TESTBIOTECH Background 20 - 6 - 2014

Testbiotech comment on the Scientific Opinion on application (EFSA-GMO-NL-2010-77) for the placing on the market of herbicide-tolerant genetically modified cotton GHB614 x LLCotton25 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience

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Introduction

The stacked genetically engineered cotton line GHB614 x LL25 was produced by crossing the genetically engineered cotton GHB614 that is resistant to glyphosate and the genetically engineered cotton LL25 that is resistant to glufosinate. Stacked events such as these are emerging as a consequence of an increase in herbicide resistant weeds in regions where genetically engineered crops are grown on a large scale.

Molecular characterisation

According to EFSA´s opinion on the parental plants, it seems that in cotton GHB614 the additional DNA was inserted near a protein coding gene of unknown function. This finding might be relevant for interaction with the second DNA construct in LL25. Furthermore, 14 new open reading frames were found in the parental plant which can give rise to RNA that is translated into proteins or might be involved in gene regulation without producing proteins (RNAi). Both pathways are relevant for assessing interactions in the stacked event.

According to EFSA´s opinion on parental cotton LL25, a fragment of genomic DNA at the target site was deleted. Furthermore, 26 new open reading frames (ORFs) were detected which can give rise to RNA that is translated into proteins or might be involved in gene regulation without producing proteins (RNAi). Both pathways are relevant for assessing interactions in the stacked event.

Despite these findings in the parental plants, possible interactions between the constructs in the stacked event were only considered theoretically. Furthermore, the only potential interactions taken into account were those of the two enzymes that render resistance to herbicides.

No further investigations into molecular characterisation were carried out. But as indicated by the compositional analysis and agronomic performance, unexpected interactions of the DNA constructs in the stacked events are likely to occur and therefore should have been investigated in much greater detail.

Furthermore, the expression of the constructs in the stacked plants was only assessed in 3 field trials, leaving aside the real range of environmental conditions and ignoring potential extreme stress conditions such as ongoing climate change. More expression data and the evaluation of the protocols used for measuring would be needed for a thorough investigation.

In conclusion, in awareness of these uncertainties and taking into account various significant findings in compositional analysis and agronomic performance, much more data would have been necessary for a thorough and robust risk assessment. These data should include information on the effects of the additional DNA on the plants genome, transcriptome, proteome and metabolome and also take into account a broad range of defined environmental stress conditions.

Comparative analysis
Various significant findings in compositional analysis and agronomic performance were observed:

In agronomic performance, plant stand, number of days to first bloom, yield, per cent lint, fibre length, fibre elongation, boll size, seed index, number of nodes, first position bolls and total bolls are listed, with per cent lint being significantly different within all comparisons. These differences were not considered relevant for food and feed safety assessment, even though they indicate interactions on the level of the genome or metabolome that might also be relevant for the overall food safety of the plants. These differences should have been investigated in more detail, taking defined environmental stress conditions into account.

In the compositional analysis, statistically significant differences were observed for crude fat, ash, calcium, potassium, magnesium, iron, zinc, phytic acid, dihydrosterculic acid and free and total gossypol. Some of the observed differences even fell outside the range of historical data that were used by EFSA to interpret these differences.

Instead of requesting further investigation into underlying causes EFSA used various explanations to declare these findings irrelevant. For example, since the content of iron in the stacked plants is 20 per cent lower than in comparators, EFSA recommends supplementing the feed with minerals. And a significant reduction in phytic acid was considered irrelevant, just because this compound is seen as an anti-nutrient. These kind of non-scientific ad hoc assumptions seem to be derived from a general bias in favour of fast-tracked market authorisation of these plants.

Consequently, all the differences that were observed, except for a higher content of gossypol (see below), were considered irrelevant for the outcome of EFSA´s risk assessment.

Food Safety Assessment

Toxicology
The only biologically relevant significant finding that was discussed in regard to food and feed safety was the content of gossypol. It is known that the content of gossypol in cotton seeds is affected by the genetic background of the plant variety as well as by environmental factors such as climate, soil type, and fertilisation. It is readily absorbed from the gastrointestinal tract, and is highly protein-bound to amino acids, especially lysine, and to dietary iron. The precise mechanism of action is not known, but gossypol renders many amino acids unavailable. Gossypol also affects enzymatic reactions critical for many biological processes, including the ability of cells to respond to oxidative stress and inhibition of oxygen release from haemoglobin. All animals are susceptible, with monogastrics, preruminants, immature ruminants, and poultry appearing to be affected most frequently. Toxic effects usually only occur after long-term exposure to gossypol, often after weeks or months. Signs of toxicity may relate to effects on the cardiac, hepatic, renal, reproductive, or other systems. (see for example: www.merckmanuals.com/vet/toxicology/gossypol_poisoning/overview_of_gossypol_poisoning.html)

But although a significantly higher level of gossypol was found in the plants, no detailed assessment of risks to health was carried out. Instead, EFSA concluded that because of general EU regulations limiting the maximum content of free gossypol in feed, the elevated content of gossypol was not a safety concern. Further, some preparations used for human food consumption are not supposed to contain free gossypol. Thus it appears that EFSA is unable to exclude toxic effects in farm animals (and humans?) when they are fed with stacked events, and is simply relying on EU controls and inspections of animal feed (which are not normally very frequent).

Such a weighing up of risk management measures has nothing to do with the scientific risk assessment of genetically engineered plants. EFSA should have requested a detailed investigation of the underlying mechanisms that cause the higher level of gossypol in the stacked event, in addition to a lot more data on the real content of gossypol under various defined environmental conditions, and after crossing with a large number of other varieties.

Despite all the uncertainties, findings and potential hazards, not a single feeding study with the whole food and feed was requested by EFSA to explore potential health effects: “Therefore, the EFSA GMO Panel does not consider additional animal safety studies with the whole GM food/feed necessary.” It should be noted that there were also no feeding studies with the parental plants (whole food and feed) to investigate health effects.

Furthermore, these crops not only inherit a new combination of DNA-constructs but also a specific pattern of residues from spraying with the 'complementary' herbicides. These residues will not just occur on occasion, but have to be expected regularly in the plants that are sprayed with glyphosate and glufosinate.

The specific pattern of residues in the plants has to be seen as a relevant plant constituent and as a specific mixture of herbicides that requires risk assessment. Since the particular pattern of residues from spraying and its potential interactions were not assessed under pesticide regulation, they have to undergo risk assessment as part of the GMO authorisation process. EFSA completely neglected to do this. No studies were requested to test the interaction between the residues from spraying in the plants.

In conclusion, this risk assessment suffers from major data gaps, is based on assumptions and considerations but not on empirical findings and facts.

Allergenicity

The “weight.of.evidence” approach as applied by the EFSA is inadequate, since it is largely based on methods such as the pepsin test that is known to be unreliable. Further, the EFSA approach does not take potential adjuvant / synergistic effects that may emerge in stacked events into account. No non-IGE-mediated immune reactions were assessed although these effects must be considered relevant (Mills et al., 2013).

Furthermore, EFSA (2010) requests detailed investigations into allergenic risks for infants and individuals with impaired digestive functions. “The specific risk of potential allergenicity of GM products in infants as well as individuals with impaired digestive functions (e.g. elderly people, or individuals on antacid medications) should be considered, taking into account the different digestive physiology and sensitivity towards allergens in this subpopulation.” However, these specific risks were left aside during EFSA risk assessment.

Environmental risk assessment

As the comments from experts from Member States show, 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 such as Spain, where cotton is grown commercially, that transgenes might be distributed in the environment. However, EFSA considers the risks for uncontrolled spread of the transgenes to be low. In doing so, EFSA has ignored data from Mexico (Wegier et al., 2012) showing that it is difficult to predict the distribution of transgenic cotton in the environment once spillage occurs. Thus the risk for contamination and uncontrolled spread of the transgenes seems to be much more relevant than the EFSA assumes.

Others

As a legal dossier compiled by Professor Ludwig Kraemer shows, EU regulations require the monitoring of effects on health at the stage of consumption. This is especially relevant in this case, because of the elevated level of gossypol that has been found, and because a specific pattern of residues from spraying with herbicides can be expected in the plants. Directive 2001/18 and Regulation 1829/2003 both require that potential adverse effects on human health from genetically modified plants are monitored during the use and consumption stage. Therefore, the EFSA opinion that monitoring the effects on health is unnecessary contradicts current EU regulations.

In any case, general surveillance as well as monitoring would require methods of detection for this particular stacked event to enable distinction from its parental plants under practical conditions. But no such methods were made available. Consequently, no market authorisation can be given.

Conclusions and recommendations

The risk assessment is inconclusive and even indicates substantial risks for animal and human health. Market authorisation for import and usage in food and feed cannot be given.

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