TESTBIOTECH Background 10 - 1- 2017

Testbiotech comment on the Scientific Opinion on application (EFSA-GMO-NL-2011-96) for the placing on the market of genetically modified insect-resistant and herbicide-tolerant cotton GHB119, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience AG

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Introduction
Cotton GHB119 expresses Bt toxin Cry2Ae, which confers resistance to certain lepidopteran species, and phosphinothricin acetyltransferase (PAT), which confers resistance to glufosinate ammonium-based herbicides.

Molecular characterisation
Gene products, such as miRNA from additional open reading frames, were not assessed. Thus, uncertainties remain about other biologically active substances emerging from the method of genetic engineering.

As Trtikova et al. (2015) show, gene regulation in the plants might be affected by stressors occurring under ongoing climate change. This might also 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 biologically active compounds, such as interfering RNA, should have been investigated under various defined stress conditions. However, no such data are available.

In addition, more varieties should have been included into the field trials since it is known that the genetic background of the varieties can influence the level of gene expression (see, for example, Trtikova et al., 2015).

Further, all parts of the plants should be taken into account for risk assessment. Since expression data have to be considered as one of the starting points in the risk assessment of the plant, the assessment of the data cannot be reduced to those parts of the plants entering the food chain.

Comparative analysis (for compositional analysis and agronomic traits and the phenotype)
According to EFSA (2016), statistically significant differences in the composition of the genetically engineered plants were found for many compounds. Many significant differences were observed in US field trials:

> *"24 significant differences between cotton GHB119 (treated with glufosinate ammonium-based herbicides) and its conventional counterpart, and 23 significant differences between cotton GHB119 (treated only with conventional herbicides) and its conventional counterpart"*

A similar outcome was reported in Spain:

> "*statistical analysis identified 29 significant differences between cotton GHB119 (treated with glufosinate ammonium-based herbicides) and its conventional counterpart, and 32 significant differences between cotton GHB119 (treated only with conventional herbicides) and its conventional counterpart*".

However, no further investigations were deemed necessary by the GMO Panel and most differences were declared irrelevant, even using non-scientific ad hoc assumptions. Certainly, many more investigations would be necessary to conclude on the underlying mechanisms and the biological relevance of these differences.

There are further gaps in the assessment of the comparative assessment:

- No omics data (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.
- Further, no data were generated representing more extreme environmental conditions, such as those caused by climate change.
- 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.

Based on the data available, the plants have to be assumed to be different in their composition compared to their conventional comparator.

Toxicology

There are several gaps in the risk assessment:

- Despite it being known that Bt toxins have an effect in several ways, only one mode of action was considered (for overview see: Hilbeck & Otto, 2015).
- Despite it being known that Bt toxins can show synergies with each other as well as with other compounds (for overview see: Then, 2010), no investigations into combinatorial effects were conducted, such as interaction with residues from spraying with glufosinate.
- There are no reliable data to assess the exposure of the food chain to the Bt toxin. The data on degradation after exposure to heat is not based on genetically engineered cotton processing data.
- No further testing of the whole plant was requested even though the comparative assessment and molecular analysis uncovered a range of uncertainties; and the sub-chronic feeding study that was provided suffered from major methodological flaws.

Such specific data would have been vital because the Cry2Ae Bt toxin is a relatively new insecticidal protein, with synthetic modifications and a different mode of action to Cry1A toxins. Thus, the characterisation of this protein, especially in terms of toxicity and specificity, should be performed in much greater depth than has been done by the applicant.

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 have 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 would also be 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: Recital 26 of Regulation 396/2005 requires Maximum Residues Levels (MRLs) for food and feed produced outside the Community if produced by different agricultural practices as regards the use of plant protection products. Article 14 of Regulation 396/2005 requires that the presence of pesticide residues arising from sources other than current plant protection uses and their known cumulative and synergistic effects are determined. Further, Article 29 of Regulation 1107/2009 states that active substances and synergists have to be approved, and the maximum residue levels for each specific agricultural product have to be determined.

In any case, both EU pesticide regulation and 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 of potential toxicity.

Consequently, the EFSA toxicological assessment is unacceptable.

Allergenicity
Several relevant issues regarding allergenicity and the immune system were left aside in the EFSA risk assessment:

- A whole range of studies indicate that adjuvant effects may be triggered by Bt toxins (see Rubio-Infante, N. & Moreno-Fierros, 2016).
- No non-IGE-mediated immune reactions were assessed, although these effects must be considered relevant (Mills et al., 2013).
- The assessment did not take the risk to more vulnerable groups of the population, such as infants (EFSA, 2010), into account.

Overall, the risk assessment is insufficient to rule out impacts on the immune system.

Environmental risk assessment
As the comments made by experts from EU Member States show (EFSA, 2016b), some plant species in Europe can cross with cotton. Apart from this, cotton is grown in several regions. Spillage from cotton seeds is likely to occur, and concerns were raised by experts from EU Member States that transgenes might be distributed in the environment. However, EFSA considers the risk of uncontrolled spread of the transgenes to be low. In taking this position, EFSA has ignored data from Mexico (Wegier et al., 2012) showing that it is difficult to predict the distribution of transgenic cot-

ton in the environment once spillage occurs. Thus, the risk of contamination and the uncontrolled spread of the transgenes seems to be much more relevant than assumed by EFSA.

Others

Monitoring should be case-specific. Exact data on the exposure to the cotton should be made available. Possible health impacts have to monitored in detail. Controls regarding residues from spraying with glufosinate have to be established. Accumulated effects that might stem from mixtures with other genetically engineered plants have to be taken into account in the monitoring plan.

Conclusions and recommendations

The risk assessment by EFSA should not be accepted. It does not identify knowledge gaps and uncertainties and fails to assess toxicity as well as the impact on the immune system and the reproductive system. The monitoring plan has to be rejected because necessary data will not be made available.

References:

EFSA (2016a) Scientific opinion on application (EFSA-GMO-NL-2011-96) for the placing on the market of genetically modified insect-resistant and herbicide-tolerant cotton GHB119, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience AG. EFSA Journal 2016; 14(10): 4586, 27 pp.

EFSA (2016b) Application EFSA-GMO-NL-2011-96 by Bayer CropScience AG, Comments and opinions submitted by Member States during the three-month consultation period, Register of Questions, http://registerofquestions.efsa.europa.eu/roqFrontend/ListOfQuestionsNoLogin?0&panel=ALL

EFSA (2010) Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010; 8(7):1700, 168 pp.

Hilbeck A. & Otto M. (2015) Specificity and Combinatorial Effects of Bacillus Thuringiensis Cry Toxins in the Context of GMO Environmental Risk Assessment. Frontiers in Environmental Science, 3: 71.

Mills, E.N.C., Marsh, J.T., Boyle, R., Hoffmann-Sommergruber, K., DuPont, D., Bartra, J., Bakalis, S., McLaughlin, J., Shewry, P.R. (2013) Literature review: ‘non-IgE-mediated immune adverse reactions to foods’, EFSA supporting publication 2013: EN-527.

Rubio-Infante, N. & Moreno-Fierros, L. (2016) An overview of the safety and biological effects of Bacillus thuringiensis Cry toxins in mammals. Journal of Applied Toxicology, 36(5): 630–648.

Trtikova, M., Wikmark, O.G., Zemp, N., Widmer, A., Hilbeck, A. (2015) Transgene expression and Bt protein content in transgenic Bt maize (MON810) under optimal and stressful environmental conditions, PLoS ONE 10(4): e0123011.

Wegier, A., Piñeyro-Nelson, A., Alarcón, J., Gálvez-Mariscal, A., Álvarez-Buylla, E. R. and Piñero, D. (2011) Recent long-distance transgene flow into wild populations conforms to historical patterns of gene flow in cotton (Gossypium hirsutum) at its centre of origin. Molecular Ecology, 20(19): 4182-4194. http://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2011.05258.x/full