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Testbiotech comment on the Scientific Opinion on an application (EFSA-GMO-NL-2010-80) for the placing on the market of herbicide-tolerant genetically modified maize NK603 × T25 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto

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Introduction

Maize NK603 × T25 was developed by Monsanto to confer tolerance to glyphosate and glufosinate-ammonium herbicides. The stacked event was created by crossing maize NK603 (glyphosate tolerant) with maize T25 (glufosinate tolerant). The application concerns import and use in food and feed.

Molecular characterisation

As EFSA states, the applicant recently provided new information regarding so-called open reading frames in maize NK603 that might lead to the expression of unintended gene products. Apparently these data provided new information that has been overlooked thus far. While previously no similarity to known allergens were detected, EFSA now states:

> *Identity of over 35 % was found with ragweed (Ambrosia artemisifolia) homologues of the Art v 1 allergen for an ORF within the NK603 insert. The putative translation product of this ORF would be generated from the reverse strand of the CP4 epsps transcriptional units.*

Since ragweed is known to be highly allergenic, EFSA should have requested empirical data if such proteins are present in the plants, and not only base its opinion on a theoretical assumption about the likelihood of expression.

The emergence of new variations, combinations and concentrations of unintended small, biological active RNA molecules such as microRNA was neither assessed in the parental plants nor in the stacked events. Small biologically active RNA molecules can be passed from the plant to humans or animals at the consumption stage. Potential biological effects will depend on similarities between the cell regulation in mammals and plants (see, for example, Zhang et al., 2011; Lukasik & Zielenkiewicz, 2014). These molecules are likely to emerge as unintended side products at the insertion sites of the additional DNA, and can show specific interactions in the stacked event. Their concentration, structure and potential biological effects should be assessed before any conclusion is drawn upon safety of the plants.

Both the expression of the enzymes that confer herbicide resistance and the concentration of small biologically active RNA molecules should have been tested under a wide range of defined environmental conditions, taking into account stressful conditions that, for example, emerge under ongoing climate change. It is known that under stress conditions, genetically engineered plants can show reactions that are not obvious under normal agricultural conditions and can be very different

from those of plants stemming from conventional breeding. For example, environmental stress can cause unexpected patterns of expression of the newly introduced DNA (Trtikova et al., 2015).

Comparative analysis (for compositional analysis and agronomic traits and the phenotype)
The field trials showed significant differences in several compounds. For example, in cross-site analysis, statistically significant differences were identified for 11 compositional endpoints, two in forage and nine in grain. In grain, two of the significant differences (palmitoleic acid and raffinose) were outside the range of the values for commercial varieties planted in the same field trial.

With regard to possible fitness advantages or persistence of maize NK603 x T25 in the environment, there are data gaps in the phenotypic assessment as values for seed germination, dormancy and pollen viability were omitted by the applicant.

Further, the assessment of the data shows major gaps and flaws:

- Material used for compositional analysis was treated with complementary herbicides, EFSA did not asses any data from non-treated plant material.
- Data used for agronomical and phenotypical analysis stems from plants that were not treated with the complementary herbicide. EFSA did not assess data from plants treated with the complementary herbicides.

As a result, a large part of relevant data is missing. Further, no production plan was made available, which is useful for assessing the robustness of the field trials.

The number of the field trials was low, the range of environmental conditions and stress factors very narrow. So no conclusion can be drawn about agronomic and phenotypical characteristics and plant composition under real field conditions as, for example, those that occur due to ongoing climate change.

EFSA mostly ignored criticism of data quality made by experts of several Member States. In the “Comments and opinions submitted by Member States during the three-month consultation period”, EFSA responded to the Member States' criticism by stating:

> *“It is correct that the application only contains compositional information on forage and grain of maize NK603 × T25 sprayed with the target herbicides glyphosate and glufosinate ammonium and not on maize NK603 × T25 not sprayed with those target herbicides. The Panel notes that the agronomic and phenotypic characteristics of maize NK603 × T25 not sprayed with target herbicides is equivalent to that of the conventional counterpart.”*

and further:

> *“The EFSA GMO Panel agreed that the optimal set of data would include information on both maize NK603 × T25 sprayed with target herbicides and maize NK603 × T25 not sprayed with target herbicides.”*

We disagree with EFSA on this assumption. It is not an optimal set of data which is missing, it is the minimum set that should have been requested according to the request from experts of Member States and EFSA guidance.

Toxicology
No feeding study to assess potential health effects was provided. This is especially relevant here since a combination of two herbicides, glufosinate and glyphosate, will be applied to genetically engineered maize in the field.

- According to the International Agency for Research on Cancer (IARC), a body of the World Health Organisation (WHO), glyphosate can be regarded as having carcinogenic potential (IARC 2015).

- Glufosinate is regarded as potentially damaging to health (EFSA, 2005). According to the German Agricultural Ministry, glufosinate will be phased out in the EU in 2017 for reasons of reproductive toxicity (BMELV, 2009).

EFSA has not requested any data on the combinatorial effects of the residues from spraying these two herbicides. The plants will contain residues from both herbicides, neither of them have been tested for specific combined toxicity. Therefore, the residues in combination should have been assessed as relevant plant constituents.

Further, commercially traded herbicide mixtures such as Roundup are considered to be much more toxic than the active ingredient alone (Mesnage et al., 2013). Even though the carcinogenic potential of glyphosate is still under discussion, these two herbicides applied in combination (and as mixtures with further adjuvant ingredients) should trigger very detailed and in-depth risk assessment before any conclusion is drawn upon the safety of the stacked events.

This was also requested by experts from Austria who stated that

> *"negative impacts on human and animal health described in scientific literature [...] have to be evaluated with regard to increased application rates of these herbicides. It must also not be forgotten that unidentified inert ingredients in formulations of glufosinate and glyphosate were shown to enhance the toxicity to human organ systems"*

This case again reveals major systemic flaws in current EFSA risk assessment. EFSA carries out the risk assessment of herbicide resistant, genetically engineered plants without taking into account the specific risks that emerge from the residues from spraying with the complementary herbicides. These risks are only partially assessed as part of EU pesticide regulation. However, if commercially traded herbicides formulas are applied in specific combinations to herbicide resistant plants, there are specific pattern of residues that need to be assessed.

Herbicide resistance in weeds is increasingly becoming a problem in areas where genetically engineered plants are cultivated. In response, several other genetically engineered plants with tolerance to various herbicides have been developed and are pending for market authorisation in the EU, or have already been authorised. This is making it necessary to develop a new systematic approach to deal with new patterns of exposure, interactions between the substances and the accumulated impact on human and animal health.

Monitoring
The applicant should provide methods to distinguish the presence of the stacked events from those of a mixture of the parental plants. Without such a method no surveillance and no monitoring can be performed on the stacked event.

As a legal dossier compiled by Professor Ludwig Kraemer (Kraemer, 2012) shows, EU regulations require the monitoring of effects on health at the stage of consumption in cases where there are uncertainties. Thus, for example, there must be a requirement for the monitoring of health effects that takes residues from spraying with herbicides into account. Epidemiological parameters that are suitable for detecting relevant health effects need to be defined.

Further, any spillage from the kernels has to be closely monitored.

Conclusion
EFSA risk assessment is failing to deal properly with findings from the comparative analysis. The assessment of toxicological effects is inadequate. Risk assessment did not take into account relevant safety issues regarding the usage of the complementary herbicide. Further, no interactions and accumulated effects from the use of such plants in food and feed have been assessed. Consequently, the application has to be rejected.

References

BMELV, Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz (2009) Neue Bewertungskriterien für Wirkstoffe in Pflanzenschutzmitteln [German language only]. http://www.greenpeace.de/fileadmin/gpd/user_upload/themen/umweltgifte/BMELV-Homepage-Liste_der_18_Pestizide.pdf

EFSA (2005) Conclusion regarding the peer review of the pesticide risk assessment of the active substance glufosinate. EFSA Scientific Report 27: 1-81.

IARC (2015) Glyphosate Monograph. http://monographs.iarc.fr/ENG/Monographs/vol112/mono112-02.pdf

Kraemer, L. (2012) The consumption of genetically modified plants and the potential presence of herbicide residues, legal dossier compiled on behalf of Testbiotech, www.testbiotech.de/sites/default/files/Legal_Dossier_Kraemer_Pesticide_RA_PMP.pdf

Lukasik, A, & Zielenkiewicz, P. (2014) In Silico Identification of Plant miRNAs in Mammalian Breast Milk Exosomes – A Small Step Forward? PLoS ONE 9(6): e99963.

Mesnage, R., Defarge, N., Spiroux, D. V. J., & Séralini, G.E. (2013) Major pesticides are more toxic to human cells than their declared active principles. BioMed Research international, 179691.

Then, C. & Bauer-Panskus, A. (2013) Testbiotech comment on the Scientific Opinion of EFSA on application EFSA-GMO-RX-T25 and EFSA-GMO-NL-2007-4 for the placing on the market of herbicide tolerant genetically modified maize T25 from Bayer. http://www.testbiotech.org/node/949

Trtikova, M., Wikmark, O.G., Zemp, N., Widmer, A., Hilbeck, A. (2015) Transgene Expression and Bt Protein Content in Transgenic Bt Maize (MON810) under Optimal and Stressful Environmental Conditions. PloS one, 10(4): e0123011. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0123011

Zhang, L., Hou, D., Chen, X., Li, D., Zhu, L., Zhang, Y., Li, J., Bian, Z., Liang, X., Cai, X., Yin, Y., Wang, C., Zhang, T., Zhu, D., Zhang, D., Xu, J., Chen, Qu., Ba, Y., Liu, J., Wang, Q., Chen, J., Wang, J., Wang, M., Zhang, Q., Zhang, J., Zen, K., Zhang, C.Y. (2011) Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. Cell Research, 22(1): 107-126.