TESTBIOTECH Background 05 - 09 - 2017

Testbiotech e. V.
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Testbiotech comment on 'Scientific Opinion on application EFSA-GMO-BE-2013-117 for authorisation of genetically modified maize MON 87427 × MON 89034 × NK603 and subcombinations independently of their origin, for food and feed uses, import and processing submitted under Regulation (EC) No 1829/2003 by Monsanto Company'

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Introduction

The GMO Panel assessed maize MON 87427 × MON 89034 × NK603 and its three subcombinations. The three single events and the two-event stack maize MON 89034 x NK603 were assessed previously. The maize contains two genes for glyphosate resistance and produces Cry1A.105 and Cry2Ab2 proteins which confer resistance to specific lepidopteran pests.
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. The reason for crossing NK603 with MON 87427 was to increase the content of EPSPS enzymes that confer resistance to glyphosate in the plants.

1. Molecular characterisation

Regarding single event MON87427:
Testbiotech had earlier observed that the process of genetic engineering involved several deletions and insertions in the maize plants. In order to assess whether the sequences encoding the newly expressed proteins or any other open reading frames (ORFs) present within the insert and spanning the junction sites raised any safety issues, it was simply assumed that the proteins that might emerge from these DNA sequences would raise no safety issues; and no detailed investigations were carried out in this regard.

Regarding MON87427xMON89034xNK603:
According to application data cited by Member State experts, relevant information necessary for a comprehensive assessment of the expression of the transgenes was not provided. Data for comparison of protein levels is missing. For example, CP4 EPSPS levels are not assessed for the untreated crop. Further, data for expression of Bt proteins contained in GM maize MON87427xMON89034xNK603 treated with glyphosate is compared with data for untreated maize MON89034.

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.

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

Mostly relevant in this context is that EFSA and the applicant completely omitted to assess the stacked event in regard to its intended purpose. The reason for crossing NK603 with MON 87427 was to increase the content of EPSPS enzymes that confer resistance to glyphosate. Indeed, the expression data reveal a much higher content of these enzymes compared to the single trait. In consequence, it has to be expected that these plants can and will be exposed to higher and also repeated dosages of glyphosate. 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. This aspect, which is the most relevant in regard to this stacked event, was completely ignored by the risk assessment as performed.

EFSA should have requested that Monsanto submit data from field trials with the highest dosage of glyphosate 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.

As a result, further investigations are necessary to assess the combinatorial genomic effects.

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

Regarding agronomic parameters,

- Maize MON 87427 x MON 89034 x NK603 <u>not treated with glyphosate</u> showed a statistically significant increase in ear height, plant height and stalk lodging, and a reduction in grain moisture. Ear height, plant height and grain moisture fell within the equivalence limits established by the non-GM reference varieties (equivalence category I), while for stalk lodging equivalence with the non-GM reference varieties was more likely than not (equivalence category II).
- Maize MON 87427 x MON 89034 x NK603 <u>treated with glyphosate</u> showed a statistically significant increase in ear and plant height. Both endpoints fell within the equivalence limits established by the non-GM reference varieties (equivalence category I).

Further, compositional data revealed many statistically significant differences:

- In maize MON 87427 x MON 89034 x NK603 not treated with glyphosate, statistically significant differences with the non-GM comparator were identified for 42 grain endpoints. Levels of carbohydrates even fell under equivalence category III and differences in calcium under category IV.
- For maize MON 87427 x MON 89034 x NK603 treated with glyphosate, statistically significant differences were identified for 16 grain endpoints and 2 forage endpoints. All the endpoints fell under equivalence category I or II except for calcium levels (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.

- Many of the observed significant changes were set aside without further more detailed and targeted investigations (EFSA, 2017a).
- 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 plant, and must be made available and assessed in detail. However, EFSA (2017a) stated that: "Considering the scope of application EFSA-GMO-BE-2013-117, interactions with the biotic and abiotic environment are not considered to be relevant issues."
- 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.

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EFSA should have requested that Monsanto submit data from field trials with the highest dosage of glyphosate 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.

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

Toxicology

Regarding parental plants:
Open questions regarding toxicity of maize MON89034 were addressed by Member State experts, but disregarded by the GMO Panel (EFSA, 2017b).

Regarding maize MON 87427 x MON 89034 x NK603:
Despite many significant changes in the composition of the plants and agronomic characteristics, no testing of the whole plant (feeding study) was requested. 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 investigation of their potential health impacts.

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. In this regard, it should be taken into account that EFSA (2015a) explicitly stated that no conclusion can be derived on the safety of residues from spraying with glyphosate occurring in genetically engineered plants resistant to this herbicide.

Further, there is a common understanding that commercially traded formulations of glyphosate, such as Roundup, can be more toxic than glyphosate itself. Therefore, the EU has already taken measures to remove problematic additives known as POE tallowmine from the market. Problematic additives are still allowed in those countries where the genetically engineered plants are cultivated. The EU Commission has confirmed the respective gaps in risk assessment:

> *"A significant amount of food and feed is imported into the EU from third countries. This includes food and feed produced from glyphosate-tolerant crops. Uses of glyphosate-based plant protection products in third countries are evaluated by the competent authorities in those countries against the locally prevailing regulatory framework, but not against the criteria of Regulation (EC) No. 1107/2009. (…)." (www.testbiotech.org/node/1637)*

The European Food Safety Authority (EFSA) agrees that further investigations and data are needed (EFSA, 2015b).

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.

Mostly relevant in this context is that EFSA and the applicant completely omitted to assess the stacked event in regard to its intended purpose. The reason for crossing NK603 with MON 87427 was to increase the content of EPSPS enzymes that confer resistance to glyphosate. In consequence, it has to be expected that these plants can and will be exposed to higher and also repeated dosages of glyphosate. Higher applications of glyphosate will not only lead to a higher burden of residues in the harvest, but may also influence the composition of the plants and agronomic characteristics. This aspect, which is the most relevant in regard to this stacked event, was completely ignored by the risk assessment as performed.

EFSA should have requested that Monsanto submit data from field trials with the highest dosage of glyphosate that can be tolerated by the plants, also including repeated spraying. The material derived from those plants should have been assessed in regard to organ toxicity, immune reactions and reproductive toxicity, also taking combinatorial effects with other plants components and the Bt toxins into account. In the context of risk assessment of this stacked event, the residues from spraying with the complementary residues must also considered to be a potent co-stressor. The impact on cells and organisms exposed to several stressors in parallel can be of great importance for the efficacy of Bt toxins. As, for example, Kramarz et al. (2007 and 2009) show, parallel exposure to chemical toxins can lead to Bt toxins having an effect on organisms that are not normally susceptible. In addition, Bøhn et al. (2016) show additive effects of several Cry toxins. Cry toxins interact with Roundup / glyphosate when co-exposed to *Daphnia magna*. These cumulative effects also have to be assessed in regard to food and feed usages.

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.

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EFSA should have requested that Monsanto submit data from field trials with the highest dosage of glyphosate that can be tolerated by the plants, also including repeated spraying. The material derived from those plants should have been assessed in regard to immune reactions, also taking combinatorial effects with the Bt toxins into account. While EFSA looked at adjuvant effects, no data were presented to assess such effects empirically.

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

Others

No data at all were presented regarding subcombinations MON 87427 × MON 89034 and MON 87427 × NK603.

Further, 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 across the fields and the environment (Trtikova et al., 2017).

Environmental risk assessment
EFSA (2017a) 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 (Trtikova et al., 2017).

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 did not have any data regarding possible toxicity and impact on the immune system, and did not identify knowledge gaps or uncertainties. 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.

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