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Testbiotech comment on ‘Assessment of genetically modified maize MZHG0JG for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2016-133)’ by company Syngenta

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Introduction

The EFSA GMO Panel assessed maize MZHG0JG, which is resistant to glyphosate and glufosinate. The maize was developed with Agrobacterium tumefaciens-mediated transformation. Compared to the previously assessed GE maize GA21 produced by Syngenta, this maize event (MZHG0JG) has a higher expression rate in the EPSPS enzyme which renders resistance to glyphosate.

1. Molecular characterisation

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; no detailed investigations were carried out in this regard.

Furthermore, other gene products, such as miRNA from additional open reading frames, were not assessed. Thus, uncertainties remain about other biologically active substances arising from the method of genetic engineering and the newly introduced gene constructs.

Environmental stress can also cause unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015). However, the expression of the additional enzymes was only measured under field conditions in the US for one year. According to the data presented, no unusual weather conditions were reported. Therefore, it was not tested to which extent specific environmental conditions, such as those caused by climate change, will influence the overall concentration of the enzymes in the plants. As EFSA states in its answer to the comments from experts from member states (EFSA, 2018b):

> “*It is well documented in the literature that protein levels can vary due to a number of factors including genetic background, environmental conditions and agricultural practices.*”

Nonetheless, 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.

More surprisingly, EFSA and the applicant omitted to assess the maize in regard to its intended purpose. Due to the increased content of EPSPS enzymes that confer resistance to glyphosate, it has to be expected that these plants can and will be exposed to higher and also repeated dosages of glyphosate (see also the comments from experts of member states, EFSA, 2018b).

Higher applications of glyphosate 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. The same aspect is relevant in regard to resistance to glufosinate, which might be applied in higher dosages due to increasing weed pressure. This aspect, which is the most relevant in regard to this specific event, was completely ignored in the risk assessment as performed. Only the so-called 'intended' usage of the complementary herbicide was taken into account, with single sprayings of each of the herbicides. However, the practical conditions under large scale cultivation and increasing weed occurrence were left aside. These missing risk assessment data are the cause of substantial flaws in following risk assessment steps.

EFSA should have requested that Syngenta submit data from field trials with the highest dosage of the complementary herbicides that can be tolerated by the plants, also including repeated spraying. The material derived from those plants should have been assessed by using Omics techniques to investigate changes in the gene activity of the transgene, as well as the natural genome of the plants.

2. Comparative analysis (for compositional analysis and agronomic traits and GM phenotype)
Field trials for compositional and agronomic assessment of maize MZHG0JG were conducted in the US during one year only (2013) and not in any other relevant maize production areas, such Brazil or Argentina.

Regarding agronomic parameters, only eight agronomic/phenotypic endpoints were submitted to statistical analysis, two of them in each group (with and without the application of the complementary herbicide) were considered to be significantly different, with one of these differences falling in equivalence category IV.

Compositional data revealed many statistically significant differences in regard to 66 constituents that were assessed:

- Statistically significant differences between the GE maize (not treated) and the non-GE comparator were identified for 29 endpoints. One was described as being in equivalence category III / IV.
- Statistically significant differences between the GE maize (treated) and the non-GE comparator were identified for 34 endpoints. One was described as being in equivalence category IV.

It has to be assumed that this event is essentially different from its comparator in regard to many compositions and biological characteristics. Even if changes taken as isolated data might not directly raise safety concerns, the overall number of effects and their strong significance has to be taken as a starting point for much more detailed investigations. It is not acceptable that EFSA failed to require further studies e.g.

- No data from Omics (proteomics, transcriptomics, metabolomics) were used to assist the compositional analysis and the assessment of the phenotypical changes.
- More powerful statistical analysis, such as multidimensional analysis, was not applied to the data.
- No field trials were conducted that lasted more than one season. Thus, based on current data, site-specific effects can hardly be assessed.
- Further, no data were generated representing more extreme environmental conditions, such as those caused by climate change. Although no application has been filed for cultivation, data on the interaction between the plants and the environment have to be considered as one of the starting points in risk assessment of the plants, and must be made available and

assessed in detail.
- In addition, more varieties carrying the transgenes should have been included in the field trials to see how the gene constructs interact with the genetic background of the plants.

As mentioned, EFSA and the applicant omitted to assess the GE maize in regard to its intended purpose. Due to the increased content of EPSPS enzymes that confer resistance to glyphosate, it has to be expected that these plants can and will be exposed to higher and also repeated dosages of glyphosate (see also the comments from experts of member states, EFSA, 2018b). Higher applications of glyphosate 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. The same aspect is relevant in regard to resistance to glufosinate, which might be applied in higher dosages due to increasing weed pressure.

This aspect, which is the most relevant in regard to this specific event, was completely ignored in the risk assessment as performed. Only the so-called 'intended' usage of the complementary herbicide was taken into account, with single sprayings of each of the herbicides. However, the practical conditions under large scale cultivation and increasing use of the complementary herbicides were left aside. These missing data are the cause of substantial flaws in following risk assessment steps.

EFSA should have requested that Syngenta submit data from field trials with the highest dosage of the complementary herbicides that can be tolerated by the plants, also including repeated spraying. The material derived from those plants should have been assessed by using Omics techniques to investigate changes in the plants composition or agronomic characteristics

Furthermore, while the GMO panel considers the assessment of the toxicity of the residues from spraying to be outside its remit, it is the duty of the GMO panel to consider and assess the specific metabolism in the plants and the specific metabolites that might occur in the plants after application of the complementary herbicides. These residues might show a specific pattern or accumulation that only occurs in this specific event. The pesticide panel can only assess the toxicity of these metabolites, if the GMO panel request specific data on metabolism and metabolites, also considering the various formulas, mixtures and combination of the complementary herbicides. So even if it is the case that the pesticide panel only has to assess the toxicity of these metabolites, it is the duty of the GMO panel to request these specific data that are needed to conclude on the safety of these plants.

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

Toxicology
We agree with EFSA that the data as presented from the 90-day feeding study do not seem to indicate adverse health effects. However, the reliability of the data is questionable:

- The diets should have been composed in way that the results from 10% and 41.5% diets can be compared.
- The stability of the test and control materials was not verified in this study.
- The material used in the diet seems to be largely different from food and feed that will result from the harvest of commercial large scale cultivation under practical conditions.

EFSA should not accept data from feeding trials that are impacted by these basic uncertainties. Furthermore, chronic feeding studies, including assessing next generation effects, were not

conducted.

There are further relevant issues: For example, the potential impact on the intestinal microbiome also has to be considered. Such effects might be caused by the residues from spraying since glyphosate has been shown to have negative effects on the composition of the intestinal flora of cattle (Reuter et al., 2007) and poultry (Shehata et al., 2013). Further, Bremmer and Leist (1997) examined the possible conversion of NAG to glufosinate in rats. Up to 10% deacetylation occurred at a low dose of 3 mg/kg bw as shown by the occurrence of glufosinate in the faeces. The authors concluded, however, that most of the conversion was caused by bacteria in the colon and rectum, although toxicity findings indicate partial bioavailability (Bremmer & Leist, 1997). In general, antibiotic effects and other adverse health effects might occur from exposure to a diet containing these plants; these were not assessed under pesticide regulation.

Further attention should be paid to the specific toxicity of the metabolites of the pesticide active ingredients that might occur specifically in the GE event. For example, glufosinate is classified in the EU as showing reproductive toxicity.[1] But there were no detailed investigations into the metabolites arising from spraying glufosinate onto these plants; these metabolites might also differ from those of the parental plants.

Nonetheless, both the EU pesticide regulation and the GMO regulation require a high level of protection for health and the environment. Thus, in regard to herbicide-resistant plants, specific assessment of residues from spraying with complementary herbicides must be considered to be a prerequisite for granting authorisation. In addition, cumulative effects have to be investigated if a plant contains or produces other compounds that are potentially toxic.

In addition, cumulative effects have to be investigated if a plant contains or produces other compounds that are potentially toxic. It should be acknowledged, that no new methodology is needed to assess the health risks emerging from the combinatorial application of the herbicides and their potential interaction with the other plant constituents. Suitable methodology to assess combinatorial effects that emerge from *simultaneous exposure* to a *fixed combination* of potential stressors via a *defined route of exposure* (as it is the case with food and feed products derived from genetically engineered plants that are resistant to several herbicides) is available and widely used. For example, chronic feeding or multigenerational studies are a well-established method to generate the relevant data.

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

Allergenicity
No data were presented to show that plant composition is unchanged in regard to allergenic potential.

There might be various reasons why the allergenic potential in the MZHG0JG event is increased: higher applications of glyphosate will not only cause a higher burden of residues in the harvest, they may also change the composition of the plants in regard to naturally occurring allergens. No data were presented to assess such potential effects.

Consequently, the assessment in regard to allergenicity cannot be regarded as conclusive.

[1] http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN

Others
According to Regulation (EU) No 503/2013, 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 maize imported into the EU,
ii) the ports and silos where shipments of the GE maize were unloaded,
iii) the processing plants where the GE maize was transferred to,
iv) the amount of the maize used on farms for feed, and
v) transport routes of the GE maize.

Environmental monitoring should be run in regions where viable kernels of the GE maize are transported, stored, packaged, processed or used for food/feed. In case of losses and spread of the GE maize, all receiving environments need to be monitored.

Furthermore, environmental exposure through organic waste material, by-products, sewage or faeces containing MZHG0JG maize during or after the production process, and during or after human or animal consumption should be part of the monitoring procedure (see also EFSA, 2018b).

Environmental risk assessment
Any spillage from the kernels has to be monitored closely. EFSA completely overlooked that populations of teosinte are abundant in Spain and France; these have to be considered to be wild relatives that enable gene flow and potential spread of the transgenes throughout the fields and the environment (Trtikova et al., 2017). 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 GE maize.

Further, as shown by Pascher (2016), EFSA has also underestimated the risks posed by occurrence of volunteers from maize plants.

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

Conclusions and recommendations
The EFSA risk assessment should not be accepted.

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