TESTBIOTECH Background 21 - 08 - 2015

Testbiotech comment on the Scientific Opinion on an application (Reference EFSA-GMO-NL-2011-100) for the placing on the market of the herbicide-tolerant, increased oleic acid genetically modified soybean MON 87705 × MON 89788 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto

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Introduction

MON 87705 x MON 89788 was produced by crossing two genetically engineered plants seemingly to produce a soybean that is doubly resistant to the herbicide glyphosate (MON89788 and MON87705 are both resistant to glyphosate), and with changed composition in nutritional characteristics / oil composition (MON87705). The EU application for MON 87705 x MON 89788 is for food and feed, import and processing.

Molecular characterisation

The purpose of this stacked event seems to be to enhance the concentration of EPSPS protein in the plants. This protein renders them resistant to glyphosate. However, the expression data of the stacked event show that the level of EPSPS is not enhanced in comparison to the parental plants. This is a surprising result that should have triggered a lot more investigation into potential silencing effects in the plants that can affect the level of EPSPS enzyme, and also the overall food quality and food safety. However, EFSA did not ask for any explanation of this surprising effect.

Further, EFSA did not assess the molecular data in regard to food safety and the occurrence of the intended small biologically active RNA molecules. The change in the oil composition in the soybeans is based on an inhibition of endogenous plant genes due to RNAi interference (RNAi). The mechanism results in reduced levels of the corresponding plant enzymes. (Short inhibitory) siRNA molecules which are part of this mechanism may both cause intended gene silencing and have off-target effects, i.e. may silence genes other than those intended (Senthil-Kumar et al., 2011). 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). Thus, for the risk assessment of plants that produce specific small double stranded RNAs, it is necessary to conduct bioinformatics studies to identify any likely unintended targets in humans or animals. But no such studies have been conducted.

Further, the emergence of new variations, combinations and concentrations of unintended small, biologically active RNA molecules such as microRNA was neither assessed in the single plants nor in the stacked event. These molecules are likely to emerge as unintended side products at the insertion sites of the additional DNA. They can interfere with each other on the level of the stacked

event. Their concentration, structure and potential biological effects should be assessed before any conclusion is drawn upon safety of the plants. Uncertainties related to the emergence of these molecules were not addressed.

Both the content of the EPSPS enzyme 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. Environmental stress can cause unexpected patterns of expression of the newly introduced DNA (Trtikova et al., 2015). Since the intended change in the oil content is related to health effects, it is important to know if genetic stability is maintained under stressful conditions. However, the plants were only tested in the US (not in other relevant soy producing countries) and only under "normal" agricultural conditions.

Comparative analysis (for compositional analysis and agronomic traits and the phenotype)
Compositional analysis revealed a high number of significant differences (in addition to the expected changes in oil composition) between the stacked event and its comparator. According to EFSA, 16 compounds in kernels were identified. Furthermore, several agronomic characteristics showed significant differences. Contrary to the opinion of EFSA, the occurrence of these high number differences together can indicate metabolic impacts and changes in MON87705 × MON89788, which may well go beyond the set of compounds selected for analysis. For example, the differences in plant components can indicate further changes affecting the level of anti-nutritionally, hormonally or immunologically active substances in the plant. These differences must therefore be investigated further to assess in detail their causes and biological relevance.

It is possible that some of the relevant changes in plant composition and plant characteristics may only be observed under specific environmental conditions. Thus, the observed differences should have triggered a request from EFSA for more studies, for example, to grow the plants under defined environmental extreme stress conditions.

However, EFSA has assumed without sufficient reason that these differences are not relevant for the food safety of soybean MON87705 x MON89788.

Toxicology
There are three main characteristics of these plants that are relevant for toxicology assessment:

- The plants are changed in their oil content
- The plants seem to be intended to render a higher level of resistance to glyphosate
- The plants produce specific siRNA, which also might be biologically active in mammals at the stage of consumption.

None of these characteristics were taken into account in toxicology assessment:

- Neither the parental plants MON87705 nor the stacked event were tested in animal feeding studies to assess health effects of the changed oil content. The feeding studies that were performed were based on usage of defatted soybeans, and therefore with only minimal concentrations of the relevant oil in the diet.
- The potentially higher dosage of glyphosate that might be sprayed on the plants was not taken into account. According to the comments of experts from Member States, the amount

of glyphosate sprayed onto the stacked event was even lower than the one applied to the parental plants. Higher levels of residues from glyphosate (which is under discussion as possibly carcinogenic, IARC, 2015) and their impact on plant composition, its nutritional characteristics and potential health effects still have to be assessed.
- The structure, concentration and potential effects of small biologically active RNA molecules produced in the plants was left aside (see molecular assessment).

As a result, the most relevant characteristics of this stacked event were not assessed in regard to toxicology.

One reason for this flaw in risk assessment is the lack of sufficient interplay between the pesticide assessment and the GMO assessment at EFSA, which in itself creates a high level of uncertainty. 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 the spraying with complementary herbicides. These risks are only partially assessed as part of EU pesticide regulation. However, if commercially traded herbicide formulas are applied in specific combinations to herbicide resistant plants, there are specific patterns 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 in dealing with new patterns of exposure, interactions between the substances and the accumulated impact on human and animal health.

Another reason for the flaws in this risk assessment is that EFSA has failed to develop specific guidance on the risk assessment of genetically engineered plants that are changed in their nutritional quality. As a recent dossier prepared by GeneWatch UK & Testbiotch (2015) shows, risk assessment of the parental plant MON87705 (and therefore also risk assessment of the stacked event) is flawed for the following reasons:
- Inadequate or missing literature reviews on health impacts
- Inadequate food safety and nutritional assessment
- Inadequate consideration of the potential impact of altered nutritional content on potentially vulnerable subpopulations
- Failure to consider all processed forms of foods
- Inadequate feed safety and nutritional assessment

Allergenicity

Most relevant for health risk assessment in this context are the naturally occurring allergens present in soybeans. A change in the plants composition might also lead to a higher concentration of the endogenic plant allergens. Further, it is known that toxicants, if applied together with the allergens, can have an adjuvant effects, triggering a stronger immune reaction to the proteins. This is a specific risk that needs to be addressed in the context of residues from spraying with the complementary herbicides.

Monsanto presented data that are meant to show that the concentration of the endogenic proteins in the plants was not enhanced. However, soybeans are known to have a substantial variation in their

natural concentrations, depending on specific varieties and on interaction with the environment. Monsanto failed to show that the level of endogenic allergens in specific varieties and/ or under specific environmental conditions is not increased. For this purpose, further crossing with other varieties should have been carried out as well as subjecting the soybeans to suitable stress tests. Further, the risk assessment completely failed to take into account potential interactions between the residues from spraying and the immune reaction to the soybean allergens.

No blood samples were taken from individuals known to have allergenic reactions in order to investigate clinical effects of the stacked event. No analysis was undertaken of the risks for individuals with an impaired immune system such as the elderly or infants, as requested by the EFSA guidance (EFSA, 2010).

Monitoring

The applicant should provide methods to distinguish the presence of the stacked events from those of the 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, where there are uncertainties EU regulations require the monitoring of effects on health at the stage of consumption. Thus, for example, requirements must include the monitoring of health effects and take 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 monitored closely.

Conclusions and recommendations

EFSA risk assessment is failing to deal properly with findings from the comparative analysis. The assessment of toxicological, hormonal and immunological effects is inadequate. Further, risk assessment did not take into account relevant safety issues regarding the usage of the complementary herbicide.

A systematic approach to risk assessment has to be developed to deal with the health effects of plants that are changed in their nutritional characteristics, that raise specific questions regarding residues from spraying with complementary herbicides and that produce small double stranded RNA.

Further, interactions and accumulated effects from the usage of such plants in food and feed have to be assessed systematically before any decision is taken on market authorisation.

In conclusion, the application has to be rejected.

References:

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