Maize GA21 x T25

Organisation: OGM dangers
Country: France
Type: Association

Comments:

Dear Commission,

My experience for the last twenty years is that Commission does not reply to consultations, does not care about the advice of the public. As a consequence, consultations are only the spectacle of a consultation to _look like_ a democracy. But it is a techno-cracy.

I have not the faintest hope that fake food be released in my environment.And the intent of the Commission to stop labels adds to my anger.

If the commission goes on faking labels on food, it will hurt the whole alimentary chain from peasans to retailers (and consumers inbetween).

I did not even propose to the members of OGM dangers to say chat they think since it is useless.

But you are also weakening the authority of all politicians. When there will be revolts, you will claim it is irrational. But it is very reasonnable!

Sincerely yours

Maize GA21 x T25

Organisation: De Gentechvrije Burgers, Europees Consumentenplatform
Country: The Netherlands
Type: Others...

Comments:

Lelystad, 24 February 2023

Subject: MON GA21 × T25 genetically modified maize

We – the GMO-Free Citizens (a European consumer platform based in Lelystad) and the Lelystad Ekopark Foundation – object to EU market authorisation for MON GA21 × T25 genetically modified maize.

Abstract (Quote)

'Genetically modified maize GA21 × T25 was developed by crossing to combine two single events: GA21 and T25. The GMO Panel previously assessed the two single maize events and did not identify safety concerns. No new data on the single maize events were identified that could lead to modification of the original conclusions on their safety.'

And quote:

'The molecular characterisation, comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological, allergenicity and nutritional assessment indicate that the combination of the single maize events and of the newly expressed proteins in maize GA21 × T25 does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that maize GA21 × T25, as described in this application, is as safe as its conventional counterpart and the non-GM reference varieties tested, and no post-market monitoring of food and feed is considered necessary.'

https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2023.7729

Our comment: We have our doubts about this. And we also consider it very important that 'post-market monitoring of food and feed' should take place. It is certainly the case now that this GM maize is being sprayed with a highly toxic substance. Many people currently have cancer or allergies – or both. See our previous comments concerning the herbicides used.

For example: https://www.gentechvrij.nl/2022/12/02/another-gm-maize-to-be-placed-on-the-eu-market-food-and-feed/#more-12278

We – the European GMO-Free Citizens and the Ekopark Foundation in Lelystad (the Netherlands) – do not wish to eat this genetically modified maize. We want to eat unsprayed food that has not been genetically manipulated. This is also better for the environment.

Nor do we want genetically modified maize as animal feed. And we do not want you to put it back on the EU market. 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.

Maize GA21 x T25

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

Comments:

1. Systematic literature review

A systematic review as referred to in Regulation (EU) No 503/2013 was not provided by the applicant. According to EFSA (2023a): “The literature searches did not identify any relevant publications on maize GA21 x T25.”

The applicant should have applied a broader range of research and also taken other glyphosate and/or glufosinate-resistant transgenic plants into account. Furthermore, at least in regard to environmental risks, it is also necessary to review literature which might indicate indirect, delayed and cumulative long-term risks, or interactions with other genetically engineered plants which might occur from spillage and further crossings. Therefore, the literature research should have taken into account potential persistence, spread and crossings with other transgenic plants which enter the environment via spillage along transport routes etc. In this context, the biological characteristics of potential offspring are also relevant for the application. Any literature research should include all relevant publications concerning the crop species and its relatives. The environmental risk assessment should, moreover, take indirect, unintended, delayed and long-term cumulative effects of animal excretions into account. Therefore, literature research should include all genetically engineered plants which may be mixed into the diet and thus cause environmental hazards.

In addition, in regard to food and feed safety, interactions with other genetically engineered plants which might be mixed with the event in diets also need to be considered. Implementing Regulation 503/2013 (point 3.2.3) requests that “the applicant shall evaluate the data generated to estimate possible short-term and long-term risks to human or animal health associated with the consumption of genetically modified food or feed with respect to the expression of new proteins/metabolites, as well as significantly altered levels of original plant proteins/metabolites.” Apparently, this legal request is not limited to the specific event. It comprises risk assessment of mixed diets in equivalence to risk assessment of stacked events, since the risks are also equivalent. A much more comprehensive literature review is, therefore, needed in order to consider potential interactions with other regulated GMOs.

2. Molecular characterisation

According to EFSA, updated bioinformatic analyses of the newly created open reading frames (ORFs) within the inserts or spanning the junctions between the insert and the flanking regions,

indicate for events GA21 and T25 that the production of a new peptide showing significant similarities to toxins or allergens for any of the events in maize GA21 x T25 is highly unlikely.

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 unintentionally produced ncRNA (non-coding RNA) from additional open reading frames, were not assessed. Thus, uncertainties remain about other biologically active substances resulting from the method of genetic engineering and the newly introduced gene constructs.

Gene expression under environmental stress conditions

Environmental stress can cause unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015). 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.

Data on herbicide application rates and their impact on gene expression

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 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 ignored in risk assessment.

Protein levels of mEPSPS and PAT were analysed in material harvested from field trials across three locations in the US during the 2012 growing season. However, from the data available to the public, there is no information on herbicide dosages. Thus, data essential for an independent assessment is missing.

Nevertheless, EFSA is of the opinion that the design of the field trials is in accordance with the expected agricultural practices. To justify this opinion, EFSA should have presented a more detailed reasoning.

Current EFSA practices are such that it is not possible to access the original data submitted by the companies within the period of consultation. Therefore, the opinion has to provide all the data necessary to allow other experts to conclude on whether the provisions of GMO regulation are fulfilled.

In light of the information available, we assume that the application and the data provided do not sufficiently represent the agricultural practices, which could include the use of single herbicide applications, high dosages and repeated spraying.

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, Linares et al., 2023). However, it seems that the data on gene expression were confined

to a single variety. Therefore, EFSA should have also requested additional data from transgenic maize varieties, e. g. those 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 of the genetic backgrounds on gene expression, as requested in EU Regulation 503/2013.

Antibiotic resistance gene

Event T25 contains parts of the bla antibiotic resistance gene. This antibiotic resistance gene inactivates critically important penicillins. According to bioinformatic analyses, it seems that is not expressed so no imminent risks may be associated with it. However, as criticised by several member state competent authorities, the use of ABR gene is outdated and should be discontinued in the EU. However, EFSA refuses to discuss the matter by repeatedly stating:

"With this application the GMO Panel assesses the GA21xT25 stack maize. The single maize event GA21 has already been assessed in other applications…" (EFSA, 2023b)

As criticised by member states experts, the applicant disregards EU Commission Implementing Regulation (EU) No 503/2013 stating that "the applicant shall endeavour to minimise the presence of inserted nucleic acid(s) sequences not essential to achieve the desired trait".

3. Comparative assessment of plant composition as well as 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 Syngenta 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 extreme weather conditions were taken into account (such as drought); (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 for one year (composition) and two years (agronomic

and phenotypic characteristics), respectively. Testbiotech welcomes the step of multi-annual field trials.

Some extreme weather conditions were reported from the field trials. These, however, remain arbitrary and not well defined, and do not allow any conclusions to be drawn on the effects of more severe climate stress due to drought, watering or high temperatures.

In order to assess changes in gene expression, the plants should have been grown in various environmental conditions and exposed to well-defined environmental stress conditions. This requirement is especially relevant in this case, as it is known that the additional epsps gene may show pleiotropic effects, thus affecting seed dormancy, growth and stress responses of the plants (see, for example, Fang et al., 2018; Wang et al., 2014; Yang et al., 2017; Beres et al., 2018, Beres, 2019).

Further, conventional varieties with a different CRM than the transgenic/isogenic variety were used by the applicant. Therefore, the interpretation of field trial data is compromised and new, valid field trials in the correct setting should be conducted.

It should not be overlooked that, for example, Brazil is among the most important countries for maize imports into the EU: Brazil is a major producer of genetically engineered maize and is one of the largest exporters of maize to the EU (Commission Committee for the Common Organisation of Agricultural Markets, 2021). Therefore, field trails should be conducted in countries such as Brazil.

Nevertheless, EFSA is of the opinion that the design of the field trials is in accordance with the expected agricultural practices. To justify this opinion, EFSA should have provided a much more detailed reasoning. 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 on whether the provisions of GMO regulation are fulfilled. In light of the information available, we assume that the application and the data provided do not sufficiently represent the agricultural practices and bio-regional conditions under which these plants are likely to be grown.

No experiments were requested showing to which extent specific environmental conditions influence plant composition and agronomic characteristics. Hence, no data were made available, as requested in Implementing regulation 503/2013, to assess 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

Due to the mode of action of the active ingredients in the complementary herbicides, it is plausible that complementary herbicide applications will cause stress responses in the plants, and thus impact gene expression and plant composition. These effects may vary with the amount of herbicide sprayed onto the crop and the various active ingredients which can be used. For example, glufosinate was shown to affect the expression levels of several genes in transgenic Arabidopsis plants (Abdeen & Miki, 2009). Glyphosate may influence plant metabolism by altering gene expression (Faus et al. 2015).

From the information available, it appears that the complementary herbicides were only applied once (post-emergent, during the growth stage of the plants) and not in combination.

There is no information regarding the dosage of the complementary herbicide.

EFSA is of the opinion that the design of the field trials is in accordance with the expected agricultural practices. To justify this opinion, EFSA should have provided a much more detailed reasoning. 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 necessary data to allow other experts to conclude on whether the provisions of GMO regulation are fulfilled. In light of the information available, we assume that the application and the data provided do not sufficiently represent the agricultural practices, such as higher dosages and repeated spraying.

EFSA should have requested the applicant to submit data from field trials on all the relevant active ingredients used in agricultural practice, including all dosages and combinations of the complementary herbicides which might be used in agricultural practice in GE maize producing countries. Without these data, no reliable conclusions can be drawn, as requested in Implementing Regulation 503/2013 (in particular for herbicide tolerant GE plants), to assess whether anticipated agricultural practices influence the expression of the studied endpoints (see also Miyazaki et al., 2019).

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 plants, as requested in EU Regulation 503/2013.

Agronomic and phenotypic characteristics

According to EFSA, statistical analysis was applied to 8 endpoints:

• For maize GA21 x T25 (treated with conventional herbicides), the test of difference identified statistically significant differences with its conventional counterpart for early stand count and final stand count. The endpoint ‘early stand count’ fell under equivalence category III, while final stand count fell under category I.

• For maize GA21 9 T25 (treated with intended herbicides), the test of difference identified a statistically significant difference for days to 50% pollen shed, which fell under equivalence category I.

Unfortunately, no information regarding herbicide dosages is given.

It is known that the genomic background of the varieties can influence both the expression of the inserted genes and plant metabolism. 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 of the genetic backgrounds on gene expression, as requested in EU Regulation 503/2013.

Data from compositional analysis show the need for further investigations

Data for the compositional analysis was taken from field trials conducted in the US for one year. 65 constituents were subjected to statistical analysis (9 in forage and 56 in grain).

• For maize GA21 x T25 not treated with the intended herbicides, statistically significant differences with its conventional counterpart were found for 12 endpoints in grain. All these endpoints fell under equivalence category I or II.

• For maize GA21 x T25 treated with the intended herbicides, statistically significant differences with its conventional counterpart were found for 14 endpoints in grain. All these endpoints fell under equivalence category I or II.

Given the above reasoning on the impact of environmental factors, herbicide applications and genetic backgrounds, as well as a number of other 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. This requirement is especially relevant in this case since it is known that the additional epsps genes may show pleiotropic effects, which also affect seed dormancy, growth and stress responses of the plants (see, for example, Fang et al., 2018; Wang et al., 2014; Yang et al., 2017; Beres et al., 2018, Beres, 2019).

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

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 in order to draw sufficiently robust conclusions to inform the next steps in risk assessment.

We further 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 factors, herbicide applications or 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 all relevant genetic backgrounds.

However, only samples from field sites located in the US were used to generate the data, and the impact of environmental factors and agricultural practices were not assessed in detail. Herbicide applications in the field trials did not represent all the relevant agricultural practices. Only one transgenic variety was grown in the field trials.

Consequently, the data presented by the applicant and accepted by EFSA are insufficient to conclude on the impact of environmental factors, herbicide applications or different genetic backgrounds on plant composition and agronomic characteristics.

Based on the available data, no final conclusions can be drawn on the safety of the plants. Therefore, the data neither fulfill the requirements of Implementing Regulation 503/2013 nor Regulation 1829/2003. This is also underlined in several statements made by experts from Member States (EFSA, 2023b).

In summary, the GE maize plants tested in the field trials do not sufficiently represent the products intended for import.

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;”

Findings from the molecular characterisation and comparative approach

As explained above, many significant changes in plant composition were identified. Even if the changes taken as isolated data might not directly raise safety concerns, the overall number of effects should have been considered as a starting point for much more detailed investigation into their potential health impacts.

However, the data presented by the applicant did not take into account cultivation of the maize under more extreme weather conditions, i. e. neither under realistic agricultural conditions nor

considering all relevant countries of cultivation. Further, the possible effects of high pesticide dosages and repeated sprayings were not taken into account. The range of differences and their significance are likely to be substantially increased in these conditions. Thus, without more data, the true range of unintended effects cannot be determined and safety cannot be demonstrated, as requested by EU regulation.

Findings from a 90-day feeding study

GA21 x T25 maize is a stacked event and the parental plants were previously assessed by EFSA, therefore a subchronic study was not requested. Nonetheless, the applicant provided such a study. The study is mentioned in the EFSA opinion, but no findings are reported. However, according to member state experts, several significant effects were found in the high-dose group, but not assessed by EFSA. Testbiotech is of the opinion that these findings should have been reason enough for a proper assessment of possible toxic effects. Instead, EFSA is hiding behind word-for-word fidelity in regard to its own guidance.

Effects of residues from spraying with complementary herbicide specific to GE plants and their mixed toxicity

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 pesticide regulation and GMO regulation both 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 a prerequisite for granting authorisation.

For example, glufosinate was shown to metabolise in a way that may lead to increased health risks for animals and consumers (Bremmer and Leist 1997).

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 for mixed toxicity (EFSA, 2019).

Glufosinate has been shown to impact or disturb the microbiome (Dong et al., 2020), which can have a substantial impact on the long-term toxicity (mixed toxicity) of whole food and feed derived from the maize. In addition, glufosinate is classified as showing reproductive toxicity (http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN) and there are indications of additive or synergistic effects of the residues from spraying (Reuter, 2015).

This is especially relevant in regard to combinatorial (accumulated) effects caused by the residues from spraying with glyphosate, which is known to cause shifts in the microbial composition and associated microbiomes of plants and animals. Glyphosate has been shown to

cause shifts not only in soil organisms (van Bruggen et al., 2018, 2021, Chávez-Ortiz et al., 2022) and rhizosphere microbiome (Cesco et al., 2021) but also in the composition of the intestinal flora of humans (Mesnage et al., 2021a), cattle (Reuter et al., 2007), poultry (Shehata et al., 2013; Ruuskanen et al., 2020), amphibians (Boccioni et al., 2021), earthworms (Owagboriaye et al., 2021) and rodents (Hu et al., 2021; Liu et al., 2022; Mao et al., 2018; Mesnage et al., 2021b, 2021c; Tang et al., 2020) as well as honey bees (Motta et al., 2020) and daphnia (Suppa et al., 2020). Therefore, antibiotic effects caused by chronic exposure to food and feed derived from glyphosate-resistant GE plants, including this GE maize, are not unlikely to trigger significant changes in intestinal bacteria (see also Testbiotech, 2021).

In general, the microbiome can be seen as a common network of life, encompassing and closely interacting with plants, animals and humans. Microbial networks are thought to have co-evolved with their hosts and have developed a mutualistic relationship that benefits both the host and microorganisms. They act at the interphase and communicate between the organisms and their wider environment while at the same time being part of an organism's closer environment. Microbiomes are considered to be vital for the health of higher organisms, i. e. humans, animals and plants.

Therefore, potential adverse effects resulting from the exposure to whole food and feed need to be tested for mixed toxicity (EFSA, 2019). This should also be considered in regard to changes in the intestinal microbiome. The described effects, which may enhance the uptake of DNA from the transgenic plants by gut bacteria, are not considered under pesticide regulation, they have to be assessed within GMO risk assessment. The reason: these effects are highly dependent on the specific dosages applied onto the GE plants, as well as on their metabolism and the resulting pattern of exposure in food and feed. In addition, cumulative effects (mixtures of GE plants in one diet) may play a decisive role. Under Directive 2001/18/EC, such effects could be considered to be indirect effects which may be immediate, delayed or cumulative. Implementing Regulation 503/2013 (point 1.4.2) requires "testing of new constituents other than proteins". In our opinion, this requirement also includes the assessment of residues from the complementary herbicides, which necessarily become constituents of all genetically engineered plants resistant to them.

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."

These findings are highly relevant to the risk assessment of the GE maize, which inherits combinations of herbicide resistance to glyphosate and glufosinate. These residues may cause gut microbiome perturbation, depending on exposure and combinatorial effects. It has to be considered a plausible hypothesis that the effects on the microbiome can trigger effects on the immune system, food uptake and body weight. This hypothesis and mixed toxicity need to be tested before any conclusion can be drawn on the health safety of food and feed. Since no such data can be derived from pesticide risk assessment, experimental data on mixed toxicity of the maize have to be requested from the applicant.

In general, antibiotic effects and other adverse health effects might occur from exposure to diets 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. Further attention should be paid to the specific toxicity of the metabolites of the pesticide active ingredients.

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. Therefore, potential adverse effects that result from combinatorial exposure of various potential stressors need specification, and their assessment needs to be prioritised. We conclude that the health risk assessment currently performed by EFSA for the maize is unacceptable. We propose testing these plants following the whole mixture approach, considering them to be "insufficiently chemically defined to apply a component-based approach" (EFSA, 2019).

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

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

Allergenicity

The EFSA assessment of allergenic risks (EFSA, 2022a) is not based on a sufficiently realistic exposure to newly introduced proteins and their interactions. Different routes of exposure, the timing of exposure, microbial exposure, oral and gut microbiota composition, epithelial barrier integrity and/or non-allergenic components of the food matrix, such as immune-modulating

components (adjuvants) of allergenic sources that facilitate immune responses, should have all been considered, but were not.

Therefore, the outcome of the allergenicity assessment cannot be regarded to be sufficient.

5. Environmental risk assessment

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

The EFSA (2022b) opinion 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 (Bauer-Panskus et al., 2020). This issue is relevant for gene flow from maize to teosinte and vice versa.

However, even in the updated risk assessment, EFSA still does not consider that epsps genes as such may confer fitness advantages (as noted, for example, by Fang et al., 2018; Wang et al., 2014; Yang et al., 2017). The updated teosinte risk assessment is therefore too narrow to conclude on possible environmental effects and provides no answers to relevant risk related questions.

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 maize.

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

Testbiotech is aware of a recent statement made by EFSA (2022b) 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."

However, even in the updated risk assessment, EFSA still does not consider that epsps genes as such may confer fitness advantages (as noted, for example, by Fang et al., 2018; Wang et al., 2014; Yang et al., 2017). The updated teosinte risk assessment is therefore too narrow to conclude on possible environmental effects and provides no answers to relevant risk related questions.

6. Others

As far as monitoring and methods to identify the specific event are concerned, 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 were 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 maize GA21 x T25 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 on 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 simply 'rubber stamping' all applications for the import of GE plants, and reduce the overall number of products entering the market, while ensuring that these products undergo much more thorough risk assessment.

References

Abdeen, A., Miki, B, (2009) The pleiotropic effects of the bar gene and glufosinate on the Arabidopsis transcriptome. Plant Biotechnol J 7(3): 266-282. https://doi.org/10.1111/j.1467-7652.2008.00398.x

Bauer-Panskus, A., Miyazaki, J., Kawall, K., Then, C. (2020) Risk assessment of genetically engineered plants that can persist and propagate in the environment. Environmental Sciences Europe, 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 in Food Science & Technology, 120: 325-337. https://doi.org/10.1016/j.tifs.2022.01.002

Beres, Z.T. (2019) Ecological and evolutionary implications of glyphosate resistance in Conyza canadensis and Arabidopsis thaliana. Dissertation presented in partial fulfillment of the requirements for the degree Doctor of Philosophy in the graduate school of the Ohio State University. http://rave.ohiolink.edu/etdc/view?acc_num=osu1555600547328876

Beres, Z.T., Yang, X., Jin, L., Zhao, W., Mackey, D.M., Snow, A.A. (2018) Overexpression of a native gene encoding 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) may enhance fecundity in Arabidopsis thaliana in the absence of glyphosate. Int J Plant Sci, 179(5): 390-401.

https://doi.org/10.1086/696701

Boccioni, A.P.P.C., García-Effron, G., Peltzer, P.M., Lajmanovich, R.C. (2021) The effect of glyphosate and ciprofloxacin exposure on the gut bacterial microbiota diversity of Rhinella Arenarum (Anura: Bufonidae) tadpoles. [Preprint]. https://www.researchsquare.com/article/rs-492368/v1

Cesco, S., Lucini, L., Miras-Moreno, B., Borruso, L., Mimmo, T., Pii, Y., ... & Trevisan, M. (2021) The hidden effects of agrochemicals on plant metabolism and root-associated microorganisms. Plant Science, 311: 111012. https://doi.org/10.1016/j.plantsci.2021.111012

Chávez-Ortiz, P., Tapia-Torres, Y., Larsen, J., & García-Oliva, F. (2022) Glyphosate-based herbicides alter soil carbon and phosphorus dynamics and microbial activity. Applied Soil Ecology, 169: 104256. https://doi.org/10.1016/j.apsoil.2021.104256

Christ, B., Hochstrasser, R., Guyer, L., Francisco, R., Aubry, S., Hörtensteiner, S., & Weng, J. K. (2017). Non-specific activities of the major herbicide-resistance gene BAR. Nature Plants, 3(12): 937-945. https://doi.org/10.1038/s41477-017-0061-1

Commission Committee for the Common Organisation of Agricultural Markets (2021) EU Cereals Trade 2021/22, Marketing Year July – September, 26 November 2021). https://circabc.europa.eu/sd/a/a1135630-e8e9-4531-a522-23670f75e2c5/cereals-trade-2017-18-marketing-year-july-december.pdf

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. Journal of Hazardous Materials, 389: 122152. https://doi.org/10.1016/j.jhazmat.2020.122152

EFSA (2015) Risk assessment of new sequencing data of GM maize event GA21. EFSA J 13(11): 4296. https://doi.org/10.2903/j.efsa.2015.4296

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

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 (2022a) Scientific Opinion on development needs for the allergenicity and protein safety assessment of food and feed products derived from biotechnology. EFSA J, 20(1): 7044. https://doi.org/10.2903/j.efsa.2022.7044

EFSA (2022b) 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 (2023a) Scientific Opinion on the assessment of genetically modified maize GA21 x T25 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2016-137). EFSA J 21(1): 7729. https://doi.org/10.2903/j.efsa.2023.7729

EFSA (2023b) Comments and opinions submitted by Member States during the three-month consultation period. OpenEFSA portal. https://open.efsa.europa.eu/

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. Front Plant Sci, 9: 233. https://doi.org/10.3389/fpls.2018.00233

Faus, I., Zabalza, A., Santiago, J., Nebauer, S.G., Royuela, M., Serrano, R., Gadea, J. (2015) Protein kinase GCN2 mediates responses to glyphosate in Arabidopsis. BMC Plant Biol, 15(1): 1-12. https://doi.org/10.1186/s12870-014-0378-0

Hu, J., Lesseur, C., Miao, Y., Manservisi, F., Panzacchi, S., Mandrioli, D., ... & Petrick, L. (2021) Low-dose exposure of glyphosate-based herbicides disrupt the urine metabolome and its interaction with gut microbiota. Scientific Reports, 11(1): 1-10. https://doi.org/10.1038/s41598-021-82552-2

Liu, J. B., Chen, K., Li, Z. F., Wang, Z. Y., Wang, L. (2022) Glyphosate-induced gut microbiota dysbiosis facilitates male reproductive toxicity in rats. Science of The Total Environment, 805: 150368. https://doi.org/10.1016/j.scitotenv.2021.150368

Liao, H., Li, X., Yang, Q., Bai, Y., Cui, P., Wen, C., ... & Zhu, Y. G. (2021) Herbicide selection promotes antibiotic resistance in soil microbiomes. Molecular Biology and Evolution, 38(6), 2337-2350. https://doi.org/10.1093/molbev/msab029

Linares, J.F., Coles, N.D., Mo, H., Habben, J.E., Humbert, S., Messina, C., ... & Brummer, E. . (2022) Transgene by germplasm interactions can impact transgene evaluation. Crop Science. https://doi.org/10.1002/csc2.20939

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

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

Mesnage, R., Calatayud, M., Duysburgh, C., Marzorati, M., Antoniou, M. (2021a) Alterations in human gut microbiome composition and metabolism after exposure to glyphosate and Roundup and/or a spore-based formulation using the SHIME® technology. BioRxiv [Preprint]. https://www.biorxiv.org/content/10.1101/2021.12.16.472928v1

Mesnage, R., Panzacchi, S., Bourne, E., Mein, C.A., Perry, M.J., Hu, J., ... & Antoniou, M.N. (2021b) Glyphosate and its formulations Roundup Bioflow and RangerPro alter bacterial and fungal community composition in the rat caecum microbiome. BioRxiv [Preprint]. https://www.biorxiv.org/content/10.1101/2021.11.19.468976v1

Mesnage, R., Teixeira, M., Mandrioli, D., Falcioni, L., Ducarmon, Q.R., Zwittink, R.D., ... & Antoniou, M.N. (2021c) Use of shotgun metagenomics and metabolomics to evaluate the impact of glyphosate or Roundup MON 52276 on the gut microbiota and serum metabolome of Sprague-Dawley rats. Environmental Health Perspectives, 129(1): 017005. https://doi.org/10.1289/EHP6990

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. Environmental Sciences Europe, 31(1): 1-21. https://doi.org/10.1186/s12302-019-0274-1

Motta, E. V., Mak, M., De Jong, T. K., Powell, J. E., O'Donnell, A., Suhr, K. J., ... & Moran, N. A. (2020). Oral or topical exposure to glyphosate in herbicide formulation impacts the gut microbiota and survival rates of honey bees. AEM, 86(18): e01150-20. https://doi.org/10.1128/AEM.01150-20

Owagboriaye, F., Mesnage, R., Dedeke, G., Adegboyega, T., Aladesida, A., Adeleke, M., ... & Antoniou, M.N. (2021) Impacts of a glyphosate-based herbicide on the gut microbiome of three earthworm species (Alma millsoni, Eudrilus eugeniae and Libyodrilus violaceus): A pilot study. Toxicol Rep, 8: 753-758. https://doi.org/10.1016/j.toxrep.2021.03.021

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. Environmental Sciences Europe, 28(1):28-30. https://doi.org/10.1186/s12302-016-0098-1

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

Reuter, W. (2015) Toxicology of glyphosate, isoxaflutole, dicamba and possible combination effects. Available via Testbiotech. https://www.testbiotech.org/content/toxicology-glyphosate-isoxaflutole-dicamba-and-possible-combination-effects

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

Shimada, N., Kim, Y.S., Miyamoto, K., Yoshioka, M., Murata, H. (2003) Effects of Bacillus thuringiensis Cry1Ab toxin on mammalian cells. J Vet Med Sci, 65(2): 187-191. https://doi.org/10.1292/jvms.65.187

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://www.nature.com/articles/s41598-017-01478-w

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

Van Bruggen, A.H.C., Finckh, M.R., He, M., Ritsema, C.J., Harkes, P., Knuth, D., Geissen, V. (2021) Indirect effects of the herbicide glyphosate on plant, animal and human health through its effects on microbial communities. Front Environ Sci, 9: 763917. https://doi.org/10.3389/fenvs.2021.763917

Wang, W., Xia, H., Yang, X., Xu, T., Si, H.J., Cai, X.X., Wang, F., Su, J., Snow, A.A., Lu, B.-R. (2014) A novel 5-enolpyruvoylshikimate-3-phosphate (EPSP) synthase transgene for glyphosate resistance stimulates growth and fecundity in weedy rice (Oryza sativa) without herbicide. New Phytol, 202(2): 679-688. https://doi.org/10.1111/nph.12428

Yang, X., Li, L., Jiang, X., Wang, W., Cai, X., Su, J., Wang, F., Lu, B.-R. (2017) Genetically engineered rice endogenous 5-enolpyruvoylshikimate-3-phosphate synthase (epsps) transgene alters phenology and fitness of crop-wild hybrid offspring. Sci Rep, 7(1): 1-12. https://doi.org/10.1038/s41598-017-07089-9