007, Bourquin et al. 2007, Hamm et al. 2007, Kozy et al. 2009, Chu et al. 2009, Berger et al. 2009

9. Proteomics not considered by EFSA

Proteomics are recommended in EFSA's own "Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA 2004).

> Especially *"To increase the chances of detecting unintended effects due to the genetic modification of organisms, profiling technologies such as transcriptomics, proteomics and metabolomics, have the potential to extend the breadth of comparative analyses (EC, 2000b; Kuiper et al., 2001; 2003; Cellini et al., 2004; ILSI, 2004). The utility and applicability of these technologies in the detection of altered gene and protein expression and metabolite composition in GM plants has been under scrutiny in specific research projects funded, for example, by EU FP5 (GMOCARE project)) and the UK Food Standards Agency (GO2 research programme)"* (EFSA 2006).

But, surprisingly, EFSA does not even mention a study which analyses MON810 with proteomics techniques.

Zolla et al. (2008) found with proteomics techniques many differences between MON810 and its near isogenic line. In particular, 7 spots were newly expressed, 14 spots were down-regulated, 13 were up-regulated, while 9 were completely repressed in the transgenic line. *"Interestingly, a newly expressed spot (SSP 6711) corresponding to 50 kDa gamma zein, a* ***well-known allergenic protein, has been detected.****"* [emphasis added] Whether these differences pose a safety threat is not clear but should be further analyzed as the authors conclude: *"However, it should be kept in mind that the detection of changes in protein profiles does not present a safety issue per se; the relevance of such changes for food safety should be assessed (also in the context of the natural variation not investigated here) by subsequent elucidation of the nature of the proteins affected."* (Zolla et al. 2008)

CONCLUSION:

It is unclear and unacceptable that EFSA does not follow its own recommendations for fully investigating the differences that may be occurring through genetic modification. Together with the detection of new "potential transgenic fusion proteins" by Rosati et al. 2008 – this is a clear safety question which has to be clarified. It is unclear why EFSA has ignored such an important publication that deals directly with this GMO.

10. Increase in cytokines not considered by EFSA

Finamore et al. (2008) evaluated the gut and peripheral immune response to genetically modified maize in mice. They fed weaning and old mice a diet containing MON810 or its parental control maize or a pellet diet containing GM-free maize for 30 and 90 days. In this study the authors identified recurrent changes in the immune system like changes in the number of a special type of lymphocytes (γδT-cells). Such T-cells are involved in the modulation of inflammatory response. The authors mention that high numbers of these (γδT-cells) have been observed in association with asthma or with untreated food allergy in children. Further alterations of the immunophenotypes induced by the transgenic maize were associated with the increase in some cytokines like (Interleukin 6 (IL-6), Interleukin 13, Interleukin 12p70 and MIP-1) which are important in the human immune response. The authors conclude*: "These cytokines (IL-6, IL-13, IL12p70, MIP-1) are involved in allergic and inflammatory responses (47-49), and although they were not strongly elevated by MON810 maize consumption, their increase is a further indicator of immune perturbations induced by MON810 maize."* (Finamore et al. 2008).

CONCLUSION:

Again, it is unclear why EFSA has not considered this publication in its opinion on MON810.

ADDITIONAL REMARKS

Contamination of conventional and organic maize crops

One of the main concerns related to GM crops is the fact that they are living organisms that can contaminate non-GM (i.e. conventional and/or organic) crops. Contamination has implications to biodiversity, farmers' livelihoods and food/feed safety. EFSA's remit does not extend to considering the potential contamination from GM maize. However, the risk managers have to be aware of issues linked to contamination.

MON810 contamination cases in Spain

There are many studies confirming long distance pollination from GM maize of up to 1000 m away (See for example: Jarosz et al. 2005, Halsey et al. 2005). In all EU reports published on gene flow and coexistence (e.g. EEA, 2002; IPTS/JRC, 2002, IPTS/JRC/ESTO, 2006) maize has been shown to be amongst the most difficult GM crops to contain (alongside oilseed rape), due to the high cross-pollination rate and the large distances that viable maize pollen can travel. GM maize is described as presenting a "medium to high risk" for cross-pollination with other crops (Treu 2000).

The small acreage of Bt corn grown in Spain is reported to be creating conflicts within society. "*The liability scheme is perceived as transferring the problem to the organic farmers. As a result, many farmers are reluctant to publicly report cases of contamination in a context where there is a need for social cohesion, as in small villages. One organic farmer said:* "as a consequence of social pressure, when farmers suffer contamination, they do not want to say so. Last year there were four contamination cases and two made it public but two did not. For fear of confronting the people in the town ... so they have to assume the economic cost, the environmental cost, and the cost of losing the organic certification but they do not say so" *(interview). Consequently, data on admixture cases are not systematically registered, although the organic certification is withdrawn in these cases*" (Binimelis 2008).

In addition, organic farming is diminishing as a result of GM contamination. "*As a result [of these cases], from 2004 (when the first analyses were done) to 2007, the area devoted to organic maize was reduced by 75% in Aragon* [where GM Bt maize is concentrated]." (Binimelis 2008).

There is a possibility that GM maize plants could survive the winter in Mediterranean Europe to contaminate future non-GM maize. Maize plants have been shown to survive over winter even in the UK, a comparatively cold part of Europe (Crawley 2001). Occasionally, maize volunteers (plants that have not been intentionally planted) have been noted from spilled seed in uncultivated fields and roadsides in the year following GM maize production (Eastham & Sweet 2002). Should any volunteer GM maize plants inadvertently grow near a maize crop, the resulting pollen could cross-pollinate, resulting in genetic contamination.

CONCLUSION:

Co-existence is highly problematic. Non-GM maize (i.e. conventional and organic) is highly likely to become contaminated in Europe. There is no liability legislation in place that would award compensation for farmers whose crops are contaminated and therefore devalued by GM maize in Europe. Indeed, Greenpeace Spain issued a report (Greenpeace 2008) detailing farmers' difficulties in remaining GM-free in Spain. This crucial aspect must be considered in terms of the cultivation of MON810.

REFERENCES

Akira, S., Uematsu. S. & Takeuchi, O. 2006. Pathogen recognition and innate immunity. Cell 124: 783-801.

Berger, M., Ablasser, A., Kim, S., Bekeredjian-Ding, I., Giese, T., Endres, S., Hornung, V, & Hartmann, G. 2009. TLR8-driven IL-12-dependent reciprocal and synergistic activation of NK cells and monocytes by immunostimulatory RNA. Journal of Immunotherapy 32: 262-271.

Binimelis, R. 2008. Coexistence of plants and coexistence of farmers: is an individual choice possible? Journal of Agricultural and Environmental Ethics 21:437–457

Bourquin, C., Schmidt, L., Hornung, V., Wurzenberger, C., Anz, D., Sandholzer, N., Schreiber, S., Voelkl, A., Hartmann, G. & Endres, S. 2007. Immunostimulatory RNA oligonucleotides trigger an antigen-specific cytotoxic T-cell and IgG2a response. Blood 109: 2953-2960.

Chu, W.M., Gong, X., Li, Z.W., Takabayashi, K., Ouyang, H.H.. Chen, Y., Chen, D.J., Li, G.C., Karin, M. & Raz, E. 2009. Retraction: DNA-PKcs is required for activation of innate immunity by immunostimulatory DNA. Cell 136: 565.

Crawley, M.J., Brown, S.L., Hails, R.S., Kohn, D.D. & Rees, M. 2001. Transgenic crops in natural habitats. Nature 409: 682-683.

Dively, G.P., Rose, R., Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Calvin, D.D. Russo, J.M. & Anderson, P.L. 2004. Effects on monarch butterfly larvae (Lepidoptera: Danaidae) after continuous exposure to Cry1Ab expressing corn during anthesis. Environmental Entomology 33: 1116-1125.

EEA 2002. Eastham, K. & Sweet, J. Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer. Expert's Corner Series, European Environment Agency, Copenhagen. http://www.eea.eu.int/

EFSA 2003. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safety of food s and food ingredients derived from herbicide-tolerant genetically modified maize NK603, for which a request for placing on the market was submitted under Article 4 of the Novel Food Regulation (EC) No 258/97 by Monsanto. The EFSA Journal 9: 1-14.

EFSA 2006. Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal 99: 1-100.

EFSA 2008a. Safety and nutritional assessment of GM plants and derived food and feed: The role of animal feeding trials - Report of the EFSA GMO Panel Working Group on Animal Feeding Trials. Food and Chemical Toxicology 46: S2-S70.

EFSA 2008b. Scientific Opinion of the Panel on Genetically Modified Organisms on a request from the European Commission related to the safeguard clause invoked by Austria on maize MON810 and T25 according to Article 23 of Directive 2001/18/EC. The EFSA Journal 891: 1-64.

EFSA 2008c. Scientific Opinion of the Panel on Genetically Modified Organisms on a request from the European Commission related to the safeguard clause invoked by France on maize MON810 according to Article 23 of Directive 2001/18/EC and the emergency measure according to Article 34 of Regulation (EC) No 1829/2003. The EFSA Journal 850: 1-45.

EFSA 2009. Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMORX-MON810) for the renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON810; (2) feed consisting of and/or containing maize MON810, including the use of seed for cultivation; and of (3) food and feed additives, and feed materials produced from maize MON810, all under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 1149: 1-84.

Finamore, A., Roselli, M, Britti, S., Monastra, G., Ambra, R., Turrini, A. & Mengheri, E. 2008. Intestinal and peripheral immune response to MON810 maize ingestion in weaning and old mice. Journal of Agricultural and Food Chemistry 56: 11533-11539.

Greenpeace 2008. La coexistencia sigue siendo imposible. http://www.greenpeace.org/raw/content/espana/reports/la-coexistencia-sigue-siendo-i.pdf

Halsey, M.E., Remund, K.M., Davis, C.A., Qualls, M., Eppard, P.J. & Berberich, S.A. 2005. Isolation of maize from pollen-mediated gene flow by time and distance. Crop Science 45: 2172-2185.

Hamm, S., Heit, A., Koffler, M., Huster, K.M., Akira, S., Busch, D.H., Wagner, H. & Bauer, S. 2007. Immunostimulatory RNA is a potent inducer of antigen-specific cytotoxic and humoral immune response in vivo. International Immunology 19(3): 297-304.

Higgins, D., Marshall, J,D,, Traquina, P., Van Nest, G. & Livingston, B.D. 2007. Immunostimulatory DNA as a vaccine adjuvant. Expert Rev Vaccines 6: 747-759.

IPTS/JRC 2002: Anne-Katrin Bock, A.-K., Lheureux, K., Libeau-Dulos, M., Nilsagård, H. & Rodriguez-Cerezo, E. Scenarios for co-existence of genetically modified, conventional and organic crops in European agriculture. Joint Research Centre (DG JRC), Institute for Prospective Technological Studies http://www.jrc.cec.eu.int/download/GMCrops_coexistence.pdf

IPTS/JRC/ESTO 2006, Messean, A., Angevin, F., Gómez-Barbero, M., Menrad, K. & Rodríguez-Cerezo, E. New case studies on the coexistence of GM and non-GM crops in European agriculture. Joint Research Centre (DG JRC), Institute for Prospective Technological Studies and European Science and Technology Observatory Technical Report EUR 22102 EN. http://www.jrc.es

Jarosz, N., Loubet, B., Durand, B., Foueillassar, X. & Huber, L. 2005 Variations in maize pollen emission and deposition in relation to microclimate. Environmental Science and Technology 39: 4377 – 4384.

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

Kozy, H.M., Garrett-Young, R., Kell, S.A., Lum, J.A., Kachura, M., Sathe, A., Biffen, M., Bell, J., Dymond, M., McHale, M., Kanzler, H., Coffman, R.L. & Hessel, E.M. 2009. Comparison of different classes of immunostimulatory DNA sequences recognizing toll-like receptor (TLR) 9. In: In Vitro and In Vivo Studies. American Journal of Respiratory and Critical Care Medicine 179(1_MeetingAbstracts): A5721.

Lang, A., Lauber, E. & Darvas, B. 2007. Early-tier tests insufficient for GMO risk assessment. Nature Biotechnology 25: 35 – 36.

Losey, J.E., Raynor, L.S. & Carter, M.E. 1999. Transgenic pollen harms monarch larvae. Nature 399: 214.

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

Mazza, R., Soave, M., Morlacchini, M., Piva, G., Marocco, A. 2005. Assessing the transfer of genetically modified DNA from feed to animal tissues. Transgenic Research 14: 775-784.

Pawar, R.D., Patole, P.S. & Wornle. M. & Anders, H.J. 2006. Microbial nucleic acids pay a Toll in kidney disease. Renal Physiology 291: F509-F516.

Pisetsky, D.S. (1997) DNA and the Immune System. Annals of Internal Medicine 126: 169-171.

Pons, X., Lumbierres, B., Lopez, C. & Albajes, R. 2005. Abundance of nontarget pests in transgenic Bt-maize: a farm scale study. European Journal of Entomology 102: 73 – 79

Rachmilewitz, D., Katakura, K., Karmeli, F., Hayashi, T., Reinus, C., Rudensky, B., Akira, S., Takeda, K., Lee, J., Takabayashi, K. & Raz, E. 2004. Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. Gastroenterology 126: 520-528.

Ramírez-Romero, R., Bernal, J.S., Chaufaux J. & Kaiser, L. 2007. Impact assessment of Bt-maize on a moth parasitoid, /Cotesia marginiventris/ (Hymenoptera: Braconidae), via host exposure to purified Cry1Ab protein or Bt-plants. Crop Protection 26: 953 – 962.

Risbey, J.S. & Kandlikar, M. (2007) Expressions of likelihood and confidence in the IPCC uncertainty assessment process. Climatic Change 85:19–31.

Rosati, A.., Bogani, P., Santarlasci, A. & Buiatti M 2008. Characterisation of 3' transgene insertion site and derived mRNAs in MON810 YieldGard maize. Plant Molecular Biology 67: 271-281.

Schlee, M., Barchet, W., Hornung, V. & Hartmann, G. 2007. Beyond double-stranded RNA-type I IFN induction by 3pRNA and other viral nucleic acids. Current Topics in Microbiology and Immunology 316: 207-230.

Schlee, M., Hornung, V. & Hartmann, G. 2006. siRNA and isRNA: two edges of one sword. Molecular Therapy 14: 463-470.

Sears, M.K., R.L. Hellmich, D.E. Stanley-Horn, K.S. Oberhauser, J.M. Pleasants, H.R. Mattila, B.D. Siegfried & G.P. Dively. 2001. Impact of Bt corn pollen on

monarch butterfly populations: A risk assessment. Proceedings of the National Academy of Sciences 98: 11937-11942.

Snow, A.A., Andow, D.A., Gepts, P., Hallerman, E.M., Power, A., Tiedje, J.M. & Wolfenbarger, L.L. 2005. Genetically engineered organisms and the environment: current status and recommendations. Ecological Applications 15: 377 – 404.

Spanish Biosafety Commission 2008. Application EFSA/GMO-RXMON810 (20.1.a) concerning the renewal of existing producst of Regulation (EC) No. 1829/2003, regarding the placing on the market of genetically modified MON810 maize for cultivation from Monsanto Europe, S.A. Spanish Biosafety Commission opinion on the envrionmental risk assessment and monitoring plan. http://registerofquestions.efsa.europa.eu/roqFrontend/?wicket:interface=:3:buttonform:questionDetailsTabs:panel:docItemForm:pageable:11:fileNameLnk:1:ILinkListener

Takahashi, N., Kitazawa, H., Iwabuchi, N., Xiao, J.Z., Miyaji, K., Iwatsuki, K. & Saito, T. 2006. Oral administration of an immunostimulatory DNA sequence from Bifidobacterium longum improves Th1/Th2 balance in a murine model. Bioscience, Biotechnology, and Biochemistry 70: 2013-2017.

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

Treu, R. & Emberlin, J. 2000. Pollen dispersal in the crops maize (*Zea mays*), oil seed rape *(Brassica napus* ssp oleifera), potatoes (*Solanum tuberosum*), sugar beet (*Beta vulgaris* ssp vulgaris) and wheat (*Triticum aestivum*). A report for the Soil Association from the National Pollen Research Unit. Available at http://www.soilassociation.org.

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

Wagner, H. & Bauer, S. 2006) All is not toll: new pathways in DNA recognition. Journal of Experimental Medicine 203: 265-268.

Zolla, L., Rinalducci, S., Antonioli, P. & Righetti, P.G. 2008. Proteomics as a complementary tool for identifying unintended side effects occurring in transgenic maize seeds as a result of genetic modifications. Journal of Proteome Research 7: 1850-1861.