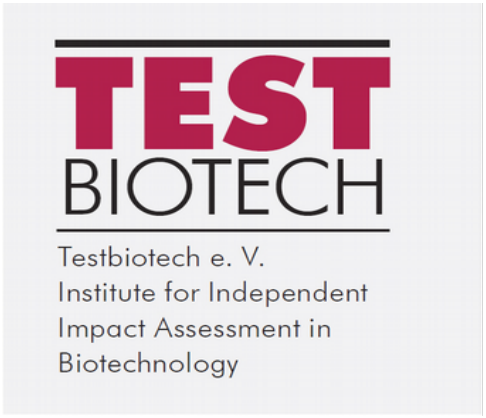
TESTBIOTECH Background 20 - 01 - 2016



Testbiotech comment on the Scientific Opinion (EFSA-GMO-DE-2009-66) for placing on the market of herbicide tolerant and insect resistant maize Bt11 × MIR162 × MIR604 × GA21 and subcombinations independently of their origin for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta

Introduction
The transgenic maize of Syngenta was created by crossing of four genetically engineered plants. The resulting stack produces three insecticidal toxins (Cry1Ab, Vip3Aa20, mCry3A) which were substantially changed in comparison to native toxins occurring in *Bacillus thuringiensis*. Further these plants are made resistant to the herbicides glyphosate and glufosinate, leaving residues in the plants. In addition they are producing a protein (phosphomannose isomerase, PMI) used as a marker for the selection of the plants.

Molecular characterisation
Many unintended changes and insertions are known to have occurred in the parental plants. For example, GA21 is known to have 3 full-length copies of the fragment. One copy has a base pair substitution in the NOS terminator region. Apart from these full-length copies 3 other copies with specific individual deletions are present. In result there is an identified disruption of a genomic maize gene and of several new putative open reading frames were created (EFSA 2015b).

Research within the open reading frames shows similarities with known allergenic proteins. EFSA (2015a) assumes that it is unlikely that these proteins are expressed in the plants. However, no empirical investigations (nor in the parental plants nor in the stacked events) were performed to proof that indeed these proteins do not occur.

Further no assessment was made (nor in the parental plants nor in the stacked event) on the various forms of interfering RNAs that are likely to emerge from the intended as well as the unintended insertion sites. Some of these interfering RNAs for example might occur in a form such as miRNA that can be taken up from the gut at the stage of consumption without loosing biological activity (Zhang et al., 2012).

There is an oingoing debate about the results of this study. While some researchers reported data consistent with the original study (Wang et al., 2012; Beatty et al., 2014), others have failed to replicate the results. But the research group reporting the initial finding has responded to some of these reports (Chen et al., 2013), and has published additional work detailing the detection of other plant derived miRNAs in humans and mice after feeding, with biological activity (Zhou et al., 2014; Liang et al., 2015). Furthermore RNAi effects might also impact the metabolism in the plant.

As Trtikova et al. (2015) show, the gene regulation in the plants might be affected by stressors occurring under ongoing climate change. This also might affect food quality or food safety. Thus

expression of intended or unintended proteins stemming from the additional DNA as well as occurrence of other new biological active compounds such as interfering RNA should have been investigated under various defined stress conditions. However, no such data are available, nor from the parental plants nor from the stacked events.

Comparative analysis (for compositional analysis and agronomic traits and the phenotype)
The parental plants as well as the stacked plants show several significant differences compared to its closest isogenic lines. According to experts of Member States (2015b), the data also show site related effects and specific trends in the stacked event compared to the parental plants. All these findings were set aside without detailed investigation. Instead the plants composition should have been assessed by further investigations taking into account defined stress conditions to examine genetic stability. These investigations should take into account conditions which for example can occur under ongoing climate change.

Further, the field trials as conducted are not in line with EFSA Guidance that requests field trials with and without the complementary herbicide (EFSA, 2015b).

Toxicology
The plants are producing several insecticidal toxins, marker proteins and additional enzymes that confer herbicide resistance and will contain residues from spraying with the complementary herbicide.

Further they are likely to produce additional interfering RNA and also might contain additional unintended proteins from the various open reading frames that were identified. The concentration of these additional proteins and biological active substances might vary substantially due to environmental conditions and stressors.

Despite these substantial uncertainties, no combined toxicological effects were empirically investigated.

Existing evidence – largely ignored by EFSA´s opinion - shows that indeed more investigations would be needed to conclude risk assessment on this stacked genetically engineered plant:

- Hilbeck & Otto (2015) give an overview on open questions regarding mode of action of Bt toxins, synergistic and additional effects. Specific synergistic effects were shown by Bergamasco et al. (2013). Further there the mode of action of Vip3A is not well characterised at all therfore should have been assessed with much more scrutiny in the stacked event as well as in the parental plants.
- Hilbeck & Otto (2015) show that there is not just one mode of action that has to be taken into account. Thus the EFSA panel can not set aside potential combinatorial effects as being relevant only for insects, simply because the mammals are supposed to lack relevant receptors (EFSA 2015a). As Rubio-Infante & Moreno-Fierros (2015) show, negative health effects of Bt toxins on mammals indeed can not be excluded, the proteins can not be regarded as being innocuous for mammals.
- Further effects on the immune system that are known to be relevant in the context of Bt toxins are not dependent on specific mode of action but on dosage effects. This is relevant in this context, since the stack shows an higher overall concentration of Bt toxins than the parental plants.
- It is also known, that degradation of Bt toxins under artificial digestion tests are not reliable when it comes to persistence of Bt toxins in the gut. For example if fed with soybeans, degradation of the Bt toxins can be delayed substantially by plant enzymes, enhancing

toxicity significantly (Pardo-López et al., 2009)

- Residues from spraying with glyphosate are suspected to show carcinogenicity effects (IARC, 2015) which might be enhanced by combinatorial effects with glufosinate but were not assessed. Glufosinate will be phased out in Europe in 2017 because of its reproductive toxicity. Thus combintorial effects have to be assessed.
- The investigations should not only cover direct effects on health but also indirect effects via changes in the microbiological composition in the gut (see for example Shehata, et al., 2012)

Thus in any case potential combinatorial health effects have to be assessed in detail before any conclusion can be drawn on food safety. No conclusion on potential health effects can be drawn from the nutritional study performed with poultry, which does not even fulfill requirements for Good Laboratory Practice (GLP).

Being aware of the complexity of the questions and the lack of standardized methods for assessing combinatorial effects, a larger research project would be needed to assess potential health effects from the stacked event, which should be conducted independently from the interests of the company.

Allergenicity

EFSA (2015a) concludes that “From the limited experimental evidence available, the EFSA GMO Panel did not find indications that the mixture of the Bt proteins in this four-event stack maize might act as adjuvants with the potential to enhance a specific IgE response and to favor the development of an allergic reaction.”

So why did EFSA not request further investigations?
It should not be ignored as done by EFSA (2015a) that the Bt toxins under real conditions will not be degraded quickly in the gut but are likely to occur in substantial concentrations in the large intestine and faeces. Since adjuvant effects are known from the single Bt toxins (see Rubio-Infante & Moreno-Fierros, 2015), it is not unlikely that a mixture of these toxins is leading to an enhanced adjuvant effects.

Further, for example combined feeding with soybeans which are known to contain a lot of allergenes should also have been assessed. Finally, the marker protein PMI which shows a substantially higher concentration in the stacked event compared to the parental plants, has similarity to allergenic parvalbumin in frogs and therefore should have been also tested in combination with the Bt toxins.

Monitoring

The JRC report (JRC 2013) confirmed that event-specific PCR-based methods validated for single events can alos be applied to DNA extracted from stack maize Bt11 x MIR162 x MIR604 x GA21. However no validated method was made available to distinguish the single event from any of the stacked named in the application. Thus no targeted monitoring or general surveillance can be performed. Therefor legal requirements for case specific identification and monitoring are not fulfilled and no market authorisation can be given.

Further, 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 to detect relevant health effects have to be defined.

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

Conclusions and recommendations
Based on the data presented and assessed, risk assessment cannot be concluded. Consequently, the application should be rejected.

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