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Testbiotech comment on EFSA GMO Panel Scientific Opinion on the assessment of genetically engineered cotton GHB614xT304-40xGHB119 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2014-122) of company Bayer

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Introduction

Cotton GHB614xT304-40xGHB119 is derived from the crossing of three events:

- Cotton GHB614 is engineered to be resistant to the herbicide glyphosate
- Cotton T304-40 produces the insecticidal protein Cry1Ab, and is resistant to glufosinate
- Cotton GBH119 produces the insecticidal protein Cry2Ae, and is resistant to glufosinate.

Regulation (EU) No 503/2013 which foresees 90-day animal feeding studies, an extended literature review, specific monitoring requirements and specific statistical analysis was applied in the risk assessment of the stacked event.

1. Molecular characterisation

Besides resistance to glyphosate, the plants are doubled in the genetic condition that confers resistance to glufosinate. This causes a higher amount of the PAT enzyme to be produced in the plants.

The cotton produces two truncated and chimeric versions of Bt toxins that do not exist naturally. No detailed consideration was undertaken regarding the extent to which the truncation of the Bt proteins will change its biological characteristics. The DNA sequences used for the expression of these proteins have not been made public, although this information is very relevant for the risk assessment of the genetically engineered cotton.

Further, the insertion of the constructs creates several new open reading frames. EFSA did not assess unintended gene products, such as miRNA, that can emerge from the insertion of the transgenes.

In order to enable further independent risk assessment, the full DNA sequence inserted into the plants should be made available, including all open reading frames.

EFSA (2018a) did not request any detailed analysis based on so-called -omics (transcriptomics, metabolomics, proteomics) to investigate changes in the overall metabolism in the plants. EFSA assumed that the data from phenotypic characteristics and compositional analysis would not indicate any need for further investigations. However, these data did show many significant changes (see below). In general, data on phenotypic characteristics and compositional analysis can be used

as complementary data, but these are not as sensitive as -omics data and cannot replace them.

It is known (Christ et al. 2017) that the PAT/bar acts upon plant endogenous amino acids leading to ectopic accumulation of two metabolites. This effect was overlooked for more than 20 years despite many relevant plants being risk assessed by EFSA. As EFSA (2018b) states, the GMO panel was not aware of the finding at the time of previous risk assessments. However, it is now at least aware of these findings and that metabolomic studies should have been requested. Such studies would be especially relevant in this case because the stacked cotton shows an increased expression of the bar gene.

Expression data provided on the newly produced proteins indicate higher rates for application of the complementary herbicides (EFSA 2018c). According to the expert opinion of Member States (EFSA 2018c), this pattern of gene expression indicates an effect of herbicide application in combination with the stacked event. Therefore, the EFSA conclusion that no indications for combinatorial effects were observed in the plants is not correct.

Furthermore, it is known that the Bt content in the plants depends on environmental impact. For example, environmental stress can cause unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015; Adamczyk & Meredith 2004). Therefore, the plants should have been subjected to a much broader range of defined environmental conditions and stressors in order to gather reliable data on gene expression and functional genetic stability.

Further, the method used to determine the amount of Bt toxins (ELISA) is known to be dependent on the specific protocols used. The data are not sufficiently reliable without further evaluation by independent labs. For example, Shu et al. (2018) highlight difficulties in measuring the correct concentration of Bt toxins produced by the genetically engineered plants (see also Székács et al., 2011). Without fully evaluated test methods to measure the expression and the concentration of the Bt toxins, risk assessment will suffer from substantial methodological gaps.

Consequently, the risk assessment of molecular characteristics is not conclusive and is not sufficient to show food and feed safety.

2. Comparative analysis (for compositional analysis and agronomic traits and GM phenotype)
Field trials were only performed in the US, at eight registered sites for only one growing season (2012), and not in any other cotton producing regions. The parental plants were not grown in parallel, thus no direct comparison can be made that would allow assessment of effects due to the process of stacking.

Around one third of the 27 parameters measured for the phenotype were found to be significantly different in comparison to the conventional plants. Some of them belong to categories III/IV which indicate major differences.

In plant composition, more than half of the 53 parameters measured were significantly different (more than 30) in comparison to the conventional plants. Again, some of them belong to categories III/IV which indicate major differences.

Taken as isolated data these differences might not directly raise safety concerns, nevertheless, the large number of effects should have led to further investigations.

Therefore, EFSA should have requested further studies e.g.

- data from omics (proteomics, transcriptomics, metabolomics),
- data representing more extreme environmental conditions such as those caused by climate change,
- data representing more areas of commercial cotton cultivation,
- more data on stress reactions under controlled conditions
- and the impact of the dosage of the complementary herbicide that was sprayed, as well as the number of times it was sprayed onto the plants under practical conditions.

Instead, EFSA (2018a) has relied solely on the newly introduced statistical method known as the "test of equivalence". This method can be helpful to make some assumptions on the relevance of the significant findings. However, it cannot replace a detailed assessment of the high number of significant differences.

Based on the available data, no final conclusions can be drawn on the safety of the plants.

Toxicology

Bayer presented data from 90-day feeding trials undertaken solely with the three parental plants. These studies suffer from methodological weaknesses. Further, in each case, a considerable number of significant effects were shown to occur in the rats. Taking into account the uncertainties from the molecular assessment and the data from composition analysis and phenotypical characteristics, it is obvious that further studies with the stacked plants should have been requested. These additional feeding studies are also necessary to assess potential combinatorial effects between stressors produced in the plants (such as the Bt proteins) and the residues from spraying the complementary herbicides (see also Then & Bauer-Panskus, 2017).

Furthermore, because truncated and synthetic versions of the Bt proteins are produced in the plants, food safety of a combination of these toxins would require a detailed investigation.

There are further relevant issues e.g. the potential impact on the intestinal microbiome also needs to be considered. Such effects might be caused by the residues from spraying since glyphosate has been shown to have negative effects on the composition of the intestinal flora of cattle (Reuter et al., 2007) poultry (Shehata et al., 2013) and rats (Mao et al., 2018). Further, Bremmer and Leist (1997) examined the possible conversion of NAG to glufosinate in rats. In general, antibiotic effects and other adverse health effects might occur from exposure to a diet containing these plants that were not assessed under pesticide regulation. However, these adverse effects on health might be triggered by the residues from spraying with the complementary herbicide (see also van Bruggen et al., 2017).

In addition, as far as the exposure of the food chain to Bt toxins is concerned, EFSA should have requested data on the overall combined exposure to Bt toxins caused by the introduction of Bt plants into the EU. Currently, there are already 30 events that produce Bt toxins authorised for import. The exposure stemming from these imports should have been added to that of the stacked cotton.

Consequently, the toxicological assessment carried out by EFSA is not sufficient to show food and feed safety.

Allergenicity
Bt toxins are known to be immunogenic. They seem to act as allergens and adjuvant effects are likely to occur. In regard to immunogenicity (non-IgE-mediated immune adverse reactions), it is generally acknowledged that Bt toxins are immunogenic (Rubio-Infante & Moreno-Fierros, 2016; Adel-Patient et.al., 2011; Andreassen et.al., 2015a,b; Andreassen et.al., 2016; see also Then & Bauer-Panskus, 2017). Thus, there are some substantial reasons for concern that reactions to allergens can be enhanced (see also EFSA 2018d, minority opinion). This is relevant since in food/feed the Bt toxins can be mixed with allergens from soybeans, amongst others. Mixing with soybeans can also substantially prolong the degradation of the Bt toxins in the gastric system (Pardo-López et al., 2009).

New findings (Santos-Vigil et al., 2018) indicate allergenic potential of Cry toxins after intra-gastric administration in a murine model. Thus, the EFSA assumption that a detailed assessment of the allergenic potential of Cry toxins is not necessary, is simply wrong.

Consequently, the assessment on allergenicity cannot be regarded as conclusive.

Others
According to Regulation (EU) No 503/2013, the applicant has to ensure that post-market monitoring is developed to collect reliable information with respect to the detection of indications of whether any (adverse) effects on health may be related to genetically modified food or feed consumption. Some experts from Member States (EFSA 2018b) have made appropriate demands regarding the implementation this obligation. Accordingly, the monitoring report should deliver detailed information on:
i) actual volumes of the cotton imported into the EU,
ii) the ports and silos where shipments of the cotton being unloaded,
iii) the processing plants where the cotton was transferred to,
iv) the amount of the stacked cotton as used on farms for feed, and
v) transport routes of the stacked cotton.

The applicant is further requested to explain how the PMM of the stacked cotton in mixed GMO commodities imported, processed or used for food/feed would be put into practice. Since traders may co-mingle the stacked cotton with other imported commercial genetically engineered cotton that is processed or used for food/feed, the applicant is requested to explain how the monitoring will be designed to distinguish between potential adverse effects caused by stacked cotton and those caused by other genetically engineered cotton, such as parental plants.

The monitoring should be run in regions where the stacked cotton will be transported, stored, packaged, processed or used for food/feed. In case of substantial losses and spread of the stacked cotton, all receiving environments need to be monitored.

Environmental risk assessment
EFSA (2018a) acknowledges that the stacked cotton seeds can give rise to volunteer plants that might persist for some time in the environment, especially in the Mediterranean region. To assess the environmental risks conferred by these genetically engineered offspring plants, experimental data are necessary. It is known that next generation effects can emerge in genetically engineered plants that are not present in the original plants (see, for example, Lu and Yang, 2009; Zhang et al., 2018). Furthermore, plants with additional expression of the EPSPS enzyme are known to show a

higher fitness even if no glyphosate is applied (Fang et al., 2018).

Consequently, environmental risk assessment carried out by EFSA is not acceptable.

Conclusions and recommendations

The EFSA risk assessment should be rejected.

When making his decision the risk manager should also take into account issues that are related to pesticide regulation. In this case, glufosinate-ammonium is about to be prohibited in the European Union.

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