Maize NK603 x T25 x DAS-40278-9

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

Comments:

We read:

“Post-market monitoring of food/feed is not considered necessary. The GMO Panel concludes that the three-event stack maize and its subcombinations are as safe as the non-GM comparator and the selected non-GM reference varieties with respect to potential effects on human and animal health and the environment.”

Assessment of genetically modified maize NK603 × T25 × DAS-40278-9 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2019-164) - - 2021 - EFSA Journal - Wiley Online Library

We do not agree with this. Our objections to this genetically modified maize concern the toxicity of the herbicide that will now contaminate the food chain with residues and metabolites, as a result of the genetically modified metabolism of the maize plant. Using the herbicide really does have an impact related to the GMO, in that the herbicide is stored in the plant in the form of a acetylated product that then enters the food chain and is again de-acetylated in the gastrointestinal tract, thus releasing the herbicide again.

Our comment:

The spraying of GM crops such as GM maize with herbicides and/or insecticides which are highly toxic for the environment, animals, insects and humans must be prohibited once and for all. We have repeatedly cited papers (via previous comments over many years - you can review these on your own website) which confirm such toxicity.

We read:

Summary

“ERM-BF415 was certified to contain the following NK603 concentrations: ERM-BF415a certified value < 0.4 g GM / kg maize; ERM-BF415b certified value 1.0 ± 0.4 g GM / kg maize;

ERM-BF415c certified value 4.9 ± 0.5 g GM / kg maize; ERM-BF415d certified value 9.8 ± 0.7 g GM / kg maize; ERM-BF415e certified value 19.6 ± 0.9 g GM / kg maize; ERM-BF415f certified value 49.1 ± 1.3 g GM / kg maize. The minimum sample intake recommended is 100 mg.“

“CERTIFICATION REPORT The Certification of Reference Materials of Dry-Mixed Maize Powder with different Mass Fractions of NK603 Maize Certified Reference Materials ERM®-BF415 (ERM®-BF415-a/ERM®-BF415-b/ ERM®-BF415-c/ ERM®-BF415-d/ ERM®-BF415-e/ERM®-BF415-f).”

Our comment:

In light of the above, we have the following question: Is this maize (non-GM and GM together) not labelled as GM in the case of a threshold value of less than 0.9%? If this is true, we consider that in those circumstances it must be made mandatory!
We agree with this statement:

"My concern about genetic engineering is that the absence of unintentional effects can never be guaranteed. It may take dozens of years before these effects reveal themselves, and we should be extremely cautious applying the technology.", Caius Rommens.

We European GM-free Citizens do not want this GM maize on our plates. We want organic farming to become the principal farming method in Europe. No authorisation for this GM crop! These objections are also raised on behalf of Stichting Ekopark in Lelystad, the Netherlands.

Maize NK603 x T25 x DAS-40278-9

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

Comments:

Testbiotech comment on EFSA's assessment of genetically engineered maize NK603 x T25 x DAS-40278-9 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2019-164) from BASF

Introduction

The EFSA GMO panel assessed the stacked maize NK603xT25xDAS-40278-9, which is derived from crossing three genetically engineered maize events (EFSA, 2021a). The parental plants were assessed by EFSA in previous opinions. The maize contains genes conferring resistance to three herbicides: glufosinate, glyphosate, 2,4-dichlorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (AOPP) herbicides:

• NK603 expressing two variants of CP4 EPSPS protein for tolerance to glyphosate-containing herbicides;

• T25 expressing PAT to confer tolerance to glufosinate-ammonium containing herbicides and a large part of a β-lactamase (bla) gene of bacterial origin which provides resistance to antibiotics was inserted, but assumed to show no expression;

• DAS-40278-9 expressing AAD-1 to catalyse the degradation of the general class of herbicides known as aryloxyphenoxypropionates (AOPP) and to confer tolerance to 2,4-D containing herbicides.

Implementing Regulation 503/2013 has been applied in the risk assessment as performed by EFSA.

1. Systematic literature review

A systematic review as referred to in Regulation (EU) No 503/2013 was not provided by the applicant. Based on preliminary information, the GMO Panel agreed that there is only limited value in undertaking a systematic review of maize NK603 x T25 x DAS-40278-9 at present (EFSA, 2021a). This not acceptable. Even if no publication on the stacked maize exists, there is a plenty of relevant material on the parental plants, e.g. Steinberg et al. (2019 and 2020) or Mesnage et al. (2016 and 2017).

2. Molecular characterisation and gene expression

Annex II of Implementing Regulation 503/2013 requests that

• "Protein expression data, including the raw data, obtained from field trials and related to the conditions in which the crop is grown (in regard to the newly expressed proteins)." (Scientific requirements 1.2.2.3)

• “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.” (Scientific requirements 1.3.1)

• “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.” (Scientific requirements 1.3.2.1)

Open reading frames and gene insertion

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, it was assumed that proteins that may emerge from these DNA sequences would raise no safety concerns. Other gene products, such as ncRNAs (non-coding RNA) from additional open reading frames, were not assessed. Thus, uncertainties remain about other biologically active substances arising from the method of genetic engineering and the newly introduced gene constructs (see also Davalos et al., 2019).

Impact of environmental factors, agricultural practice and genetic backgrounds

The data presented by BASF 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 may be cultivated, and extreme weather conditions were taken into account systematically; (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, stress conditions and their impact on gene expression

Data was only presented from field trials carried out at six sites in the US and Canada for just one year. Environmental stress can cause unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015; Benevenuto et al., 2021). However, the data from the field trials do not allow conclusions to be drawn on how gene expression will, for example, be affected by climate stress due to drought, watering or high temperatures. Therefore, to assess gene expression, the plants should have been grown in different environmental conditions and exposed to defined environmental stress conditions. 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).

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 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 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.

Furthermore, the data presented raise some questions. It appears that the original data showed significant differences but were subsequently corrected by introducing additional factors, such as the efficiency of the method to extract the material (see EFSA 2021b). This might have been correct and necessary. However, the data presented in the opinion are now surprisingly homogeneous. We strongly recommend establishing a system with independent controls to repeat the trials and double check the data.

The need for more a detailed and independent assessment is also backed by a comparison of data for the expression of CP4 EPSPS in maize NK603 in various stacked events such as MON89034 x 1507 x NK603 x DAS-40278-9; NK603 x T25; 59122 x NK603; MON89034 x NK603; MON89034 x 1507 x NK603; 1507 x MON810 x MIR162 x NK603 and 1507 x 59122 x MON810 x NK603. These data (see also tabled overview) show a wide range of means (dry weight) especially in

• pollen: 187-460 µg/g;

• leaf (V2-V4): 162 – 310 µg/g

• leaf (R1): 98-220 µg/g

• root (R1): 40-93 µg/g

• forage: 53-120 µg/g

• grain: 8-14 µg/g.

It is not clear whether this wider range of expression, which in many cases exceeds 100%, is caused by errors in measuring, stacking, agronomical practices, environmental factors or varietal backgrounds. To the best of our knowledge, such patterns of large variations in gene expression have not been found in other events (such as DAS-40278-9). Whatever the case, the EPSPS production in maize is clearly complex, with two genes contributing to the overall concentration of the protein.

These observations show that experiments under defined stress conditions, e.g. in a growth chamber, are needed. This is especially relevant in the light of the findings of Fang et al. (2018), Wang et al. (2014), Yang et al. (2017), Beres et al. (2018) and Beres (2019), which show that the level of EPSPS expression can impact a wide range of biological characteristics of plants.

In conclusion, the GE maize plants tested in field trials may not sufficiently represent the products intended for import. The data presented by the applicant are insufficient to conclude on the impact of environmental factors or stress conditions on gene expression as requested by EU Regulation 503/2013.

Data on herbicide application rates and their impact on gene expression

From the available information, it looks like the complementary herbicides were only applied in combination, and only sprayed once. Nevertheless, EFSA is of the opinion that the design of the field trials is in accordance with the expected agricultural practices (see EFSA, 2021b). To justify this opinion, EFSA should have presented more detailed reasoning. Furthermore, data for both the treated and untreated plants should be presented in the Annex.

Due to current EFSA practices, 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 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, higher dosages and repeated spraying.

Therefore, EFSA should have requested the applicant to submit data from field trials that included all the relevant agricultural practices, all active ingredients, all dosages and all combinations of the complementary herbicides that might be used in agricultural practice of the GE maize producing countries. Without these data, no reliable conclusion 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 plant as requested 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, it appears that the data on gene expression were confined to a single variety. Therefore, EFSA should also have requested additional data from transgenic maize varieties, e.g. those cultivated in South America.

However, EFSA has not taken 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.

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 BASF 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 only conducted in the US and Canada (each site only for one year). A smaller number of field trials (eight sites in the US) were used to assess plant composition compared to the number of field trials to assess agronomic and phenotypic characteristics (11 sites in the US and one site in Canada). 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 how gene expression will be affected by more severe climate stress due to drought, watering or high temperatures. In order to assess changes 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, since it is known that the additional epsps genes may show pleiotropic effects, also 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).

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).

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 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.

However, no experiments were requested to show 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.

From the available information, it looks like that the complementary herbicides were only applied in combination, with only one post-emergent (during the growth of the plants) spraying. 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 necessary data to allow other experts to conclude 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, i.e. single herbicide use, 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 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). However, it appears that the data on gene expression were confined to a single variety. Therefore, EFSA should also have requested additional data from transgenic maize varieties that are, for example, cultivated in South America.

However, EFSA has not taken 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

72 constituents were subjected to statistical analysis (10 in forage and 62 in grain). For those plants which were not treated with the complementary herbicides, statistically significant differences were found for 27 endpoints (four in forage and 23 in grains); for plants sprayed with the complementary herbicides, 33 endpoints (five in forage and 28 in grains) were found to be significantly different. EFSA concludes that these differences are not of biological relevance (equivalence category I and II).

Given the above reasoning on the impact of environmental factors, herbicide applications and genetic backgrounds as well as a higher number of significant findings in fields treated with the complementary herbicides, 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).

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

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 (see literature review, EFSA 2021a), 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 factors, 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 all relevant genetic backgrounds.

However, only samples from field sites located in the US (and one in Canada) 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 by several statements made by experts from Member States (EFSA, 2021b).

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;"

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.

Both, EU pesticide regulation and GMO regulation, require a high level of protection for health and the environment. Thus, in regard to herbicide-resistant plants, specific assessment of residues from spraying with complementary herbicides must be considered a prerequisite for granting authorisation.

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, 2019b).

All three herbicides, 2,4-D, glufosinate and glyphosate, have been shown to impact or disturb the microbiome, which can have substantial impact on the long-term toxicity (mixed toxicity) of whole food and feed derived from the stacked event. Dong et al. (2020) show that glufosinate can severely impact the microbiome; Tu et al. (2019) provide evidence on the adverse effects of 2,4-D. 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. human, animal and plants.

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 for the risk assessment of the GE maize, which inherits combinations of herbicide resistance to glyphosate, glufosinate and 2,4-D. 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 the 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 stacked maize have to be requested from the applicant.

In general, antibiotic effects and other adverse health effects might occur from exposure to a diet containing these plants that were not assessed under pesticide regulation. These adverse effects on health might be triggered by the residues from spraying with the complementary herbicide (see also van Bruggen et al., 2018). Further attention should be paid to the specific toxicity of the metabolites of the pesticide active ingredients that might occur specifically in the stacked event.

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 stacked maize is unacceptable. We propose testing these plants following the whole mixture approach, considering them to be "insufficiently chemically defined to apply a component-based approach" (EFSA, 2019).

Despite all these open questions regarding potential health impacts, we are not aware of a single sub-chronic or chronic feeding study performed with whole food and feed derived from the stacked maize. This observation is supported by the literature review carried out by the company which did not yield any peer reviewed publication. In this context, it is relevant to consider that the outcome of the feeding studies with the parental plants raised several questions concerning their results, methodology and reliability (see comments from the experts of Member States, EFSA, 2021b)

Testbiotech is also aware that the findings from feeding studies with NK603 of (Steinberg et al., 2019; Steinberg et al., 2020; Mesnage et al., 2017; Seralini et al., 2014) show the need for further in-depth analysis. For example, Steinberg et al. (2019 and 2020) found a correlation between higher uptake of NK603 in rats was associated with higher mortality. They they try to explain these findings, however, they can not remove uncertainty in regard to adverse effects. The corrected version of these findings (Steinberg et al., 2020) which is similar to the original publication (Steinberg et al., 2019) reads:

"In weeks 52–78, the male rats fed the NK603 + Roundup diet at an inclusion rate of 33% showed a higher mean body weight and feed consumption when compared to the corresponding control group, while the mean body weight and feed consumption of the female rats were similar in all five experimental groups (Figs. 5 and 6)".

"The increased mortality observed between the 12th and 24th month of the feeding trial in male rats fed the 33% NK603 + Roundup diet coincided with an increase in the body weight and feed consumption".

These effects which were related to a specific diet, a specific period of time and male animals can, for example, be caused or fostered by a disturbance in the microbiome of the animals. Whatever the case, these should have been mentioned and discussed by the applicant and EFSA. It should be taken into account that these effects seem to escape the duration of a subchronic feeding study (90 days).

In conclusion, the EFSA opinion on the application for authorisation of the stacked maize (EFSA, 2021a) cannot be said to fulfil the requirements for assessment of potential synergistic or antagonistic effects resulting from the combination of the transformation events in regard to toxicology.

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

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

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 from Palaudelmàs et al. (2009) in Spain or from Pascher (2016) in Austria. Further, in awareness of the biological characteristics of the GE maize and the findings of Fang et al. (2018), the stacked maize needs to be examined in detail regarding next generation effects, volunteer potential (persistence) and gene flow. Under these circumstances, even a rare single outcrossing that goes unnoticed can have a huge long-term impact on the agro-ecosystems.

Furthermore, in the EFSA (2021a) opinion is also wrong for several reasons:

• Without more data on the teosinte species growing in the EU, the likelihood of gene flow from the maize to teosinte cannot be assessed (Trtikova et al., 2017). The same is true for gene flow from teosinte to genetically engineered plants.

• Furthermore, the characteristics of potential hybrids and next generations have to be investigated and cannot be predicted simply from the data of the original event. It is well known that there can be next generation effects and interference from genetic background that cannot be predicted from the assessment of the original event (Bauer-Panskus et al., 2020). This issue is relevant for gene flow from maize to as well from teosinte to maize.

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

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

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

Others

For monitoring and methods to identify the specific event, Implementing Regulation 503/2013 requests:

The method(s) shall be specific to the transformation event (hereafter referred to as 'event-specific') and thus shall only be functional with the genetically modified organism or genetically modified based product considered and shall not be functional if applied to other transformation events already authorised; otherwise the method cannot be applied for unequivocal detection/identification/quantification. This shall be demonstrated with a selection of non-target transgenic authorised transformation events and conventional counterparts. This testing shall include closely related transformation events.

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

If approval for import is given, the applicant has to ensure that post-market monitoring (PMM) is developed to collect reliable information on the detection of indications showing whether any (adverse) effects on health may be related to GM food or feed consumption. Thus, the monitoring report should at very least contain detailed information on: i) actual volumes of the GE products imported into the EU, ii) the ports and silos where shipments of the GE products were unloaded, iii) the processing plants where the GE products was transferred to, iv) the amount of the GE products used on farms for feed, and v) transport routes of the GE products. Environmental monitoring should be run in regions where viable material of the GE products such as kernels are transported, stored, packaged, processed or used for food/feed. In case of losses and spread of viable material (such as kernels) all receiving environments need to be monitored. Furthermore, environmental exposure through organic waste material, by-products, sewage or faeces containing GE products during or after the production process, and during or after human or animal consumption should be part of the monitoring procedure (see also comments from Member States experts, EFSA, 2021b).

We agree with comments made by experts from Member States (EFSA, 2021b) that monitoring requires more improvements, such as those suggested by German authority, BVL:

"The monitoring plan does not relate the monitoring activities to relevant protection goals. Even more it is not described which routine observations (including parameters or monitoring characters) are carried out in relation to the protection goals. Only reporting on 'any unanticipated effect' is solely not an appropriate parameter, because it already anticipates an evaluation. This evaluation process should be based on a distinct set of parameters and a scientific sound data analysis. It is requested that the applicant specifies in detail, how and which information will be pro-actively queried, gathered, and how they will be evaluated. In addition, it might be useful to integrate information about the use of the product in food and feed to deliver supplementary helpful data (of exposure to consumers and animals) for general surveillance. Therefore, the applicant should specify monitoring activities in the field of human and animal health. He should describe in

detail how animal and human health surveillance is integrated in the monitoring plan."

In addition, the example of the stacked 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) The Commission should not allow the import of GE crops which inherit DNA from bacteria which can trigger resistance to antibiotics (see comments from Experts of Member States, EFSA 2021b)

(3) 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. Environmental Sciences Europe, 32(1): 1-15. https://doi.org/10.1186/s12302-020-00301-0

Benevenuto, R.F., Zanatta, C.B., Guerra, M.P., Nodari, R.O., Agapito-Tenfen, S.Z. (2021) Proteomic profile of glyphosate-resistant soybean under combined herbicide and drought stress conditions. Plants, 10(11): 2381. https://doi.org/10.3390/plants10112381

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

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

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

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

EFSA (2021a) Scientific Opinion on the Assessment of genetically modified maize NK603 × T25 × DAS-40278-9 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2019-164). EFSA Journal, 19(12): 6942. https://doi.org/10.2903/j.efsa.2021.6942

EFSA (2021b) Application , Comments and opinions submitted by Member States during the three-month consultation period. OpenEFSA portal. https://open.efsa.europa.eu/questions/EFSA-Q-2019-00808

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

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

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., Agapito-Tenfen, S.Z., Vilperte, V., Renney, G., Ward, M., Séralini, G.E, Nodari, R.O., Antoniou, M.N. (2016) An integrated multi-omics analysis of the NK603 Roundup-tolerant GM maize reveals metabolism disturbances caused by the transformation process. Sci Rep, 6: 37855. https://doi.org/10.1038/srep37855

Mesnage, R., Arno, M., Séralini, G.E., Antoniou, M.N. (2017) Transcriptome and metabolome analysis of liver and kidneys of rats chronically fed NK603 Roundup-tolerant genetically modified maize. Environmental Sciences Europe, 29(1): 1-9. https://doi.org/10.1186/s12302-017-0105-1

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

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. Toxicology Reports, 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 Research, 18(4): 583-594. https://doi.org/10.1007/s11248-009-9250-7

Séralini, G.E., Clair, E., Mesnage, R., Gress, S., Defarge, N., Malatesta, M., ... & de Vendômois, J.S. (2014) Republished study: Long-term toxicity of a Roundup herbicide and a Roundup-tolerantgenetically modified maize. Environmental Sciences Europe, 26(1): 1-17. https://doi.org/10.1186/s12302-014-0014-5

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

Steinberg, P., van der Voet, H., Goedhart, P. W., Kleter, G., Kok, E. J., Pla, M., ... & Wilhelm, R. (2019) Lack of adverse effects in subchronic and chronic toxicity/carcinogenicity studies on the glyphosate-resistant genetically modified maize NK603 in Wistar Han RCC rats. Archives of Toxicology, 93(4): 1095-1139. https://doi.org/10.1007/s00204-019-02400-1

Steinberg, P., van der Voet, H., Goedhart, P. W., Kleter, G., Kok, E. J., Pla, M., ... & Wilhelm, R. (2020). Correction to: Lack of adverse effects in subchronic and chronic toxicity/carcinogenicity studies on the glyphosate-resistant genetically modified maize NK603 in Wistar Han RCC rats. Archives of Toxicology, 94: 1779-1781. https://doi.org/10.1007/s00204-020-02751-0

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., et al. (2015) Transgene expression and Bt protein content in transgenic Bt maize (MON810) under optimal and stressful environmental conditions. PLoS ONE 10(4): e0123011. 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. Scientific Reports, 7:1560. https://www.nature.com/articles/s41598-017-01478-w

Tu, P., Gao, B., Chi, L., Lai, Y., Bian, X., Ru, H., & Lu, K. (2019) Subchronic low-dose 2, 4-D exposure changed plasma acylcarnitine levels and induced gut microbiome perturbations in mice. Scientific reports, 9(1), 1-11. https://www.nature.com/articles/s41598-019-40776-3

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

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. Frontiers in Environmental Science, 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