

TESTBIOTECH Background 4 - 12 - 2019

Testbiotech comment on ‘Statement complementing the EFSA Scientific Opinion on application (EFSA-GMO-UK-2006-34) for authorisation of food and feed containing, consisting of and produced from genetically modified maize 3272’ by company Syngenta

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Introduction

The EFSA GMO Panel assessed maize 3272, which produces an artificial enzyme belonging the group of alpha-amylase. This enzyme (thermos-tolerant alpha-amylase AMY797E) is supposed to have a positive effect on the processing of the maize kernels at high temperatures, especially when intended for use in producing agro-fuels. Maize 3272 was developed for use in the dry-grind fuel ethanol process whereby the starch contained in cereal grains is hydrolysed into glucose, which is subsequently converted to ethanol by fermentation. The final product is used as the substrate for yeast (*Saccharomyces cerevisiae*) fermentation to produce ethanol. The AMY797E protein is chimeric encoded by gene segments derived from three parental alpha-amylase genes originating from strains of the archeal order *Thermococcales*, including marine organisms of unclear taxonomy.

Further, the maize produces the protein PMI protein (phosphomannose isomerase) derived from *Escherichia coli*. Expression of PMI enables transformed maize cells to utilise mannose and therefore to survive on specific media used for selecting the maize plants after the process of genetic engineering (so-called marker gene).

The integration of the additional DNA was performed by using *Agrobacterium tumefaciens*.

According to EFSA (2013a), the 3272 maize event is intended for cultivation and use in the dry-grind fuel ethanol process outside the EU. However, it cannot be excluded that the crop originally intended for industrial use could inadvertently enter the food and feed chain. In addition, by-products of the dry-grind ethanol process produced from maize and other cereal are widely used as feed (e.g. distillers’ dried grains with solubles).

The scope of the application is for food and feed uses, import and processing of maize 3272 and all derived products.

The EFSA opinion was published in 2013. However, it was found to be non-conclusive due to missing data. In 2017, the EU Commission asked EFSA to finalise the risk assessment, and the statement complementing the opinion was published in 2019 (EFSA, 2019).

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. 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 cause unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015). The data presented in the original dossier assessed by EFSA (2013a), show a high amount of AMY797E is produced in the kernels with a wide range of concentration, from 1004 – 3365 µg/g dw (EFSA, 2013b). Only a very low number of individual maize plants were analysed, which do not allow a solid statistical analyses (EFSA, 2013b). No further expression data were presented in the statement for complementation of the opinion (EFSA, 2019), even though new field trials were performed by Syngenta.

From the data presented, it cannot be concluded to which extent specific environmental conditions, such as those caused by climate change, will influence the overall concentration of the enzymes in the plants.

In regard to expression of the additionally inserted genes, Implementing Regulation 503/2013 requests “*Protein expression data, including the raw data, obtained from field trials and related to the conditions in which the crop is grown*”.

However, the few data presented between 2003 and 2007 (EFSA, 2013a) do not represent the conditions in which the plants would be grown, since no extreme weather conditions were taken into account.

While in the previous assessment (EFSA, 2013a), the old EFSA guidance was applied, there is no excuse why EFSA (2019), after being requested by the EU Commission in 2017, did not use Implementing Regulation 503/2013, which has to be applied to all applications filed after December 2013.

Whatever the case, 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, taking into account more extreme drought conditions. In addition, they should have been tested in the maize producing countries in South America. EFSA should also have requested data from several varieties, including those cultivated in South America. Furthermore, data from the parental plants need to be presented.

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, as well as changes in metabolic pathways and the emergence of unintended biologically active gene products. Such in-depth investigations should not depend on findings indicating potential adverse effects, they should always be necessary to come to sufficiently robust conclusions to inform the next steps in risk assessment.

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

For the assessment published by EFSA (2019), new field trials for compositional and agronomic assessment of maize 3272 were conducted in the US during one year only (2014) and not in any other relevant maize production areas, such as Brazil or Argentina.

The statistical analysis presented (EFSA, 2019) showed several significant differences between the conventional comparator and maize 3272, some of these were in the highest category.

No data were presented to assess the overall fitness of the plants (for example, seed dormancy, germination rate and survivability at higher or lower temperatures).

Overall, it is not plausible that the data as presented are sufficient to assess the real biological characteristics of the plants, since the production of the two enzymes in the kernels (one of them in high amounts) is likely to change more than one metabolic pathway in the plants.

Under these circumstances, it is not acceptable that EFSA failed to require further studies even though:

- No data from omics (proteomics, transcriptomics, metabolomics) were used to assist the compositional analysis and the assessment of the phenotypical changes.
- No field trials were conducted for the final assessment 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.
- No data were generated that represent the growing conditions in other relevant maize growing regions outside the US.

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.

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

Toxicology

As mentioned, the original opinion of EFSA was published in 2013. However, it was found to be non-conclusive due to missing data. In 2017, the EU Commission asked EFSA to finalise the risk assessment. The statement complementing the opinion was published in 2019 (EFSA, 2019).

While in the previous assessment (EFSA, 2013a), the old EFSA guidance was applied, there is no excuse why EFSA (2019), after being requested by the EU Commission in 2017, did not apply Implementing Regulation 503/2013, which has to be applied to all applications filed after December 2013.

At the same time, EFSA did not request a 90-day feeding study as requested by Implementing Regulation 503/2013 for the complementation of its assessment.

According to EFSA (2019) the presence of the thermo-tolerant AMY797E protein in maize 3272 might result in processed food (e.g. ready-to-eat-cereals) and feed (e.g. canned pet food or by-products of the wet-milling) being different from that produced from conventional maize. Under certain processing conditions (e.g. temperature, moisture, pH), the AMY797E protein might cause the hydrolysis of maize starch into dextrins, maltose and other oligosaccharides, changing the composition and texture of the processed commodities as compared to those produced from

conventional maize. Therefore, more specific data, for example, including feeding studies with ruminants, would have been necessary.

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

Allergenicity

As experts from Member States (EFSA, 2013b) as well as EFSA (2013a and 2019) point out, allergenicity is a highly relevant topic for the assessment of maize 3272. It is well known that alpha amylase can trigger allergic reactions, especially via the respiratory system.

We support the conclusion from EFSA that in this regard, safety could not be demonstrated by the applicant and substantial uncertainties remain regarding the allergenicity of the newly introduced protein for which safe use is not reported:

“The GMO Panel notes that previous concerns on the potential capacity for de novo sensitisation of the AMY797E still remain not completely addressed. An aspect for concern is the potential of this protein to sensitise and provoke respiratory disorders in humans. Furthermore, elicitation of allergic reactions upon oral ingestion of maize 3272 in potentially sensitised individuals to the AMY797E protein is unlikely to occur but it cannot be excluded. In the case of animals, the available literature on allergy to alpha-amylases is more limited.”

Therefore, the opinion of EFSA remains inconclusive and the application for market authorisation for import, covering all uses for food and feed, cannot be approved.

Others

Being aware of the specific genetic changes in maize 3272 i.e. establishing unprecedented metabolic pathways in the plants and producing artificial proteins with any record of safe use, EFSA, after being requested by the Commission in 2017, should have requested a full new dossier, taking into account the criteria of Implementing Regulation 503/2013 and also requesting much more specific data to demonstrate safety for health and the environment.

Environmental risk assessment

Any spillage from the kernels has to be monitored closely. EFSA and Syngenta 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. Finally, the actual ability of maize 3272 to persist and propagate in the environment after spillage was not assessed, for example, data on dormancy and survivability are missing.

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

Conclusions and recommendations

The EFSA risk assessment is still not conclusive. No approval for import can be issued.

References

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