Maize MON 87427 x MON 89034 x MIR162 x MON 87411

Organisation: The European GMO-free Citizens (De Gentechvrije Burgers)
Country: The Netherlands
Type: Others...

---

a. Assessment:
b. Food Safety Assessment:
Toxicology

About Cry1F:

Revista Facultad Nacional de Agronomía Medellín

Print version ISSN 0304-2847

On-line version ISSN 2248-7026

Populations of Spodoptera frugiperda (Lepidoptera: Noctuidae) cause significant damage to genetically modified corn crops Abstract JARAMILLO-BARRIOS, Camilo Ignacio; QUIJANO, Eduardo Barragán and ANDRADE, Buenaventura Monje. Populations of Spodoptera frugiperda (Lepidoptera: Noctuidae) cause significant damage to genetically modified corn crops. Rev. Fac. Nac. Agron. Medellín [online]. 2019, vol.72, n.3, pp.8953-8962. ISSN 0304-2847.

http://dx.doi.org/10.15446/rfnam.v72n3.75730

Quote:"This behavior suggests that if refuge areas and strategies such as pest monitoring are not established, these insects could generate higher resistances to the plants with the endotoxin Cry1F."

Keywords : Fall armyworm Larvae; Pest insects Population dynamics; Transgenic.

http://www.scielo.org.co/scielo.php?script=sci_abstract&pid=S0304-28472019000308953

http://www.scielo.org.co/pdf/rfnam/v72n3/2248-7026-rfnam-72-03-08953.pdf

---
Roundup

Petition text from *STOP*SPRAYING OUR*CHILDRENS*FOOD &*PLAYPARKS*&*FARM-CROPS WITH CANCEROGENIC**"ROUND-UP"** https://www.change.org/p/stop-spraying-our-childrens-food-playparks-farm-crops-with-cancerogenic-round-up

We are On A Mission To Raise AWARENESS About Severe Toxic Exposure, To Carcinogenic {Roundup} Herbicides etc. being Sprayed in Scotland,& Around the *World**SIGN*THIS*PETITION* For Yourself &*Children's*Health*& "Pets"*Cats, dogs etc.& Our Environments" W.H.O. & I.A.R.C & "OUR OWN SCIENTISTS STATED" THAT Roundup Weedkiller! WAS *CARCINOGENIC & Cause Diseases TO HUMANS & ANIMALS?

MANY INDEPENDENT STUDIES* SHOW MANY OTHER DISEASES! NON-Hodgkin-lymphoma,Obesity,Birth defects,infertility microcephaly,Autism, Asthma, Respiratory disease, Copd IBS, fibromyalgia, Sinus infections,ADHD,Diabetes,Celiac Disease, Endocrine Disruptor, hormonal disturbances, etc. SCIENTIFIC Peer reviewed Seralini Studies found Severe liver & Kidney disease, PEER REVIEWED & Published See Below:

& Farmers are Spraying Our Food Crops? & City Councils are Spraying Our Environments? it's costing TAXPAYERS Over £95,000 per year?*PLEASE WATCH THE VIDEO'S ABOVE*&CLICK SCIENCE REFERENCES INFO BELOW. OUR WEBSITE: FOR PUBLIC AWARENESS>>>>>> https://glyphosateisinsidious.weebly.com/references--data-evidence.html

MOST Cities, & Countries throughout the world have taken steps to either restrict or ban glyphosate, the active ingredient in Monsanto’s Roundup weed killer.The following countries have issued outright bans on glyphosate, imposed restrictions or have issued statements of intention to ban or restrict glyphosate-based herbicides, including Roundup, over health concerns and the ongoing Roundup cancer litigation:

https://www.baumhedlundlaw.com/toxic-tort-law/monsanto-roundup-lawsuit/where-is-glyphosate-banned/

Please spread awareness about this and be careful where you let your Children play, you wouldn’t let your children play with the chemicals under your kitchen sink, or with your medicine cabinet so keep them away from all areas which may of been sprayed. (Council parks, pathways,grass verges.. e.t.c) OUR *Dogs*&*Cats*eat the orange grass? & We all get very sick Cancers*liver & Kidney diseases etc.& MOST Cities & Farmers are Still SPRAYING IT? it's costing TAXPAYERS Over £95,000 per year? FOR Our CITY COUNCIL Alone?

It is not acceptable that we are are being routinely exposed to dangerous, life threatening chemicals, whether we like it or not. In 2015 the International Agency

for Research on Cancer, the World Health Organisation's cancer agency, concluded that it was "probably carcinogenic to humans. In a landmark case (August 2018), a Californian jury found that Monsanto knew its Roundup and RangerPro weedkillers were dangerous and failed to warn consumers.Monsanto has just been ordered to pay £226m damages to a man Jurors found that the company had acted with "malice" and that its weedkillers contributed "substantially" to Mr Johnson's terminal illness. Glyphosate is the active ingredient in many weedkillers, including Monsanto's *Roundup* Its used in Farming to Burn down farmers fields & to "desiccate" dry out our Crops? as it makes it easier for farmers to harvest? then we eat the food produced by farmers & the farmers cattle, cows, sheep, pigs, dogs, etc. eats the residues in their Feed? & we eat The Meats & vegetables from farms* with residues of glyphosate, which accumulates in our bones & Organs & plays havoc with all our health's, also Sprayed in Public places like Play-Parks, Streets, Schools, Care homes, also by pregnant woman with children in their gardens, Around people's homes* BY CITY COUNCILS? It is the World's Most widely Sold Weedkiller.

We Need this"insidious Chemical"out our foods etc. FOR PUBLIC AWARENESS>>>>>> https://glyphosateisinsidious.weebly.com/references--data-evidence.html THE Decision makers,EFSA,ECHA,EPA etc. only look mainly at the company's {Biased} evidence 95% funded by monsanto scientists, they will not allow OUR independent peer reviewed,published scientific studies & they have been sued for defamation & forgery & LIES & its Studies on **"ROUND-UP"** in hundreds of Court Cases worldwide! Monsanto put pressure on Scientists & Regulatory bodies to re-consider or reclassify to help get their Chemical "ROUND-UP" Re-licensed every few years. THEY MAKE BILLIONS WORLDWIDE? More Than 20 Countries Have Banned Its Use! France is to Vote against it being"Re-licensed" & is stopping its use! Can you imagine the Public's Response if they knew that glyphosate is being sprayed on their oats & in their Cheerios & Wheat,Vegetables & Meats etc.

Only Weeks before its manufactured & Put in shops? & it's impossible to wash or scrub this Chemical off, or cook it off our food, as it's in the cells of all OUR Meats & Veg except Organic. Along with wheat and oats, glyphosate is used to desiccate a wide range of other crops including lentils, peas, non-GMO soybeans, corn, flax, rye, triticale, buckwheat, millet, canola, sugar beets & Cabbage, carrot. Sunflower seed* IS also treated pre-harvest with glyphosate, according to the National Sunflower Association, & peanuts, tea, coffee beans etc. +++*References*+++ http://glyphosateisinsidious.weebly.com/references--data-evidence.html

Transcriptome profile Analysis Reflects rat liver&kidney Damage following chronic ultra-low dose glyphosate Roundup Exposure https://ehjournal.biomedcentral.com/articles/10.1186/s12940-015-0056-1

Seralini's team wins defamation & forgery court cases on pesticide research

http://www.gmoseralini.org/seralinis-team-wins-defamation-and-forgery-court-cases-on-gmo-and-pesticide-research/ IF ANYONE STILL "doubts this they Should Simply Read the Published {peer reviewed} SERALINI paper's" RE-SEARCH OPEN ACCESS BELOW: {three}expert reviews

https://ehjournal.biomedcentral.com/articles/10.1186/s12940-015-0056-1

Using **"ROUND-UP"** and glyphosate-based products, as a Pre-harvest treatment is fundamentally wrong, and We are calling for an end to it Being Sprayed on Our children's foods & playparks & environment Now!!! The critical period of development for most organisms is between the transition from a fertilized egg into a fully formed infant. As the cells begin to grow and differentiate, there are critical balances of hormones and protein changes that must occur. Therefore, a dose of {disrupting chemicals} may do substantial damage to a {developing fetus}

http://earthopensource.org/wp-content/uploads/RoundupandBirthDefectsv5.pdf

Government figures show its use in UK farming has increased by a shocking 400% in the last 20 years. Nearly a third of UK cereal crops were sprayed with glyphosate *Glyphosate {Roundup B.S.H.} toxicology* *fact sheets* http://fundacionterrazul.org/Archivo/Glyphosate_Fact_Sheets.pdf

Alongside the Concerns laid out by IARC, & W.H.O That its probably Cancerogenic to Humans, NEW long term Studies Have Been Done... 3x Seralini Studies

REPUBLICATION of the SERALINI STUDY LED BY PROF-GILLES-ERIC SERALINI SPEAKS FOR ITS SELF,DR MICHAEL ANTONIOU A MOLECULAR GENETICIST BASED IN LONDON

COMMENTED Transcriptome profile Analysis Reflects rat liver&kidney Damage following chronic ultra-low dose Roundup Exposure https://ehjournal.biomedcentral.com/articles/10.1186/s12940-015-0056-1

Glyphosate etc. DOES indeed pose a health risk. According to UK government data, the average level of #glyphosate found in Bread is Around 0.2mg in up to a third of bread. Given the average amount of bread eaten daily and the Average body weight of a UK adult, the average person therefore consumes around 78ng of glyphosate per kg body weight every day. This is nearly 20 times the level found to {cause liver and kidney damage} in one of the recent animal studies (only 4ng per kg body weight per day! https://en.wikipedia.org/wiki/Endocrine_disruptor

The chemical companies encourage wheat farmers to use #glyphosate not only as a weedkiller but also as a pre-harvest desiccant (i.e a drying agent) But even the

industry-funded Agriculture and Horticulture Development Board advises farmers that there is no advantage & huge risks in using glyphosate this way, Overuse also causes Super weeds? & Super bugs?

THEY MAKE BILLIONS WORLDWIDE! farmers routinely use Roundup and other herbicides to clear their fields of weeds before crops emerge in the spring. But what's more Alarming is they're also using glyphosate on Crops Shortly Before they are harvested, in order to desiccate (dry out) the plants & this makes them easier to harvest #Glyphosate is A pesticide* regularly found in routine testing of *British bread* appearing in over 60% of wholemeal bread samples tested by the Defra committee on Pesticide Residues in Food. also studies are showing Evidence of Celiac Sprue and Gluten intolerance? *Celiac Sprue and Guten intolerance* Stephanie seneff & Anthony samsel

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3945755/

Trials in Scotland & England showed no advantage - in terms of grain and Straw Moisture content, harvest efficiency or grain quality where weed-free wheat crops were Treated. Serious yield losses can occur when much of the grain is well above 30% moisture content. This highlights the potential risk of using pre-harvest glyphosate to even up harvesting. Residues are likely to be higher if glyphosate is applied to such moist grain.

"REPUBLICATION of the SERALINI STUDY" LED BY PROF-GILLES-ERIC SERALINI SPEAKS FOR ITS SELF,DR MICHAEL ANTONIOU A MOLECULAR GENETICIST BASED IN LONDON COMMENTED, few Studies would survive such intense scrutiny by fellow scientists, the republication of the study after {three}expert reviews, is a testament to its rigour, as well as to the integrity of the researchers. The study found {severe liver & kidney damage} & hormonal disturbances in rats fed food with minute amounts of glyphosate herbicide Roundup, that was below the permitted amount in drinking water. Glyphosate {Roundup B.S.H.}{CAUSES AN IMBALANCE Of {GUT FLORA} Kills All GOOD Bacteria Causes OVER-GROWTH Of Bad Bacteria In Your Gut Which Is 80% Your Immune System!!!

http://glyphosateisinsidious.weebly.com/

#glyphosate {induces human breast cancer} ROUNDUP* FOUND IN HIGH LEVELS IN BREAST MILK?

https://www.ncbi.nlm.nih.gov/pubmed/23756170

Molecular, geneticist Dr Michael Antoniou,& Prof Gilles-Eric-Seralini, Dr Stephanie Seneff & Anthony Samsel ETC. Below some "Peer" Reviewed Papers Stephanie Seneff & Anthony Samsel*Celiac sprue and gluten intolerance

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3945755/

SERALINI STUDY https://enveurope.springeropen.com/articles/10.1186/s12302-014-0014-5 *TOGETHER *WE* ARE *POWERFUL*SIGN* UP *&*YOU*MAKE THE *CHANGE HAPPEN* "WE HAVE RIGHTS" TO FEED *OUR CHILDREN* CHEMICAL FREE FOOD & TO HAVE SAFE ENVIRONMENTS FOR THEM TO PLAY IN AGAIN. PLEASE *SIGN* THIS PETITION & MAKE*THE HEALTHY CHANGE* Petition by PAMELA CASSIE.

change.org:NOV 22, 2019 —

Roundup [glyphosate] and birth defects: Is the public being kept in the dark? Quote: "The pesticide industry and EU regulators knew as long ago as the 1980s-1990s that Roundup, the world's bestselling herbicide, causes birth defects but they failed to inform the public." https://www.researchgate.net/publication/258416831_Roundup_and_birth_defects_Is_the_public_being_kept_in_the_dark ---------- Jeffrey Smith Webinar: How Glyphosate Causes Cancer

https://vimeo.com/366811206

Webinar Highlights:

4:00 – What is sulforaphane? 8:00 – What are gap junctions? 18:00 – How glyphosate reduces gap junction function 22:30 – How sulforaphane improves gap junction function 32:30 – What is the Nrf-2 pathway? 41:40 – How glyphosate negatively impacts Nrf-2 and how sulforaphane improves

In this revealing interview by Jeffrey Smith with Dr. John Gildea, and Dr. Martin Katz, you will discover their breaking research on how glyphosate wreaks havoc in our bodies by disrupting the communication network between cells and how sulforaphane - the good chemical from broccoli - prevents this. ----------

Study Links Widely Used Pesticides to Antibiotic Resistance

BY ELIZABETH GROSSMAN / CIVILEATS.COM

MARCH 24, 2015

Quote: Now, the chemical has another strike against it. A new study published by the American Society of Microbiology’s journal mBio has linked glyphosate and two other widely-used herbicides–2,4-D and dicamba–to one of the most pressing public health crises of our time: antibiotic resistance. This study found that

exposure to these herbicides in their commercial forms changed the way bacteria responded to a number of antibiotics, including ampicillin, ciprofloxacin, and tetracycline–drugs widely used to treat a range of deadly diseases.

https://time.com/3756870/pesticides-antibiotic-resistance/

Sublethal Exposure to Commercial Formulations of the Herbicides Dicamba, 2,4-Dichlorophenoxyacetic Acid, and Glyphosate Cause Changes in Antibiotic Susceptibility in Escherichia coli and Salmonella enterica serovar Typhimurium

Brigitta Kurenbach,a Delphine Marjoshi,a Carlos F. Amábile-Cuevas,b Gayle C. Ferguson,c William Godsoe,d Paddy Gibson,a Jack A. Heinemanna School of Biological Sciences, University of Canterbury, Christchurch, New Zealanda; Fundación Lusara, Mexico City, Mexicob; Institute of Natural and Mathematical Sciences, Massey University, Palmerston North, New Zealandc; Bio-Protection Centre, Lincoln University, Lincoln, New Zealand

IMPORTANCE Increasingly common chemicals used in agriculture ,domestic gardens, and public places can induce a multiple antibiotic resistance phenotype in potential pathogens

https://mbio.asm.org/content/mbio/6/2/e00009-15.full.pdf Quote:"Although this study only looked at two laboratory strains of human pathogens, the antibiotics examined represent what he calls “broad classes” of drugs we’ve come to depend on to fight infections and the herbicides are three of the most-used worldwide" #glyphosate via Twitter ----------

Tweet Non gmo rapport: Pakistan has banned the import of #geneticallymodified maize seeds on health grounds.

buff.ly/2pBAKHe https://tribune.com.pk/story/2081973/2-pakistan-banned-import-genetically-modified-maize-seeds-health-grounds/?amp=1&__twitter_impression=true

'Pakistan banned import of genetically modified maize seeds on health grounds' By APP Published: October 18, 2019

The ministry official remarked that the Bio-Safety Committee had approved the import of GM seeds for tests and trials but imposed a ban on the import of GM maize seeds in 2018. The committee wanted to engage in further deliberations on the health and environmental impact and effects of cross-pollination MORE in other varieties. Published in The Express Tribune, October 18th, 2019. ----------

EU report on weedkiller safety copied text from Monsanto study This article is from 2017

Exclusive: EU’s food safety watchdog recommended that glyphosate was safe but pages of report were identical to application from pesticide maker The European food safety authority (Efsa) based a recommendation that a chemical linked to cancer was safe for public use on an EU report that copied and pasted analyses MORE from a Monsanto study, the Guardian can reveal.

https://www.theguardian.com/environment/2017/sep/15/eu-report-on-weedkiller-safety-copied-text-from-monsanto-study ----------

Quote; "Critics say that the Roundup formula used in the U.S. also contains a surfactant that makes the herbicide far more toxic than the variation of the spray sold in the European market."

https://theintercept.com/2019/08/23/monsanto-republicans-cancer-research/ ---------- The Monsanto Papers

Updated 11 Oct 2018, 7:57amThu 11 Oct 2018, 7:57am

The secret tactics used by global chemical giant Monsanto, to protect its billion-dollar business and its star product, the weed killer, Roundup. Quote: "Monsanto has engaged in a systematic and deliberate campaign to attack any science that says their product is not safe and to attack any scientist that has the MORE courage to say something." Lawyer https://www.abc.net.au/news/2018-10-08/the-monsanto-papers/10352384 ----------

Quote:“…75% of all the glyphosate ever used – since it was introduced in the 1970s – has been used in the last ten years…”

http://waronwildlife.co.uk/2019/11/15/new-podcast-nick-mole-pesticide-action-network-uk/

… Agrobacterium tumefaciens in agroinfected plants. Molecular Plant – Microbe Interactions 1993, 6(50), 673-5. 19. Ho MW and Cummins J. Horizontal gene transfer from GMOs does happen. Science in Society 38.

The association of Morgellons Disease with dirt and soil where Agrobacterium lives, the widespread use of Agrobacterium in genetic engineering of plants, and the ability of Agrobacterium to infect human cells, all point towards a possible role of genetic engineering in the aetiology of Morgellans disease via Agrobacterium.

http://www.i-sis.org.uk/agrobacteriumAndMorgellons.php

Allergenicity

The EFSA GMO panel has not had the combination of events investigated, and no feed tests have been done. Only the four stand-alone events (GM maize MON 87427 x MON 89034 x MIR162 x Mon 87411 are four events) have been investigated. This is unacceptable!

Others

South Africa bans cultivation of MON89034.

03 October 2019

https://www.acbio.org.za/sites/default/files/documents/Minister%27s_final_decision_on_Monsanto_appeal.pdf

Quote:"MINISTER'S FINAL DECISION ON THE APPEAL LODGED BY MONSANTO SOUTH AFRICA (PTY) LIMITED UNDER THE GMO ACT, 1997 Minister of Agriculture, Forestry and Fisheries has made a final decision on the appeal lodged by Monsanto South Africa (Pty) Limited against the decision taken by the Executive Council regarding the general release application of a genetically modified maize event MON87460 x MON89034 x NK603. The maize is genetically modified to be tolerant to drought as well as resistant to certain insects".

The Executive Council (EC) took a decision to refuse the application and the reasons for the refusal included the following: • Kernel count per row and kernel count per ear showed that there were no statistically significant differences between the MON87460 x MON89034 x NK603 maize event and conventional maize in water limited conditions.

• The yield benefits associated with the MON87460 x MON89034 x NK603 maize event were inconsistent and in some trials the MON87460 x MON89034 x NK603 maize event had lower yields than the conventional maize,

• The insect resistance data presented was insufficient since it was only collected from one trial site for two planting seasons.

Decision by South Africa

https://www.acbio.org.za/sites/default/files/documents/EXECUTIVE_COUNCIL-DECISION_DOCUMENT-MONSANTO_GENERAL_RELEASE_MON87460XMO....pdf ----------

---------- Fragment
Consideration of the complaints and appeals to the Ministry of Housing, Spatial Planning and the Environment and the Council of State, Amsterdam, 11 August 2002.

THIS AND THAT

"And exhibiting a plate with the words “NO ENTRY” or “DO NOT USE”, or keeping the public at a safe distance from it by surrounding it with vegetation, like they used to do with anthrax-infected carcases which were buried in the ground. Poor CTB! [*Presumably the Institute for the Authorisation of Pesticides – translator.*]

(Bt, Bc – bacillus cereus, Ba – bacillus anthracis – are related to each other and can acquire each other’s characteristics. Life in the soil is not static!)"

AND

"I am sceptical of crops which have been genetically modified to make them resistant to pesticides. The companies which introduce GM crops which are resistant to substances found in pesticides are responsible for the damage which they do to our health. The biggest company in the sector in the Netherlands has assured me that it has no idea what substances the herbicides contain, even though they are the very substances which they are seeking to make their plants resistant to. “That’s a question for Hoechst”, I was told. Hoechst returns that particular ball by saying that whoever introduces a new variety is responsible for the consequences. Even Monsanto eventually said that its bears no responsibility for the consequences of its products being used in crop cultivation. Is it that easy?

"Now there’s a funny thing: A Bt insecticide, Foray 48B, contains methylparaben as an “active substance”. At the time, it was registered as such by the EPA. This substance is also found in ointments, etc. So, we’re supposed to rub it into our skin to heal wounds! Can someone explain that one to me, please?" L. Eijsten (reproduced with permission).

https://www.gentechvrij.nl/dossiers/archief-lily-eijsten/een-en-ander/ ----------

---

4. Conclusions and recommendations

How can people who wish to leave a comment be well-informed if the consultation is only held in English, and not in other EU languages, not even major ones like German, French or Spanish? This oversight must be remedied! This is why there are so few comments from countries where English is not spoken! We repeat: We don’t want GM maize!

cry2Ab2

cry1A.105

vip3Aa20

cry3Bb1

dvsnf7

Glyphosate;

Poison, poison everywhere! Insects and other creatures are being killed by built-in poison. And we're ingesting it, too. It's war on nature. But you can't beat nature over the long term. The answer is to work WITH her. An example is the push-pull method.

The African solution: push-pull (taken from 16A). *In Kenya, the Indian scientist Dr Zeyaur Khan has developed an alternative to Bt maize. Every year, about half of Kenya's maize harvest is wiped out by a joint invasion of witchweed (Striga) and stem borers.

These destructive insects belong to the maize borer family, at which the multinationals have targeted their GM Bt maize. Khan's "push-pull" method combats both weed and insect, without using chemical pesticides or genetic manipulation.

Khan and his team tested more than 400 varieties of grass before hitting on Napier grass, a variety which proved to be very attractive for stem borers. A hedge of Napier grass planted around a field of maize lures the insects away from the maize. And to make the maize unattractive to the stem borers, Khan's team sowed desmodium in between the stalks.

The desmodium repels the insects and at the same time combats the witchweed. It also fertilises the soil with natural nutrients. This is the "push-pull" method: the desmodium keeps the stem borers out of the maize field and the Napier grass attracts them.

Page 11, *"Recept voor een markttoelating"* (*"Recipe for market authorisation"*), author: Miep Bos, December 2007.

https://www.gentechvrij.nl/wp-content/uploads/2017/10/Recept-voor-markttoelating-2007-ISBN-EAN-9789081263818-.pdf

5. Others

This week, U.S. Right to Know's Carey Gillam reports that as the litigation drags on, "several plaintiffs have died or are nearing death, or have suffered such extreme health problems that their ability to undergo the rigors of depositions and trials has become limited."

Quote:"Some of those deceased or dying plaintiffs will be represented at trial by family members, under a legal process called "Suggestion of Death." From Organic Consumers Association ---------- This application is being routed via the Netherlands.

The CA writes: "The Dutch CA has assessed the dossier with respect to the food and feed safety of MON 87427 x MON 89034 x MIR162 x MON 87411 maize and has no comments or requests for additional information in relation to the safety of this GM event".

But also: "The applicant claims that all the information in the application is confidential. Information which is crucial to assess potential risks of a GM crop should not be declared confidential, because a lack of transparency undermines public trust in the risk assessment. This is in conflict with the Aarhus Convention, which regularises the right of the public to access environmental information and has been implemented in the European legislation. According to Article 30 of Regulation (EC) No 1829/2003 information on amongst others the composition of a GMO, physico-chemical and biological characteristics, and effects on human and animal health and the environment cannot be declared confidential. The Dutch CA on Regulation (EC) No 1829/2003 will send an email on this matter to the European Commission."

We can't get our heads round this contradiction.

6. Labelling proposal

If you were to take the terrible decision not to ban this genetically modified maize (which can never be the same as "ordinary" maize, given that it has been, well, modified!), then the most effective label would be a skull inside a warning triangle. And not only starting at 0.9% of the ingredients, but wherever GM organisms are present.

These replies are being sent to you jointly on behalf of Stichting Ekopark, Donaustraat 152, Lelystad, Netherlands.

Organisation: The European GMO-free Citizens (De Gentechvrije Burgers)
Country: The Netherlands
Type: Others...

---

a. Assessment:
Others

28-11-2019. Supplement to our earlier complaints: We read:

ONE: Quoted in the New York Times Magazine (October 25, 1998, "Playing God in the Garden"), Philip Angell, Monsanto's director of corporate communications, famously stated: "Monsanto shouldn't have to vouchsafe the safety of biotech food. Our interest is in selling as much of it as possible. Assuring its safety is the FDA's job."

TWO: From the Federal Register, Volume 57, No.104, "Statement of [FDA] Policy: Foods Derived from New Plant Varieties," here is what the FDA had to say on this matter: "Ultimately, it is the food producer who is responsible for assuring safety."

Both quotes taken from: Jon Rappoport, No more fake news, 25-11-2019
https://blog.nomorefakenews.com/2019/11/25/monsanto-science-and-fraud-are-same-thing/

---

4. Conclusions and recommendations

These GM crops are not assessed by the US Government! Yet you approve them solely on the basis of data provided by the multinationals! It’s not on!

Organisation: The European GMO-free Citizens (De Gentechvrije Burgers)
Country: The Netherlands
Type: Others...

---

a. Assessment:
Others

28-11-2019. Supplement to our earlier complaints: We read:

ONE: Quoted in the New York Times Magazine (October 25, 1998, "Playing God in the Garden"), Philip Angell, Monsanto's director of corporate communications, famously stated: "Monsanto shouldn't have to vouchsafe the safety of biotech food. Our interest is in selling as much of it as possible. Assuring its safety is the FDA's job."

TWO: From the Federal Register, Volume 57, No.104, "Statement of [FDA] Policy: Foods Derived from New Plant Varieties," here is what the FDA had to say on this matter: "Ultimately, it is the food producer who is responsible for assuring safety."

Both quotes taken from: Jon Rappoport, No more fake news, 25-11-2019 https://blog.nomorefakenews.com/2019/11/25/monsanto-science-and-fraud-are-same-thing/

---

4. Conclusions and recommendations

We don’t understand how the Netherlands can approve this!

Organisation: The European GMO-free Citizens (De Gentechvrije Burgers)
Country: The Netherlands
Type: Others...

---

a. Assessment:
Others

4-12-2019. Second supplement to our earlier complaints and those from Stichting Ekopark, Lelystad, Netherlands.

Austria is the first country in the EU to ban glyphosate (as from 1 January 2020).

From GMWatch (Twitter) Quote:“EU Commission gives green light to Austria’s glyphosate ban! Austria will become the 1st country in the EU to phase out glyphosate on 1 January 2020″. @global2000 call for support for farmers to help them transition away from #glyphosate. Source (German).

Dutch translation:

Van GMWatch (Twitter). Fragment:“De EU Commissie geeft het groene licht voor de ban van glyfosaat in Oostenrijk. Oostenrijk zal het eerste land zijn in de EU omdat het glyfosaat vanaf 1-1-2020 uit zal faseren. @global2000 vraagt om steun

voor boeren om hen te helpen glyfosaat uit te faseren. Bron. (Duits). https://www.gentechvrij.nl/2019/12/03/oostenrijk-ban-gly/

Organisation: The European GMO-free Citizens (De Gentechvrije Burgers)
Country: The Netherlands
Type: Others...

---

a. Assessment:
4. Conclusions and recommendations

Supplement 4-12-2019. Quote: After so many years of EFSA's poor implementation and partial disregard of repeated EU Parliament requests to fix its independence policy, the new Parliament would be wise to step up the pressure on this EU agency. https://corporateeurope.org/en/2019/06/efsa-gene-drive-working-group-fails-independence-test

---

Organisation: Testbiotech
Country: Germany
Type: Non Profit Organisation

---

a. Assessment:
Molecular characterisation

The process of genetic engineering involved several deletions and insertions in the parental GE maize plants. In order to assess the sequences encoding the newly expressed proteins or any other open reading frames (ORFs) present within the insert and spanning the junction sites, it was assumed that the proteins that might emerge from these DNA sequences would raise no safety issues; therefore, no detailed investigations were carried out in this regard. Furthermore, other gene products such as dsRNA from additional open reading frames were not assessed. Thus, uncertainties remain about other biologically active substances arising from the method of genetic engineering and the newly introduced gene constructs.

Previous research has indicated that expression of Cry1A.105, Cry2Ab2 and EPSPS proteins in genetically engineered maize can induce changes in the overall proteome of the respective GE maize line, with impacts on associated endogenous

metabolic pathways (Agapito-Tenfen et al. 2014). These transgenes are also present in the stacked maize. Thus, robust data should have been presented to assess whether metabolic changes with relevance to biosafety occur in the stacked maize.

Therefore, EFSA (2019f) should have requested much more detailed investigation into potential biologically active gene products and changes in metabolic pathways.

In regard to the expression of the additionally inserted genes, Implementing Regulation 503/2013 requests "protein expression data, including the raw data, obtained from field trials and related to the conditions in which the crop is grown".

However, there are three reasons why the data presented do not represent the conditions in which the plants are grown: (1.1) the field trials were not conducted in all relevant regions where the maize will be cultivated, and no extreme weather conditions were taken into account; (1.2) the field trials did not take current agricultural management practices into account; (1.3.) only one transgenic variety was included in the field trials.

1.1 Environmental stress can cause unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015). There is plenty of evidence that drought or heat can significantly impact the content of Bt in the plant tissue (Adamczyk & Meredith, 2004; Adamczyk et al., 2009; Chen et al., 2005; Dong & Li, 2006; Luo et al., 2008; Then & Lorch, 2008; Trtikova et al., 2015). Therefore, to assess gene expression, the plants should have been grown under conditions of severe drought, with and without irrigation, with and without application of the complementary herbicide and in comparison to more moderately severe climate conditions. However, no such data were requested or used for detailed comparison to assess the genome x environment interactions.

Furthermore, Fang et al. (2018) showed that stress responses can lead to unexpected changes in plant metabolism inheriting additional EPSPS enzymes. However, the expression of the additional enzymes was only measured under field conditions in the US for one year. The plants should have been subjected to a much broader range of defined environmental conditions and stressors to gather reliable data on gene expression and functional genetic stability. Whatever the case, they should have been tested in the maize producing countries in South America.

In consequence, the available publications strongly indicate that plants inheriting a combination of EPSPS and CSPB are likely to show strong reactions in their gene expression when grown under stress conditions, such as drought. These effects are also likely to impact plant composition and biological characteristics crucial for the assessment of food and feed safety. However, no specific data were requested or used for detailed comparison to assess the genome x environment interactions.

Whatever the case, the plants should have been subjected to a much broader range of defined environmental conditions and stressors (which for example have to expected under ongoing climate change) to gather reliable data on gene expression and functional genetic stability.

1.2 Due to increased weed pressure, it has to be expected that these plants will be exposed to high and also repeated dosages of glyphosate. Higher applications of the herbicide will not only lead to a higher burden of residues in the harvest, but may also influence the expression of the transgenes or other genome activities in the plants. This aspect was completely ignored in the EFSA risk assessment. EFSA should have requested the applicant to submit data from field trials with the highest dosage of glyphosate that can be tolerated by the plants, including repeated spraying.

As mentioned by the experts of Member States, application of higher rates the complementary herbicides can cause stress reactions in the plants and impact gene expression (EFSA 2019d). However, this aspect was ignored in the EFSA risk assessment.

1.3 It is known that the genomic background of the variety can influence the expression of the inserted genes (see, for example, Trtikova et al., 2015). Therefore, EFSA, should have requested additional data from several varieties, including those cultivated in South America.

Additional findings The findings (1.1 – 1.3) on flaws in risk assessment are supported by data from previous applications with the same parental events. Data presented in Table 1 show widely differing gene expression and content of Vip3Aa20.

Table 1: Gene expression and content of Vip3Aa20 present in maize MIR162 in grain (µg/g dry weight, mean values)

Application (EFSA opinion) Details from field trials Content of Vip3Aa20

MON 87427 x MON 89034 x MIR162 x MON87411 (EFSA 2019f) Field trials at five locations in the USA in 2014 (sprayed with glyphosate) 52

MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 (EFSA 2019a) Field trials at five locations in the USA in 2014 (sprayed with glyphosate) 38

MON 87427 x MON 89034 x MIR162 x NK603 (EFSA 2019b) Field trials at five locations in the USA in 2013 (sprayed with glyphosate) 59

Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 (EFSA 2019c) Field trials at three locations in the US in 2012 (not sprayed with complementary herbicides) 100

Bt11 x MIR162 x 1507 x GA21 (EFSA 2018a) Field trials at one single location in the US 2008 (sprayed?) 28

Bt11 x MIR162 x MIR604 x GA21 (EFSA 2015a) Single location in the US in 2006 (sprayed?) 140

MIR162 (EFSA 2012) Bloomington, Illinois 2005, Hybrid A 46 York, Nebraska, 2005, Hybrid B 41 Bloomington, Illinois, 2006, Hybrid A 124 Bloomington, Illinois, 2006, Hybrid B 84 Brazil, Ituiutaba, 2007 62 Brazil, Uberlandia , 2007 59

These data show a range of mean values between 28 µg/g and 140 µg/g for Vip3Aa20 in the grain, while in other cases even 166 µg/g were measured as maximum range in the grain (EFSA 2012); this is evidence of highly variable gene expression, with the actual content of the additional protein being unpredictable.

The factors influencing the content might seem variable. As EFSA (2012) stated in previous opinions "a year-to-year and site-to-site variation is evident". In addition, genetic backgrounds of different varieties and effects from stacking seem to be relevant as well. There is no justification for not requesting additional data on the impact of drought conditions on Vip3Aa20 gene expression.

In general, EFSA fails to give a full overview of existing data from previous applications and findings to facilitate an examination of the range of gene expression in more detail, and to derive a conclusive and sufficiently robust risk assessment.

Further findings dsRNA can have many functions and interact with gene regulation in many ways. In most cases, gene activity will be blocked or down regulated (silenced). In many cases, there may be cross-kingdom activity. They are known to interact with gene regulation in microorganisms, insects, plants and mammals. Its specificity is dependent on several factors such as its stability, further splicing and regions within DNA where it can interact.

To assess potential off-target effects, the structure of the dsRNA can be compared with genomic regions in organisms that might come into contact with the molecules. Regulation (EU) No 503/2013 says that when silencing approaches with RNAi are used in genetically engineered plants, a bioinformatics analysis is required in order to identify potential 'off-target' genes. An important starting point is the collection of relevant data to make comparisons with the RNA networks of non- target organisms, including mammals and humans that are exposed to the plants via food and feed.

However, in the assessment of the parental plant MON87411 (EFSA 2018d), the additional dsRNA produced in the plants was compared only with RNA as expressed in plants. EFSA concluded that there was similarity that would raise

concerns. However, no comparison was made in regard to mammals and microorganisms.

This gap in risk assessment was also expressed in comments from the experts of Member States (EFSA, 2018c), such as the BVL (Germany): "The applicant has not provided data on potential RNAi-targets of DvSnf7 dsRNA in non-target organisms, including humans. (…) Thus, additional data like bioinformatic evaluations should be considered. As demonstrated by a history of safe consumption of dsRNAs with high homology in conventional food and feed, the identification of sequence similarities between the dsRNA produced by MON 87411 and transcripts of exposed species would not directly indicate an increased risk of adverse effects. Nevertheless, a bioinformatic search for potential targets in transcripts of human and likely exposed non target species (farm animals) would back the weight of evidence approach if no matching targets where identified. The German Competent Authority therefore recommends a bioinformatic evaluation, comparable to study no.: RAR-2015-0373, to identify potential target genes in human and other relevant non target species. Additional information might be recommended according to the outcome of the bioinformatics evaluation."

However, no such data were requested by EFSA for the parental plants nor the stacked events. Instead, EFSA seems to be of the opinion that such data would not allow reliable prediction of the potential effects of such molecules. The protocol of the EFSA panel meeting (EFSA, 2017) states: "In plants a set of parameters allows for a reasonable prediction of RNAi off-target genes while in human and animals the extent of complementarity between the small RNA and the target is more limited and therefore these prediction tools do not allow for sufficiently reliable predictions (Pinzón et al., 2017). Therefore the GMO Panel considers that only the search for small RNA off-targets in the GM plant could have value for the risk assessment of GM plants."

This is an interesting statement since it exposes some limitations in current knowledge. Pinzón et al. (2017) show that further research would be needed to make reliable predictions in regard to dsRNA effects in mammals. This publication can not be used as justification not to assess health risk in the case of MON87411. But EFSA neither tries to overcome these limitations of current knowledge, nor does it consider that risk assessment cannot be concluded without sufficient data and meaningful analysis.

Instead, EFSA (2018b) simply accepts these limitations by restricting its considerations and risk assessment to potential off-target effects in the plants, leaving aside effects in humans and livestock and their gut microbiomes that are exposed to the maize via the food and feed chain. This is akin to someone who has lost something in the dark and then only searches where street lamps shed light because that is where the light is available.

A similar approach was taken by EFSA in assessing the concentration of dsRNA and its downstream metabolic products in the plants. EFSA (2018b) states: “The applicant provided a measure of the levels of DvSnf7 dsRNA in different tissues including grain and forage. However, the dsRNA is an intermediate molecule which is processed by dicer to siRNA molecules and the levels of dsRNA are not a good proxy for the levels of the active siRNAs in the plant (Paces et al., 2017). Therefore, the levels of the DvSnf7 dsRNA were not considered relevant for the risk assessment of maize MON 87411.”

As a result, the data on the concentration of the biologically active molecules in the plants were not assessed. However, such data are necessary to assess the risks for the food chain and the fate of these molecules in the environment (see below).

Instead of performing detailed risk assessment, EFSA (2018b), in contradiction to scientific publications (see below) simply assumes that: “the amount of RNAs taken up and absorbed after oral ingestion is considered negligible in humans and animals (mammals, birds and fish).”

EFSA´s risk assessment of the dsRNA expressed in the plants on a molecular level might be described as the perfect example of a ‘don´t look – don´t find’ strategy incompatible with existing regulation.

There are further gaps in risk assessment: EFSA did not assess additional unintended gene products, such as other unintended dsRNA, that can emerge from the insertion of the transgenes.

Further, no detailed consideration was undertaken regarding the extent to which the modification of the Bt protein Cry3Bb1 will change biological characteristics. In order to enable further independent risk assessment, the full DNA sequence inserted into the plants should be made available, including all open reading frames.

EFSA also did not request any detailed analysis based on so-called ‘Omics’ (transcriptomics, metabolomics, proteomics) to investigate changes in the overall metabolism in the plants. EFSA assumed that the data from phenotypic characteristics and compositional analysis would not indicate any need for further investigations. However, these data did show many significant changes (see below). In general, data on phenotypic characteristics and compositional analysis can be used as complementary data, but these are not as sensitive as -omics data and cannot replace them.

Expression data were provided on the new intended proteins. It is known that the Bt content in the plants depends on environmental impact. For example, environmental stress can cause unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015). Therefore, the plants

should have been subjected to a much broader range of defined environmental conditions and stressors in order to gather reliable data on gene expression and functional genetic stability. The same investigations should be performed in regard to dsRNA produced in the maize.

Further, the method used to determine the amount of Bt toxins (ELISA) is known to be dependent on the specific protocols used. The data are not sufficiently reliable without further evaluation by independent labs. For example, Shu et al. (2018) highlight difficulties in measuring the correct concentration of Bt toxins produced by the genetically engineered plants (see also Székács et al., 2011). Without fully evaluated test methods to measure the expression and the concentration of the Bt toxins and the dsRNA (and its metabolites), risk assessment will suffer from substantial methodological gaps. Based on such poor and inconclusive data, the dietary exposure to Bt toxins within the food chain cannot be determined as required by Regulation (EU) No 503/2013. A similar problem emerges from the dsRNA produced in the plants.

Consequently, the risk assessment of molecular characteristics is not conclusive and is not sufficient to show food and feed safety.

Conclusion on molecular characterisation We conclude that the available data strongly indicate gene expression of several of the newly introduced genes is likely to depend on, or be influenced by, stacking, varietal background, the spraying of the herbicide or environmental conditions such as drought.

Therefore, the plants should have been subjected to a much broader range of defined environmental conditions and stressors to gather reliable data on gene expression and functional genetic stability, taking into account more extreme drought conditions. In addition, they should have been tested in the maize producing countries in South America. Furthermore, EFSA should have requested the applicant to submit data from field trials with the highest dosage of the complementary herbicides that can be tolerated by the plants, including repeated spraying. In addition, EFSA should have requested data from several varieties, including those cultivated in South America.

The material derived from the plants should have been assessed by using ‘Omics-techniques’ to investigate changes in the gene activity of the transgene and the plants genome, as well as changes in metabolic pathways and the emergence of unintended biological active gene products. Such in-depth investigations should not depend on findings indicating potential adverse effects, they should always be necessary to come to sufficiently robust conclusions to inform the next steps in risk assessment.

Finally, it is not acceptable that the molecular characterisation of the dsRNA as produced in the plants does not allow an assessment of its non-target across

kingdom effects and the concentration of the toxin the plants can not be determined.

Adamczyk Jr, J.J., & Meredith Jr, W.R. (2004) Genetic basis for variability of Cry1Ac expression among commercial transgenic Bacillus thuringiensis (Bt) cotton cultivars in the United States. Journal of Cotton Science, 8(1): 433-440. http://www.cotton.org/journal/2004-08/1/17.cfm

Adamczyk, J.J., Perera, O., Meredith, W.R. (2009) Production of mRNA from the cry1Ac transgene differs among Bollgard® lines which correlates to the level of subsequent protein. Transgenic Research, 18: 143-149. https://doi.org/10.1007/s11248-008-9198-z

Agapito-Tenfen SZ, Vilperte V, Benevenuto RF, et al. (2014) Effect of stacking insecticidal cry and herbicide tolerance epsps transgenes on transgenic maize proteome. BMC Plant Biology 14: 346. doi: 10.1186/s12870-014-0346-8

Chen, D., Ye, G., Yang, C., Chen Y., Wu, Y. (2005) The effect of high temperature on the insecticidal properties of Bt Cotton. Environmental and Experimental Botany, 53: 333–342.

Dong, H.Z., & Li, W.J. (2006) Variability of endotoxin expression in Bt transgenic cotton. J. Agronomy & Crop Science, 193: 21-29.

EFSA (2012) Scientific Opinion on application (EFSA-GMO-DE-2010-82) for the placing on the market of insect-resistant genetically modified maize MIR162 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta. EFSA Journal 10(6):2756. doi: 10.2903/j.efsa.2012.2756

EFSA (2015a) Scientific Opinion on an application by Syngenta (EFSA-GMO-DE-2009-66) for placing on the market of herbicide tolerant and insect resistant maize Bt11 × MIR162 × MIR604 × GA21 and subcombinations independently of their origin for food and feed uses, import and processing under Regulation (EC) No 1829/2003. EFSA Journal 13(12):4297. doi: 10.2903/j.efsa.2015.4297

EFSA (2015b) Scientific Opinion on the application (EFSA-GMO-BE-2012-110) for the placing on the market of tissue-selective herbicide-tolerant genetically modified maize MON 87427 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 13(6):4130. doi: 10.2903/j.efsa.2015.4130

EFSA (2017) Scientific Panel on GMO Minutes of the 118th Plenary meeting of the Scientific Panel on GMO, 25-26 October 2017, Parma (Agreed on 28 November 2017).

EFSA (2018a) Assessment of genetically modified maize Bt11 x MIR162 x 1507 x GA21 and three subcombinations independently of their origin, for food and feed uses under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2010-86). EFSA Journal 16(7): e05309. doi: 10.2903/j.efsa.2018.5309

EFSA (2018b) GMO Panel (EFSA Panel on Genetically Modified Organisms), Scientific Opinion on the assessment of genetically modified maize MON 87411 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2015-124). EFSA Journal 2018;16(6):5310, 29 pp. https://doi.org/10.2903/j.efsa.2018.5310

EFSA (2018c) Comments from the experts of Member States on GMO Panel (EFSA Panel on Genetically Modified Organisms), GMO Panel (EFSA Panel on Genetically Modified Organisms), Scientific Opinion on the assessment of genetically modified maize MON 87411 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2015-124), accessed via the register of questions at EFSA. http://registerofquestions.efsa.europa.eu/roqFrontend/login?0

EFSA (2019a) Scientific Opinion on the assessment of genetically modified maize MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2016-134). EFSA Journal 2019;17 (8): 5774, 36 pp. https://doi.org/10.2903/j.efsa.2019.5774

EFSA (2019b) Assessment of genetically modified maize MON 87427 × MON 89034 × MIR162 × NK603 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2016-131). EFSA Journal 17(7):e05734. doi: 10.2903/j.efsa.2019.5734

EFSA (2019c) Assessment of genetically modified maize Bt11 × MIR162 × MIR604 × 1507 × 5307 × GA21 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2011-103). EFSA Journal 17(4):e05635. doi: 10.2903/j.efsa.2019.5635

EFSA (2019d) Application EFSA-GMO-NL-2017-144, Comments and opinions submitted by Member States during the three-month consultation period, Register of Questions, http://registerofquestions.efsa.europa.eu/roqFrontend/ListOfQuestionsNoLogin?0&panel=ALL

EFSA (2019f) GMO Panel (EFSA Panel on Genetically Modified Organisms), Scientific Opinion on the assessment of genetically modified maize MON 87427 9 MON 89034 9 MIR162 9 MON 87411 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2017-

144). EFSA Journal 2019;17 (11):5848, 33 pp. https://doi.org/10.2903/j.efsa.2019.5848

Fang, J., Nan, P., Gu, Z., et al. (2018) Overexpressing exogenous 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) genes increases fecundity and auxin content of transgenic arabidopsis plants. Front Plant Sci 9: 233. doi: 10.3389/fpls.2018.00233

Luo, Z., Dong, H., Li, W., Ming, Z., & Zhu, Y. (2008) Individual and combined effects of salinity and waterlogging on Cry1Ac expression and insecticidal efficacy of Bt cotton. Crop protection, 27(12): 1485-1490. https://www.sciencedirect.com/science/article/pii/S0261219408001257

Pinzón, N., Li, B., Martinez, L., Sergeeva, A., Presumey, J., Apparailly, F., Seitz, H. (2017) MicroRNA target prediction programs predict many false positives. Genome Research, 27(2): 234-245. http://genome.cshlp.org/content/27/2/234.short

Shu, Y., Romeis, J., Meissle, M. (2018) No interactions of stacked Bt maize with the non-target aphid Rhopalosiphum padi and the spider mite Tetranychus urticae. Front. Plant Sci. 9: 39. doi: 10.3389/fpls.2018.00039

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

Then, C. & Lorch, A. (2008), A simple question in a complex environment: How much Bt toxin do genetically engineered MON810 maize plants actually produce? In: Breckling, B., Reuter, H. & Verhoeven, R. (2008) Implications of GM-Crop Cultivation at Large Spatial Scales. Theorie in der Ökologie 14. Frankfurt, Peter Lang: 17-21. http://www.mapserver.uni-vechta.de/generisk/gmls2008/beitraege/Then.pdf

Trtikova, M., Wikmark, O.G., Zemp, N., et al. (2015) Transgene expression and Bt protein content in transgenic Bt maize (MON810) under optimal and stressful environmental conditions. PLoS ONE 10(4): e0123011. doi: 10.1371/journal.pone.0123011

Comparative analysis (for compositional analysis and agronomic traits and GM phenotype)

Implementing Regulation 503/2013 requests: “In the case of herbicide tolerant genetically modified plants and in order to assess whether the expected agricultural practices influence the expression of the studied endpoints, three test materials shall be compared: the genetically modified plant exposed to the intended herbicide; the

conventional counterpart treated with conventional herbicide management regimes; and the genetically modified plant treated with the same conventional herbicide management regimes."

"The different sites selected for the field trials shall reflect the different meteorological and agronomic conditions under which the crop is to be grown; the choice shall be explicitly justified. The choice of non-genetically modified reference varieties shall be appropriate for the chosen sites and shall be justified explicitly."

However, the data presented do not represent expected agricultural practices or the different meteorological and agronomic conditions under which the crop is to be grown. There are three reasons: (2.1) the field trials were not conducted in all relevant regions where the maize will be cultivated, and no extreme weather conditions were taken into account; (2.2) the field trials did not take the current agricultural management practices into account; (2.3) only one transgenic variety was included in the field trials.

2.1 Field trials for compositional and agronomic assessment of the stacked maize were conducted in the US for only one year and not in other relevant maize production areas, such as Brazil and Argentina. As shown in the EFSA opinion (2019f), "no exceptional weather conditions were reported at any of the selected field trial sites". In addition, and contrary to the expected agricultural practices or the different meteorological and agronomic conditions under which the crop is to be grown, EFSA states it "considers that the selected sites reflect commercial maize-growing regions in which the test materials are likely to be grown."

Taking into account the purpose of the genetic engineering in this case, it is not acceptable that EFSA failed to require further studies e.g. • No field trials were conducted that lasted more than one season. Thus, based on current data, it is hardly possible to assess site-specific effects. However, as our analysis on gene expression shows, specific site by site and year by year effects have to be expected. • No data were generated representing more extreme environmental conditions, such as those caused by climate change resulting in more extreme droughts. • No data were generated that represent the growing conditions in other relevant maize growing regions outside the US.

In addition, Fang et al. (2018) showed that stress responses can lead to unexpected changes in plant metabolism inheriting additional EPSPS enzymes. Available publications strongly indicate that plants producing additional EPSPS enzymes are likely to show strong reactions in gene expression under stress conditions, such as drought. These effects are also likely to impact plant composition and biological characteristics that are crucial for the assessment of food and feed safety. However, no specific data were requested or used for detailed comparison to assess genome x environment interactions.

Therefore, the plants should have been subjected to a much broader range of defined environmental conditions and stressors to gather reliable data.

2.2 Due to high weed pressure in many maize growing regions, it has to be expected that these plants will be exposed to higher amounts and repeated dosages of glyphosate. It has to be taken into account that the herbicides can be sprayed with high dosages and repeated sprayings. These agricultural practices have to be taken into account to assess whether the expected agricultural practices will influence the expression of the studied endpoints. However, this requirement was mostly ignored by EFSA and the company: glyphosate was only sprayed at an early stage of vegetation and at comparably low dosages.

Industry recommendations suggest dosages to be sprayed on herbicide resistant maize of up to approx. 3,5 kg a.i./ha glyphosate post-emergence, 9 kg per season, and even higher rates (www.greenbook.net/monsanto-company/roundup-weathermax; www.greenbook.net/monsanto-company/roundup-ultra). From the available data, it has to be assumed that the specific patterns of complementary herbicide applications will not only lead to a higher burden of residues in the harvest, but may also influence the composition of the plants and agronomic characteristics. This aspect, which is supported by the analysis of the gene expression provided above, was ignored in the EFSA risk assessment.

EFSA should have requested the company to submit data from field trials with the highest dosage of the complementary herbicides that can be tolerated by the plants, including repeated spraying with each active ingredient individually as well as in combination. Taking into account the specific characteristics of the stacked maize, only the application of high and repeated dosages of glyphosate should have been regarded as representative for expected agricultural practices.

2.3 It is known that the genomic background of the variety can influence the expression of the inserted genes (see, for example, Trtikova et al., 2015). Therefore, EFSA should have requested additional data from several varieties, including those cultivated in South America, to examine how the gene constructs interact with the genetic background of the plants. This approach is supported by the analysis of the gene expression provided above but was ignored in the EFSA risk assessment.

Further findings Only data from a low number of agronomic parameters (10) were subjected to statistical analysis in accordance with EFSA guidance, 5 (without and without spraying of the complementary herbicide) of these were found to be statistically and significantly different.

Compositional analysis of 54 endpoints in the grains revealed many (and partly major) statistically significant differences: 32 endpoints were statistically significantly different in plants spayed with the complementary herbicides, 42 in plants not sprayed with glyphosate (but other conventional herbicides).

Even if changes taken as isolated data might not directly raise safety concerns, the overall high number of significant effects has to be taken as a starting point for much more detailed investigations: half of the parameters measured in regard to agronomic characteristics and more than half concerning plant composition were significantly different.

As explained above, EFSA should have requested further tests with repeated spraying with higher herbicide dosages and exposure to a much wider range of environmental conditions, taking more extreme drought conditions into account. Furthermore, the plant material should have been assessed by using 'Omics-techniques' to investigate changes in plant composition or agronomic characteristics in more detail.

However, instead of assessing the overall pattern of changes in plant components, their causes and possible impacts in more detail, EFSA only assessed the observed changes in isolation in regard to evidence of potential harm. This approach turns the comparative approach into a trivial concept of assessing bits and pieces, and it ignores questions concerning the overall safety of the whole food and feed. However, more in-depth investigations should not depend on findings indicating adverse effects, they should always be necessary to come to sufficiently robust conclusions to inform the next steps in risk assessment.

Based on the available data, no final conclusions can be drawn on the safety of the plants. The data do not fulfill the requirements of Implementing Regulation 503/2013.

EFSA (2019f) GMO Panel (EFSA Panel on Genetically Modified Organisms), Scientific Opinion on the assessment of genetically modified maize MON 87427 9 MON 89034 9 MIR162 9 MON 87411 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2017-144). EFSA Journal 2019;17 (11):5848, 33 pp. https://doi.org/10.2903/j.efsa.2019.5848

Fang, J., Nan, P., Gu, Z., et al. (2018) Overexpressing exogenous 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) genes increases fecundity and auxin content of transgenic arabidopsis plants. Front Plant Sci 9: 233. doi: 10.3389/fpls.2018.00233

Trtikova, M., Wikmark, O.G., Zemp, N., Widmer, A., Hilbeck, A. (2015) Transgene expression and Bt protein content in transgenic Bt maize (MON810) under optimal and stressful environmental conditions. PloS One, 10(4): e0123011.

---

b. Food Safety Assessment:
Toxicology

Implementing Regulation 503/2013 requests: “Toxicological assessment shall be performed in order to: (a) demonstrate that the intended effect(s) of the genetic modification has no adverse effects on human and animal health; (b) demonstrate that unintended effect(s) of the genetic modification(s) identified or assumed to have occurred based on the preceding comparative molecular, compositional or phenotypic analyses, have no adverse effects on human and animal health;”

“In accordance with the requirements of Articles 4 and 16 of Regulation (EC) No 1829/2003, the applicant shall ensure that the final risk characterisation clearly demonstrates that: (a) the genetically modified food and feed has no adverse effects on human and animal health;”

As explained above, many significant changes were identified: half of the parameters measured in regard to agronomic characteristics and more than half of plant composition were significantly different. Even if the changes taken as isolated data might not directly raise safety concerns, the overall high number of effects should have been considered as a starting point for much more detailed investigation of their potential health impacts.

Despite these findings, and in awareness of the lack of more specific data and the resulting major uncertainties (such as combinatorial effects, the effects caused by the dsRNA and the artifically synthesized Bt proteins), no testing of the whole stacked plant (feeding study) was requested.

In more detail, many uncertainties are surrounding the risk assessment of the parental plant MON87411: For the single plant, the company conducted a 90-day feeding trial with maize MON87411 in rats. In this feeding trial only one dosage of maize (33 %) was included as part of the diet, instead of several dosages as requested by existing guidance. Nevertheless, EFSA still accepted the data.

The stability of the test and control materials was not tested; therefore it remains unclear if the diet is comparable to diets fed under practical conditions if, for example, the maize is fed to animals closer to the date of harvest.

The most relevant finding was weight depression in the rats fed with the maize. As EFSA (2018b) summarises : “Statistically significant lower mean feed consumption (as g/cage per day only) were observed in males fed test diet (~ 9% in study week intervals 5–6, 9–10, 10–11, 11–12). This was associated with a statistically significant decrease in mean body weights, compared to the concurrent control (~ 7% in weeks 11 and 12) and in mean cumulative body weight (~ 12% in study week intervals 0–10, 0–11 and 0–12). Moreover, statistically significant lower mean weekly body weight change was also observed in males (study week intervals 0–1, 3–4, and 6–7) and in females (study week interval 7–8) fed the test diet, compared to the concurrent controls.”

However, in the absence of test diet-related clinical signs and histopathological changes in the digestive tract, the GMO panel considered the changes to be non-adverse. Further, EFSA (2018b), without citing specific references, very generally questions whether the uptake of the dsRNA can be expected at all: “Dietary ncRNAs [non coding RNAs] are generally rapidly denaturated, depurinated and degraded shortly after ingestion due to enzymes and conditions (e.g. pH) in the gastrointestinal tract lumen; in addition, the presence of barriers (e.g. mucus, cellular membranes) limits the cellular uptake of ncRNAs by gastrointestinal cells, and a rapid intracellular degradation of possible uptaken ncRNA occurs. Due to the above, the amount of RNAs taken up and absorbed after oral ingestion is considered negligible in humans and animals (mammals, birds and fish).”

This assessment of toxicology has to be rejected for several reasons: • In 2012, it was reported for the first time that miRNA produced by plants can enter the bloodstream of mammals (including humans) at the stage of consumption (Zhang et al, 2012). These findings were called into question by several experts (see, for example, US EPA 2014; EFSA, 2014). However, looking at more recent publications, one has to assume that plant miRNA can indeed enter the bloodstream, organs, milk and urine of mammals after ingestion (Yang et al., 2015; Liang et al., 2015; Hirschi et al, 2015, Lukaski & Zielenkiewicz, 2014). • There is evidence that small RNAs taken up from the intestine do indeed interfere with gene regulation in humans and animals. For example, it was found that miRNA transferred via milk shows biological activity (Baier et al., 2014). Small RNAs produced by plants are able to interfere with the immune system in humans and animals (Zhou et al., 2015; Cavalieri et al., 2016). • It is also known from several studies that uptake of miRNA from the mammalian gut and its detection is dependent on specific factors. For example, Liang et al. (2015) describe mechanisms for uptake and measurement that need to be taken into account to successfully quantify the uptake, Yang et al. (2015) as well as Wang et al. (2012) show that the health status of the recipient can be decisive; Baier et al. (2014) show that packaging in liposomes enhances uptake; Yang et al. (2015) show that dosage and also prolonged duration of exposure is important.

None of these issues were discussed or assessed by EFSA (2018b). Further, an external study commissioned by EFSA (Paces et al., 2017) overlooked several relevant studies. Moreover, in its conclusions it does not support the position of EFSA that uptake cannot generally be expected. Paces et al. (2017) summarise the discussion as follows: “Thus, it is apparent that four years after the original report (Zhang et al., 2012(...)), the field remains split. The essential questions concerning the existence of the proposed mechanism emerged already in 2012. Further research is necessary to clarify the basis of the aforementioned contradictory observations.”

Paces et al. (2017) also mention that the findings (Zhang et al., 2012), which although disputed are not in contradiction to the general findings in this field: “In

2012, the article by Zhang et al. proposed that miRNAs from ingested plants could traverse into the bloodstream and suppress genes in the liver (Zhang et al., 2012 (...)). The report sparked an ongoing debate because of potential implications these data could have. It should be pointed out that, while the article reported unexpected and surprising results, it was not breaking any conceptual dogma. The idea that information could be transmitted from food in a form of a large organic molecule that would traverse into the human organism has been an integral part of the prion hypothesis, which brought a concept of food-borne infectious particles made only of proteins (...). The prion hypothesis, for which Stanley Prusiner received a Nobel Prize in 1997, is nowadays a biology textbook knowledge. Furthermore, cross-kingdom regulation by small RNAs was discovered in RNA silencing field already in its early years – long dsRNA expressed in bacteria could induce repression of worm genes with complementary sequences when worms were fed with such bacteria (...). Furthermore, in 2012 it was already well known that feeding on a plant carrying an RNAi-inducing transgene can induce RNAi in nematodes, insects, or fungi (...). Thus, the article by Zhang et al. was not bringing any major shift in existing paradigms. The article essentially extended knowledge of RNA silencing spreading by reporting an example of a miRNA activity transferred from plants to mammals through feeding."

There are at least two ways in which the additional dsRNA expressed in the plants can impact mammalian health:

(1) Uptake from the gut into the bloodstream in the same way as other plant miRNAs as described (see, for example, Yang et al., 2015; Liang et al., 2015; Hirschi et al, 2015; Beatty et al., 2014). If the bioactive molecules produced in the plants start to interfere with mammalian gene regulation, the effects might be drastic: in humans dysfunction of the ESCRT complex is associated with numerous pathologies, including cancer and various neurodegenerative diseases (Henne et al., 2012).

Based on current knowledge, this scenario cannot be excluded. This is especially true in the light of the specific circumstances described by Liang et al. (2014), Zhang et al. (2012) and Yang (2015) that are relevant for the uptake of miRNA from the gut. The need for further investigation is supported by the outcome of a FIFRA scientific panel workshop held in the US in 2014, maintaining that in particular the risks for immune-compromised individuals should be tested (US EPA, 2014): "The stability of dsRNA should be tested in individuals that manifest specific diseases (e.g., Crohn's, colitis, irritable bowel syndrome, etc.), the immune compromised, elderly, as well as children. These individuals may have compromised digestion or increased sensitivity to dsRNA exposure."

(2) It is well known that miRNA plays a key role in gene regulation in the gut microbiome, as well as in the communication between the mammalian host and its gut microbiome (see, for example, Williams et al., 2017). It is plausible that the

dsRNA produced in maize MON87411 can interact with the gut microbiome directly without direct uptake from the gut. At least for yeast, the essential role of the Snf7 as part of the ESCRT pathway is well described (see www.yeastgenome.org/locus/S000004015). Thus, there is a plausible hypothesis on how the additional dsRNA might affect the gut microbiome community.

Interaction with the microbiome also might explain the findings from animal feeding studies showing weight differences without pathological effects.

These aspects were mostly overlooked by EFSA (2018b) in its risk assessment even though a 2014 EFSA workshop (ESFA 2014) identified the following issues as relevant for risk assessment of health effects: "Throughout the different discussion topics, the following issues were identified as knowledge gaps, where more research could be warranted: - The RNAi and metabolic profiling in RNAi-based plants could be further explored and corroborated to support risk assessment. In this context, 'omics' techniques should be further investigated as supporting tools. - The use of bioinformatics to predict potential off target effects in consumers should be further explored. - Possible changes in microbiota, residing in human or animal guts, following consumption of food and feed products derived from RNAi-based plants could be a research topic."

As the BSE crisis showed, the risk of bioactive compounds being transmitted via the food and feed chain poses a high risk for farm animals and humans (see Paces et al., 2017). Therefore, uncertainties and knowledge gaps identified in the current risk assessment cannot be accepted.

In addition, the need for more detailed assessment is underlined by publications showing that the Bt toxins also raise further questions in regard to feed and food safety:

(1) There are several partially diverging theories about the exact mode of action of the Bt toxins at the molecular level (see Then, 2010; Hilbeck & Otto, 2015). Thus, it cannot be excluded a priori that the toxins are inert in regard to human and animal health as maintained under risk assessment for food and feed.

(2) There are further uncertainties regarding the specificity of Bt toxins (Venter and Bøhn, 2016). Changes in specificity may emerge from structural modifications performed to render higher efficacy. For example, the proteins are truncated to become activated (see Hilbeck and Schmidt, 2006).

(3) In addition, there are findings in mammalian species showing that Bt toxicity is a relevant topic for detailed health risk assessment: some Cry toxins are known to bind to epithelial cells in the intestines of mice (Vázquez-Padrón et al., 1999).

(4) As far as potential effects on health are concerned, several publications (Thomas and Ellar 1983; Shimada et al., 2003; Mesnage et al., 2013; Huffman et al., 2004; Bondzio et al., 2013) show that Cry proteins may indeed have an impact on the health of mammals. For example, de Souza Freire et al., (2014) confirm haematological toxicity of several Cry toxins. Some of these effects seem to occur where there are high concentrations and tend to become stronger over longer periods of time.

(5) Further, the toxicity of Bt toxins can be enhanced through interaction with other compounds, such as plant enzymes (Zhang et al., 2000, Zhu et al., 2007; Pardo-López et al., 2009); other Bt toxins (Sharma et al., 2004; Tabashnik et al., 2013; Bøhn et al. 2016, Bøhn 2018); gut bacteria (Broderick et al., 2009); residues from spraying with herbicides (Bøhn et al. 2016, Bøhn 2018) and other (Kramarz et al., 2007; Kramarz et al., 2009; Khalique and Ahmed, 2005; Singh et al., 2007; Zhu et al., 2005; Mason et al., 2011; Reardon et al., 2004).

In this context, it is relevant that Bt toxins can survive digestion to a much higher degree than has been assumed by EFSA. Chowdhury et al., (2003) and Walsh et al. (2011) showed that when pigs were fed with Bt maize, Cry1A proteins could frequently and successfully still be found in the colon of pigs at the end of the digestion process. This means that Bt toxins are not degraded quickly in the gut and can persist in larger amounts until digestion is completed; and that there is enough time for interaction between various food compounds.

Further, as far as the exposure of the food chain with Bt toxins is concerned, EFSA should have requested data on the overall combined exposure to Bt toxins resulting from the introduction of Bt plants in the EU. Currently, there are already 40 events that produce Bt toxins authorised for import. The accumulated exposure stemming from these imports should have been taken into account. For example a new study testing corn with a combination of Bt toxins (Cry1Ab and Cry34Ab1) indicates health impacts in rats (Zdziarski et al., 2018).

We conclude the need for more detailed investigation. Further, more detailed (e.g. using several dosages) and long-term feeding studies, taking into account the functioning of the microbiome, would be necessary to assess potential health impacts. These studies should include -omics data from animals, as well as detailed assessment of the impact of higher dosages of glyphosate sprayed on the plants (as can be expected under practical conditions).

In any case, the toxicological assessment carried out by EFSA (2018b and 2019f) is not sufficient to show food and feed safety.

Beyond that, the residues from spraying were considered to be outside the remit of the GMO panel. However, without detailed assessment of these residues, no conclusion can be drawn on the safety of the imported products: due to specific

agricultural practices in the cultivation of these herbicide resistant plants, there are, for example, specific patterns of applications, exposure, occurrence of specific metabolites and emergence of combinatorial effects that require special attention (see also Kleter et al., 2011).

More detailed assessment is also in accordance with pesticide regulation that requires specific risk assessment of imported plants if the usage of pesticides is different in the exporting countries compared to the usage in the EU. In this regard, it should be taken into account that EFSA (2019g) explicitly stated that no conclusion can be derived on the safety of residues from spraying with glyphosate occurring in genetically engineered plants resistant to this herbicide.

Further, there is a common understanding that commercially traded formulations of glyphosate, such as Roundup, can be more toxic than glyphosate itself. Therefore, the EU has already taken measures to remove problematic additives known as POE tallowamine from the market. Problematic additives are still allowed in those countries where the genetically engineered plants are cultivated. The EU Commission has confirmed the respective gaps in risk assessment: "A significant amount of food and feed is imported into the EU from third countries. This includes food and feed produced from glyphosate-tolerant crops. Uses of glyphosate-based plant protection products in third countries are evaluated by the competent authorities in those countries against the locally prevailing regulatory framework, but not against the criteria of Regulation (EC) No. 1107/2009. (…)." (www.testbiotech.org/content/eu-commission-request-consider-impact-glyphosate-residues-feed-animal-health-february-2016)

Consequently, EFSA should have requested the company to submit data from field trials with the highest dosage of the complementary herbicides that can be tolerated by the plants, including repeated spraying. The material derived from those plants should have been assessed in regard to organ toxicity, immune system responses and reproductive toxicity, also taking combinatorial effects with other plant components into account.

There are further relevant issues: for example, the potential impact on the intestinal microbiome also has to be considered. Such effects might be caused by the residues from spraying since glyphosate has been shown to have negative effects on the composition of the intestinal flora of cattle (Reuter et al., 2007), poultry (Shehata et al., 2013) and rodents (Mao et al., 2018). In general, antibiotic effects and other adverse health effects might occur from exposure to a diet containing these plants, which were not assessed under pesticide regulation.

In general, antibiotic effects and other adverse health effects might occur from exposure to a diet containing these plants that were not assessed under pesticide regulation. These adverse effects on health might be triggered by the residues from spraying with the complementary herbicide (see also van Bruggen et al., 2017).

Further attention should be paid to the specific toxicity of the metabolites of the pesticide active ingredients that might occur specifically in the stacked event. Whatever the case, both the EU pesticide regulation and the GMO regulation require a high level of protection for health and the environment. Thus, in regard to herbicide-resistant plants, specific assessment of residues from spraying with complementary herbicides must be considered to be a prerequisite for granting authorisation.

EU legal provisions such as Regulation 1829/2003 (as well as Implementing Regulation 503/2013) state that "any risks which they present for human and animal health and, as the case may be, for the environment" have to be avoided. Therefore, potential adverse effects that result from combinatorial exposure of various potential stressors need specification, and their assessment needs to be prioritised. We conclude that the health risk assessment currently performed by EFSA for the stacked maize is unacceptable. We propose testing these plants following the whole mixture approach, considering them to be "insufficiently chemically defined to apply a component-based approach" (EFSA, 2019e).

Despite all these open questions regarding potential health impacts, we are not aware of a single sub-chronic or chronic feeding study performed with whole food and feed derived from the stacked maize. This observation is supported by the literature review carried out by the company which did not yield any peer reviewed publication. In this context, it is relevant to consider that the outcome of the feeding studies with the parental plants raised several questions concerning their results, methodology and reliability (see comments from the experts of Member States, EFSA, 2019d)

Testbiotech is also aware that feeding studies with similar stacked maize indicated potential health impacts such as inflammatory responses in the stomach (Zdziarski et al., 2018). Inflammatory responses are an alarm signal typical of many chronic diseases and therefore require close attention. While the applicant provided some data in regard to celiac disease, other diseases associated with symptoms of chronic inflammation were not considered at all.

In conclusion, the EFSA opinion on the application for authorisation of the stacked maize (EFSA 2019f) cannot be said to fulfil the requirements for assessment of potential synergistic or antagonistic effects resulting from the combination of the transformation events in regard to toxicology.

For this purpose, EFSA should have requested the company to submit data from field trials with the highest dosage of complementary herbicides that can be tolerated by the plants, including repeated spraying. The material derived from the plants should have been assessed in regard to organ toxicity, immune responses and reproductive toxicity, also taking combinatorial effects with other plants components into account.

As a result, the toxicological assessment carried out by EFSA is not acceptable.

Baier, S.R., Nguyen, C., Xie, F., Wood, J.R. Zempleni, J. (2014) MicroRNAs Are Absorbed in Biologically Meaningful Amounts from Nutritionally Relevant Doses of Cow Milk and Affect Gene Expression in Peripheral Blood Mononuclear Cells, HEK-293 Kidney Cell Cultures, and Mouse Livers. The Journal of nutrition, 144(10): 1495-1500. https://academic.oup.com/jn/article-abstract/144/10/1495/4575107

Beatty, M., Guduric-Fuchs, J., Brown, E., Bridgett, S., Chakravarthy, U., Hogg, R.E., et al. (2014) Small RNAs from plants, bacteria and fungi within the order hypocreales are ubiquitous in human. Plasma, 15: 1–12. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4230795/

Bøhn, T., Rover, C.M., Semenchuk, P.R. (2016) Daphnia magna negatively affected by chronic exposure to purified Cry-toxins. Food Chem. Toxicol., 91: 130–140.

Bøhn, T. (2018) Criticism of EFSA's scientific opinion on combinatorial effects of ‘stacked’ GM plants. Food and Chemical Toxicology. https://www.sciencedirect.com/science/article/pii/S0278691517306907

Bondzio, A., Lodemann, U., Weise, C., Einspanier, R. (2013) Cry1Ab Treatment Has No Effects on Viability of Cultured Porcine Intestinal Cells, but Triggers Hsp70 Expression. PLOS ONE 8, e67079. https://doi.org/10.1371/journal.pone.0067079

Broderick, N.A., Robinson, C.J., McMahon, M.D., Holt, J., Handelsman, J., Raffa, K.F. (2009) Contributions of gut bacteria to Bacillus thuringiensis-induced mortality vary across a range of Lepidoptera. BMC Biol. 7: 11. https://doi.org/10.1186/1741-7007-7-11

Cavalieri, D., Rizzetto, L., Tocci, N., Rivero, D., Asquini, E., Si-Ammour, A., Bonechi, E., Ballerini, C., Viola, R. (2016) Plant microRNAs as novel immunomodulatory agents. Scientific Reports, 6: 25761. https://www.nature.com/articles/srep25761

Chowdhury, E.H., Kuribara, H., Hino, A., Sultana, P., Mikami, O., Shimada, N., ... & Nakajima, Y. (2003) Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11. Journal of animal science, 81(10): 2546-2551.

de Souza Freire, I., Miranda-Vilela, A.L., Barbosa, L.C.P., Martins, E.S., Monnerat, R.G., Grisolia, C.K. (2014) Evaluation of Cytotoxicity, Genotoxicity and Hematotoxicity of the Recombinant Spore-Crystal Complexes Cry1Ia,

Cry10Aa and Cry1Ba6 from Bacillus thuringiensis in Swiss Mice. Toxins 6, 2872–2885. https://doi.org/10.3390/toxins6102872

EFSA (2014) Event report: International scientific workshop 'Risk assessment considerations for RNAi based GM plants' (4-5 June 2014, Brussels, Belgium). EFSA supporting publication 2014:EN-705. www.efsa.europa.eu/de/events/event/140604

EFSA (2018b) GMO Panel (EFSA Panel on Genetically Modified Organisms), Scientific Opinion on the assessment of genetically modified maize MON 87411 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2015-124). EFSA Journal 2018;16(6):5310, 29 pp. https://doi.org/10.2903/j.efsa.2018.5310

EFSA (2019d) Application EFSA-GMO-NL-2017-144, Comments and opinions submitted by Member States during the three-month consultation period, Register of Questions, http://registerofquestions.efsa.europa.eu/roqFrontend/ListOfQuestionsNoLogin?0&panel=ALL

EFSA (2019e) Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA Journal 2019;17(3): 5634, 77 pp. https://doi.org/10.2903/j.efsa.2019.5634

EFSA (2019f) GMO Panel (EFSA Panel on Genetically Modified Organisms), Scientific Opinion on the assessment of genetically modified maize MON 87427 9 MON 89034 9 MIR162 9 MON 87411 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2017-144). EFSA Journal 2019;17 (11):5848, 33 pp. https://doi.org/10.2903/j.efsa.2019.5848

EFSA (2019g) European Food Safety Authority, Review of the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005 – revised version to take into account omitted data. EFSA Journal 2019;17(10):5862, 211 pp. https://doi.org/10.2903/j.efsa.2019.5862

Henne, W.M., Buchkovich, N.J., Zhao, Y., Emr, S.D. (2012) The Endosomal Sorting Complex ESCRT-II Mediates the Assembly and Architecture of ESCRT-III Helices, Cell 151: 356–371. www.sciencedirect.com/science/article/pii/S0092867412011208

Hilbeck, A., Otto, M. (2015) Specificity and Combinatorial Effects of Bacillus Thuringiensis Cry Toxins in the Context of GMO Environmental Risk Assessment. Front. Environ. Sci. 3. https://doi.org/10.3389/fenvs.2015.00071

Hilbeck, A., Schmidt, J.E.U. (2006) Another View on Bt Proteins – How Specific are They and What Else Might They Do? Biopesticides international, 2(1): 1-50. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.319.4683&rep=rep1&type=pdf

Hirschi, K. D., Pruss, G. J. & Vance, V. (2015) Dietary delivery: a new avenue for microRNA therapeutics?. Trends Biotechnol. 33: 431–432. www.sciencedirect.com/science/article/pii/S0167779915001250

Huffman, D.L., Abrami, L., Sasik, R., Corbeil, J., Goot, F.G. van der, Aroian, R.V. (2004) Mitogen-activated protein kinase pathways defend against bacterial pore-forming toxins. Proc. Natl. Acad. Sci. U. S. A. 101, 10995–11000. https://doi.org/10.1073/pnas.0404073101

Khalique, F. & Ahmed, K. (2005) Compatibility of bio-insecticide with chemical insecticide for management of Helicoverpa armigera Huebner. Pak. J. Biol. Sci. 8, 475-478.

Kleter, G.A., Unsworth, J.B., Harris, C.A. (2011) The impact of altered herbicide residues in transgenic herbicide-resistant crops on standard setting for herbicide residues. Pest Manag Sci 67(10): 1193-1210. doi: 10.1002/ps.2128

Kramarz, P., de Vaufleury, A., Gimbert, F., Cortet, J., Tabone, E., Andersen, M.N., Krogh, P.H. (2009) Effects of Bt-maize material on the life cycle of the land snail Cantareus aspersus. Appl. Soil Ecol. 42, 236–242.

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

Liang, H., Zhang, S., Fu, Z., Wang, Y., Wang, N., Liu, Y., ... & Chen, X. (2015) Effective detection and quantification of dietetically absorbed plant microRNAs in human plasma. The Journal of nutritional biochemistry, 26(5): 505-512. www.sciencedirect.com/science/article/pii/S0955286315000169

Lukaski, A. & Zielenkiewicz, P. (2014) In Silico Identification of Plant miRNAs in Mammalian Breast Milk Exosomes – A Small Step Forward? PloS one, 9(6): e99963. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0099963

Mao, Q., Manservisi, F., Panzacchi, S., Mandrioli, D., Menghetti, I., Vornoli, A., Bua, L., Falcioni, L., Lesseur, C., Chen, J., Belpoggi, F., Hu, J. (2018) The Ramazzini Institute 13-week pilot study on glyphosate and Roundup administered at human-equivalent dose to Sprague Dawley rats: effects on the microbiome. Environmental Health, 17: 50. https://doi.org/10.1186/s12940-018-0394-x

Mason, K.L., Stepien, T.A., Blum, J.E., Holt, J.F., Labbe, N.H., Rush, J.S., Raffa, K.F., Handelsman, J. (2011) From commensal to pathogen: translocation of Enterococcus faecalis from the midgut to the hemocoel of Manduca sexta. mBio 2: e00065-00011. https://doi.org/10.1128/mBio.00065-11

Mesnage, R., Clair, E., Gress, S., Then, C., Székács, A., Séralini, G.-E. (2013) Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. J. Appl. Toxicol. 33, 695–699. https://doi.org/10.1002/jat.2712

Paces, J., Nic, M., Novotny, T.. Svoboda, P. (2017) Literature review of baseline information to support the risk assessment of RNAi-based GM plants. EFSA supporting publication 2017:EN-1246, 314 pp. https://doi.org/10.2903/sp.efsa.2017.en-1246

Pardo-López, L., Muñoz-Garay, C., Porta, H., Rodríguez-Almazán, C., Soberón, M., Bravo, A., (2009). Strategies to improve the insecticidal activity of Cry toxins from Bacillus thuringiensis. Peptides, Invertebrate Neuropeptides, IX 30: 589–595. https://doi.org/10.1016/j.peptides.2008.07.027

Reardon, B.J., Hellmich, R.L., Sumerford, D.V., Lewis, L.C. (2004) Growth, Development, and Survival of Nosema pyrausta -Infected European Corn Borers (Lepidoptera: Crambidae) Reared on Meridic Diet and Cry1Ab. J. Econ. Entomol. 97: 1198-1201. https://doi.org/10.1093/jee/97.4.1198

Reuter T, Alexander TW, Martínez TF, McAllister TA (2007) The effect of glyphosate on digestion and horizontal gene transfer during in vitro ruminal fermentation of genetically modified canola. J Sci Food Agric 87(15):2837–2843. doi: 10.1002/jsfa.3038

Sharma, H.C., Sharma, K.K., Crouch, J.H., 2004. Genetic Transformation of Crops for Insect Resistance: Potential and Limitations. Crit. Rev. Plant Sci., 23: 47-72. https://doi.org/10.1080/07352680490273400

Shehata, A.A., Schrödl, W., Aldin, A.A., et al. (2013) The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in Vitro. Curr Microbiol, 66(4): 350-358. doi: 10.1007/s00284-012-0277-2

Shimada, N., Kim, Y.S., Miyamoto, K., Yoshioka, M., Murata, H. (2003) Effects of Bacillus thuringiensis Cry1Ab Toxin on Mammalian Cells. J. Vet. Med. Sci. 65, 187–191. https://doi.org/10.1292/jvms.65.187

Singh, G., Rup, P.J., Koul, O., (2007) Acute, sublethal and combination effects of azadirachtin and Bacillus thuringiensis toxins on Helicoverpa armigera

(Lepidoptera: Noctuidae) larvae. Bull. Entomol. Res., 97: 351–357. https://doi.org/10.1017/S0007485307005019

Tabashnik, B.E., Fabrick, J.A., Unnithan, G.C., Yelich, A.J., Masson, L., Zhang, J., Bravo, A., Soberón, M. (2013) Efficacy of genetically modified Bt toxins alone and in combinations against pink bollworm resistant to Cry1Ac and Cry2Ab. PloS One 8: e80496.

Then, C. (2010) Risk assessment of toxins derived from Bacillus thuringiensis - synergism, efficacy, and selectivity. Environ Sci Pollut R 17(3):791–797. https://link.springer.com/article/10.1007/s11356-009-0208-3

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

US EPA (2014) RNAi Technology: Problem Formulation for Human and Ecological Risk Assessment. SAP minutes No. 2014-02, http://wssa.net/wp-content/uploads/RNAi-report_EPA-May-2014.pdf

van Bruggen, A.H.C., He, M.M., Shin, K., Mai, V., Jeong, K. C., Finckh, M.R., Morris, J.G. (2018) Environmental and health effects of the herbicide glyphosate. Science of The Total Environment, 616: 255-268. https://www.sciencedirect.com/science/article/pii/S0048969717330279

Vázquez-Padrón, R.I., Moreno-Fierros, L., Neri-Bazán, L., de la Riva, G.A., López-Revilla, R. (1999) Intragastric and intraperitoneal administration of Cry1Ac protoxin from Bacillus thuringiensis induces systemic and mucosal antibody responses in mice. Life Sci. 64, 1897–1912. https://doi.org/10.1016/S0024-3205(99)00136-8

Venter, H.J., Bøhn, T. (2016) Interactions between Bt crops and aquatic ecosystems: A review. Environ. Toxicol. Chem. 35, 2891–2902. https://doi.org/10.1002/etc.3583

Walsh, M.C., Buzoianu, S.G., Gardiner, G.E., Rea, M.C., Gelencsér, E., Jánosi, A., ... & Lawlor, P. G. (2011) Fate of transgenic DNA from orally administered Bt MON810 maize and effects on immune response and growth in pigs. PLoS One, 6(11): e27177. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0027177

Wang, K., Li, H., Yuan, Y., Etheridge, A., Zhou, Y., Huang, D., et al. (2012) The complex exogenous RNA spectra in human plasma: an interface with human gut biota? PloS one, 7(12): e51009.

Williams, M.R., Stedtfeld, R.D., Tiedje, J.M. Hashsham, S.A. (2017) MicroRNAs-Based Inter-Domain Communication between the Host and Members of the Gut Microbiome. Front. Microbiol. 8:1896. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0051009

Yang, J., Farmer, L. M., Agyekum, A.A.A. Hirschi, K.D. (2015) Detection of dietary plant-based small RNAs in animals. Cell Res. 25, 517–520. www.nature.com/articles/cr201526

Zdziarski, I.M., Carman, J.A. and Edwards, J.W. (2018) Histopathological Investigation of the Stomach of Rats Fed a 60% Genetically Modified Corn Diet. Food and Nutrition Sciences , 9, 763-796. www.scirp.org/journal/PaperInformation.aspx?paperID=85687

Zhang, J., Wang, C., Qin, J. (2000) The Interactions between Soybean Trypsin Inhibitor and δ-Endotoxin of Bacillus thuringiensis in Helicoverpa armigera Larva. J. Invertebr. Pathol. 75: 259-266. https://doi.org/10.1006/jipa.2000.4936

Zhang, L., Hou, D., Chen, X., Li, D., Zhu, L., Zhang, Y., Li, J., Bian, Z., Liang, X., Cai, X., Yin, Y., Wang, C., Zhang, T., Zhu, D., Zhang, D., Xu, J., Chen, Qu., Ba, Y., Liu, J., Wang, Q., Chen, J., Wang, J., Wang, M., Zhang, Q., Zhang, J., Zen, K., Zhang, C.Y. (2012) Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. Cell Research, 22(1): 107-126. www.nature.com/articles/cr2011158

Zhou, Z., Li, X., Liu, J., Dong, L., Chen, Q., Liu, J., ... & Zhang, L. (2015) Honeysuckle-encoded atypical microRNA2911 directly targets influenza A viruses. Cell research, 25: 39–49. www.nature.com/articles/cr2014130

Zhu, Y.C., Adamczyk, J.J., West, S. (2005) Avidin, a Potential Biopesticide and Synergist to Bacillus thuringiensis Toxins Against Field Crop Insects. J. Econ. Entomol. 98: 1566-1571. https://doi.org/10.1093/jee/98.5.1566

Zhu, Y.C., Abel, C.A., Chen, M.S. (2007) Interaction of Cry1Ac toxin (Bacillus thuringiensis) and proteinase inhibitors on the growth, development, and midgut proteinase activities of the bollworm, Helicoverpa zea. Pestic Biochem Phys 87(1): 39-46. www.sciencedirect.com/science/article/pii/S0048357506000848

Allergenicity

Implementing Regulation 503/2013 requests: “In cases when known functional aspects of the newly expressed protein or structural similarity to known strong adjuvants may indicate possible adjuvant activity, the applicant shall assess the possible role of these proteins as adjuvants. As for allergens, interactions with other

constituents of the food matrix and/or processing may alter the structure and bioavailability of an adjuvant and thus modify its biological activity."

"In accordance with the requirements of Articles 4 and 16 of Regulation (EC) No 1829/2003, the applicant shall ensure that the final risk characterisation clearly demonstrates that: (a) the genetically modified food and feed has no adverse effects on human and animal health;"

However, EFSA did not request the applicant to provide data to verify whether the source of the transgene is allergenic. According to Santos-Vigil et al (2018), the Bt toxin Cry1Ac can act as an allergen if ingested. This publication is highly relevant: the Bt toxin Cry1Ac was used as a source for the synthesis of Cry1A.105 expressed in the stacked maize. Therefore, the synthetically derived Cry1A.105 toxin produced in the maize has structural similarity with Cry1Ac. If Cry1Ac is suspected of being an allergen, the source of Cry1A.105 has to be verified as allergenic and therefore investigated in detail.

The EU Commission initially noted that the Santos-Vigil et al (2018) publication was relevant for the risk assessment of genetically engineered plants producing Bt toxins, and therefore requested the European Food Safety Authority (EFSA) for an assessment. However, EFSA (2018e) came to the conclusion that the Santos-Vigil et al. (2018) publication does not provide any new information and suffers from methodological flaws. However, this EFSA opinion is based on a rather biased interpretation of existing publications, and it does not provide any evidence that the Santos-Vigil (2018) findings are invalid or irrelevant (Moreno-Fierros et al., 2018).

In conclusion, the EFSA assessment of the stacked maize cannot be said to fulfil the requirements for assessing allergenicity of the source of the transgene. The Santos-Vigil et al. (2018) publication has to be considered valid and not properly assessed by EFSA (Moreno-Fierros et al., 2018). In awareness of the high concentrations of insecticidal proteins produced in the stacked maize and products derived thereof, EFSA should have started with the hypothesis that the consumption of products derived from the maize can trigger allergic reactions – and should therefore have requested empirical investigations.

Furthermore, there are several studies indicating that immune responses such as adjuvanticity in mammals are triggered by Bt toxins and have to be considered in this context. Studies with the Cry1Ac toxin (Moreno-Fierros et al., 2000; Vázquez et al. 1999; Legorreta-Herrera et al., 2010; Jarillo-Luna et al. 2008; González-González et al., 2015; Ibarra-Moreno et al., 2014; Guerrero et al. 2007; Guerrero et al., 2004; Moreno-Fierros et al. 2013; Rubio-Infante et al. 2018) are especially relevant (for review also see Rubio-Infante et al. 2016).

All the responses described in the above publications are likely to be dependent on the dosage to which the mammals were exposed. In this regard, and again as

mentioned above, the investigation of potential immune responses triggered by the maize is highly relevant, it has to be considered that the concentration of the insecticidal proteins is much higher in gluten meal produced from the maize, and that it can reach a much higher concentrations compared to the kernels. Therefore, the food and feed products derived from the stacked maize need to be much more carefully risk assessed in regard to their impact on the immune system and potential adjuvanticity compared to those genetically engineered plants producing just one Bt toxin.

In its risk assessment, EFSA did not consider that under real conditions and contrary to what is suggested by the findings of in-vitro studies, Bt toxins will not be degraded quickly in the gut but are likely to occur in substantial concentrations in the large intestine and faeces (Chowdhury et al., 2003; Walsh et al., 2011).

In regard to the degradation of the Bt toxins during ingestion, there is specific cause for concern that the maize or gluten is likely to be fed together with soybeans that naturally produce enzymes, which can substantially delay the degradation of Bt toxins in the gut (Pardo-López et al., 2009). In addition, soybeans are known to produce many food allergens. Therefore, the immune system responses caused by the allergens in the soybeans might be considerably enhanced by the adjuvant effects of the Bt toxins.

Our findings on gene expression show that no reliable conclusion on the content of insecticidal proteins can be derived from the available data. Furthermore, in processed products, such as maize gluten, the toxins can even show a much higher concentration. These higher overall concentrations of the three insecticidal proteins is relevant for the assessment of overall toxicology as well as for the immune system; nevertheless, there were no empirical investigations. This is especially relevant for Vip3Aa20, which so far was not subjected to more detailed analysis regarding immunological or other toxicological effects, and that can be present in comparably high concentrations in the grain.

Furthermore, it also has to be taken into account that so far only very few Bt toxins produced in genetically engineered plants have been investigated in regard to their potential impact on the immune system. As yet, only two Bt toxins (Cry1Ac and Cry1Ab) have been tested for their possible effects on the immune system; none of the toxins produced in the maize were investigated in this regard in empirical research. The effects caused by a combination of these toxins also remain untested. The need for more detailed investigations in regard to potential immunogenic effects is further underlined in the minority opinion in another EFSA opinion (Annex II of EFSA, 2018a). While the applicant provided some data in regard to celiac disease, other diseases associated with symptoms of chronic inflammation were not considered at all.

In their answers to experts from Member States (EFSA, 2019d), EFSA admits only that “limited experimental evidence” is available to conclude the safety of Bt toxins in regard to immune system reactions.

Given the fact that potential effects of Bt toxins on the immune system have meanwhile been discussed for many years (for overview see, for example, Then & Bauer-Panskus, 2017), and already around 40 GE crops events producing Bt toxins have been approved for the EU market, any further delay in resolving these crucial questions cannot be accepted. In accordance with EU Regulation 1829/2003, safety of whole food and feed has to be demonstrated before approval for import can be issued. Since this is not the case with the stacked maize, the risk assessment is not conclusive and no market authorisation can be granted.

In summary, the EFSA assessment of the stacked maize cannot be said to fulfill the requirements for assessing risks to the immune system.

Chowdhury, E. H., Kuribara, H., Hino, A., Sultana, P., et al. (2003) Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11. Journal of Animal Science, 81(10): 2546-2551. https://academic.oup.com/jas/article-abstract/81/10/2546/4789819

EFSA (2018a) Assessment of genetically modified maize Bt11 x MIR162 x 1507 x GA21 and three subcombinations independently of their origin, for food and feed uses under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2010-86). EFSA Journal 16(7): e05309. doi: 10.2903/j.efsa.2018.5309

EFSA (2018e) Relevance of new scientific information (Santos-Vigil et al., 2018) in relation to the risk assessment of genetically modified crops with Cry1Ac. EFSA supporting publication 2018:EN- 1504. 13 pp. doi:10.2903/sp.efsa.2018.EN-1504. https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2019.EN-1504

EFSA (2019d) Application EFSA-GMO-NL-2017-144, Comments and opinions submitted by Member States during the three-month consultation period, Register of Questions, http://registerofquestions.efsa.europa.eu/roqFrontend/ListOfQuestionsNoLogin?0&panel=ALL

González-González, E., García-Hernández A.L., Flores-Mejía, R., López-Santiago, R., Moreno-Fierros, L. (2015) The protoxin Cry1Ac of Bacillus thuringiensis improves the protection conferred by intranasal immunization with Brucella abortus RB51 in a mouse model. Vet. Microbiol. 175: 382–388, http://dx.doi.org/10.1016/j.vetmic.2014.11.021

Guerrero, G.G., Dean, D.H., Moreno-Fierros, L. (2004) Structural implication of the induced immune response by Bacillus thuringiensis cry proteins: role of the N-terminal region, Molecular Immunology, 41(12): 1177-1183. http://dx.doi.org/10.1016/j.molimm.2004.06.026

Guerrero, G.G. & Moreno-Fierros L., (2007) Carrier potential properties of Bacillus thuringiensis Cry1A toxins for a diphtheria toxin epitope, Scandinavian Journal of Immunology, 66(6): 610–618. http://dx.doi.org/10.1111/j.1365-3083.2007.01992.x

Jarillo-Luna, A., Moreno-Fierros L., Campos-Rodríguez R., Rodríguez-Monroy, M.A., Lara-Padilla, E., Rojas-Hernández, S. (2008) Intranasal immunization with Naegleria fowleri lysates and Cry1Ac induces metaplasia in the olfactory epithelium and increases IgA secretion. Parasite Immunol., 30: 31-38. http://dx.doi.org/10.1111/j.1365-3024.2007.00999.x

Legorreta-Herrera, M., Oviedo Meza, R., Moreno-Fierros L. (2010) Pretreatment with Cry1Ac protoxin modulates the immune response, and increases the survival of plasmodium-infected CBA/Ca mice. J Biomed Biotechnol: 198921. http://dx.doi.org/10.1155/2010/198921

Moreno-Fierros, L., García N., Gutiérrez, R., López-Revilla, R., Vázquez-Padrón, R.I., (2000) Intranasal, rectal and intraperitoneal immunization with protoxin Cry1Ac from Bacillus thuringiensis induces compartmentalized serum, intestinal, vaginal and pulmonary immune responses in Balb/c mice. Microbes Infect., 2(8): 885-890. http://dx.doi.org/10.1016/S1286-4579(00)00398-1

Moreno-Fierros, L., García-Hernández, A.L., Ilhuicatzi-Alvarado, D., Rivera-Santiago, L., Torres-Martínez, M., Rubio-Infante N., Legorreta-Herrera, M. (2013) Cry1Ac protoxin from Bacillus thuringiensis promotes macrophage activation by upregulating CD80 and CD86 and by inducing IL-6, MCP-1 and TNF-α cytokines, Int. Immunopharmacol. 17(4):1051-1066. http://dx.doi.org/10.1016/j.intimp.2013.10.005

Moreno-Fierros, L., Santos-Vigil, K., Ilhicatzi-Alvarado, D. (2018) Response to assessment of the Relevance of new scientific information (Santos-Vigil et al., 2018) in relation to the risk assessment of genetically modified crops with Cry1Ac of European Food Safety Authority (EFSA). www.testbiotech.org/node/2304

Pardo-López, L., Muñoz-Garay, C., Porta, H., Rodríguez-Almazán, C., Soberón, M., Bravo, A. (2009) Strategies to improve the insecticidal activity of Cry toxins from Bacillus thuringiensis. Peptides, 30(3): 589–595. https://www.sciencedirect.com/science/article/pii/S0196978108003264

Rubio Infante, N., & Moreno-Fierros, L. (2016) An overview of the safety and biological effects of Bacillus thuringiensis Cry toxins in mammals. Journal of Applied Toxicology, 36(5): 630-648. http://onlinelibrary.wiley.com/doi/10.1002/jat.3252/full

Rubio-Infante, N., Ilhuicatzi-Alvarado, D., Torres-Martínez, M., et al. (2018) The macrophage activation induced by Bacillus thuringiensis Cry1Ac protoxin involves ERK1/2 and p38 pathways and the interaction with cell-surface-HSP70. Journal of Cellular Biochemistry, 119:580-598. doi: 10.1002/jcb.26216

Santos-Vigil, K.I., Ilhuicatzi-Alvarado, D., García-Hernández, A.L., Herrera-García, J.S., Moreno-Fierros, L. (2018) Study of the allergenic potential of Bacillus thuringiensis Cry1Ac toxin following intra-gastric administration in a murine model of food-allergy. International immunopharmacology, 61: 185-196. https://www.sciencedirect.com/science/article/pii/S1567576918302467

Vázquez-Padrón RI, Moreno-Fierros L, Neri-Bazán L, et al. (1999) Intragastric and intraperitoneal administration of Cry1Ac protoxin from Bacillus thuringiensis induces systemic and mucosal antibody responses in mice. Life Sciences 64(21): 1897-1912. doi: 10.1016/S0024-3205(99)00136-8

Walsh, M.C., Buzoianu, S.G., Gardiner, G.E., Rea, M.C., et al. (2011) Fate of transgenic DNA from orally administered Bt MON810 maize and effects on immune response and growth in pigs. PLoS One, 6(11): e27177. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0027177

Others

(1) From studying the statements of the experts from Member States (2019d), we have the impression that EFSA is not aware of more recent publications showing a higher degree of horizontal gene transfer (HGT) than previously thought. Further, in their interpretation of the data, EFSA seems to be adopting a biased approach based on the assumption that no HGT should be expected.

In addition, given the fact that stacked events always show a higher overall amount of additionally inserted DNA, the statistical expectation of HGT involving this specific DNA needs more consideration. We conclude that the EFSA conclusions in regard to HGT to the intestinal gut of livestock and humans as well as the fate of the DNA in the environment will need further assessment.

(2) For monitoring and methods to identify the specific event, Implementing Regulation 503/2013 requests: The method(s) shall be specific to the transformation event (hereafter referred to as ‘event-specific’) and thus shall only be functional with the genetically modified organism or genetically modified based

product considered and shall not be functional if applied to other transformation events already authorised; otherwise the method cannot be applied for unequivocal detection/identification/quantification. This shall be demonstrated with a selection of non-target transgenic authorised transformation events and conventional counterparts. This testing shall include closely related transformation events.

However, no such method for identification was made available. Based on the information available, it will not be possible to distinguish the stacked event from a mixture of single parental events or stacked events that overlap with the actual stack.

If approval for import is given, the applicant has to ensure that post-market monitoring (PMM) is developed to collect reliable information on the detection of indications showing whether any (adverse) effects on health may be related to GM food or feed consumption. Thus, the monitoring report should at very least contain detailed information on: i) actual volumes of the GE products imported into the EU, ii) the ports and silos where shipments of the GE products were unloaded, iii) the processing plants where the GE products was transferred to, iv) the amount of the GE products used on farms for feed, and v) transport routes of the GE products. Environmental monitoring should be run in regions where viable material of the GE products such as kernels are transported, stored, packaged, processed or used for food/feed. In case of losses and spread of viable material (such as kernels) all receiving environments need to be monitored. Furthermore, environmental exposure through organic waste material, by-products, sewage or faeces containing GE products during or after the production process, and during or after human or animal consumption should be part of the monitoring procedure (see also comments from Member States experts , EFSA, 2019d). (3) We agree with comments made by experts from Member States (EFSA 2019d), that the applicant should be asked to provide a detailed analysis of the fate of the Bt proteins in the environment and a quantitative estimate of subsequent exposure of non-target organisms.

Besides methods of detection, other methods for quantifying exposure to the insecticidal proteins need to be made publicly available in order to facilitate monitoring. Food and feed producers, farmers as well as experts dealing with environmental exposure (for example which waste material, spillage and manure) have to be able to gather independent information on their exposure to the toxins via independent laboratories. As yet, these methods are regarded as confidential business information and are not made available upon request by EFSA. Thus, the Commission should ensure that the relevant data are both publicly available and also reliable.

As existing evidence shows (Székács et al., 2011; Shu et al., 2018), the methods need to be carefully evaluated to ensure that the results are reliable, comparable and reproducible. Therefore, fully evaluated methods have to be published that allow

the Bt concentration in the maize to be measured by independent scientists, as is the case for other plant protection compounds used in food and feed production. This is necessary to make sure that the environment as well as human and animals coming into contact with the material (for example, via dust, consumption or manure) are not exposed to higher quantities of Bt toxins than described in the application.

(4) Finally, in regard to the literature research, we do not agree with the way it was carried out. The review should take into account all publications on the parental plants and provide all relevant information regarding gene expression, findings from field trials and feeding studies. Further, monitoring data should be provided on imports of parental plants into the EU.

EFSA (2019d) Application EFSA-GMO-NL-2017-144, Comments and opinions submitted by Member States during the three-month consultation period, Register of Questions, http://registerofquestions.efsa.europa.eu/roqFrontend/ListOfQuestionsNoLogin?0&panel=ALL

Shu, Y., Romeis, J., Meissle, M. (2018) No interactions of stacked Bt maize with the non-target aphid Rhopalosiphum padi and the spider mite Tetranychus urticae. Frontiers in Plant Science, 9: 39. https://www.frontiersin.org/articles/10.3389/fpls.2018.00039

Székács, A., Weiss, G., Quist, D., Takács, E., Darvas, B., Meier, M., Swain, T., Hilbeck, A. (2011) Interlaboratory comparison of Cry1Ab toxin quantification in MON 810 maize by ezyme-immunoassay. Food and Agricultural Immunology, 23(2): 99-121. www.tandfonline.com/doi/abs/10.1080/09540105.2011.604773

---

3. Environmental risk assessment

The appearance of teosinte in Spain and France (see Testbiotech, 2016; Trtikova et al., 2017) has to be considered in more detail. In its assessment of the volunteer potential, the information provided by Monsanto is largely outdated. As Pascher et al (2016) show, the volunteer potential of maize is higher than assumed by Monsanto. Further, in awareness of the biological characteristics of the maize and the findings of Fang et al. (2018), the stacked maize needs to be examined in detail regarding next generation effects, volunteer potential (persistence) and gene flow.

Furthermore, in the EFSA (2019f) opinion is also wrong for several reasons: Without more data on the teosinte species growing in the EU, the likelihood of gene flow from the maize to teosinte cannot be assessed (Trtikova et al., 2017). The same is true for gene flow from teosinte to genetically engineered plants. The characteristics of potential hybrids and next generations have to be investigated and cannot be predicted simply from the data of the original event. It is well known that

there can be next generation effects and interference from genetic background that cannot be predicted from the assessment of the original event (Kawata et al., 2009; Cao et al., 2009; Yang et al., 2017; Bollinedi et al., 2017; Lu and Yang, 2009; Vacher et al., 2004; Adamczyk & Meredith, 2004; Adamczyk et al., 2009). This issue is relevant for gene flow from maize to as well from teosinte to maize.

EFSA should have requested data from the applicant to show that no adverse effects can occur through gene flow from the maize to teosinte and / or from teosinte to the maize volunteers. In the absence of such data, the risk assessment and the authorisation have to be regarded as not valid.

Without detailed consideration of the hazards associated with the potential gene flow from maize to teosinte and from teosinte to maize, no conclusion can be drawn on the environmental risks of spillage from the stacked maize.

Consequently, environmental risk assessment carried out by EFSA is not acceptable.

Adamczyk Jr, J.J., & Meredith Jr, W.R. (2004) Genetic basis for variability of Cry1Ac expression among commercial transgenic Bacillus thuringiensis (Bt) cotton cultivars in the United States. Journal of Cotton Science, 8(1): 433-440. http://www.cotton.org/journal/2004-08/1/17.cfm

Adamczyk, J.J., Perera, O., Meredith, W.R. (2009) Production of mRNA from the cry1Ac transgene differs among Bollgard® lines which correlates to the level of subsequent protein. Transgenic Research, 18: 143-149. https://doi.org/10.1007/s11248-008-9198-z

Bollinedi, H., S. G.,K, Prabhu, K.V., Singh, N.K., Mishra, S., Khurana, J.P., Singh, A.K. (2017) Molecular and functional characterization of GR2-R1 event based backcross derived lines of Golden Rice in the genetic background of a mega rice variety Swarna. PLoS ONE 12(1): e0169600. https://doi.org/10.1371/journal.pone.0169600

Cao, Q.-J., Xia, H., Yang, X., Lu, B.-R. (2009) Performance of hybrids between weedy rice and insect-resistant transgenic rice under field experiments: implication for environmental biosafety assessment. Journal of Integrative Plant Biology 51(12): 1138–1148. doi: 10.1111/j.1744-7909.2009.00877.x

Fang, J., Nan, P., Gu, Z., et al. (2018) Overexpressing exogenous 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) genes increases fecundity and auxin content of transgenic arabidopsis plants. Front Plant Sci 9: 233. doi: 10.3389/fpls.2018.00233

Kawata, M., Murakami, K., Ishikawa, T. (2009) Dispersal and persistence of genetically modified oilseed rape around Japanese harbors. Environmental Science and Pollution Research 16(2):120-126. doi: 10.1007/s11356-008-0074-4

Lu, B.-R., Yang, C. (2009) Gene flow from genetically modified rice to its wild relatives: Assessing potential ecological consequences. Biotechnol. Adv., Biotechnology for the Sustainability of Human Society Invited Papers from IBS 2008, 27(6), 1083–1091. https://doi.org/10.1016/j.biotechadv.2009.05.018

Pascher, K. (2016) Spread of volunteer and feral maize plants in Central Europe: recent data from Austria. Environmental Sciences Europe, 28(1):28-30. https://link.springer.com/article/10.1186/s12302-016-0098-1

Testbiotech (2016) Cultivation of genetically engineered maize: Risks not under control - Overview: Why the EU should not allow the cultivation of transgenic maize engineered to produce insecticidal toxins. Testbiotech Background, https://www.testbiotech.org/node/1759

Trtikova, M., Lohn, A., Binimelis, R., Chapela, I., Oehen, B., Zemp, N., Widmer, A., Hilbeck, A. (2017) Teosinte in Europe – Searching for the Origin of a Novel Weed. Scientific Reports, 7:1560. https://www.nature.com/articles/s41598-017-01478-w

Vacher, C., Weis, A.E., Hermann, D., Kossler, T., Young, C., Hochberg, M.E. (2004) Impact of ecological factors on the initial invasion of Bt transgenes into wild populations of birdseed rape (Brassica rapa). Theor. Appl. Genet. 109(4), 806–814. https://doi.org/10.1007/s00122-004-1696-7

Yang, X., Li, L., Jiang, X., et al. (2017) Genetically engineered rice endogenous 5-enolpyruvoylshikimate-3-phosphate synthase (epsps) transgene alters phenology and fitness of crop-wild hybrid offspring. Scientific Reports, 7. doi: 10.1038/s41598-017-07089-9

4. Conclusions and recommendations

The EFSA risk assessment cannot be accepted.