Maize MON 89034 x 1507 x NK603 x DAS-40278-9

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

Please refer to our previous complaints.

International Immunopharmacology Volume 61, August 2018, Pages 185-196

Study of the allergenic potential of Bacillus thuringiensis Cry1Ac toxin following intra-gastric administration in a murine model of food-allergy Author links open overlay panel Karla I.Santos-Vigil et All https://doi.org/10.1016/j.intimp.2018.05.029

Allergenicity

Study conducted by Hoechst (Dr Arno Schulz) into the substrates of phosphinothricin acetyltransferase (PAT).

PAT present in herbicide (PPT)-resistant crops.

Amsterdam, 7 November 1999

Two studies producing opposing conclusions, namely:

1. Charles J. Thompson, 1987: Characterization of the herbicide-resistant gene bar from Streptomyces hygroscopicus;

2. Dr Arno Schulz, 1993: L-Phosphinothricine N-Acetyl-transferase -Biochemical Characterization - a report incorporated into Wehrmann, 1996 (Schulz is co-author). The subject is the characterisation of the enzyme phosphinotricin acetyltransferase (PAT), and in particular the specificity of the substrates. The first study concerns the reaction of phosphinothricin with acetyl co-enzyme A under the influence of PAT and compares this with a number of structural analogues of phosphinothricin (PPT). One of the analogues was L-glutamate. The products of the reaction were identified using a mass spectrogram and the equilibrium constants (affinity) were determined. In addition to phosphinothricin (PPT), a number of structural analogues were tested to determine whether there was an acetylation reaction. L-glutamine acid was one of the substances studied. Compared with PPT, the

affinity of most of the substances was limited: one substance did not react at all. In the light of this test, in which there was a reaction to an identified product (the detection threshold is not at issue here) which can be reported in numerical terms, there seems to be no reason to doubt that glutamine acid is a substrate of PAT.

The second study concerns the reaction of a large number of amino acids, including L-glutamine acid, which was also involved in the first study, in a reaction mix with a 100% excess of PPT in relation to acetyl co-enzyme A, which is a source of acetyl, and PAT. The products of the reaction were identified using chromatography. Even with a very large excess of L-amino acid, no products of reaction with the amino acids were detected. Only acetyl phosphinothricin was found. The authors concluded that PAT very specifically has only PPT as a substrate. The following criticisms can be levelled at this conclusion, which contradicts the findings of the first study. (Incidentally, the first study is cited in the literature used in the second study):

1. No detection threshold was determined for acetylated L-glutamine acid.

2. The possibility of acetylated glutamine acid being a source of acetyl for the acetylation of PPT was ignored. This could have been tested in the study by adding acetylated glutamine acid to the reaction mix in a quantity above the detection threshold and examining whether this added quantity disappears during the reaction. Based on the results of the first study, its disappearance is a foregone conclusion!!

3. The study was conducted using a reaction mix in which a large excess of a competing substrate, PPT, was present. There were no observations regarding the pure amino acids.

4. There is no discussion whatsoever of the results of the first study, in particular as to why those results were so different.

5. Essentially, the authors of the second study accuse the authors of the first of fabrication and fraud (the first study contains a wealth of numerical data; the second contains no figures). In the second study this aspect is not developed satisfactorily. The background to the conclusion that PAT has only one substrate (PTT) is as follows:

PAT is present in herbicide-resistant (i.e. PPT-resistant) crops. In order to have products approved for the market, the toxicity of this GM product must be examined. Could this GM product react with the CONTENT OF OUR GUT, e.g. with the important amino acid L-glutamine acid? Research demonstrating that the dangers were minimal would be enormously expensive. Total denial appears to be the preferred strategy at HOECHST! We believe that the conclusion drawn in the second study is completely unfounded and that the investigation does not merit the descriptor “research”. It is an incompetent study and persons who cite it need to be made aware of this. J. van der Meulen, L. Eijsten. http://www.gentechvrij.nl/rvs9911.html

EU to restrict herbicide glufosinate

Category: Crop Protection Products Tags: EU , restrict , herbicide , glufosinate The European Commission has announced the restrictions for the use of the herbicide glufosinate, which will be effective from Nov 13, 2013.

The decision is based on the additional information provided by the notifier, the Commission considered that the further confirmatory information required had not been provided and that a high risk for mammals and non- target arthropods could not be excluded except by imposing further restrictions.

The active ingredient will only be authorised for band or spot application at rates not exceeding 750 g ai/ha (treated surface) per application, with a maximum of two applications per year.

EU member states must amend or withdraw existing product authorizations in accordance with Regulation (EC) No 1107/2009 by Nov 13, 2013 .They may set a grace period of up to one year for use of existing stocks.New approvals should include the application of drift-reducing nozzles and spray shields, together with relevant labelling.

Glufosinate obtained EU approval for use in apple orchards in 2007. Source: EUR-Lex http://news.agropages.com/News/NewsDetail---9598.htm

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4. Conclusions and recommendations

Statements by mothers in the USA, where GMOs are not labelled.

"When my son was born he fussed a lot, the whole day, wouldn't nap. I breast fed until he was three months old. And because his gut was not right, he fussed and I could never console him. I tried all the gassy meds, not sure they are considered meds. Once on formula the fussy continued, we switched to different formulas, but not until we switched to parents choice organic, Walmart, his fussy stopped, he began taking naps. As a toddler, I fed him cheerios, a main staple in our house. The tantrums began; two hours at a time couple times a day. This is with head banging or slamming his head into the wall repeatedly. He wouldn't let me hold him, not even touch him. Can you imagine not cuddling your baby? I cried everyday. I had watched the movie Food Inc. It touched on a subject I wasn't familiar with. After watching Genetic Roulette, I cleaned out the cupboards. After doing this, within two weeks my sons tantrums stopped completely, he started smiling, crawling into my lap for cuddles. I had no idea that was the issue. Even now when he gets something conventionally/ GMO poison, he'll have another tantrum like his past. So if there's a question as to where it's from-what kind of seed, I don't take it. So for me and my family, we bow out from being a guinea pig."- Stephanie Vanderyacht

"My husband was in the hospital 5 times last year. Doctors wanted to remove part of his intestine because it was so infected instead doctors pumped him full of antibotics for a week when he got out of hospital I changed his diet and all our family food choices to NON- GMO foods WOW what a diffrence he’s doing great and food never tasted so good! I will march sign petitions anything to reclaim our healthy labeled food choices. God Speed JUST SAY NO TO GMO’S ….MAAM! " Rhonda Bryne, MAA

My 7 year old son was diagnosed with asthma and needed glasses inside of two weeks. I started learning about asthma and natural ways to control it. Then I found out about GMO. I removed my family from GMO foods/drinks. My 7 year old went from needing a nebulizer

3x’s a day to not at all. His asthma disappeared. He also no longer had the stigmatism that required glasses. The eye Dr. said he must have had ‘some sort of inflammation’ that is now gone for whatever reason. The reason was removing GMO from our diets. He was recommended for retention last year. This year, he is at the top of his class. Karen L.~Moms Across America The above testimonials are a sampling of the hundreds of testimonials which Moms have sent to us. More see: http://www.momsacrossamerica.com/zenhoneycutt/mom_s_testimonials

GMO-free Citizens do not want GMOs on their plates, nor do they want them as medicines, nor in biologicals, vaccines or crops on the fields. We eat organic food.

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5. Others

Rising demand for organic and non-GMO grains outpaces U.S. production By Ken Roseboro Published: February 22, 2017 Issue: March Category: Organic/Sustainable Farming

Organic imports rise sharply as U.S. corn and soybean growers contemplate premiums, risk-reward scenarios Increasing consumer demand for organic and non-GMO foods led to a sharp rise in organic grain imports in 2016—prompting food manufacturers to explore new incentives for U.S. growers transitioning to organic production, according to a new report from CoBank. While U.S. production of non-GMO crops has risen, domestic production of organic corn and soybeans remains well short of demand. CUT http://non-gmoreport.com/articles/rising-demand-organic-non-gmo-grains-outpaces-u-s-production/

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

Supplement: New research confirms GM causes massive off-target damage to plant genomes Details Published: 28 January 2019 A new open-access paper (see link) by researchers at the Salk Institute in the US confirms that the GM transformation process in plants is extraordinarily damaging at a genetic and epigenetic level. The researchers found that inserting new genes into a plant using the bacterium Agrobacterium tumefaciens as a shuttle creates major unintended effects in the genome. The authors studied four different GM lines of the standard laboratory model plant Arabidopsis. https://www.gmwatch.org/en/news/latest-news/18730

Organisation: nolde
Country: The Netherlands
Type: Industry

a. Assessment:
Molecular characterisation

Guys, stop placing these dangerous crops on the market! The world is sick enough as it is.

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

a. Assessment:
b. Food Safety Assessment:
Toxicology

Supplement 02-10-2019, Review. Exposure to Glyphosate-Based Herbicides and Risk for Non-Hodgkin Lymphoma: A Meta-Analysis and Supporting Evidence AuthorLuopingZhanga IemaanRanaa Rachel M.Shafferb EmanuelaTaiolic LianneSheppardbdmore Three of authors EPA scientists!!! Quote of Abstract: “Overall, in accordance with evidence 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.” https://www.sciencedirect.com/science/article/pii/S1383574218300887?via%3Dihub

Cry1Ab

2011:” Scientists from the University of Sherbrooke, Canada, have detected the insecticidal protein, Cry1Ab, circulating in the blood of pregnant as well as non-pregnant women.” Paper https://www.ncbi.nlm.nih.gov/pubmed/21338670. Source: India Today. https://www.indiatoday.in/india/north/story/toxin-from-gm-crops-found-in-human-blood-133483-2011-05-11 Quote:” Earlier studies had found trace amounts of the Cry1Ab toxin in gastrointestinal contents of livestock fed on GM corn. This gave rise to fears that the toxins may not be effectively eliminated in humans and there may be a high risk of exposure through consumption of contaminated meat.” Source GMWatch.

Organisation: Testbiotech
Country: Germany
Type: Non Profit Organisation

a. Assessment:
Molecular characterisation

The process of genetic engineering involved several deletions and insertions in the parental 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; and therefore no detailed investigations were carried out in this regard. Furthermore, other gene products, such as miRNA 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 indicated that expression of Cry1A.105, Cry2Ab2 and EPSPS proteins in genetically engineered maize can induce changes in the overall proteome of the respective GM maize line, with impacts on associated endogenous metabolic pathways (Agapito-Tenfen et al. 2014). Similar transgenes are also present in the stacked maize MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9. 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 effect on their own or potentiate adverse effects of histamine (see also comments form the Experts of Member States).

Environmental stress can cause unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015). More specifically, Fang et al (2018) showed that stress responses especially can lead to unexpected changes in plant metabolism, if they inherit additional EPSPS enzymes. However, the expression of the additional enzymes was only measured under field conditions in the US for one year. It is unclear, to which extent specific environmental conditions will influence the overall concentration of the enzymes in the plants. 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.

Due to increased weed pressure, it has to be expected that these plants can and will be exposed to high and also repeated dosages of glyphosate alone and / or in combination with the other complementary herbicides. Higher applications of herbicides 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 risk assessment even though compositional changes were most noticeable after treatment with complementary herbicides; this supports the premise that a potential unexpected aggregation

of herbicides increases the impact of genetic modification on plant metabolism (see also comments form the Experts of Member States).

Industry in its own recommendations suggests dosages on herbicide resistant maize up to 92 g ai/ha quizalofop 1,5 kg ai/ha glufosinate 7 l / ha 2,4-D 3,6 l / ha glyphosate

EFSA should have requested that Monsanto submit data from field trials with the highest dosage of the complementary herbicides that can be tolerated by the plants, also including repeated spraying and the application of each of the relevant herbicides alone and in combination. The material derived from those plants should have been assessed by using omics techniques to investigate changes in the gene activity of the transgene, as well as the natural genome of the plants. Agapito-Tenfen SZ, Vilperte V, Benevenuto RF, Rover CM, Traavik TI, Nodari RO, 2014. Effect of stacking insecticidal cry and herbicide tolerance epsps transgenes on transgenic maize proteome. BMC plant biology 14: 346.

Fang, J., Nan, P., Gu, Z., Ge, X., Feng, Y.-Q., Lu, B.-R. (2018) Overexpressing Exogenous 5-Enolpyruvylshikimate-3-Phosphate Synthase (EPSPS) Genes Increases Fecundity and Auxin Content of Transgenic Arabidopsis Plants. Frontiers in Plant Sciences, 9: 233. https://doi.org/10.3389/fpls.2018.00233

Mesnage et al. (2016). An integrated multi-omics analysis of the NK603 Roundup-tolerant GM maize reveals metabolism disturbances caused by the transformation process. Scientific Reports, 6:37855, DOI: 10.1038/srep37855

Trtikova, M., Wikmark, O.G., Zemp, N., Widmer, A., Hilbeck, A. (2015) Transgene expression and Bt protein content in transgenic Bt maize (MON810) under optimal and stressful environmental conditions. PloS one, 10(4): e0123011. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0123011

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

Field trials for compositional and agronomic assessment of the stacked maize were conducted in the US for only one year (2010) and not in other relevant maize production areas, such Brazil or Argentina.

Only data from a low number of agronomic parameters (14), were subjected to statistical analysis in accordance with EFSA guidance, 6 were found to be statistically different in untreated plants, and 7 in plants sprayed with the complementary herbicides. These significant changes fall into categories I, II and III. Considering the many (and major) significant differences in this small set of data, EFSA should requested more data.

Compositional analysis revealed many (and major) statistically significant differences: • Statistically significant differences between the four-event stack maize (not treated) and the non-GM comparator were identified for 19 out of 65 endpoints, with several endpoints in category III / IV. • Statistically significant differences between the four-event stack maize

(treated with complementary herbicides) and the non-GM comparator were identified for 23 of 65 endpoints, with several endpoints falling into category III / IV.

The most relevant differences that were identified concern cystine, isoleucine, phenylalanine, raffinose, manganese and b-carotene in grain and in levels of total fat in forage.

Since the maize treated with the complementary herbicides shows many more significant differences compared to maize that was not treated, it is likely that this has an impact on plant composition. However, EFSA did not request any further tests (toxicological data, repeated spraying with higher herbicide dosages or exposure to a wider range of environmental conditions). Instead EFSA simply concluded: Cystine, isoleucine, phenylalanine, raffinose, manganese and b-carotene in grain and total fat in forage were significantly different in the four-event stack maize when compared to its comparator and showed lack of equivalence with the set of non-GM reference varieties (...). Taking into account the known biological role of these compounds, these differences are considered of no toxicological concern by the GMO Panel."

Consequently, instead of assessing the overall pattern of changes in plant components as well as their causes and possible impacts, EFSA only assessed each of the compounds in isolation (!!). This approach turns the comparative approach into a trivial concept of assessing bits and pieces, and ignores questions concerning the overall safety of the whole food and feed.

It has to be assumed that this event is essentially different from its comparator in regard to many compositions and biological characteristics, especially if sprayed with the complementary herbicide. Even if changes taken as isolated data might not directly raise safety concerns, the overall number of effects and their clear significance has to be taken as a starting point for much more detailed investigations. 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. • Further, no data were generated representing more extreme environmental conditions, such as those caused by climate change.

Due to high weed pressure in many maize growing regions, it has to be expected that these plants can and will be exposed to higher amounts and also repeated dosages of the herbicides. Industry in its own recommendations suggests dosages on herbicide resistant maize up to 92 g ai/ha quizalofop 1,5 kg ai/ha glufosinate 7 l / ha 2,4-D 3,6 l / ha glyphosate

Due to high weed pressure in many maize growing regions, it has to be expected that these plants can and will be exposed to higher amounts and also repeated dosages of the herbicides. From the data that is available, 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 was ignored in the risk assessment. EFSA should have requested that Monsanto submit data from field trials with the highest dosage of the complementary herbicides that can be tolerated by the plants, also including repeated spraying with each active ingredient in isolation as well as in combination. In addition, more varieties carrying the transgenes should have been included in the field trials to see how the gene constructs interact with the genetic background of the plants.

The material derived from those plants should have been assessed by using omics techniques to investigate changes in plant composition or agronomic characteristics. Further more powerful statistical analysis, such as multidimensional analysis, was not applied to the data.

Moreover, the comments made by Austrian, French and German experts clearly show that the existing data are not sufficient to conclude on the safety of the stacked maize.

Based on the available data, no final conclusions can be drawn on the safety of the plants.

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b. Food Safety Assessment:
Toxicology

Despite many highly significant changes in the composition of the plants and agronomic characteristics, no testing of the whole plant (feeding study) was requested. It has to be assumed that this event is essentially different from its comparator in regard to many compositions and biological characteristics. Even if changes taken as isolated data might not directly raise safety concerns, the overall number of effects and their clear significance has to be taken as a starting point for much more detailed investigation of their potential health impacts. In addition, as mentioned, a higher number of applications of the complementary herbicide is not likely to just 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 due to interaction with the additionally inserted gene constructs (see also comments form the Experts of Member States; Vivancos et al., 2011; Ayyadurai et al. 2016).

Furthermore, the stacked maize differs from the parental lines in regard to the overall amount of toxin produced, which is much higher than in the parental lines. In processed products, such as maize gluten, the toxins can even show a more than tenfold higher concentration. These higher concentrations are relevant for the assessment of overall toxicology as well as for the immune system; nevertheless there were no empirical investigations.

In regard to toxicology and potential synergistic or other combinatorial effects, 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; and are therefore not identical to their natural templates (Hilbeck & Otto, 2015). These facts were completely ignored by EFSA in their opinion which states: “The Cry1A.105, Cry2Ab2 and Cry1F proteins are delta endotoxins with high specific insecticidal properties acting through cellular receptors found in target insect species. It is reported that the gastrointestinal tract of mammals, including humans, lacks receptors with high specific affinity to Cry proteins (...).”

Despite what is claimed by EFSA, not all mode of actions are dependent on the specific mechanisms that only occur in the target insect species. Only very few Bt toxins (especially Cry1Ab, for overview see, for example, 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. (2012) 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, also combination with material that was sprayed with the complementary herbicides. In this context, it is important that the stacked maize is also resistant to the herbicides glyphosate, glufosinate 2-4D and quizalofop, which should be seen as potential co-stressors (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; Vivancos et al., 2011; Ayyadurai et al. 2016).

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 usage in the EU. In this regard, it should be taken into account that EFSA (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, in the case of 2,4-D, there are publications suggesting that carcinogenic metabolites are produced in genetically modified plants (Lurquin, 2016), but these were not assessed by EFSA. Further, as stated by experts from member states, the metabolism of quizalofop in quizalofop-resistant plants was not assessed in quizalofop risk assessment (EFSA 2008). Since, in addition, glufosinate is classified as showing reproductive toxicity (http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN) EFSA should have at least requested data on the combined toxicity of the residues from spraying with the complementary herbicides.

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 tallowmine 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/node/1637)

Consequently, EFSA should have requested that Monsanto submit data from field trials with the highest dosage of the complementary herbicides that can be tolerated by the plants, also including repeated spraying. It should further be taken into account that not always a mixture of all complementary herbicides will be used in the fields where the maize is cultivated; in some cases just one of them will be used. This might lead to an increase in dosages of the respective complementary herbicides. The choice of herbicide will depend on the price of the herbicide formulations, the respective weed problem and regional agricultural practices. For example, it can be expected that in Argentina, Brazil and the US, there will be different prices, different herbicide formulations and varying regimes of herbicide applications under which the maize is cultivated. None of these specific agronomic practices were considered in the design of the field trials or in EFSA risk assessment.

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 and the Bt toxins 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). Such effects might be also be caused by the residues from spraying with glufosinate since glufosinate interferes with bacterial growth, and in certain circumstances acts as an antimicrobial agent causing shifts in bacterial community structures (Ahmad and Malloch 1995; Hsiao et al. 2007; Pampulha et al. 2007; Kopcáková et al. 2015). 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. Further, Bremmer and Leist (1997) examined the possible conversion of NAG to glufosinate in rats. Up to 10% deacetylation occurred at a low dose of 3 mg/kg bw as shown by the occurrence of glufosinate in the faeces. The authors concluded that most of the conversion was caused by bacteria in the colon and rectum, although toxicity findings indicate partial bioavailability (Bremmer & Leist, 1997).

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.

In addition, cumulative effects have to be investigated if a plant contains or produces other compounds of potential toxicity. It should be acknowledged, that no new methodology is

needed to assess the health risks emerging from the combinatorial application of the herbicides and their potential interaction with the other plant constituents. Suitable methodology to assess combinatorial effects that emerge from simultaneous exposure to a fixed combination of potential stressors via a defined route of exposure (as is the case with food and feed products derived from genetically engineered plants that are resistant to several herbicides) is available and widely used. For example, chronic feeding or multigenerational studies are a well-established method to generate the relevant data.

Despite all these open questions regarding potential health impacts, we are not aware of a single sub-chronic or chronic feeding study being performed with whole food and feed derived from the stacked maize.

In conclusion, the EFSA opinion on the application for authorisation of the stacked maize 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 and allergenicity. The hypothesis which should have been used as a starting point is that there will be synergistic effects between the various Bt toxins and between the various Bt toxins and other stressors, such as residues from spraying. Therefore, the effects of the Bt toxins in regard to mammalian cell systems and intestinal microbiomes should have been tested in combination with other stressors. Furthermore, combinatorial (adjuvant) effects triggered by Bt toxins occurring in high concentrations in the stacked maize and especially in gluten prepared from the maize, have to be tested in interaction with known allergens, such as the one occurring in soybeans. For this purpose, EFSA should have requested that Monsanto submit data from field trials with the highest dosage of glyphosate that can be tolerated by the plants, also including repeated spraying. The material derived from those plants should have been assessed in regard to organ toxicity, immune responses and reproductive toxicity, also taking combinatorial effects with other plants components and the Bt toxins into account.

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

Ahmad I, Malloch D, 1995. Interaction of soil microflora with the bioherbicide phosphinothricin. Agriculture, Ecosystems and Environment 54(3): 165-174.

Ayyadurai et al. (2016). In-Silico Analysis & In-Vivo Results Concur on Glutathione Depletion in Glyphosate Resistant GMO Soy, Advancing a Systems Biology Framework for assessment frame Safety Assessment of GMOs. American Journal of Plant Sciences, 7, 1571-1589.

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. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0067079

Bremmer, J.N. and Leist, K.-H. (1997) Disodium-N-acetyl-L-glufosinate; AE F099730 – Hazard evaluation of Lglufosinate produced intestinally from N-acetyl-L-glufosinate. Hoechst Schering AgrEvo GmbH, Safety Evaluation Frankfurt. TOX97/014. A58659. Unpublished.

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Allergenicity

According to Santos-Vigil et al (2018), the Bt toxin Cry1Ac can act as an allergen if ingested. This publication highly relevant: the Bt toxin Cry1Ac was used as a source for the synthesis of Cry1A.105 as 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, 2018b) came to the conclusion that the Santos-Vigil et al. (2018) publication does not provide any new information and suffers from methodological flaws. This EFSA opinion, however, 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 to be both valid, and not properly assessed by EFSA (Moreno-Fierros et al., 2018). In awareness of the high concentrations of Bt toxins 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) are especially relevant (for review also see Rubio-Infante et al. 2016).

As mentioned, the Bt toxin Cry1Ac was used as a source for the synthesis of Cry1A.105 expressed in the maize.1 Therefore, the synthetically derived Cry1A.105 toxin produced in the maize has structural similarity with Cry1Ac. If Cry1Ac is immunogenic, Cry1A.105 is also likely to be immunogenic.

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 Bt toxins is much higher in gluten meal produced from the maize and can reach a more than tenfold higher concentration 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 responses caused by the allergens in the soybeans might be considerably enhanced by the adjuvant effects of the Bt toxins. Such effects are likely to lead to detrimental effects on health.

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 upon 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 any 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 also underlined in the minority opinion in another EFSA opinion (Annex II of EFSA, 2018c).

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

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Others

We support the statement made by the German and Hungarian experts that much more detailed monitoring should be required (see comments form the Experts of Member States).

Besides the methods of detection, other methods for quantifying exposure to Bt toxins 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, via 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 humans 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.

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3. Environmental risk assessment

Monsanto completely ignored the appearance of teosinte in Spain and France (see Testbiotech, 2016; Trtikova et al, 2017). Thus, the statement that no wild relatives of maize would occur in Europe is simply wrong. 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 findings of Fang et al. (2018), the glyphosate-resistant maize needs to be examined in detail regarding next generation effects, volunteer potential (persistence) and gene flow. There are substantial reasons for following a hypothesis that the maize can show higher fitness compared to conventional maize.

In its opinion, EFSA was aware of the occurrence of teosinte in the EU and tried to assess the risks of gene flow. However, EFSA is 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.

As the German experts (BfN) summarise (see comments form the Experts of Member States): “The potential for gene flow between teosinte and maize is high (Ellstrand et al. 2007, Chavez et al. 2012). Chavez et al. concluded that biosafety regulators in regions where teosinte occurs should not only consider outcrossing from maize to teosinte but also the possibility of teosinte acting as a genetic bridge back to maize. Teosinte grains are very difficult to control. The kernels have got a high duration in the seedbank and long dormancy. Teosinte flowers earlier and longer than maize and pollen of both species can spread over long distances. Teosinte is considered an agricultural pest which needs management.”

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.

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4. Conclusions and recommendations

The EFSA risk assessment cannot be accepted.
