TESTBIOTECH Background 23 - 5 - 2018

Testbiotech comment on EFSA GMO Panel, 2018, Assessment of genetically engineered cotton GHB614 x LLCotton25 x MON 15985 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2011-94) from Bayer CropScience

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Introduction

Stacked GHB614 x LL25 cotton was produced by crossing genetically engineered cotton lines to make the stacked event resistant to glyphosate (GHB614) and glufosinate (LL25). Owing to further crossings with MON15985 cotton, the final stacked plants produce two insecticidal proteins (Cry1Ac and Cry2Ab2). In addition, the plants produce proteins that confer resistance to antibiotics (NPTII and AAD) as well as the GUS protein that was used as a histochemical marker during product development.

Molecular characterisation

The assessment of the parental plants shows there are many open reading frames created by the insertion of the additional genes. These multiple new open reading frames were found in the parental plants and can give rise to RNA that is translated into DNA, or which might be involved in gene regulation without producing proteins (RNAi). However, EFSA only assessed the probability of unintended proteins being produced by the DNA sequences – other biologically active compounds, such as miRNA, were not assessed.

Data regarding the sites of insertion and open reading frames are highly relevant for risk assessment. The sequence data should be made public to allow independent experts to perform further assessments. Uncertainties regarding possible gene products should be fully identified.

Further, the way in which the expression data from the constructs were assessed is inconclusive. There is plenty of evidence that genetic background, soil and climate conditions substantially impact Bt expression in the plants (Adamczyk & Meredith, 2004; Adamczyk et al., 2008; Beura & Rakshit, 2013; Chen et al., 2005; Chen et al., 2012; Luo et al., 2008; Wang et al., 2015; Zhu et al., 2018). Therefore, the expression of the constructs in the stacked plants should have been assessed under a wide range of defined environmental conditions, taking into account potential extreme stress conditions such as those caused by ongoing climate change. In addition, more varieties should have been included in the field trials since it is known that the genetic background of the varieties can influence the level of gene expression.

Apart from this, the protocols used for measuring Bt content in the plants should have been evaluated to make sure a sufficiently robust method was used to generate the data. As Székács, et al., 2011 point out, without further evaluation, the Bt content in the plants cannot be reliably determined.

The Bt expression data are not only relevant for the agronomic performance of the Bt cotton in the fields, but also necessary for assessment of exposure in the food and feed chain. A lack of further data means that the genetic and biological stability of the plants as well as the content of Bt toxins cannot be determined.

Further investigations should include data on the effects of the additional DNA on the plants' genome, transcriptome, proteome and metabolome.

Comparative analysis

Various significant findings in compositional analysis and agronomic performance were observed in the parental plant as well as in the stacked event, including, amongst others, a much higher content in gossypol, which is known to be highly toxic.

In assessing these data, EFSA completely overlooked the biological effects of the EPSPS enzyme as expressed in the stacked cotton. As evidenced by research undertaken by Fang et al., (2018), Wang et al. (2014) and Yang et al. (2017), the EPSPS enzyme can render higher fitness in the plants and their offspring. These effects are also dependent on environmental stressors (Fang et al., 2018; Luo et al., 2008). These observations are not only relevant for environmental risk assessment (below) but also for food safety.

As Fang et al (2018) show, the EPSPS enzyme is very likely to interfere with the auxin content in the plant. Auxin is known to be involved in many metabolic pathways in cotton (Xu et al., 2013). Further, there is evidence that enhanced content of auxin also causes higher accumulation of gossypol in the cells (Baksha et al., 2006). Thus, the data provided by the applicant indicate some interference in the endogenous metabolism in the plants probably leading to changes in the auxin content and, therefore, resulting in changes in plant composition and a higher content of gossypol.

There are concerns over the enhanced content of gossypol that are increased due to the additional EPSPS enzymes. These concerns are additionally intensified by the fact that the stacked event GHB614 x LL25 also showed a higher content of gossypol, while MON15985 did not. Our hypothesis is further supported by a more detailed investigation of the data from field trials made available by German experts (EFSA, 2018 b):

> "Those plants including the modified 2mEPSPS showed less phosphorus but more gossypol compared to those plants including the unmodified EPSPS and this result was continuous for all sites tested. (…) the results indicate potential difference in compositional and agro-phenotypic performance at events including the modified 2mEPSPS."

These findings make it necessary for EFSA to reassess the available data and, if needed, request further data to investigate the changes in plant composition and agronomic performance in order to determine food and environmental safety - thereby taking into account the effects of the EPSPS enzyme, the auxin content, the increase in phytoalexins, such as gossypol, and other relevant compounds. Investigation of these issues should also take into account a wide range of defined environmental stress conditions since the gossypol content will very likely be influenced by genome x environmental interactions.

Food Safety Assessment

Toxicology

The toxicological risk assessment is based on assumptions and biased considerations but not on facts. There are strong indications that further toxicological studies are needed because changes in plant composition, the potential combinatorial and cumulative effects of new biologically active substances produced in the plants (such as Bt toxins) as well as residues from spraying with high dosages of the complementary herbicides and relevant herbicidal formulations are to be expected. For example, specific patterns of residues from spraying with the complementary herbicides and their impact on plant constituents and potential combinatorial effects require detailed risk assessment (see, for example, Then & Bauer Panskus, 2017). In addition, the possible interaction of the two Bt toxins and other substances require more detailed investigations (see, for example, Bøhn,et al., 2016 and Bøhn, 2018; Hilbeck & Otto, 2015).

The overall combinatorial effects can only be assessed if the stacked event is subjected to an assessment of the whole food and feed. However, only one nutritional feeding study was performed with the whole food and feed and this was not accepted by EFSA (2018a) due to an overall high mortality of the animals. There were no studies carried out with the whole food and feed to investigate any potential health effects from the consumption of products derived from the stacked cotton.

Strong indications that the composition of the plants was unintentionally changed by biochemical mechanisms involving the auxin hormone and the gossypol content were ignored. Therefore, the risk assessment has to be rejected.

Allergenicity

Based on the data provided, no conclusion can be drawn on the overall allergenicity of food and feed derived from the stacked cotton. Immune system responses must be investigated since the Bt proteins are known for adjuvant properties – something that the EFSA (2018b) has acknowledged and which is evidenced in several publications (see, for example, Rubio-Infante & Moreno-Fierros, 2016).

Other

The risk manager should take into consideration that this stacked event produces enzymes which render resistance to antibiotics. NptII provide resistance to neomycin and kanamycin. Both antibiotics are classified by the WHO as “highly important” (see EFSA 2018b). EFSA (2018 a) even considers there to be some likelihood that the DNA sequences for NptII will be transferred to microorganisms. The risk manager should answer the question of whether from a global human health perspective the cultivation and consumption of plants conferring resistance to antibiotics should still be encouraged (by allowing imports of products such as the stacked cotton), whilst, at the same time, EU regulation actually requires that this technology should have been phased out years ago.

Environmental risk assessment

It was not considered that the stacked cotton is very likely to show enhanced fitness (see Fang et al., 2018), therefore, the environmental risk assessment is inconclusive and has to be rejected.

Others

General surveillance as well as monitoring requires specific methods to trace and detect this particular stacked event under practical conditions. But no such methods were made available. Thus, market authorisation cannot be issued.

Conclusions and recommendations

The risk assessment is inconclusive and there are indications of substantial risks for animal and human health. Market authorisation for import and usage in food and feed cannot be given.

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