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Impact Assessment in
Biotechnology

Testbiotech comment on EFSA Scientific Opinion on application (EFSA-GMO-DE-2010-82) for the placing on the market of insect-resistant genetically modified maize MIR162 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta

Molecular data

The data as provided by Syngenta make it evident that there is a year-to-year and site-to-site variation in the content of the additional proteins expression, and therefore shows that environmental conditions will impact the level of gene expression. Nevertheless, the expression of the gene construct and the functional stability of the gene construct were, for example, not tested under extreme climate conditions such as drought and flooding which are likely to occur under present ongoing climate change. Investigations under controlled environmental conditions should have been performed to determine the actual range of variation and to identify relevant impact factors.
Further, the effects of the additional genes on the activity of the plants´ genome and the plants´ metabolism should have been investigated using methods such as metabolic profiling.

The methods for measuring the content of VIP3A toxin and the phosphomannose isomerase (PMI) enzyme in parts of the plants were not evaluated by independent laboratories. Hence, the data by Syngenta should not be considered as reliable.

Comparative assessment (for compositional analysis and agronomic traits and GM phenotype)

The data as provided by Syngenta show several significant differences between MIR162 and its conventional counterparts in composition and agronomic performance. These differences have been declared irrelevant by referring to historical data from the ILSI Database, which is known to be unreliable.

Instead of using these historical data, the actual differences should have investigated further under various environmental conditions and by applying methods such as metabolic profiling.

The data as presented by Syngenta does not represent the relevant geo-climatic regions where this maize will be grown. The data from Brazil, for instance, lacked basic information on relevant plant constituents. But instead of repeating the trials in Brazil, additional data were generated in the US. As a result, relevant data from other regions (beside the US) are missing.

Toxicology

The VIP3Aa20 toxin as produced in the plants was never assessed by pesticide authorisation. EFSA assumes that humans and animals, and even consumers might have been exposed to VIPs before:

> „One biopesticide contained an immunoreactive protein with a molecular weight equal to that of Vip3Aa20 as present in maize MIR162. This leads the EFSA GMO Panel to conclude that the data indicate that humans and animals, including consumers and agricultural workers, may already have been exposed to low levels of Vips.“

However, this is not only a very vague but also an inadequate assumption, with no factual basis for the risk assessment of VIPs as produced in the plants. There are several important differences between the toxin as produced in the plants and its usage in traditional mixtures. In contrast to the very targeted use of traditional sprays, the toxin is applied throughout the whole period of vegetation, thus the exposure in the food chain will be different. Further, for the toxin to be effective it has to be used in higher concentrations than traditional mixtures: In the mixtures, additive and synergistic effects will require only a low level of the single compounds. Additionally, several details of the DNA sequence were changed during the process of transferring the DNA into the plants´ genome.

It should also be acknowledged that the mode of action of VIP proteins is still unknown and simply based on preliminary assumptions derived from the outcome of just a few studies. Particular details that render selectivity and toxicity of the VIP proteins are not understood. Without such knowledge, no conclusion can be drawn upon its (long time) effects for the food chain.

It should be a matter of concern that in comparison with Cry1Ab the toxicity of VIP3A does not appear to depend upon specific receptors. As Lee at al., 2003 explains:

> “Planar lipid bilayer experiments validate and extend the conclusions from our isolated midgut voltage clamp data in that processed Vip3A demonstrates the ability to form distinct ion channels in the absence of any receptors.”

Specific receptors in the gut of insects are used in the case of Cry toxins to assume selectivity and safety for mammalians. Since toxicity of VIP3A does not appear to depend these receptors, selectivity cannot be assumed.

The toxicological studies as performed in vivo and in vitro are in no way sufficient to prove selectivity of the toxin and safe use in food and feed. These investigations cannot be seen as a replacement for missing data under EU pesticide regulation. Based on the existing data, no conclusion can be drawn on the safety of the toxin and the genetically engineered maize.

In the Council Conclusions on Genetically Modified Organisms (GMOs) from the Council meeting on 4 December 2008, Member States requested a revision of current EU regulations to close the loopholes between the pesticide regulation and the regulation on genetically engineered plants. This demand is in no way confined to the usage of the genetically engineered plants in agriculture, but

includes all relevant products, which might be authorised on the market[1]:

> "(...) the mandate includes examination of the criteria and requirements for assessing all GMPs, including GMPs that produce active substances covered by directive 91/414/EEC and herbicide-tolerant GMPs with a view to reviewing them if necessary; (…) RECALLS that the use of plant protection products implies authorisations at national level and EMPHASISES THE NEED for competent authorities involved with the implementation of Directive 2001/18/EC and of Council Directive 91/414/EEC concerning the placing of plant protection products on the market, within the Commission and at national level, to co-ordinate their action as far as possible;"

EFSA risk assessment completely ignores these demands.

Looking at the comments made by experts from Member States, it is evident that the outcome of the in vitro and in vivo studies can be interpreted in different ways. For example, there are several significant findings in the 90 days study such as tumors and a lower number of cell counts in the group fed with the genetically engineered maize. These findings should have been investigated further.

There are also several publications indicating that Bt proteins such as VIP3A are likely to show synergies and interactions with other Bt toxins, other stressors and plant enzymes (for overview see Schnepf et al., 1998; Then, 2010). But no tests were performed to determine potential combinatorial or accumulated effects of the toxins, nor of any other factors such as other toxic compounds, bacteria, plant enzymes and pesticides in mammals. Since these plants will be used in food and feed and might be mixed with other genetically engineered plants, further tests need to be carried out to determine potential accumulative or combinatorial effects.

Data is also missing on the effects that processing has on the proteins produced in the plants. Since there might be some interactivity between the stability of the proteins and their surroundings in the plants, the experiments on the heat stability of the proteins should have been conducted using whole plants.

References:

Lee, M.K., Walters, F.S., Hart, H., Palekar, N., Chen, J.-S. (2003) The mode of action of the Bacillus thuringiensis vegetative insecticidal protein Vip3A differs from that of Cry1Ab δ-endotoxin. Applied and Environmental Microbiology, 69(8): 4648–4657.

Schnepf, E., Crickmore, N., van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R. & Dean, D.H. (1998) Bacillus thuringiensis and its pesticidal crystal proteins, Microbiol Mol Biol Rev., 62 (3): 775–806.

Then, C. (2010) Risk assessment of toxins derived from Bacillus thuringiensis-synergism, efficacy, and selectivity. Environ Sci Pollut Res Int; 17(3):791-7.

Allergenicity

Allergenicity was assessed by applying a pepsin digestion assay. But it is known that these studies are insufficient to judge on the degradation of the protein under real conditions. For example, Cry1Ab toxins are shown to be degraded quickly in pepsin digestion, but can nevertheless can survive the passage through the intestine under real conditions, (Chowdhury et al., 2003; Walsh et

[1] http://www.consilium.europa.eu/ueDocs/cms_Data/docs/pressdata/en/envir/104509.pdf

al., 2011). But no tests were performed to investigate the degradation of the additional proteins under real conditions.

Furthermore, besides potential allergic reactions, no targeted investigations were performed to investigate the impact on the immune systems. Since proteins from bacteria are, in general, likely to cause immune responses in humans, the additional proteins should have been subjected to more detailed testing. For example, VIP3A might act like an adjuvans as known from some Cry proteins, and could, therefore, boost immune reactions to the plants´ endogenous proteins. If this were the case then allergic reactions to the maize proteins could be significantly enhanced.

No investigations were conducted to find out whether the content of allergenic proteins in the maize plants is changed in comparison to its comparators.

References:

Chowdhury, E. H., Kuribara, H., Hino, A., Sultana, P., Mikami, O., Shimada, N., Guruge, K. S., Saito, M., Nakajima, Y. (2003) Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11. J. Anim. Sci., 81: 2546-2551.

Walsh, M.C., Buzoianu, S.G., Gardiner, G.E., Rea M.C., Gelencsér, E., Jánosi A., Epstein M.M., Ross, R.P., Lawlor, P.G. (2011) Fate of Transgenic DNA from orally odministered Bt MON810 maize and effects on immune response and growth in pigs. PLoS ONE 6(11): e27177, doi:10.1371/journal.pone.0027177.

Environmental risk assessment

EFSA states that the exposure to the environment of maize MIR162 would be through manure and faeces from animals. However, no data are given or assessed about the fate of the additional proteins during digestion, their content in manure and its fate in the environment. Thus EFSA completely fails to conduct the required environmental risk assessment.

Others

As a recent legal dossier compiled by Professor Ludwig Kraemer shows, the decision not to monitor any health effects violates the requirements of EU regulations: Directive 2001/18 and Regulation 1829/2003 both require that potential adverse effects on human health from genetically modified plants are controlled during the use and consumption stage, including those cases where such effects are unlikely to occur.

Thus EFSA's opinion that monitoring health effects is unnecessary, is wrong.

References:

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

Conclusion and recommendations: The opinion of EFSA should be rejected.