Maize DP23211

Organisation: Doapea
Country: Luxembourg
Type: Individual

Comments:

Here https://webgate.ec.europa.eu/dyna2/gm-consultations/199

Maize DP23211

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

Comments:

23-1-24

We – The European GMO-Free Citizens and the Ekopark Foundation in Lelystad (the

Netherlands) – do not wish to eat this genetically modified maize as oil and other ingredients.

We want to eat unsprayed food that has not been genetically manipulated. This is also better for the environment and for our health and that of animals.

Nor do we want genetically modified maize as animal feed. If you were to approve it (which

we would regret), we would want every product and every end product , to be labelled as a GMO, even if GMOs can no longer be detected in an end product.

See all our comments on GM maize of an earlier date.

Maize DP23211

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

Comments:

Introduction

The GMO Panel assessed maize DP23211 (EFSA, 2024a). The maize produces

• IPD072Aa toxin from the soil bacterium Pseudomonas chlororaphis (against corn rootworm), which is assessed by EFSA here for the first time;

• DvSSJ1 dsRNA (against corn rootworm), which is assessed here by EFSA for the first time;

• the phosphinothricin acetyl transferase (PAT) protein for tolerance to glufosinate-ammonium-containing herbicides;

as well as the PMI protein (as selectable marker). Consequently, the maize is resistant to glufosinate herbicides and toxic to corn rootworm. The genetic intervention involved a multistep process to introduce a 'landing pad' at the target site, where the gene constructs for the production of new proteins (new traits) are subsequently inserted.

1. Systematic literature review

A systematic review as requested in Regulation (EU) No 503/2013 was not provided by the applicant. The submitted scoping review appears to not include a peer-reviewed publication with a clear explanation of the mode of action of the newly expressed insecticidal IPD072Aa toxin. Further, it has to be stated that there is a clear lack of independent science, i.e. all available scientific papers regarding event DP23211 were written by the applicant.

2. Molecular characterisation

Maize DP23211 was developed using a multi-step method. In the first step, a specific genomic integration site (landing pad) was created by microprojectile bombardment. In step 1, three plasmids were introduced (one for the specific integration site and two plasmids for regeneration of the plants). A meganuclease induces a double strand break in a defined region of chromosome 1. Step 2 consists of Agrobacterium transformation to introduce the dsRNA as well as the gene for insecticidal activity and the pat gene (for resistance to glufosinate).

Unintended modifications, e.g. genome rearrangements or indels, may occur in Step 1 as a result of off-target double strand cuts due to endonucleases (EFSA, 2012). Member states' experts therefore asked for an analysis of the whole genome sequence by next generation sequencing to ensure that all possible unintended modifications altering the genome in the process of transformation can be identified. EFSA responded by saying that, for example, "any unintended changes with potential impact on product are indirectly assessed in the comparative risk assessment" (EFSA, 2024b). This does not seem to be a convincing scientific reason .

The genetic engineering process led to the emergence of many new open reading frames in the genome of the maize. 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, EFSA states that proteins that may emerge from these DNA sequences would not raise safety concerns:

"In addition, bioinformatic analyses of the newly created open reading frames (ORFs) within the insert and spanning the junctions between the insert and genomic DNA revealed six ORFs (DP23211_220, DP23211_466, DP23211_724, DP23211_832, DP23211_833 and DP23211_994) showing a sequence similarity to potentially allergenic proteins exceeding a 35% identity over an 80 amino acid window and one short ORF (DP23211_524) that presents an exact 8 amino acid match to known allergens. Two of these ORFs (DP23211_220 and DP23211_466) are within the transcriptional unit of mo-pat gene and ipd072Aa gene, in the same orientation but in a different reading frame. The remaining ORFs are in reverse orientation, outside transcribed regions, and lack promoters and start codons for proper expression."(EFSA, 2024a)

The large number of ORFs indicates that there are signs of genomic perturbations caused by the multi-step process of genetic engineering - this should have prompted a higher level of scrutiny.

Further, there are open questions regarding the molecular characterisation of the RNAi construct. Different member state authorities raised questions about the off-target effects of the RNAdb directed against the DvSsj1 gene. They requested more data on the sequence homology between siRNA and human and animal transcriptomes using less restrictive parameters to identify partial identities (EFSA, 2024b). Other aspects of RNAi are discussed in the "Toxicity" chapter.

Thus, uncertainties remain about further biologically active substances arising from the method of genetic engineering and the newly introduced gene constructs.

Gene expression under stress conditions

Environmental stress can cause unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015). Expression of the additional enzymes was, nevertheless, only measured under field conditions in six locations in the US and Canada for one year (2018), with no exceptional weather conditions being reported. It is evident that these weather conditions are not representative of all growing conditions, e. g. in 2023 the temperatures were much higher due to ongoing climate change. Therefore, new data on gene expression need to be made available. Data should also cover a much broader range of defined biotic and abiotic stressors to demonstrate stability in gene expression under sufficiently realistic conditions.

Consequently, the GE maize plants tested in field trials do not sufficiently represent the products intended for import. The data presented by the applicant are insufficient to conclude on the impact that environmental factors may have on gene expression, as laid down in EU Regulation 503/2013.

Impact of genetic backgrounds on gene expression

It is known that the genomic background of the varieties can influence both the expression of the inserted genes and plant metabolism (see, for example, Lohn et al., 2020; Trtikova et al., 2015). However, data on gene expression were confined to a single variety. Therefore, EFSA should have also requested additional data from different transgenic maize varieties, e. g. those cultivated in South America. Gao et al (2020) showed that also genes expression with a landing pad can largely be impacted by the genetic backgound.

EFSA did not consider any of these issues. Consequently, the GE maize plants tested in the field trials do not sufficiently represent the products intended for import. As such, the data presented by the applicant are insufficient to conclude on the impact the genetic backgrounds may have on gene expression, as laid down in EU Regulation 503/2013.

Summary of molecular analysis

EFSA should have requested that the applicant use suitable methods to detect unintended genetic changes, and to assess all the biologically active molecules occurring at novel open reading frames. Data collection on gene expression should include the highest dosage of the complementary herbicides that may be used in the countries of cultivation. Transgenic plants with differing genetic backgrounds should be grown in the field trials, and a broad range of defined environmental conditions should be applied. The plant material derived from such trials should be assessed with 'Omics' techniques to investigate changes in the gene activity of the transgenes, as well as changes in the plants' own genes.

3. Comparative assessment of plant composition, and agronomic and phenotypic characteristics

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."

The data presented by Corteva do not meet the requirements of Implementing Regulation 503/2013: (1) the field trials were not conducted in all relevant regions where the GE maize will be cultivated, and no defined extreme weather conditions were taken into account; (2) the field trials did not take all relevant agricultural management practices into account; (3) not all relevant genetic backgrounds were taken into account.

Data on environmental factors and stress conditions - and their impact on plant composition and phenotype

Field trials to assess plant composition as well as agronomic and phenotypic characteristics of the GE maize were conducted in the US and Canada for one year (2018) at eight (compositional analysis) or twelve (agronomic performance) sites. Some extreme weather conditions were reported from some fields, but no targeted investigation was carried out to for example investigate the impact of climate change conditions. In order to assess possible compositional or agronomic changes, the plants should have been grown in various environmental conditions and exposed to well-defined environmental stress conditions by also taking into account maize growing regions such as Brazil.

In light of the information available, we assume that the data provided do not sufficiently represent the agricultural practices and bio-regional conditions under which these plants are likely to be grown. For example, the plants are supposed to be grown also in countries like Brazil, under different environmental conditions.

No experiments were requested to show to which extent specific environmental conditions influence plant composition and agronomic characteristics. Hence, the data made available do not allow conclusions (as requested in Implementing regulation 503/2013) whether the expected environmental conditions under which the plants are likely to be cultivated will influence the expression of the studied endpoints.

Data on herbicide application rates and their impact on plant composition as well as agronomic and phenotypic characteristics

The complementary herbicide (glufosinate) was only applied once during the field trials. The dosage was chosen in accordance with the label recommendations (EFSA 2024a). However, as Myiazaki et al (2019) show, the herbicide applications are likely to differ across regions and in response to pressure by herbicide resistant plants.

Therefore, in the light of the information available, we assume that the data provided do not sufficiently represent the agricultural practices, like higher dosages and repeated spraying.

Consequently, the GE maize plants tested in field trials do not sufficiently represent the products intended for import. The data presented by the applicant are insufficient to conclude on the impact of the herbicide applications on gene expression, plant composition or biological characteristics of the plant as requested in EU Regulation 503/2013.

Impact of genetic backgrounds on plant composition as well as on agronomic and phenotypic characteristics

It is known that the genomic background of the varieties can influence both the expression of the inserted genes and plant metabolism (see, for example, Lohn et al., 2020; Trtikova et al., 2015; Gao et al., 2020). However, it appears that the data on gene expression were confined to a single variety. Therefore, EFSA should have also requested additional data from transgenic maize varieties that are, for example, cultivated in South America.

EFSA did not take these issues into consideration. Consequently, the GE maize plants tested in field trials do not sufficiently represent the products intended for import. The data presented

by the applicant are, therefore, insufficient to conclude on the impact that the genetic backgrounds may have on gene expression, as laid down in EU Regulation 503/2013.

Agronomic and phenotypic characteristics

Of the 9 endpoints, the agronomic assessment resulted in 5 significant differences for the DP23211 maize not treated with the intended herbicide, and 4 significant differences for the IHT DP23211 maize treated with the intended herbicide, as compared to the control maize line.

It should be stated that four/five statistical differences out of nine endpoints are a fairly high number. These findings, together with the large number of significant differences, as shown in the compositional analysis, should have prompted further investigations in regard to the equivalence of maize DP23211 with its isogenic parent.

Data from compositional analysis

Maize DP23211 forage and grains harvested from the field trials were analysed for 71 different constituents (10 in forage and 61 in grains).

• For maize DP23211 not treated with the intended herbicide, statistically significant differences were identified with respect to the conventional counterpart for 13 constituents (1 in forage and 12 in grains). The test of equivalence between maize DP23211 and the non-GM maize reference varieties indicated that all those constituents fell under equivalence category I or II.

• For maize DP23211 treated with the intended herbicide, statistically significant differences with the conventional counterpart were identified for 21 constituents (2 in forage and 19 in grains). All these constituents fell under equivalence category I or II except for histidine, phenylalanine, magnesium and phosphorus in grains, which fell under equivalence category III or IV, while the test of equivalence was not applied for folic acid in grains.

In summary, statistical differences were found in almost one third of all constituents in maize DP23211 treated with the recommended dose of glufosinate (grain). Four of these constituents even fell under equivalence category III or IV. These findings, together with the large number of significant differences as shown in the agronomic assessment, should have prompted further investigations in regard to the equivalence of maize DP23211 with its isogenic parent.

Given the above reasoning on the impact of environmental factors, herbicide application and genetic backgrounds as well as the large number of significant findings, EFSA should have requested more data: data on agronomic and phenotypic endpoints should be generated from a wider range of clearly defined stress factors, including all relevant agricultural practices and genetic backgrounds.

A more detailed analysis would have been necessary to investigate changes in plant composition and phenotype, and also to investigate potential unintended changes in metabolic pathways and the emergence of unintended biologically active gene products.

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 plant genome, and also to investigate changes in metabolic pathways and the emergence of unintended biologically active

gene products (see Benevenuto et al., 2022). Such in-depth investigations should not depend on findings indicating potential adverse effects, they should always be necessary to draw sufficiently robust conclusions to inform the next steps in risk assessment.

In addition, in awareness of the absence of any independent data on this maize, we strongly recommend establishing a system with independent controls to repeat the trials and double check the data on plant composition and agronomic characteristics.

Conclusion on the comparative assessment of plant composition as well as on phenotypic and agronomic characteristics

The data provided by the applicant and accepted by EFSA are insufficient to conclude on the impact of environmental stressors, herbicide applications and genetic backgrounds on gene expression, plant metabolism, plant composition, or on agronomic and phenotypic characteristics.

To gather reliable data on compositional analysis and agronomic characteristics, the plants should have been subjected to a much broader range of defined environmental conditions and stressors. Furthermore, EFSA should have requested the applicant to submit data from field trials which reflect current agricultural practices, including all relevant complementary herbicides and several genetic backgrounds.

4. Toxicity

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;”

a) IPD072Aa protein

As far as the IPD072Aa protein is concerned, there are some important differences, but also some similarities, in comparison to Bt toxins currently produced by transgenic plants. These need to be taken into account in risk assessment.

1. The source

Introducing these proteins into agriculture and the food chain cannot fully rely on previously gained experience. The source organism of the gene coding for the IPD072Aa protein

(Pseudomonas chlororaphis) is used in agriculture to protect plants by producing compounds that inhibit fungal growth insects and nematodes. EFSA assessed a strain of P. chlororaphis in 2017 and identified several data gaps regarding mammalian toxicity and ecotoxicology, amongst others (EFSA, 2017).

2. Mode of action

While the mode of action of Bt toxins, before introduced into transgenic plants, was already a matter of investigations and explored to some detail, this is not the case with the toxin from P. chlororaphis. So far, the mode of action of this insecticide is only partly understood. A recent paper by Corteva scientists (not quoted in EFSA, 2024a) on this subject gives some details, but still has to admit that:

"Attempts to observe pore formation by IPD072Aa in artificial lipid membranes failed to reveal pore forming activity (Nelson, unpublished observations). [….] Similarly, attempts to identify the plasma membrane receptor for IPD072Aa using co-precipitation and yeast two-hybrid approaches failed to identify a protein interactor that serves as a valid functional receptor (data not shown). [….] The molecular mechanism of IPD072Aa toxicity to WCR larvae, including the identity of the membrane receptor to which it binds will continue to be evaluated" (Jiménez-Juárez et al., 2023).

In addition, all data available seem to stem from experts working for the applicant. The lack of insight into the mode of action of IPD072Aa toxin is also underlined by comments from the experts from the Member States (EFSA, 2024b).

3. Combinatorial or synergistic factors impacting toxicity and allergenicity

It is known from toxicity of Bt toxins that plants constituents such as protein inhibitors or other cofactors substances can largely enhance its toxicity (MacIntosh et al., 1990; Pardo-López et al., 2009). Therefore, to determine 'no observed effect concentration' or 'no observed effect dose', it is not sufficient to use the proteins in isolation as produced by bacteria. Instead it is necessary to take into account the real conditions of exposure, such as in combination with plant protein inhibitors. Also the residues from spraying with glufosinate should be considered. This is obligatory for determining chronical and subchronical toxicity, immunogenicity (allergenicity), impact on microorganisms (intestinal microbiome or soil organisms) as well as the effects on non-target organisms.

b) Effects of residues from spraying with complementary herbicide specific to GE plants

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 management practices in the cultivation of the herbicide-resistant plants, there are, for example, specific patterns of spraying, exposure, occurrence of specific metabolites and emergence of combinatorial effects that require special attention.

EU legal provisions, such as Regulation 1829/2003 (and 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 resulting from combinatorial exposure of various potential stressors need to be tested.

In regard to food and feed safety, EFSA (2020) considers microbiomes to be highly relevant to the health status of their hosts. Therefore, it is desirable to understand the importance of their role in risk assessment. EFSA expects that gut microbiome research (not only in the case of GE plants) will play a relevant role in regulatory science with potential implications for future risk assessments and predictive risk models. As EFSA states: "considering that the gut microbiome is a biological component directly and indirectly involved in the metabolism of food/feed components and chemicals and in the protection of the host against adverse environmental exposure, it would be useful to establish criteria on how to evaluate the potential adverse impacts of perturbators on this defensive barrier, and consequently, on human/animal health."

A 2019 study commissioned by EFSA on adjuvanticity / immunogenicity assessment of proteins included the role of the microbiome. Parenti et al. (2019) state that "one of the most important drivers of immune response is the gut microbiota and other microbial constituent of the human body which are able to regulate host-pathogen balance and to produce systemic pro-inflammatory stimuli. The lifelong antigenic load represented by foods and bacteria/bacterial products leads to a profound remodeling of the gut microbiota and these changes are emerging as a driving force of the functional homeostasis of the immune system. As a matter of fact, a perturbation of the gut microbiota homeostasis due to irregular lifestyles, stress and age may lead to gut microbiota dysbiosis. This condition may predispose the host to metabolic disorders and inflammation."

However, no attempts have been made to integrate the microbiome into the risk assessment of food and feed derived from the GE maize. This is in direct contradiction to Regulation 1829/2003 which requests "genetically modified food and feed should only be authorised for placing on the Community market after a scientific evaluation of the highest possible standard, to be undertaken under the responsibility of the European Food Safety Authority (Authority), of any risks which they present for human and animal health and, as the case may be, for the environment." (Recital 9).

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.

In addition, cumulative effects (mixtures of GE plants in one diet) may play a decisive role. For example, insecticidal toxins or residues from spraying with other herbicides may contribute to synergistic effects that can be decisive for the overall toxicity of a given diet.

Furthermore, findings by Christ et al. (2017) showing that the PAT/BAR enzyme may also acetylate endogenous amino acids, should have been the starting point for further investigations.

c) Toxicity of DvSSJ1 dsRNA

A report commissioned and published by EFSA in 2019 (Davalos et al., 2019) considers the role of ncsRNA in the risk assessment of GE plants. Davalos et al. summarise current findings on ncsRNAs produced by plants; they discuss to which extent they can be taken up via food or feed consumption and show cross kingdom activity due to unintentional interaction with human or animal gene regulation.

Potential off-target genes in mammals

As Davalos et al. (2019) show, there are many matches between the ncsRNA produced in food and medical plants and regulatory pathways in human and animals. There is no doubt that in cases where relevant plant molecules are transmitted into the cells of humans and animals, RNAi effects, such as gene silencing, can occur and, for example, genes in animals can be downregulated by plant nscRNA.

Therapeutic effects from the uptake of ncsRNA from the gut have been evidenced in several publications. Some of the research shows that biological effects can be achieved with very low dosages (for references see Davalos et al., 2019).

As seen above (molecular characterisation), several experts from member states agreed that there are open questions regarding the off-target effects of DvSSJ1 dsRNA (EFSA, 2024b).

Stability of ncsRNA

It appears that some findings depend on the specific type of ncsRNA. For example, naked synthetic ncsRNA used by some researchers, is degraded very quickly compared to ncsRNA produced by plants (for references see Davalos et al., 2019).

The Davalos et al. (2019) study found strong indications that plant miRNAs are more stable than previously anticipated. This is due to structural properties influencing their stability and turnover "However, when assessing the stability of plant ncRNAs outside the plant, compelling evidence exists that plant miRNAs are highly stable under different conditions including food storage, processing, cooking, or simulated digestion. Moreover, they seem to survive after long incubation in serum, or are detected in the gastric content of mice, suggesting that plant miRNAs are more resistant to degradation than synthetic or animal miRNAs."

ncsRNA uptake from the gut

Contrary to assumptions made by EFSA, research by Davalos et al. (2019) shows that the uptake of ncsRNA from plants and microorganisms via the gut into the cells of humans and animals is an established fact.

It is known that there are many barriers between the intestine, the blood stream, the cells and the cell nuclei, which lower the likelihood of such RNAi effects occurring. However, according to Davalos et al. (2019), there are mechanisms that can allow the molecules to pass through these barriers: plant ncsRNA is protected against degradation by methylation, it can be excreted and taken up in vesicles (such as exosomes); nano-particles are also produced by plants which can serve as transport elements.

Several studies discuss the uptake of small RNAs in body liquids, e.g. in humans and pigs (Heinemann et al. 2013, Luo et al. 2017, Nawaz et al. 2018). Indeed, the ncsRNA molecules originating from plants can reportedly be found in many bodily fluids of humans and animals, including blood and milk. Similar findings have been reported by Nawaz et al., (2019): “Strong evidence suggested that plant-food-miRNAs can survive digestion, enter the body and affect gene expression patterns.”

In this context, Davalos et al. (2019) see the need for further research to explore the uptake and biological effects of ncsRNA: “Exogenous plant-derived ncRNAs have been found in exosomes or macrovesicles. How they reach these types of structures in biological fluids is unknown. In summary, supporting and contradicting evidence concerning the existence of systemic effects of dietary plant-derived exogenous ncRNAs is heavily debated. Important aspects such as the precise mechanism/s of transport of plant ncRNAs from food into the systemic circulation, the amount of exogenous ncRNAs reaching tissues or the molecular mechanisms of cellular uptake need to be determined.”

Interactions on the level of the microbiome

There is strong evidence that ncsRNAs originating in the host (e.g. produced by intestinal epithelial cells) are taken up by the gut microbiota and can manipulate its gene regulation. The same evidence is available for ncsRNA produced in the gut microbiome: it can be taken up by the host and enact RNAi in its cells, demonstrating the existence of bidirectional ncRNAs based host-microbial interactions (for details see Davalos et al., 2019).

In this context, Davalos et al. (2019) show that plant-derived ncsRNA does not necessarily have to be taken up from the intestine to exert its effects. Instead, interaction with the intestinal microbiome can emerge which, in a next step, may impact the health of the animal or human host.

Thus, there is a plausible hypothesis on how the additional dsRNA might affect the gut microbiome community after ingestion, and further research is needed to understand the impact of exogenous dsRNA in mammalian host microbiota composition and identify microbial targets along with their effect on physiological conditions.

There is broad consensus on the role of the gut microbiome in human and animal health. For example, in 2019, in the study commissioned by EFSA, Parenti et al. (2019) states that “one of the most important drivers of immune response is the gut microbiota and other microbial constituent of the human body which are able to regulate host-pathogen balance and to produce systemic pro-inflammatory stimuli. The lifelong antigenic load represented by foods and bacteria/bacterial products leads to a profound remodeling of the gut microbiota and these changes are emerging as a driving force of the functional homeostasis of the immune system. As a matter of fact, a perturbation of the gut microbiota homeostasis due to irregular lifestyles, stress and age may lead to gut microbiota dysbiosis. This condition may predispose the host to metabolic disorders and inflammation.”

Therefore, the interaction between the ncsRNAs produced by GE plants and the microbiome of humans or animals has to be considered in food and feed safety assessment. In this context, the barrier for ncsRNA to pass from plants to gut microorganisms seems to be much lower compared to those identified in the human or animal body.

Conclusions on toxicity assessment of DvSSJ1 dsRNA

In summary, it is clear that interference with gene regulation following the absorption and processing of dsRNAs to siRNA within humans and animals after ingestion of RNAi-based GM crops is both feasible and plausible. As Nawaz et al. (2019) conclude: “Based on the currently available evidence, off-target effects from the ingestion of novel siRNA present in

foods derived from either GM crops or foliar insecticidal or anti-viral spray application, cannot be ignored and thus should form an integral part of the risk assessment of these products."

As shown by Davalos et al., (2019) and Nawaz et al. (2019), the uptake of ncsRNA from plants via ingestion in sufficient amounts to exert effects on gene regulation in mammalian cells must be seen as a certainty. Further, the impact on the host via its microbiome is another way in which human or animal health could be affected.

Therefore, further risk assessment has to be performed

• to trace the fate of the artificial ncsRNA after ingestion;

• to identify the potential target site in the microbial community in the gut and mammalian cells;

• to assess the magnitude of potential effects if identified.

Additional questions have arisen from risk assessment in respect to the mixed toxicity of the stacked Maize. These questions are highly relevant for demonstrating the safety of the plants because other newly expressed proteins, residues from spraying or plant constituents, can influence the impact on the microbiome in the gut or the uptake from the gut.

5. Environmental risk assessment

a) IPD072Aa toxin

The specificity of the IPD072Aa toxin was tested on several non-target insects and some grain-feeding mammals (Boeckmann et al., 2021). However, the test material itself was produced in bacteria in isolated form. Therefore, this approach leaves aside the real routes of exposure via plant material, which will always include protein inhibitors that may strongly increase toxicity. In addition, other potential co-factors and stressors typically present in the receiving environments were ignored. No data were made available on whether the protein can accumulate in the food webs, or persist and accumulate in the environment (e. g. the soil). Therefore, basic information needed for environmental risk assessment is absent.

In peer-reviewed papers published by the applicant, there were several significant findings in regard to non-target organisms (Boeckmann et al., 2019, 2021). Negative effects were found for example in springtails and species of ladybird beetles. These results should be the starting point for risk assessment by non-corporate scientists.

Available data shows that IPD072Aa is stable and biologically active after heat treatment up to 95°C (Carlson et al. 2019). Therefore, environmental exposure via waste material from processing must be anticipated. Gastric fluids in mammals are likely to degrade the toxin but studies with a qualitative proof (i.e. bioassays) are missing. In addition, the information on non-target soil organisms, which would be main group affected by waste and manure containing IPD072Aa, are lacking.

b) Teosinte

The appearance of teosinte in Spain and France (see Testbiotech, 2016; Trtikova et al., 2017) has to be considered in more detail. Maize volunteers can be found in the EU on a regular basis, as reported by Palaudelmàs et al. (2009) in Spain or Pascher (2016) in Austria.

Testbiotech is aware of an EFSA (2022) opinion regarding the teosinte situation in France and Spain. Here, EFSA comes to the conclusion:

"The new evidence retrieved confirms that where maize and EU teosinte plants co-occur and flower synchronously, maize alleles (transgenic or not), can move into teosinte populations at rates that depend on different factors. Hence, the possible introgression of transgenes from maize MON810, Bt11, 1507 and GA21 into EU teosinte may only provide a selective advantage to GM teosinte hybrid progeny under high infestation of target pests and/or when glufosinate-ammonium- and/or glyphosate-based herbicides are applied. However, this fitness advantage will not allow GM teosinte hybrid progeny to overcome other biological and abiotic factors limiting their persistence and invasiveness. Therefore, EFSA considers that the growth habits of EU teosinte plants and teosinte hybrid progeny are such that the acquisition of insect resistance and/or herbicide tolerance is unlikely to change their relative persistence and invasive characteristics under EU conditions."

This opinion not sufficiently backed by science: 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 also interference from the genetic background that cannot be predicted from the assessment of the original event (Bauer-Panskus et al., 2020). This issue is relevant for gene flow from maize to teosinte, and from teosinte to maize.

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

6. 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.

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.

In addition, the example of the maize highlights some general problems. These are:

(1) Due to current EFSA practices it is not possible to access the original data from the companies within the period of consultation. Therefore, the opinion has to provide all the necessary data to allow other experts to conclude whether the provisions of GMO regulation (esp. 503/2013) are fulfilled. We are making this comment after our recent experiences in requesting access to documents, which in many instances took months to achieve. The Commission should advise EFSA to improve transparency.

(2) A Testbiotech report published in 2021 (Testbiotech, 2021), shows how the European Food Safety Authority (EFSA), which is responsible for risk assessment of GE plants, intentionally puts crucial issues aside. This careless approach exemplifies the overall decrease in general food safety standards that has been ongoing since the introduction of GE plants. The number of events authorised for import has, at the same time, steadily increased. In light of these findings, the Commission should try to avoid ‘rubber stamping’ all applications for import of GE plants, and thus reduce the overall number of products entering the market, while ensuring that these products undergo much more thorough risk assessment.

References

Bauer-Panskus A., Miyazaki J., Kawall K., Then C. (2020) Risk assessment of genetically engineered plants that can persist and propagate in the environment. Environ Sci Europ, 32(1): 1-15. https://doi.org/10.1186/s12302-020-00301-0

Benevenuto R.F., Venter H.J., Zanatta C.B., Nodari R.O., Agapito-Tenfen S.Z. (2022) Alterations in genetically modified crops assessed by omics studies: Systematic review and meta-analysis. Trends Food Sci Technol, 120: 325-337. https://doi.org/10.1016/j.tifs.2022.01.002

Boeckman C. J., Huang E., Sturtz K., Walker C., Woods R., Zhang J. (2019) Characterization of the spectrum of insecticidal activity for IPD072Aa: a protein derived from Pseudomonas chlororaphis with activity against Diabrotica virgifera virgifera (Coleoptera: Chrysomelidae). J Econ Entomol, 112(3): 1190-1196. https://doi.org/10.1093/jee/toz029

Boeckman C.J., Anderson J.A., Linderblood C., Olson T., Roper J., Sturtz K., ... & Woods R. (2021) Environmental risk assessment of the DvSSJ1 dsRNA and the IPD072Aa protein to

non-target organisms. GM Crops & Food, 12(1): 459-478. https://doi.org/10.1080/21645698.2021.1982348

Carlson A.B., Mathesius C.A., Ballou S., Boeckman C.J., Gunderson T.A., Mirsky H.P., ... & Delaney B. (2019) Safety assessment of coleopteran active IPD072Aa protein from Pseudomonas chlororaphis. Food Chem Toxicol, 129: 376-381. https://doi.org/10.1016/j.fct.2019.04.055

Dávalos A., Henriques R., Latasa M.J., Laparra M., Coca M. (2019) Literature review of baseline information on non-coding RNA (ncRNA) to support the risk assessment of ncRNA-based genetically modified plants for food and feed. EFSA Supporting Publication, 16: EN-1688. https://doi.org/10.2903/sp.efsa.2019.EN-1688

Dong T., Guan Q., Hu, W., Zhang, M., Zhang, Y., Chen, M., ... & Xia, Y. (2020). Prenatal exposure to glufosinate ammonium disturbs gut microbiome and induces behavioral abnormalities in mice. J Hazard Mat, 389: 122152. https://doi.org/10.1016/j.jhazmat.2020.122152

EFSA (2012): Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function. In: EFSA J, 10(10): 2943. https://doi.org/10.2903/j.efsa.2012.2943

EFSA (2017) Conclusion on the peer review of the pesticide risk assessment of the active substance Pseudomonas chlororaphis strain MA 342. EFSA J, 15(1): 4668. doi:10.2903/j.efsa.2017.4668

EFSA (2020) Editorial: Exploring the need to include microbiomes into EFSA's scientific assessments. EFSA J, 18(6): e18061. https://doi.org/10.2903/j.efsa.2020.e18061

EFSA (2022) Statement on the update of environmental risk assessment conclusions and risk management recommendations of EFSA (2016) on EU teosinte. EFSA J, 20(4): 7228. https://doi.org/10.2903/j.efsa.2022.7228

EFSA (2024a) Assessment of genetically modified maize DP23211 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2019-163). EFSA J, 22(1): e8483. https://doi.org/10.2903/j.efsa.2024.8483

EFSA (2024b) Comments and opinions submitted by Member States during the three-month consultation period [Supporting information]. https://doi.org/10.2903/j.efsa.2024.8483

Gao H., Mutti J., Young J.K., Yang M., Schroder M., Lenderts B., ... & Chilcoat N.D. (2020) Complex trait loci in maize enabled by CRISPR-Cas9 mediated gene insertion. Front Plant Sci, 11: 535. https://doi.org/10.3389/fpls.2020.00535

Heinemann J.A., Agapito-Tenfen S.Z., Carman J.A. (2013) A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments. Environ Int, 55: 43-55. https://doi.org/10.1016/j.envint.2013.02.010

Jiménez-Juárez N., Oral J., Nelson M.E., Lu A.L. (2023) IPD072Aa from Pseudomonas chlororaphis targets midgut epithelial cells in killing western corn rootworm (Diabrotica

virgifera virgifera). Appl Environ Microbiol, 89(3): e01622-22. https://doi.org/10.1128/aem.01622-22

Lohn A.F., Trtikova M., Chapela I., Van den Berg J., du Plessis H., Hilbeck A. (2020) Transgene behavior in Zea mays L. crosses across different genetic backgrounds: Segregation patterns, cry1Ab transgene expression, insecticidal protein concentration and bioactivity against insect pests. PloS ONE, 15(9): e0238523. https://doi.org/10.1371/journal.pone.0238523

Luo, Y. Wang P., Wang X., Wang Y., Mu Z., Li Q., ... & Li M. (2017) Detection of dietetically absorbed maize-derived microRNAs in pigs. Sci Rep, 7(1): 645. https://doi.org/10.1038/s41598-017-00488-y

MacIntosh, S.C., Kishore, G.M., Perlak, F.J., Marrone, P.G., Stone, T.B., Sims, S.R., Fuchs, R.L (1990) Potentiation of Bacillus thuringiensis insecticidal activity by serine protease inhibitors. J. Agric Food Chem, 38: 1145-1152. https://doi.org/10.1021/jf00094a051

Miyazaki J., Bauer-Panskus A., Bøhn T., Reichenbecher W., Then C. (2019) Insufficient risk assessment of herbicide-tolerant genetically engineered soybeans intended for import into the EU. Environ Sci Eur, 31(1): 1-21. https://doi.org/10.1186/s12302-019-0274-1

Nawaz M.A, Mesnage R., Tsatsakis A.M., Golokhvast K.S., Yang S.H., Antoniou M.N., Chung G. (2019) Addressing concerns over the fate of DNA derived from genetically modified food in the human body: a review. Food Chem Toxicol, 124: 423-430. https://doi.org/10.1016/j.fct.2018.12.030

Palaudelmàs M., Peñas G., Melé E., Serra J., Salvia J., Pla M., Nadal A., Messeguer J. (2009) Effect of volunteers on maize gene flow. Transgenic Res, 18(4): 583-594. https://doi.org/10.1007/s11248-009-9250-7

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://doi.org/10.1016/j.peptides.2008.07.027

Parenti M.D., Santoro A., Del Rio A., Franceschi C. (2019) Literature review in support of adjuvanticity/immunogenicity assessment of proteins. EFSA Supporting Publications, 16(1): 1551E. https://doi.org/10.2903/sp.efsa.2019.EN-1551

Pascher K. (2016) Spread of volunteer and feral maize plants in Central Europe: recent data from Austria. Environ Sci Eur, 28(1): 28-30. https://doi.org/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

Testbiotech (2021) Risk assessment of GE plants in the EU: Taking a look at the ‘dark side of the moon’. https://www.testbiotech.org/content/risk-assessment-ge-plants-eu-taking-look-dark-side-moon

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. https://doi.org/10.1371/journal.pone.0123011

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. Sci Rep, 7: 1560. https://doi.org/10.1038/s41598-017-01478-w