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# Testbiotech comment on EFSA Scientific Opinion on an application by DOW AgroSciences LLC (EFSA-GMO-NL-2010-89) for placing on the market the genetically modified herbicide-tolerant maize DAS-40278-9 for food and feed uses, import and processing under Regulation (EC) No 1829/2003

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## Introduction

Maize DAS-40278-9 is genetically engineered to confer resistance to 2,4-dichlorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (AOPP) herbicides. The maize is part of a biotech industry strategy to introduce more and more herbicide resistances into crop plants in order to combat herbicide-resistant weeds that are particularly problematic in the US.

## 1. Molecular characterisation

Gene products such as miRNA from additional open reading frames were not assessed. Thus uncertainties remain about other biologically active substances emerging from the method of genetic engineering.

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. 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 environmental conditions to gather reliable data on gene expression and functional genetic stability.

In addition, more varieties should have been included into the field trials since it is known that the genetic background of the varieties can influence the level of gene expression (see Trtikova et al., 2015).

Further, all parts of the plants should be taken into account for risk assessment. Expression data have to be considered as one of the starting points in risk assessment of the plant, so the assessment of the data cannot be reduced to those parts of the plants entering the food chain.

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

There are major gaps in the assessment of the comparative assessment:

- Many of the observed significant changes were set aside without further more detailed and targeted investigations (EFSA, 2016a).

- 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.
- Experts from Member States were unable to access the raw data during the period in which comments were allowed (see EFSA 2016b), this meant that they could not carry out their own analyses.
- 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 plant, and must be made available and assessed in detail. However, EFSA (2016a) stated that: “Considering the scope of the application, interactions with the biotic and abiotic environment were not considered an issue.”
- In addition, more varieties should have been included into the field trials to see how the gene constructs interact with the genetic background of the plants.

Based on the available data, it has to be assumed that the plants differ in their composition in comparison to their conventional comparator.

**Toxicology**

There are several gaps in the risk assessment:

- Despite major uncertainties remaining from comparative assessment and molecular analysis, no testing of the whole plant (feeding study) was requested.
- No long-term or accumulated effects were considered; the impact on reproductive systems was not discussed.
- The animal studies made available by the applicant should not have been accepted due to methodological flaws (see EFSA 2016b).

Beyond that, 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 the specific agricultural practices that go along with the cultivation of these herbicide resistant plants, there are, for example, specific patterns of applications, exposure, occurrence of specific metabolites and emergence of combinatorial effects that require special attention.

Herbicide-resistant plants are meant to survive the application of the complementary herbicide while most other plants will die after short time. Thus, for example, residues of glyphosate, its metabolites and additives to the formulated product might accumulate and interact in the plants. As the publication by Kleter et al. (2011) shows, using herbicides to spray genetically engineered herbicide-resistant plants does indeed lead to patterns of residues and exposure that need to be assessed in detail. According to a reasoned legal opinion drawn up by Kraemer (2012), residues from spraying with complementary herbicides have to be taken into account in the risk assessment of genetically engineered plants from a regulatory point of view.

More detailed assessment is also in accordance with pesticide regulation, which requires specific

risk assessment of imported plants if the usage of pesticides is different in the exporting countries compared to the one in the EU: Recital 26 of Regulation 396/2005 requires maximum residue levels (MRLs) are set for food and feed produced outside the Community if produced by different agricultural practices as regards the use of plant protection products. Article 14 of Regulation 396/2005 requires that the presence of pesticide residues arising from sources other than current plant protection uses and their known cumulative and synergistic effects are determined. Further, Article 29 of Regulation 1107/2009 states that active substances and synergists have to be approved, and the maximum residue levels for each specific agricultural product have to be determined.

In any case, 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 with potential toxicity.

Regarding metabolisation of 2,4-D in maize DAS-40278-9, a recent study by Dow scientists states negligible risk from residues (Zhou et al., 2016). However, as this is the only peer-reviewed paper on this topic, there's a complete lack of industry independent data.

From scientific literature (not acknowledged by EFSA) it is known that metabolisation in crops tolerant towards 2,4-D may lead to the production of the compound 2,4-DCP. According to a review by Lurquin (2016), 2,4-DCP may cause negative metabolic and genotoxic effects, and, like 2,4-D, is listed as “a possible carcinogen based on inadequate evidence in humans and limited evidence in experimental animals” by IARC. Therefore, much more detailed data on toxicity should have been requested by EFSA.

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

**Allergenicity**
No data were presented to show that plant composition in regard to allergenic components is unchanged. Further, it should be considered that residues from spraying with the complementary herbicides can lead to enhanced immune system reactions due to plant ingredients. It is known that toxicants applied together with allergens, can have adjuvant effects, triggering a stronger immune system response to the proteins.

Consequently, the assessment of the impact on the immune system cannot be regarded as conclusive.

**Others**
Monitoring should be case specific. Exact data on the exposure to the maize should be made available. Possible health impacts have to be monitored in detail. Controls regarding residues from spraying with the complementary herbicides have to be established. Accumulated effects that might stem from admixtures of other genetically engineered plants have to be taken into account in the monitoring plan.

**Environmental risk assessment**
EFSA (2016a) risk assessment is extensively flawed since the authority refers to completely outdated literature on the occurrence of wild relatives in Europe: “Populations of sexually compatible indigenous wild relatives of maize are not known in Europe (Eastham and Sweet, 2002;

OECD, 2003), therefore vertical gene transfer is not considered to be 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 about its occurrence that might encompass further regions in the EU. Further, as shown by Pascher (2016), the EFSA is underestimating the risks posed by occurrence of volunteers.

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

**Conclusions and recommendations**
EFSA risk assessment should not be accepted. It does not identify knowledge gaps and uncertainties and fails to assess toxicity, impact on the immune system and reproductive system. The environmental risk assessment is based on wrong assumptions. The monitoring plan has to be rejected because it will not make the necessary data available.

**References:**
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