Maize MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603

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

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a. Assessment:
Molecular characterisation

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

Monsanto's Roundup® Used With GMOs Linked to Pregnancy & Reproductive Problems & Endocrine Disruption Situation: ♣

Seralini – France ♣

In vitro study with placental-derived tumor cells ♣ Decrease in aromatase activity (enzyme involved in the synthesis of estrogen).
https://dn9ly4f9mxjxv.cloudfront.net/app/uploads/2018/05/08084451/34-Monsanto-PowerPoint-Shows-Company-Awareness-of-Roundup-Cancer-Plausibility.pdf Does this fall outside the GM panel's remit? We don't think so!

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b. Food Safety Assessment:
Toxicology

Roundup banned by ever more countries and cities:

https://www.ad.nl/wonen/oostenrijk-verbiedt-als-eerste-land-onkruidverdelger-glyfosaat~a4673db2/?referrer=https://www.google.nl/ Oostenrijk verbiedt als eerste land onkruidverdelger glyfosaat

Austria is the first European country to ban the weed-killer glyphosate. The new legislation could well set Austria on a collision course with the European Union.

London Set to Ban Glyphosate Use over Public and Occupational Health Concerns

Posted on Jul 7 2019 - 11:47am by Sustainable Pulse

https://sustainablepulse.com/2019/07/07/london-set-to-ban-glyphosate-use-over-public-and-occupational-health-concerns/?fbclid=IwAR3bqOaNRJhYgtBR46VIvKO9lCxR4t-8PmtxJVo6L6fZyfjzV2uWYeyMIhY#.XSrg0m5uKUl

On Thursday, the London Assembly called on the Mayor to cease the use of the herbicide on Greater London Authority (GLA) land and the Transport for London (TfL) estate.

July 16, 2019

Sick Children Among Cancer Victims Suing Monsanto Over Roundup

Posted on July 16, 2019 by Carey Gillam

A 12-year-old boy suffering from cancer is among the newest plaintiffs taking on Monsanto and its German owner Bayer AG in growing litigation over the safety of Roundup herbicides and Monsanto's handling of scientific concerns about the products.
https://usrtk.org/monsanto-roundup-trial-tacker/sick-children-among-cancer-victims-suing-monsanto-over-roundup/

Cancer Maps and Glyphosate – Zach Bush MD – Farmer's Footprint Behind the Scenes

"According to triple board certified doctor Zach Bush….the cause is primarily glyphosate. This is a bold statement, and one that the agriculture industry and our government would not want us to believe. However the problem is pervasive. Glyphosate is contaminating our water, urine, breast milk, food, vaccines and cotton products."

Cancer Maps and Glyphosate – Zach Bush, MD https://vimeo.com/315920699

Glyphosate is even polluting the air we breathe!

Glyphosate Causes Serious Multi-Generational Health Damage to Rats – New WSU Research Washington State University (WSU) researchers have found a variety of diseases and other health problems in the second- and third-generation offspring of rats exposed to glyphosate, the world's most used weed killer.

https://responsibletechnology.org/glyphosate-causes-serious-multi-generational-health-damage-to-rats-new-wsu-research/

Assessment of Glyphosate Induced Epigenetic Transgenerational Inheritance of Pathologies and Sperm Epimutations: Generational Toxicology

• Deepika Kubsad, • Eric E. Nilsson, • […] • Michael K. Skinner Quotation:

Ancestral environmental exposures to a variety of factors and toxicants have been shown to promote the epigenetic transgenerational inheritance of adult onset disease. One of the most widely used agricultural pesticides worldwide is the herbicide glyphosate (N-(phosphonomethyl) glycine), commonly known as Roundup.

Scientific Reports volume 9, Article number: 6372(2019) | Download Citation

Received

02 October 2018 Accepted 09 April 2019 Published 23 April 2019

DOI https://doi.org/10.1038/s41598-019-42860-0

Is glyphosate safe?

If we are to believe the breweries and the governments, the quantity of glyphosate in beer is too low to have a harmful effect. Some researchers believe that glyphosate is dangerous even in low doses.

https://www.ahealthylife.nl/veel-bier-bevat-bestrijdingsmiddel-glyfosaat-uitslag-onderzoek/

https://www.nature.com/articles/srep39328?WT.feed_name=subjects_molecular-biology Article | OPEN ACCESS | Published: 09 January 2017 Multiomics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide.

• Robin Mesnage • , George Renney • , Gilles-Eric Séralini • , Malcolm Ward • & Michael N. Antoniou

Scientific Reports volume 7, Article number: 39328 (2017) 17 August 2018

A correction to this article has been published and is linked from the HTML and PDF versions of this paper. The error has not been fixed in the paper. (22). Their sales pitch is that the substance only blocks a few amino acids because it has a similar molecule. That's why the plant dies.

https://www.biernet.nl/nieuws/bier-in-nederland-bevat-onkruidverdelger

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Others

Data from the applicant

"The GMO Panel considered that the scope of the post market environmental monitoring (PMEM) plan provided by the applicant is consistent with the intended uses of maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603. "

Again, considerable faith is being placed in data provided by the applicant. It's like a student being allowed to mark his own test paper.

But:

South Africa government rejects Monsanto's triple stacked GM drought-tolerant maize

Details

Published: 15 November 2018

Created: 15 November 2018

Last Updated: 20 November 2018

"The ACB and more than 25,000 people from South Africa, the rest of the continent and around the world signed a petition rejecting Monsanto's application on the grounds that:

* The application was not backed by peer-reviewed scientific data and evidence supporting Monsanto's claim that MON87460 will confer drought tolerance".

More:

https://www.gmwatch.org/en/news/archive/2018/18587-south-africa-government-rejects-monsanto-s-triple-stacked-gm-drought-tolerant-maize

* The experimental design to assess the efficacy of the trait was flawed, and * Potential socio-economic risks posed by MON87460 to smallholder and resource poor farmers had not been considered.

The product in question is MON87460 x MON89034 x NK603.

Drought-Tolerant Maize MON 87460 This maize has only once been admitted to the European market, in 2015 (MON 87460).

But not in combination with other GM maize. Via the Netherlands, too.

https://webgate.ec.europa.eu/dyna/gm_register/index_en.cfm

Like MON 87460, MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 produces the CspB protein, derived from Bacillus subtilis, which provides reduced yield loss under water-limited conditions compared to conventional maize; and the NptII protein, derived from E. coli, which provides resistance to kanamycin, which acts as a selectable marker.

From: Application for authorisation to place on the market MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 maize in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed

Member State comments.

Hungary: 1.1.3 “There is a history of safe use of conventional maize, but there is no history of safe use of GM maize. GM maize is being grown for 21 years the most, which is too short a time to call “history”.

Reply from the EFSA: “The GMO Panel takes note of the comment. It is also noted that there is no internationally agreed definition of history of safe use which causes controversial discussions among risk assessors around the world.” Pag. 95. Application EFSA-GMO-NL-2016-134

Over horizontal gene transfer

Extract from comments by the Netherlands:

So-called horizontal gene transfer between plants and bacteria has not been observed in field experiments.

The nptII gene is present in bacteria in the environment, and the above-mentioned antibiotics are of minor importance to human medicine. Therefore, the presence of nptII in GM plants poses a negligible risk to the environment. In view of public perception, the presence of the nptII antibiotic resistance gene in MON87427xMON87460xMON89034xMIR162xNK603 may, however, be considered undesirable.”

“So-called horizontal gene transfer between plants and bacteria has not been observed in field experiments”. Dit is onjuist, dit is wel ontdekt bij veldexperimenten met gentech suikerbieten en al in 1998 beschreven:

Abstract, fragment:

The transgenic sugar beets contained the marker genes nptII and bar under the control of the bidirectional TR1/2 promoter conferring kanamycin (Km) and glufosinate ammonium resistance to the plant.”

Monitoring field releases of genetically modifed sugar beets for persistence of transgenic plant DNA and horizontal gene transfer.

Frank Gebhard, Kornelia Smalla * Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Pflanzenvirologie, Mikrobiologie und biologische Sicherheit (Federal Biological Insitute for Agriculture and Forestry, Institute for Plant Virology, Microbiology and Biological Safety),

Messeweg 11^12, D-38104 Braunschweig, Germany

Received 24 July 1998; received in revised form 20 November 1998; accepted 21 November 1998.

https://www.semanticscholar.org/paper/Monitoring-¢-eld-releases-of-genetically-modi-¢-ed-Gebhard-Smalla/9b39d91b0f865a511e5f8d4d83292a9a98bc7842

EN

Insertion-site mutations and horizontal gene transfer

Insertion-site mutations involving the integration of specific types of superfluous DNA pose an additional risk. Insertions of superfluous bacterial DNA flanking the transgene (e.g. vector backbone, marker DNA, and particularly origins of replication) have the potential to facilitate horizontal gene transfer of transgenes into soil or gut bacteria by providing opportunities for homologous recombination (De Vries and Wackernagel, 2002; Prudhomme et al., 2002).

Transformation-induced mutations in transgenic plants: Analysis and biosafety implications. ALLISON K. WILSON1*, JONATHAN R. LATHAM1 AND RICARDA A. STEINBRECHER2 1Bioscience Resource Project, P0 Box 66, Ledbury, HR8 9AE, UK and 2EcoNexus, P.O. Box 3279, Brighton, BN1 1TL, UK 2006

https://www.researchgate.net/publication/224834291_Transformation-induced_Mutations_in_Transgenic_Plants_Analysis_and_Biosafety_Implications

CONNER, A.J., WILLIAMS, M.K., ABERNETHY, D.J., FLETCHER, P.J. AND GENET, R.A. (1994). Field performance of transgenic potatoes. New Zealand Journal of crop and Horticultural Science 22, 361-371.

See also:

PLOS ONE

Complete Genes May Pass from Food to Human Blood

Sándor Spisák, Norbert Solymosi,Péter Ittzés, András Bodor, Dániel Kondor, Gábor Vattay, [...view 9 more...], István Csabai Fragment: “Though, there are animal studies, mainly focusing on the GMO issue [4], supporting the idea that small fragments of nucleic acids may pass to the bloodstream and even get into various tissues.”

https://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0069805&type=printable

The Stability and Degradation of Dietary DNA in the Gastrointestinal Tract of Mammals: Implications for Horizontal Gene Transfer and the Biosafety of GMOs

4. Rizzi A, Raddadi N, Sorlini C, Nordgrd L, Nielsen KM, et al. (2012) The stability and degradation of dietary DNA in the gastrointestinal tract of mammals: implications for horizontal gene transfer and the biosafety of GMOs. Crit Rev Food Sci Nutr 52: 142–161. https://www.tandfonline.com/doi/abs/10.1080/10408398.2010.499480

Foreign (M13) DNA ingested by mice reaches peripheral leukocytes, spleen, and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA.

Schubbert R, Hohlweg U, Renz D, Doerer W (1998) On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transmission to the fetus. Mol Gen Genet 259: 569–576.

https://www.ncbi.nlm.nih.gov/pubmed/9023365

Fragment: “A combined analysis of four other independent studies involving more than 1,000 human samples and a team of researchers from universities in Hungary, Denmark and the U.S. looked at the assimilation process for GMOs as they are currently consumed throughout the world.”

https://www.naturalnews.com/045710_GMOs_gene_transfer_DNA.html?fbclid=IwAR139uk3nxA52kUK1KfftahpH2lZZGBvzLWqCxAA3UytbBQ86KHMmv0Rz1M

Availability of genetically modified feed ingredient: investigations of ingested foreign dna in rainbow trout oncorhynchus mykiss.

Chainark P, Satoh S, Hirono I, Aoki T, Endo M (2008) Availability of genetically modified feed ingredient: investigations of ingested foreign dna in rainbow trout oncorhynchus mykiss;. Fisheries Science 74: 380–390.

https://link.springer.com/article/10.1111/j.1444-2906.2008.01535.x

Assessing the transfer of genetically modified DNA from feed to animal tissues.

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

“A small fragment of the Cry1A(b) transgene was detected in blood, liver, spleen and kidney of the animals raised with the transgenic feed.”

J Agric Food Chem. 2006 Mar 8;54(5):1699-709. Detection of transgenic and endogenous plant DNA in digesta and tissues of sheep and pigs fed Roundup Ready canola meal.

Sharma R, Damgaard D, Alexander TW, Dugan ME, Aalhus JL, et al. (2006) Detection of transgenic and endogenous plant DNA in digesta and tissues of sheep and pigs fed Roundup Ready canola meal. J Agric Food Chem 54: 1699– 1709.

Fragment abstract: “This study confirms that feed-ingested DNA fragments (endogenous and transgenic) do survive to the terminal GI tract and that uptake into gut epithelial tissues does occur. A very low frequency of transmittance to visceral tissue was confirmed in pigs, but not in sheep.”

https://www.ncbi.nlm.nih.gov/pubmed/16506822

The Dutch CA writes:

In view of public perception, the presence of the nptII antibiotic resistance gene in MON87427xMON87460xMON89034xMIR162xNK603 may, however, be considered undesirable.”

Our response: So it’s not only consumers who are worried: more and more scientists are showing that the DNA of GM crops can be found in humans and laboratory animals. The idea that the DNA of GMOs is broken down in the gastro-intenstinal tract is plain wrong. The antobiotic-resistant genes (one confers Neomycin/Kanamycin resistance and the other

Ampicillin resistance.) can also be found in the DNA of calves which have undergone gene-editing and were born without horns. See below.

Antibiotic Resistance Genes

Like MON 87460, MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 produces the CspB protein, derived from Bacillus subtilis, which provides reduced yield loss under water-limited conditions compared to conventional maize; and the NptII protein, derived from E. coli, which provides resistance to kanamycin, which acts as a selectable marker.

From: Application for authorisation to place on the market MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 maize in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed

It has recently come to light (only in calves, but even so …) that:

Unexpected Antibiotic Resistance Genes

FDA Finds Unexpected Antibiotic Resistance Genes in 'Gene-Edited' Dehorned Cattle

……while the DNA of both calves contained two antibiotic resistance genes, along with various other gene sequences of bacterial origin. The inadvertently introduced bacterial sequences were found close to the editing site. Of the two antibiotic resistance genes found by FDA, one confers Neomycin/Kanamycin resistance and the other Ampicillin resistance.

https://www.nature.com/articles/nbt.3560

Testbiotech over MON 87460, extract from 2012:

Molecular data (1) The plants contain a DNA sequence that confers resistance to antibiotics (npt II). Despite the fact that gene excision technology was used in this case (Cre/lox site-specific recombination system of bacteriophage origin), the DNA was not removed from the plants.

Please regard this as an integral part of the present comments.

https://www.testbiotech.org/sites/default/files/Comment_Mon87460_Testbiotech.pdf

EU Directive 2001/18, however, requires this outdated technology to be phased out because it can have the opposite effect to what is intended.

Zie ook: (Chen, J., Jin, M., Qiu, Z.G., Guo, C., Chen, Z.L., Shen Z.Q. Wang X.W., Li J.W. (2012) A Survey of Drug Resistance bla Genes Originating from Synthetic Plasmid Vectors in Six Chinese Rivers, Environ. Sci. Technol. 2012, 46, 13448−13454). The EFSA should take a closer look.

Fragment abstract: In this study, PCR and real-time quantitative PCR were used to investigate the synthetic plasmid vector-originated ampicillin resistance gene, β-lactam antibiotic (blá), in microbes from six Chinese rivers with significant human interactions. Various levels of blá were detected in all six rivers, with the highest levels in the Pearl and …..

Meer: https://pubs.acs.org/doi/pdf/10.1021/es302760s?rand=cmsgqitw Article Published: 19 August 2019

A global overview of pleiotropy and genetic architecture in complex traits

Kyoko Watanabe, et All. “After a decade of genome-wide association studies (GWASs), fundamental questions in human genetics, such as the extent of pleiotropy across the genome and variation in genetic architecture across traits, are still unanswered.” https://www.nature.com/articles/s41588-019-0481-0

“A new study in the Netherlands has shown that tinkering with our genes is even riskier than previously thought. This is because a single gene often contains the code for countless different characteristics, which means that tinkering with them can have unforeseen consequences.”

Source: NPO Radio “Waarom sleutelen aan DNA soms onvoorziene gevolgen heeft” (“Why tinkering with our genes has unforeseen consequences”)

https://www.nporadio1.nl/wetenschap-techniek/18199-hoe-honderden-genen-je-eigenschappen-bepalen?fbclid=IwAR2k9bUtrhlh9QeFIBG5rY8eh7gGeabkhSp_TrVPqg2YVyq_kgJZr32QzSg

Our comment: The same goes for tinkering with crops.

More responses from the Dutch CA:

“The applicant claims that the information in the application is confidential. The Aarhus Convention 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 EFSA has informed the European Commission on the claim for confidentiality of the application and awaits its decision. 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.”

Our comment: You don’t say!

But there’s more:

“The Dutch CA has assessed the dossier with respect to the food and feed safety of MON87427 x MON87460 x MON89034 x MIR162 x NK603 maize and has no comments or requests for additional information in relation to the safety of this GM event.”

It’s odd that the Dutch should adopt this critical stance even though the intention is for this GM maize to come onto the market via the Netherlands. Can you explain this to us? It’s neither fish nor flesh!

We agree with the critical comments made by Austria, Belgium, France, Germany, Hungary, Italy, the Netherlands (apart from the above comment) and Norway.

Please regard the above as an integral part of our comments. We are ashamed of the most recent comment by our countrymen. It is no coincidence that the authorisation is being channelled via the Netherlands.

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4. Conclusions and recommendations

The inevitable conclusion is that you must not allow this genetically modified maize on the market.

4. Conclusions and recommendations

The link to your “Open Consultations” web site doesn’t work.

How can people who wish to post a response be well informed if the link to the EFSA’s SO doesn’t work? We have contacted the EFSA about this but have not yet received an answer. The deadline should be put back for as long as the correct link is unavailable (which is still the case as at today, 22 August 2019)! ----------

Email dated 12 August 2019; reply from the Helpdesk and our question.

“Thank you for your interest in the European Food Safety Authority. We do our best to answer all questions by email within 15 working days and are normally able to respond in a shorter period.

Applications Helpdesk

European Food Safety Authority

Via Carlo Magno 1A, 43126 Parma, Italy

http://www.efsa.europa.eu/en/applicationshelpdesk/askaquestion.htm

--- Your question---

L.S.,

Re:

Open Consultations

Maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 Scientific Opinion on the assessment of genetically modified maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and subcombinations for food and feed uses submitted under Regulation

(EC) No 1829/2003 by Monsanto (EFSA- GMO-NL-2016-134) EFSA opinion: 8 August 2019 Deadline: 8 September 2019

Make comments

https://ec.europa.eu/food/plant/gmo/public_consultations_en

In our opinion the wrong EFSA opinion is put on this site. It must be: https://efsa.onlinelibrary.wiley.com/action/doSearch?AllField=Maize+MON+87427+%C3%97+MON+87460+%C3%97+MON+89034+%C3%97+MIR162+%C3%97+NK603&SeriesKey=18314732&startPage=&Ppub=%5B20190710%20TO%2020190810%5D

And than we like to have a link to the whole opinion, which we cannot find, and the comments of the Member States. End of email. ----------

Appeal by Christoph Then at Testbiotech to the new President of the European Commission, Ursula von der Leyen:

"The EU-Commission can no longer ignore scientifically-based concerns about the approval of GE plants. We are calling on the new President of the EU Commission, Ursula von der Leyen, to make it clear that the protection of health and the environment is more important than the interests of big corporations and the US economy," Christoph Then says for Testbiotech".

https://www.testbiotech.org/en/news/eu-commission-gives-green-light-approval-seven-new-genetically-engineered-plants

We agree with Christoph Then and call upon the new President of the European Commission, Ursula von der Leyen, not to approve this maize or other GM crops which are in the pipeline until independent researchers investigate how the toxicity of herbicides and insecticides impacts the health of humans, animals, insects and the environment. There must also be a ban on making crops resistant to antibiotics. It's crazy that the EFSA has not been instructed to research the toxic impact of herbicides and insecticides! Until it does, these GM crops must not be allowed to end up on our plates.

This genetically modified maize must not be allowed on the market!

We, the GMO-free Citizens, don't want to eat this stuff. Glyphosate and Roundup are being banned by more and more countries as time goes by, and countries should refuse to accept this toxic maize! It is GM maize and contains a controversial herbicide and a number of controversial insecticides, i.e. poison, yet more poison, resistance to antibiotics and drought-tolerant maize which doesn't do what the manufacturer promises, and whose commercial cultivation has been banned by the South African Government. And this is maize with just three variants! Who in their right mind wants to eat this stuff?!

You are way behind the curve!

We are sending you these complaints jointly on behalf of Stichting Ekopark, Donaustraat 152, 8226 LC Lelystad, Netherlands; Ms Wieteke van Dort, Mesdagstraat 61, 2596 XV Den Haag, Netherlands, info@wietekevandort.nl; Mr Wilbrord Braakman, Transition Coalition Food Organisation (a non-profit organisation), braakman@wilbrord-nature.nl, De Verbinding 5, 1741 DB Schagen, Netherlands; and Ms L. Mast, Nieuwstraat 62, 1404 JN Bussum, Netherlands, "Praktijk voor Natuurgeneeskunde In Goede Handen" ("Natural Medicine in Good Hands"). The GMO-free Citizens have instructed us to inform you that they share our objections to this application

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5. Others

Quote from the EFSA: " We have not received any instruction to do this"

It is time to focus on the toxicity of the herbicides and insecticides to which this GM maize and other GM crops have been made resistant. Saying that you have no remit to do this is outrageous.

(" The assessment of herbicide residues and metabolites is not in the remit of the GMO Panel." Response to a comment from Austria, p. 73, Application EFSA-GMO-NL-2016-134). So, whose remit is it, then? And why do we never get to read about it?

GM crops resistant to toxic herbicides and insecticides

Because this means that this and other GM crops are highly toxic for future generations! Quite apart from the fact that the DNA, the very blueprint of life, has been tampered with, we can never predict what the end result will be; these GM crops are being made so toxic that they should never be allowed on the European market! Another reason for banning them is that they include a maize which has been made drought-tolerant and possesses resistance to antibiotics, and which can be found in the DNA of animals.

The question is what these strains of GM maize do in combination, but it is one which has not been studied! What is more, scientists have found the DNA of GM crops in various animal organs. This means that HGT is also taking place in crops (See also Smalla et al.), even if the Dutch Competent Authority maintains that this has not been shown in field experiments.

Finally, an example of a failed GM test in Africa:

New Scientist. Monsanto failure. 7 February 2004.

7 February 2004

A SHOWCASE project to develop a genetically modified crop for Africa has failed. Three years of field trials have shown that GM sweet potatoes modified to resist a virus were no less vulnerable than ordinary varieties, and sometimes their yield was lower, according to the Kenya Agricultural Research Institute. Embarrassingly, in Uganda conventional breeding has produced a high-yielding resistant variety more quickly and ….

Read more: https://www.newscientist.com/article/mg18124330-700-monsanto-failure/

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6. Labelling proposal

If you do not decide to ban this maize, which we think would be disastrous, a warning triangle with a death head would be the most effective choice. And not only upwards of 0.9% of the ingredients, but wherever genetically modified organisms are present.

Organisation: The European GMO-free Citizens (De Gentechvrije Burgers)
Country: The Netherlands
Public: Yes

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a. Assessment:
b. Food Safety Assessment:
Toxicology

23 september 2019. Aanvulling op onze eerdere reactie.

In the United States, three juries have decided that #glyphosate causes cancer and that the manufacturer, #Bayer-Monsanto, has concealed the risk. Today, I'll be showing how the supervisory authorities, including those in the Netherlands, gave the green light for glyphosate, despite the proof that it's no good to anyone. Tweet by Vincent Harmsen

Together with two other members of the EPA panel, she (sic) discovered a 'conclusive link' between glyphosate and non-Hodgkin lymphoma, an aggressive cancer of the lymph glands. Her (sic) research was published in February of this year in Mutation Research and was widely reported in the world press: 'Weed-killer increases risk of non-Hodgin lymphoma by 41 per cent'. For more information, see,' MEER: https://www.ftm.nl/artikelen/monsanto-bayer-glyfosaat-toezichthouders?utm_medium=social&utm_campaign=Vincent-Harmsen&utm_source=twitter

Abstract, quote: Overall, in accordance with findings from experimental animal and mechanistic studies, our current meta-analysis of human epidemiological studies suggests a compelling link between exposures to GBHs and increased risk for NHL. Paper: https://www.sciencedirect.com/science/article/pii/S1383574218300887

Rejection of GMOs in European Parliament welcomed by Greens 7 October 2016 Greens, who are campaigning for a GMO-free Europe, are celebrating after the European Parliament objected to 5 pending GMO authorisations. A vote in the Parliament yesterday sent a clear signal to the Council body tasked with deciding on the authorisations. The new objections by

MEPs follow previous objections to authorizations for GM maize, soybeans and carnations. https://mollymep.org.uk/2016/10/07/rejection-of-gmos-in-european-parliament-welcomed-by-greens/

https://sustainablepulse.com/2016/06/08/european-parliament-rejects-gmos-in-europe-and-africa/#.XYXcuW5uKUk

The Authorization Process for Imports is Not Democratic Two objections to the authorization for import of a GM carnation and a GM maize[1 Maize Bt11 × MIR162 × MIR604 × GA21] were voted on Wednesday. These were the 5th and 6th objections (all initiated by the Greens/EFA) that have been submitted to the plenary since December 2015. https://sustainablepulse.com/2016/06/08/european-parliament-rejects-gmos-in-europe-and-africa/#.XYXcuW5uKUk

A critical evaluation of EFSA's environmental risk assessment of genetically modified maize MON810 for honeybees and earthworms Veronika Chvátalová Environmental Sciences Europevolume 31, Article number: 52 (2019) https://enveurope.springeropen.com/articles/10.1186/s12302-019-0238-5 (open access)

Chvátalová looked at EFSA's risk assessment of MON810, focusing on two non-target organisms, honeybees and earthworms.

Chvátalová found that "EFSA omits relevant available studies, selectively cites information, misquotes studies, fails to acknowledge uncertainties, fails to call for further research where needed, and fails to critically interpret studies and their findings".

Pathogenic, genetically modified maize is going mainstream

The TV series "Onze Boerderij" ("Our Farm"). Sunday, 8 September, 20:25 • KRO-NCRV • 49 min. Yvon receives a disturbing phone call from milk farmer Riks in Canada. A large number of his milkers are seriously ill.

Our comment:

We happened upon a video of Yvon Jaspers' TV series "Onze Boerderij". At last, a Dutch farmer in Canada who says that he thinks his cows are being made ill by feed which contains GM maize. At least 20 cows had nearly died after calving. And only in winter. GM maize in their roughage. He named Roundup, which the GM maize had been made resistant to. The cows were doing fine in the summer, when they weren't being given roughage. Other Canadian farmers are experiencing the same problem. But it's often blanketed in silence. It's a sensitive issue. The farmer thinks this is because people in North and South America believe that GM crops will eliminate world hunger. It is costing him a fortune now that he is unable to sell the milk produced by those 20 cows. Hopefully, farmers in the Netherlands will also come to realise that they are not only making their cows ill, but also the humans who eat their meat. Their animals are ingesting GM maize gluten and GM soy chunks found in the concentrate which they are fed. Our Canadian farmer says that the roughage fed to cows in the Netherlands doesn't contain GM ingredients. When will the government protect humans and animals from the multinationals? An episode broadcast on 22 September shows how, when the farmer in question brings up the subject of GM feed and the dead cows with other Canadian farmers, he gets called a tree-hugger, an econerd, a Nazi and a traitor. They feel that

they are under siege. Everyone uses this feed, don't they? It can't be true. The farmers are too scared too talk about it, but this farmer knows that others are experiencing the same problems. But they keep saying "it can't be true" because they are constantly being told that it isn't. Our farmer decides that from now on, he will make his own feed: not monocultures, but mixed crops. He has come to the conclusion that he has to stop using all that poison. Partly for the sake of our families and future generations. He finds it hard being the odd man out, but he has no choice. https://www.npostart.nl/onze-boerderij/08-09-2019/KN_1708879?utm_medium=refferal&utm_source=tvblik https://www.npostart.nl/KN_1708879 https://www.youtube.com/watch?v=pUTiIBRe7IE

Notabene:

Monday, 12 FEBRUARY 2018 For the first time since records began, the number of milk farms in Canada has fallen below the 11,000 mark. According to the Canadian Dairy Information Centre, in 2017 there were 10,951 dairy farms in Canada which supplied milk to dairies. In 2016, the number was 11,289. https://veeteelt.nl/nieuws/minder-dan-11000-melkveebedrijven-canada

Unique Hair Testing Project Reveals High Levels of Glyphosate in Members of the Japanese Parliament

"A unique testing project involving 23 members of the Japanese Parliament, has shocked the country after it was revealed that the majority of the politicians had long-term exposure to a variety of pesticides, including the world's most used herbicide, glyphosate."

The pesticides, insecticides, fungicides and herbicides which were found are Cyprodinil, Fipronil Sulfone, Iprovalicarb, Metolachlor, Propiconazole, Pyraclostrobin, Spiroxamine, Tebuconazole, Tetramethrin, Transfluthrin, Trifloxystrobin, Glyphosate, AMPA and Glufosinate.

https://sustainablepulse.com/2019/08/29/unique-hair-testing-project-reveals-high-levels-of-glyphosate-and-ampa-in-members-of-the-japanese-parliament/#.XWfpxW5uKUk Posted on Aug 29 2019 - 4:21pm by Sustainable Pulse

Removal of glyphosate from global usage 31.07.2019 In: FIGO Statements, Environmental Health Removal of glyphosate from global usage: A Statement by the FIGO (International Federation of Gynecology and Obstetrics) Committee on Reproductive and Developmental Environmental Health "We strongly endorse Precautionary Principle that "When an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically." We recommend that glyphosate exposure to populations should end with a full global phase out." https://www.figo.org/statement-glyphosate-removal

INSIDERS "Glyphosate can cause cancer" COMMENTS By Euronews • last updated: 01/07/2016 https://www.euronews.com/2016/07/01/glyphosate-can-cause-cancer

euronews: "Why isn't glyphosate banned considering these findings which are damning?" Kurt Straif: "This is really an independent review of all the published literature that then leads to a classification about what we know about the substance and particularly its cancer-causing effects, but then it's up to other agencies, meer zie link hierboven.

Extract from a tweet by Vincent Harmsen

Three juries in the United States have decided that #glyphosate can cause cancer and that the manufacturer, #Bayer-Monsanto, is concealing the risk. Today I'll be showing how the supervisory authorities, including those in the Netherlands, gave the green light to glyphosate, despite the proof that it's no good to anyone. https://www.ftm.nl/artikelen/monsanto-bayer-glyfosaat-toezichthouders?utm_medium=social&utm_campaign=Vincent-Harmsen&utm_source=twitter

Following an Email Trail: How a Public University Professor Collaborated on a Corporate PR Campaign. https://usrtk.org/gmo/following-an-email-trail-how-a-public-university-professor-collaborated-on-a-corporate-pr-campaign/

. "He also doesn't talk much about the hundreds of thousands of dollars Monsanto donated to the university as (Bruce) Chassy was helping promote GMOs, or Monsanto's secretive role in helping Chassy set up a nonprofit group and website to criticize individuals and organizations who raise questions about GMOs."Posted on January 29, 2016 by Carey Gillam https://www.baumhedlundlaw.com/pdf/monsanto-documents-2/MONGLY00940553-REVISED-REDACTIONS.pdf

On June 28, 2013, the group's executive director, Zen Honeycutt, posted an open letter to Hugh Grant, then-CEO of Monsanto, on the organization's blog. The letter asked Grant to stop selling Roundup-ready seeds, citing concerns about the chemical glyphosate, which is sprayed on the crops that are genetically modified to …MORE

https://newfoodeconomy.org/monsanto-bayer-roundup-moms-across-america/
https://www.momsacrossamerica.com/open_letter_to_monsanto_from_moms

DER SPIEGEL: Anti-Monsanto Lawyer 'Monsanto's History Is One Full of Vast Lies'

Interview Conducted by Nils Klawitter August 22, 2018 Yet Monsanto apparently also conducted studies on Round Up itself. Wisner: Yes, Monsanto hired the company TNO to conduct a study in 2002. It showed that the stuff permeated the skin of rats at three times the rate permitted in Germany. Monsanto said this is a big problem, whereupon TNO offered to repeat the study at no extra cost. Meer: https://www.spiegel.de/international/world/anti-monsanto-lawyer-monsanto-s-history-is-full-of-lies-a-1223756.html

Brent Wisner Roundup Cancer Attorney

"Monsanto's history is one full of vast lies. They mislead people, promise that their products are safe and make a lot of money by doing so. And when things get uncomfortable, they simply move on to another product. This strategy has proven successful for over 100 years." Brent Wisner Interview with Der Spiegel https://www.baumhedlundlaw.com/toxic-tort-law/monsanto-roundup-lawsuit/

We achieved this historic result by taking the weakness of the case, i.e., that the [Environmental Protection Agency] does not think Roundup causes cancer, and using that fact to show how invasive and successful Monsanto's 40 years of corruption and fraud has been.

https://www.law.com/nationallawjournal/2019/08/26/nlj_sept2019_wl_r-brent-wisner/?cmp=share_twitter

Brazil public prosecutor demands ban on three commercialised GM maize varieties

Published: 27 September 2018

Brazil's federal public prosecutor has filed a public civil action requiring the suspension of commercial release of three GM maize seed varieties: Monsanto's MON87411 and MON87460; and Syngenta's 3272. The lawsuit cites several problems with the authorisations, including the lack of studies showing that these GMOs are safe to eat….Meer: https://www.gmwatch.org/en/news/latest-news/18481-brazil-public-prosecutor-demands-ban-on-three-commercialised-gm-maize-varieties

EMAILS SHOW MONSANTO ORCHESTRATED GOP EFFORT TO INTIMIDATE CANCER RESEARCHERS Lee Fang

August 23 2019, 12:00 p.m. And in 2017, one of the most stunning disclosures revealed that a senior EPA staffer, Jess Rowland, had quietly tipped Monsanto off to the fact that federal agencies had sought to reevaluate the safety of glyphosate, boasting to the company that he could "kill" federal investigations into glyphosate. Meer: https://theintercept.com/2019/08/23/monsanto-republicans-cancer-research/

Others

The Netherlands is helping to build a 'soy route' which will compound the destruction of the Amazon. According to the Trouw daily newspaper, the Netherlands has already been cooperating with Brazil for ten years on the construction of a fast soy route through the Amazon. Thousands of kilometres of railway tracks and new asphalt will eventually plough right through it, and more than 60 new ports will be built. https://www.trouw.nl/duurzaamheid-natuur/nederland-bouwt-mee-aan-een-sojaroute-die-de-amazone-nog-verder-vernielt~b5dc16b7/?fbclid=IwAR3e5OHAPqBMWrvFKmqxVFyxqmDj6v8dvMMZyI5Jr6Dm_WUqWN-eftLM1fQ

Woodland is being cut down and native communities are being displaced so that soy from central Brazil can be transported quickly to seaports via the Amazon. From there, the soy is taken by ship to ports like Rotterdam. The soy ends up as cattle feed on farms in the Netherlands and other countries. Trouw reports that Dutch companies are actively involved in the project: Arcadis helped with the construction of the soy port. Boskalis pilots ships carrying soy in two Amazon ports, while the shipbuilders Damen Shipyards and Alewijnse have provided tugs. With the sole excpetion of Arcadis, none of these shipbuilders want to talk about their role in the soy route, beyond confirming that they are or have been involved in it. Source: https://www.facebook.com/EenVeiligNest/?__tn__=kCH-R&eid=ARC40vbm34OKwGnzm8be-9BAoGTf-XHxCwU984joD06loGX3lDQhdmQ9aJWOI0z03lruYFihwDgJK6Sq&hc_ref=ARRdUOHBApduwvEeLvPS8ZhbE6lMM8qYCasYXJVC0DAqKPAR-

q5kaxqHNP1zZVv8q68&fref=nf&__xts__[0]=68.ARCnx2Zvab4WO3TSW7LxcSKW3h-MKqzNLBYWrgRPQPkaGoKstJJGJbR0CRdqhUkp8X_SdValUtmjbHRW4w4KGKJJ7KEAgZ_a0_2reVFd-bCDLoKydZnGviyq9DFyQMekBml4XXaMQAuXI-WZRfD2_TGYQXew2KxBzpV5aJ7e_rj5KVO2k74EUqxf1gNtYYUQoyv9QzjrfJtdo7IS9pgTqWUlosV2PK3hV9CgxlzF7Zk2CIqGJWgAwZj1NEGdhHkxaxgRNB0A6bMqArdT6sI129-SJ0xG7GsAEwMqxaMN4gXTj2FVDwHzuFAycuL8d7YWQou-pCiq5EazxiWuWFEt1nRV

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4. Conclusions and recommendations

How can people who wish to make a comment be well-informed if the text of the consultation is only made available in English and not in any of the other languages spoken in the EU, not even in major languages like German, French or Spanish? This shortcoming has to be rectified! This is why so few responses have been received from countries where English is not spoken! We repeat: We don't want GM maize!

Organisation: The European GMO-free Citizens (De Gentechvrije Burgers)
Country: The Netherlands
Public: Yes

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a. Assessment:
Others

Second supplement (corrections of previous complaints).

The text which we wrote was incorrect: Quote from the EFSA: "We have not received any instruction to do this"

" It is time to focus on the toxicity of the herbicides and insecticides to which this GM maize and other GM crops have been made resistant. Saying that you have no remit to do this is outrageous ("The assessment of herbicide residues and metabolites is not in the GMO Panel's remit." Response to a comment from Austria, p. 73, Application EFSA-GMO-NL-2016-134). Whose remit is it, then? And why do we never get to read anything about it?

GM crops resistant to toxic herbicides and insecticides "

The text should read as follows:

Quote from the EFSA: "We have not received any instruction to do this."

It is time to focus on the toxicity of the herbicides to which this GM maize and other GM crops have been made resistant, on the insecticides which are incorporated in them, and on built-in resistance to antibiotics. Saying that you have no remit to do this is outrageous ("The assessment of herbicide residues and metabolites is not in the GMO Panel's remit.") Response to a comment from Austria, p. 73, Application EFSA-GMO-NL-2016-134). Whose remit is it, then? And why do we never get to read anything about it?

"GM crops resistant to toxic herbicides and built-in insecticides

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Organisation: individual
Country: The Netherlands
Type: Individual

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a. Assessment:
5. Others

Our cows are getting sick and mortality is increasing a lot after feeding them with gm food. We don't know the consequences and can't for see the impacts in our selves, the food chain and the ecosystem! So when really impartial research of many years lack, we can't allow it anywhere!

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

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a. Assessment:
b. Food Safety Assessment:
Toxicology

26-9-2109. This is our third supplement.

1. Introduction Genetically modified (GM; transgenic) crops have been grown for human and animal consumption since the 1990's [1]. Most currently-grown crops have been developed through the transfer and incorporation of a gene cassette into the #Judy A. Carman is currently affiliated with IHER (Affiliation 2) and was previously affiliated with Flinders University (Affiliation 3). How to cite this paper: 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.

https://doi.org/10.4236/fns.2018.96058 Received: April 28, 2018 Accepted: June 26, 2018 Published: June 29, 2018
http://www.scirp.org/pdf/FNS_2018062813423624.pdf

Abstract

Genetic modification (GM) represents new opportunities for enhanced crop features such as improved insect resistance and herbicide tolerance. The technology allows for cross-species alterations, therefore potentially allowing a vast array of novel traits. Many GM crops have been developed and approved for human and animal consumption. The present study investigated a triple-stacked GM corn variety containing modifications for insect resistance (via cry 1 Ab and cry 3 Bb 1 genes) and herbicide tolerance (via an EPSPS gene), which was fed to rats for six months. The study investigated the mucosa of the stomach. Alterations to tight junction apposition, gland dilatations with epithelial elongation and dysplasia in the GM-fed rats were observed. These results indicate that GM-corn may have an effect on rat stomach mucosa, which may have health implications.

CUT

2.1. Diet The triple-stacked GM corn variety, containing the MON863, MON810 and NK603 genes, was obtained from a farmer who had grown it in the United States. I. M. Zdziarski et al. DOI: 10.4236/fns.2018.96058 767 Food and Nutrition Sciences

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5. Others

2.1. Diet The triple-stacked GM corn variety, containing the MON863, MON810 and I. M. Zdziarski et al. DOI: 10.4236/fns.2018.96058 767 Food and Nutrition Sciences NK603 genes, was obtained from a farmer who had grown it in the United States

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Organisation: Testbiotech e.V. - Institute for Independent Impact Assessment of Biotechnology
Country: Germany
Type: Non Profit Organisation

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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). Similar 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. Further, Mesnage et al. (2016) demonstrated alteration in stress-related metabolic pathways for NK603, which were, amongst others, accompanied by increased levels of polyamines. The authors stated that polyamines can provoke toxicological effects on their own or potentiate adverse effects of histamine.

Therefore, EFSA 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., 2005). 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.

Some comments made by experts of Member States (EFSA, 2019d) point out this gap in risk assessment. In response, EFSA stated: "Considering that there is no indication of an interaction between the events (see section 3.4.1.4 of the Scientific Opinion), it was not necessary to request the inclusion of field trials under drought conditions for the five-event stack maize."

This statement is scientifically problematic. It has to be expected that the stacked maize will be grown under drought conditions to an extent that most of the parental GE plants were not tested for in their previous risk assessment. Moreover, it is the first time that the combination of artificial gene constructs will be exposed to more extreme drought conditions. It is obvious that in the absence of adequate data, there can be no assessment of whether interactions will occur under stress conditions. Therefore, there is no scientifically sound way of arguing why much more specific data would not be necessary. Consequently, the stacked 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.

In consequence, the EFSA response to the requests from member states as quoted above is not acceptable. The GMO Panel appears to be abusing its position in order to exert a dominant rhetorical position that ignores sound scientific arguments and escapes the challenges of a sufficiently robust risk assessment.

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.

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): MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 (EFSA 2019a) Details from field trials: Field trials at five locations in the USA in 2014 (sprayed with glyphosate) Content of Vip3Aa20: 38

Application (EFSA opinion): MON 87427 x MON 89034 x MIR162 x NK603 (EFSA 2019b) Details from field trials: Field trials at five locations in the USA in 2013 (sprayed with glyphosate) Content of Vip3Aa20: 59

Application (EFSA opinion): Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 (EFSA 2019c) Details from field trials: Field trials at three locations in the US in 2012 (not sprayed with complementary herbicides) Content of Vip3Aa20: 100

Application (EFSA opinion): Bt11 x MIR162 x 1507 x GA21 (EFSA 2018a) Details from field trials: Field trials at one single location in the US 2008 (sprayed?) Content of Vip3Aa20: 28

Application (EFSA opinion): Bt11 x MIR162 x MIR604 x GA21 (EFSA 2015a) Details from field trials: Single location in the US in 2006 (sprayed?) Content of Vip3Aa20: 140

Application (EFSA opinion): MIR162 (EFSA 2012) Details from field trials: Bloomington, Illinois 2005, Hybrid A Content of Vip3Aa20: 46 Details from field trials: York, Nebraska, 2005, Hybrid B Content of Vip3Aa20: 41 Details from field trials: Bloomington, Illinois, 2006, Hybrid A Content of Vip3Aa20: 124 Details from field trials: Bloomington, Illinois, 2006, Hybrid B Content of Vip3Aa20: 84 Details from field trials: Brazil, Ituiutaba, 2007 Content of Vip3Aa20: 62 Details from field trials: Brazil, Uberlandia , 2007 Content of Vip3Aa20: 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 (2012), “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 The stacked maize inherits the antibiotic resistant marker nptII that renders resistance to clinically important antibiotics, such as neomycin and kanamycin, which are of therapeutic relevance. Although, many experts are of the opinion that a transfer of antibiotic resistance genes from GM plants to bacteria associated with use of GM plants is unlikely, this is a risk that should nevertheless be avoided, as requested by EU Directive 2001/18. Furthermore, as many statements made by Experts of Member States (2019d) show, there are numerous uncertainties surrounding such a potential gene transfer and its possible consequences. Since existing non-industry data on the usage of maize MON87460 give no

reason to expect major advantages in comparison to drought-tolerant maize derived from conventional breeding (Nielsen, & Schneekloth, 2018;), the question arising for the risk manager is why the economic interests of the applicant should be given more weight than the provisions of EU Directive 2001/18. According to Nuccio et al. (2018), MON87460 (DroughtGard®) “has not had a significant impact in the marketplace and does not appear to exhibit an advantage over non-GM efforts to improve drought tolerance that would justify the cost of registration and research.”

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.

References:

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

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 (2019d) Application EFSA-GMO-NL-2016-134, 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

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

Mesnage, R., Agapito-Tenfen, S.Z., Vilperte, V., et al. (2016) An integrated multi-omics analysis of the NK603 Roundup-tolerant GM maize reveals metabolism disturbances caused by the transformation process. Sci Rep, 6: 37855. doi: 10.1038/srep37855

Nielsen, D.C. & Schneekloth, J.P. (2018) Drought Genetics Have Varying Influence on Corn Water Stress under Differing Water Availability. Agronomy Journal, 110(3): 983-995. https://dl.sciencesocieties.org/publications/aj/abstracts/110/3/983

Nuccio, M.L., Paul, M., Bate, N.J., Cohn, J., Cutler, S.R. (2018) Where are the drought tolerant crops? An assessment of more than two decades of plant biotechnology effort in crop improvement. Plant science, 273, 110-119. https://www.sciencedirect.com/science/article/pii/S016894521731213X

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 (2019a), “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 inheriting a combination of EPSPS and CSPB are likely to show strong reactions in gene expression of the newly introduced genes, as well as in the plant’s own genes 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, to assess changes in plant composition and biological characteristics, 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. Moreover, the plants should have been subjected to a much broader range of defined environmental conditions and stressors to gather reliable data.

However, no experiments were requested to show to which extent specific environmental conditions, such as more extreme drought conditions, will influence plant composition and agronomic characteristics. Some comments made by experts of Member States (EFSA, 2019d) point to this gap in risk assessment. In response, EFSA stated: “Considering that there is no indication of an interaction between the events (see section 3.4.1.4 of the Scientific Opinion), it was not necessary to request the inclusion of field trials under drought conditions for the five-event stack maize.”

This statement is scientifically problematic. It has to be expected that the stacked maize will be grown under drought conditions to an extent that most of the parental GE plants were not tested for in their previous risk assessment. Moreover, it is the first time that the artificial gene constructs in combination will be exposed to more extreme drought conditions. It is obvious that in the absence of adequate data, it cannot be assessed whether interactions will occur under stress conditions. Therefore, there is no scientifically sound way of arguing why much more specific data would not be necessary. For this reason, the stacked 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.

In consequence, the EFSA response to the requests from Member States as quoted above is not acceptable. The GMO Panel appears to be abusing its position in order to exert a dominant rhetorical position that ignores sound scientific arguments and escapes the challenges of a sufficiently robust risk assessment.

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 (13) were subjected to statistical analysis in accordance with EFSA guidance, 6 (without spraying) and 8 (with

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: 39 endpoints were statistically significantly different in plants spayed with the complementary herbicides, 47 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 number of significant effects has to be taken as a starting point for much more detailed investigations: more than half of the parameters measured in regard to agronomic characteristics as well as those concerning plant composition were significantly different.

Furthermore, the data as presented did not take into account cultivation of the stacked maize under more extreme drought conditions, even though these are the most relevant environmental conditions in which the plants will be cultivated. Under such environmental conditions, the range of differences and their significance are likely to be substantially increased.

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.

References:

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 (2019d) Application EFSA-GMO-NL-2016-134, 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

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., 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

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

Furthermore, the data as presented did not take into account cultivation of the stacked maize under more extreme drought conditions; these are the most relevant environmental conditions in which the plants will be cultivated. Under such conditions, the range of differences and their significance are likely to be substantially increased.

Despite these findings, and in awareness of the lack of more specific data and the resulting major uncertainties, no testing of the whole stacked plant (feeding study) was requested.

Furthermore, our findings on gene expression show that no reliable conclusion on the content of insecticidal proteins can be derived from the available data. The overall concentration?? 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 was never subjected to more detailed analysis regarding immunological or other toxicological effects, and that can be present in comparably high concentrations in the grain. The safety of Cry1A.105 (artificially synthesized) and Cry2Ab2 is an issue since these can trigger health effects (see below).

In regard to toxicology and potential synergistic or other combinatorial effects, the negative impacts of Bt toxins on human and animal health cannot be excluded a priori. Bt toxins have

several modes of action and are altered in their biological quality; therefore, they are not identical to their natural templates (Hilbeck & Otto, 2015). It should not be overlooked that the mode of action of Vip3Aa20 is described as similar to Bt toxins. This has, however, not so far been assessed in detail.

In is known that not all modes of action of the insecticidal proteins produced in the plants depend on the specific mechanisms that only occur in the target insect species. Only very few Bt toxins (especially Cry1Ab, for overview see, Then, 2010) were investigated in more detail in regard to their exact mode of action, and there is no data on the Bt toxins produced in the maize. Further, no data were presented to show that the toxins produced in the plants are only activated and become effective in insects. On the other hand, several publications exist showing the effects of Bt toxins in mammals: some Cry toxins are known to bind to epithelial cells in the intestine of mice (Vázquez-Padrón et al., 1999, Vásquez-Padrón et al., 2000). As far as potential effects on health are concerned, Thomas and Ellar (1983), Shimada et al. (2003) Huffmann et al. (2004), Ito et al. (2004), Mesnage et al. (2013) and Bondzio et al. (2013) show that Cry proteins could potentially have an impact on the health of mammals. Two recent publications (de Souza Freire et al., 2014; Mezzomo et al., 2014) confirm hematotoxicity of several Cry toxins, including those being used in genetically engineered plants such as Cry 1Ab and Cry1Ac. These effects seem to occur after high concentrations and tend to become stronger after several days. Such observations call for the study of effects after long-term exposure to various dosages, including in combination with material sprayed with the complementary herbicides. In this context, it is important that the stacked maize is also resistant to the herbicide glyphosate, and the resulting residues should be seen as potential co-stressors at the stage of consumption (see also Then & Bauer-Panskus, 2017).

Moreover, it is evident that Bt toxins can survive digestion to a much higher degree than has been assumed by EFSA: Chowdhury et al., (2003) as well as Walsh et al. (2011) have found that Cry1A proteins can frequently and successfully still be found in the colon of pigs at the end of digestion when they were fed with Bt maize. The Cry1A proteins can show much higher stability at least in monogastric species than predicted by current in vitro digestion experiments. This shows that Bt toxins are not degraded quickly in the gut and can persist in larger amounts until digestion is completed, and there is enough time for interaction between various food compounds. Consequently, there is substantiated concern that especially the stacked event can trigger immune system responses and have adverse health effects.

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 (2015c and 2018) 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 2019a) 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.

References:

Bondzio, A., Lodemann, U., Weise, C., Einspanier, R. (2013) Cry1Ab treatment has no effects on viability of cultured porcine intestinal cells, but triggers hsp70 expression. PLOS ONE 8(7): e67079. doi: 10.1371/journal.pone.0067079

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

de Souza Freire, I., Miranda-Vilela, A.L., Barbosa, L.C.P., et al. (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(10): 2872-2885. doi: 10.3390/toxins6102872

European Food Safety Authority (EFSA) (2015c) Request for the evaluation of the toxicological assessment of the co-formulant POE-tallowamine. EFSA J 13(11): 4303. doi: 10.2903/j.efsa.2015.4303

EFSA (2018b) Reasoned Opinion on the review of the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2018;16(5): 5263, 230 pp. https://doi.org/10.2903/j.efsa.2018.5263)

EFSA (2019d) Application EFSA-GMO-NL-2016-134, 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

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: 71. doi: 10.3389/fenvs.2015.00071

Huffman, D.L., Abrami, L., Sasik, R., et al. (2004) Mitogen-activated protein kinase pathways defend against bacterial pore-forming toxins. Proc Natl Acad Sci U S A 101(30): 10995-11000. doi: 10.1073/pnas.0404073101

Ito, A., Sasaguri, Y., Kitada, S., et al. (2004) A Bacillus thuringiensis crystal protein with selective cytocidal action to human cells. J Biol Chem 279(20):21282-21286. doi: 10.1074/jbc.M401881200

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

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

Mesnage, R., Clair, E., Gress, S., et al. (2013) Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. Journal of Applied Toxicology 33(7): 695-699. doi: 10.1002/jat.2712

Mezzomo, B.P. (2013) Hematotoxicity of Bacillus thuringiensis as spore-crystal strains Cry1Aa, Cry1Ab, Cry1Ac or Cry2Aa in Swiss albino mice. J Hematol Thromb Dis, 1: 1. http://repositorio.unb.br/handle/10482/18532

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

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(2): 187-191. https://www.jstage.jst.go.jp/article/jvms/65/2/65_2_187/_article/-char/ja/

Then, C. (2010) Risk assessment of toxins derived from Bacillus thuringiensis synergism, efficacy, and selectivity. Environ Sci Pollut Res, 17(3):791-797. doi: 10.1007/s11356-009-0208-3

Then, C., & Bauer-Panskus, A. (2017) Possible health impacts of Bt toxins and residues from spraying with complementary herbicides in genetically engineered soybeans and risk assessment as performed by the European Food Safety Authority EFSA. Environmental

Sciences Europe, 29(1):1. https://enveurope.springeropen.com/articles/10.1186/s12302-016-0099-0

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

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 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

Vázquez-Padrón, R.I., Gonzáles-Cabrera, J., García-Tovar, C., et al. (2000) Cry1Ac protoxin from Bacillus thuringiensis sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. Biochemical and Biophysical Research Communications, 271(1): 54-58. doi: 10.1006/bbrc.2000.2584

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

Zdziarski, I.M., Carman, J.A., 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. https://doi.org/10.4236/fns.2018.96058

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 (EFSA, 2018c) 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; E. 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. However, the need for more detailed testing is acknowledged: "EFSA has previously highlighted that the testing of adjuvant and allergenic potential of proteins requires stronger and fit-for-purpose standardised study design, and that future studies should consider limitations of current models, using relevant routes and methods of administration, doses, appropriate control proteins, and realistic exposure regimes. These aspects will require a broader discussion with the involvement of the international scientific community and its stakeholders to define a consensus on a fit-for-purpose study design for this assessment.

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 38 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.

References:

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 (2018c) 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-2016-134, 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

Ibarra-Moreno, S., García-Hernández, A.L., Moreno-Fierros L. (2014) Coadministration of protoxin Cry1Ac from Bacillus thuringiensis with metacestode extract confers protective immunity to murine cysticercosis. Parasite Immunol. 36(6): 266-270. http://dx.doi.org/10.1111/pim.12103

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

Then, C., & Bauer-Panskus, A. (2017) Possible health impacts of Bt toxins and residues from spraying with complementary herbicides in genetically engineered soybeans and risk assessment as performed by the European Food Safety Authority EFSA. Environmental Sciences Europe, 29(1):1. https://enveurope.springeropen.com/articles/10.1186/s12302-016-0099-0

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

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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 maize MON87460 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. In doing so, the hypothesis that the maize and its offspring will show a higher fitness compared to conventional maize, is evident. This might also concern adaption to colder climate due to the biological characteristics of the cold shock

protein (CSPB). Under these circumstances, even a rare single outcrossing that goes unnoticed can have a huge long-term impact on the agro-ecosystems.

Moreover, the conclusion of EFSA is simply wrong, stating that: “Even if cross-pollination would occur, the GMO Panel is of the opinion that environmental effects as a consequence of the spread of genes from occasional feral GM maize plants in Europe will not differ from that of conventional maize varieties.”

Furthermore, in the EFSA (2019a) 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. • Furthermore, 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. • Finally, it is well established under EU regulation that it is the applicant who has to present data sufficient to show that the respective event is safe before the application can be considered to be valid (see Kraemer, 2016). Thus, an application with incorrect or missing information on crucial aspects of environmental risk assessment cannot be accepted as a starting point for EFSA risk assessment.

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.

References:

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

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

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

Kraemer, L. (2016) Teosinte plants in the European environment and its implication for market authorisation of genetically engineered maize Legal analysis commissioned by Testbiotech, https://www.testbiotech.org/sites/default/files/Teosinte_legal%20dossier.pdf

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.

5. Others

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). 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.

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.

References:

EFSA (2019d) Application EFSA-GMO-NL-2016-134, 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