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Testbiotech comment on "Scientific Opinion on application (EFSA-GMO-DE-2011-95) for the placing on the market of genetically modified maize 5307 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Crop Protection AG"

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Introduction

Maize 5307 produces a novel insecticidal protein classified as eCry3.1Ab, which is meant to kill corn rootworm effectively. The toxin is derived from a fusion and rearrangement of toxins that naturally occur in soil bacteria, known as *Bacillus thuringiensis*. As publications show, the synthetic toxin produced in all parts of the plants, including the kernels, is meant to work in a new way. However, not all the details of its mode of action are understood.

EFSA did not finalise their risk assessment of maize 5307 in 2015 (EFSA, 2015). Instead, the GMO panel stated that they could not conclude on the toxicity of the new protein because a 28-day feeding trial was not performed in accordance with required scientific standards. In 2018, an additional assessment was published after Syngenta provided further data; EFSA thereupon concluded their risk assessment with a positive opinion (EFSA, 2018).

1. Molecular characterisation

Maize 5307 was genetically engineered with the help of *Agrobacterium tumefaciens*. It expresses the chimeric eCry3.1Ab toxin (based on fusion and rearrangement of Cry3A from *Bacillus thuringiensis* subsp. *tenebrionis* and the Cry1Ab from *B. thuringiensis* subsp. *kurstaki* strain HD-1. While Cry1Ab is understood to be toxic to *lepidoptera* (a taxon which comprises insects such as butterflies) the fused protein toxic is understood to be toxic in *coleoptera* (a taxon which comprises beetles). Specifically, the toxin is meant to kill the larvae of the corn rootworm, which is a plant pest living in the soil in some maize growing areas. The exact mode of action of the new toxin is not known, however, it is likely to be effective via a new mode of action (Walters et al., 2010).

Furthermore, the plants contain a promotor to confer high activity to the inserted gene (derived from *Cestrum Yellow Leaf Curling Virus*) and an enzyme known as phosphomannose isomerase (PMI), which can be used for the selection of the transformants. It is used to replace the antibiotic resistance genes that are used in other cases. The PMI is biologically active and involved in carbohydrate metabolism.

Several open reading frames were identified; some of which could also give rise to new proteins with unknown functions and with some similarities to known allergens. However, EFSA regarded the likelihood of these proteins being produced as low.

EFSA did not request empirical investigations into whether these proteins were in fact produced or into any biological activity they might show. In addition, EFSA did not consider the emergence of any other gene products, such as miRNA, which might be transferred at the stage of consumption and, for example, interact with the intestinal microbiome. Neither did EFSA request any omics data to investigate unintended changes in plant metabolism.

On the expression of the intended new proteins PMI and the eCry3.1Ab toxin, EFSA accepted data that were only based on low number of samples, and they did not ask for data on expression rates in further genetic backgrounds or under specific environmental conditions.

For example, environmental stress can also cause unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015). Therefore, it is unclear to which extent specific environmental conditions will influence the overall concentration of the enzymes in the plants. The plants should have been subjected to a much broader range of defined environmental conditions and stressors in order to gather reliable data on gene expression and functional genetic stability.

Further, the method used to determine the amount of Bt toxins (ELISA) is known to be dependent on the specific protocols used. It is possible that data might be insufficiently reliable without further evaluations by independent labs. For example, Shu et al. (2018) highlight difficulties in measuring the correct concentration of Bt toxins produced by the genetically engineered plants (see also Székács et al., 2011). Without fully evaluated test methods to measure the concentration of the Bt toxins, risk assessment for food and the environment will suffer from substantial methodological gaps.

Consequently, substantial uncertainties remain concerning the quality and quantity of biologically active substances arising from the method of genetic engineering and from the newly introduced gene constructs. The data as provided and assessed by EFSA do not allow any conclusions to be drawn on the safety of the products derived from these plants if used for food and feed.

2. Comparative analysis (for compositional analysis and agronomic traits and GM phenotype)
Field trials for the assessment of agronomic and phenotypical characteristics were only conducted in the US and not in other relevant maize growing areas, such Brazil or Argentina. Compositional analysis included data from Argentina. Nevertheless, we welcome the fact that the trials were conducted over two years and not just for one growing period.

However, the design of the field trials is questionable: The line used for comparison is not the one which was used for genetic engineering. The additional reference varieties were not grown within the plots of the specific field trial, but separately from those and only for one year. The specific environmental conditions (soil, climate, biotic and abiotic stressors) were not described.

Some differences were noted in maize 5307 in comparison to its conventional counterpart (i.e. higher ‘heat units to 50 % pollen shed’, higher grain moisture and higher plant height in the 2007 field trials; higher grain yield in the 2008 field trials). The EFSA GMO panel concluded that *“none of the differences identified in the composition, agronomic and phenotypic characteristics of grain and forage obtained from maize 5307 required further assessment regarding food and feed safety.”*

However, even if changes taken as isolated data might not directly raise safety concerns, these effects should have led to further investigations. The need for further investigation is underlined by the fact that specific data assessing the role of the PMI enzyme revealed significant changes in the

content of several carbohydrates in the plants; these were set aside without any explanation of the causes.

Therefore, EFSA should have requested further studies e.g.

- data from omics (proteomics, transcriptomics, metabolomics)
- data representing more extreme environmental conditions such as those caused by climate change.
- 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

Whole food and feed was not tested for safety and there was no 90-day feeding trial. Syngenta only provided a nutritional study on poultry, which was rejected by EFSA due to methodological flaws.

The 28-day feeding study was repeated at the request of EFSA, however, only the isolated protein was tested. The data provided and assessed by EFSA left out the most relevant hypotheses known for potential impact on human and animal health:

- Firstly, Bt toxins are known to be immunogenic. They seem to act as allergens and adjuvant effects are likely to occur. In regard to immunogenicity (non-IgE-mediated immune adverse reactions), it is generally acknowledged that Bt toxins are immunogenic (Rubio-Infante & Moreno-Fierros, 2016; Adel-Patient et.al., 2011; Andreassen et.al., 2015a,b; Andreassen et.al., 2016; see also Then & Bauer-Panskus, 2017). Thus, there are some substantial reasons for concern that reactions to allergens can be enhanced. This is relevant since in food/feed the Bt toxins can be mixed with allergens from soybeans amongst others. It is inexplicable and unacceptable that adjuvant effects were only discussed in the case of the PMI, but not in the case of the Bt toxin.
- Secondly, the toxicity of Bt toxins can be enhanced through interactions with other compounds such as plant enzymes (Zhang et al., 2000, Zhu et al., 2007; Pardo-López et al., 2009), other Bt toxins (Sharma et al., 2004; Tabashnik et al., 2013; Bøhn et al. 2016, Bøhn 2018), gut bacteria (Broderick et al., 2009), residues from spraying with herbicides (Bøhn et al. 2016, Bøhn 2018) and others (Kramarz et al., 2007; Kramarz et al., 2009; Khalique and Ahmed, 2005; Singh et al., 2007; Zhu et al., 2005; Mason et al., 2011; Reardon et al., 2004). Thus, testing the Bt toxin alone and in isolated form does not allow any conclusion to be drawn on its real health impacts after consumption.
- Thirdly, the applicant identified significant similarities between the amino acid sequence of eCry3.1Ab and parasporin proteins. According to EFSA, parasporal proteins can show cytotoxic activity on mammalian cells. However, the parasporal characteristics of the synthetic toxin were not investigated.

In this context, it is very relevant that Bt toxins can survive digestion to a much higher degree than has been assumed by EFSA: Chowdhury et al., (2003) as well as Walsh et al. (2011) have found that when pigs were fed with Bt maize, Cry1A proteins could frequently and successfully still be found in the colon of pigs at the end of the digestion process. Thus, Cry1A proteins can show much higher stability, at least in monogastric species, than predicted by current in vitro digestion experiments. This means that Bt toxins are not degraded quickly in the gut and can persist in larger amounts until digestion is completed, and that there is enough time for interaction between various food compounds.

Given all the remaining uncertainties from the molecular analysis, including the mode of action of the Bt toxin, the content of the Bt content in the harvest and the compositional analysis, there should have been feeding studies with the whole food accompanied by data from the application of omics and in-vitro studies on combinatorial effects. Furthermore, detailed investigations into the immunogenic properties of the chimeric Bt toxins are indispensable.

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

Allergenicity
See comments on toxicological assessment. Furthermore, uncertainties remain regarding the real allergenic potential of the new proteins produced by the plants.

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

Others
The newly synthesised Bt toxin should be fully tested according pesticide regulation before any decision is taken on market authorisation for maize 5307.

Environmental risk assessment
Any spillage from the kernels must be closely monitored. EFSA has 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).

In this regard, the opinion of EFSA (2015) is extensively flawed since the authority refers to completely outdated literature on the occurrence of wild relatives in Europe, claiming that populations of sexually compatible indigenous wild relatives of maize are not known in Europe and therefore, vertical gene transfer should not be considered an environmental issue in the EU. However, since 2009, teosinte, a wild relative of maize, is known to occur in Spain. There are further reports from France on its occurrence that might encompass other regions in the EU (Trtikova et al., 2017).

Thus, 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.

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

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

Conclusions and recommendations
The EFSA risk assessment for health risks cannot be accepted.

The environmental risk assessment is based on false assumptions.

The monitoring plan must be rejected because it does not allow control of spillage, gene flow or

what happens to Bt toxins in the environment. Neither is it suitable for the identification of potential health effects.

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