**TESTBIOTECH Background 20 - 07 - 2015**

# Testbiotech comment on the Scientific Opinion on application (EFSA-GMO-NL-2012-108) for the placing on the market of the herbicide-tolerant genetically modified soybean MON 87708 × MON 89788 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto

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## Introduction

MON 87708 x MON 89788 was produced by crossing two genetically engineered plants to produce combined herbicide resistance in the stacked soybeans. This is the first time that it will be possible to apply a combination of the two herbicides, dicamba and glyphosate, to soybean cultivated commercially. Monsanto plants are a consequence of problems with the increasing number of herbicide resistant weeds in countries where genetically engineered plants are cultivated. The parental plant MON 87708 is genetically engineered to be resistant to the herbicide, dicamba. The degradation of dicamba leaves residues such as 3.6-dichlorosalicylic acid (DCSA) and formaldehyde in the plants (EFSA, 2013). The other parental plant MON 89788 is genetically engineered to be resistant to the herbicide glyphosate. The degradation of glyphosate leaves residues such as AMPA (aminomethylphosphonic acid) in the plants. The EU application for MON 87708 x MON 89788 market authorisation is for food and feed, import and processing.

## Molecular characterisation

The molecular characterisation of the plants did not take into account the emergence of new double stranded miRNA that might be transmitted as a biologically active substance at the consumption level to humans or animals. miRNA might be transmitted to the consumer and there are indications that it interacts with gene regulation in mammalian cells (see, for example, Zhang et al., 2011; Lukasik & Zielenkiewicz, 2014). The emergence of new versions, combinations and concentrations of miRNA was neither assessed in the single plants nor in the stacked event. Uncertainties related to the emergence of these molecules were not addressed.

The gene expression of the gene constructs in some parts of the stacked plants showed substantial differences compared to those in the single plants. This is an indication of genomic effects caused by the crossing of the plants, and should have prompted further investigation

There was no assessment of the expression of the constructs in the plants under conditions that could represent the true range of environmental conditions, taking into account stressful conditions such as that caused by ongoing climate change.

**Comparative analysis (for compositional analysis and agronomic traits and GM phenotype)**
The outcome of the comparative analysis shows that several of the endpoints measured were significantly and consistently different. Differences were observed, for example, in the oil composition of the plants.

Further significant differences were observed for agronomic and phenotypical characteristics. In particular, differences in the 100-seed weight can be an indication for unintended genomic effects due to the genetic engineering of the plants.

Genomic x environment interactions were shown for several parameters. The effects might be much stronger under more extreme environmental conditions. However, the data presented by Monsanto only contains data from US fields (none from South America) and only for one year while the plants were grown under 'normal' agricultural conditions.

To summarise, there are indications that unintended effects are due both to the process of genetic engineering of the single plants and to the crossing of the plants. Further, environmental interactions are likely to play a role in triggering these significant differences.

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. Such conditions can also reveal genetic potential for instability in the expression of the newly introduced DNA (see, for example, Trtikova et al., 2015).

However, EFSA has assumed without sufficient reason that these differences are not relevant for the food safety of soybean MON87708 x MON89788. Thus, none of these issues were assessed in detail. Instead of requesting more data, EFSA accepted that Monsanto had failed to provide data as required by EFSA guidance. In fact, EFSA accepted the incomplete data based on explanations that are mostly vague and do not allow any conclusions.

**Toxicology**
The outcome and quality of the 90-day feeding studies with the single plants triggered several critical comments from the experts of the Member States. This should have been followed up by a request for further feeding studies with the stacked events. Furthermore, the findings related to the composition of the plants and their agronomic and phenotypic characteristics should also have triggered further studies on potential health impacts. However, no further feeding studies with the stacked event were requested.

Even though this is the first time that a combination of two herbicides, dicamba and glyphosate, will be applied to genetically engineered soybeans in the field, EFSA has not requested any data on the combinatorial effects of the residues from spraying these two herbicides. The plants will contain residues such as 3.6-dichlorosalicylic acid (DCSA), formaldehyde (see EFSA, 2013), glyphosate and AMPA, none of which have been tested for specific combined toxicity. These residues in combination should have been assessed as relevant plant constituents. According to the International Agency for Research on Cancer (IARC), a body of the World Health Organisation

(WHO), both active ingredients and / or their metabolites can be regarded as having carcinogenic potential (IARC 2012, Guyton et al., 2015). 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.

There was no assessment of any interaction between plant components such as immunologically or anti-nutritionally, hormonally or immunologically active substances with the residues from spraying. Besides carcinogenicity, other interactions have to be assumed as being relevant: For example, mixtures of glyphosate are suspected of inducing hormonal activity (see for example Thongprakaisang et al., 2013). Thus, these compounds might enhance the hormonal effects of the plant estrogens present in soybeans.

This case 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 the complementary herbicides. These risks are only assessed partially within the framework of EU pesticide regulation and only for the active ingredients. However, if these herbicides are applied to herbicide resistant plants and become plant constituents then there are additional specific risks (as shown above) that need to be assessed.

Several other genetically engineered plants with tolerance to various herbicides have pending market authorisations for the EU or have already been authorised, making a systematic approach necessary to deal with new patterns of exposure, interactions between the substances and the accumulated impact on human and animal health. Thus risk assessment of generically engineered plants always should take into account potential interactions and accumulated effects.

**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.

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 performed 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**
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 case of uncertainties. Thus for example monitoring of health effects, taking into account residues from spraying with herbicides must be required. Epidemiological parameters that are suitable to detect relevant health effects have to be defined.

The applicant should provide methods to distinct the presence of the stacked events from those of the mixture of the parental plants.

**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 does not take the many safety issues regarding the combined usage of the complementary herbicide into account. In conclusion, the application should be rejected.

A systematic approach has to be developed to deal with interactions and accumulated effects from the usage of such plants in food and feed before any further decision is taken on market authorisation of genetically engineered plants that are resistant to herbicides.

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