TESTBIOTECH Background 31 - 8 - 2014

Testbiotech comment on Scientific Opinion on applications (EFSA-GMO-UK-2008-57 and EFSA-GMO-RX-MON15985) for the placing on the market of insect-resistant genetically modified cotton MON 15985 for food and feed uses, import and processing, and for the renewal of authorisation of existing products produced from cotton MON 15985, both under Regulation (EC) No 1829/2003 from Monsanto.

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Introduction

Cotton 15985 (Bollgard II) is a stacked insect-resistant plant producing the two Bt toxins Cry1Ac and Cry2Ab2. Unlike most other stacked events, cotton 15985 was not obtained by conventional crossing of two transgenic plants but by genetic modification (retransformation) of transgenic cotton 531 (producing Cry1Ac toxin). Cotton 15985 contains antibiotic resistance marker genes.

Molecular characterisation

According to the GMO Panel, molecular characterisation of MON 15985 did not give rise to safety issues.

However, 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.

Further, a potential new allergen is produced in cotton MON 15985. According to EFSA, an allergen search identified a 10-amino acid-long stretch showing identity to beta-conglycinin-alpha storage protein. EFSA finds it is highly unlikely that this protein is expressed in the plants but fails to ask the applicant for more detailed investigations.

Overall, the information on molecular data given in EFSA's opinion is scarce and therefore difficult to provide further comment. For example, EFSA stated that open reading frames have been identified, however, unlike in previous opinions given, no further details (such as the number of ORFs) are provided.

Testbiotech urges for more clarity and additional details on risk relevant issues to be provided in EFSA's opinions.

Comparative assessment (for compositional analysis and agronomic traits and phenotype)
Due to the minor quality of the applicant's data, EFSA was not able to conclude on possible differences in agronomic traits and phenotype. Yet, instead of asking Monsanto to produce valid data, EFSA simply states that no assessment of unintended effects was possible. Hence, a major part of the EFSA risk assessment is missing and lacking detailed explanation.

Data presented in the ERA part of the EFSA opinion shows that an assessment of phenotypic and agronomic traits should be conducted. Referring to a field trial conducted in 1998, EFSA states:

> *The agronomic and phenotypic analysis identified seven statistically significant differences (of 11 parameters tested) in the across location statistical analysis. Cotton MON 15985 had a higher stand count at 14 and 30 days after planting, a higher number of flowers at visits 3, 4, 5 and 6 during the flowering period and an increased yield than its conventional counterpart. Experimental data provided by the applicant showed that seed germination of cotton MON 15985 was in some cases significantly lower than that of its conventional counterpart.*

It has to be reaffirmed that an investigation of agronomic traits and phenotype is an essential part of food and feed risk assessment. Data derived from these trials can show interactions between the environment and the genome, or indicate unexpected changes in biological functions of the transgenic plants, which may impact food safety. Without reliable data, it is not possible to draw conclusions from a risk assessment.

<u>Composition:</u>
The results of two field trials (conducted in 1999 and 2007) were the basis for the comparative assessment. However, it should be noted that both of these trials were conducted over one season only. In order to investigate possible genome x environment interactions, these field trials should have been conducted in different climatic regions and over more than one season.

<u>Composition of MON 531</u>
According to EFSA, significant differences to the parental line were already observed in MON 531. Accordingly, during field trials conducted in 1992 and 1993,

> *"significant differences in cottonseeds were observed for myristic acid, stearic acid and oleic acid (1992), glutamic acid, valine, methionine, isoleucine, tyrosine, lysine and histidine (1993) and total fat, carbohydrates, palmitic acid, linoleic acid, calcium and iron (1999). However, these differences were not consistent and were found for only some growing seasons."*

<u>Composition of MON 15985</u>
In field trials in 1999, significant differences were found in different compounds like

> *"dihydrosterculic acid, calcium and the fatty acids myristic acid, stearic acid and arachidic acid, as well as decreased levels of gossypol (free and total), the fatty acids palmitic acid and linoleic acid, copper, iron, phosphorus and potassium".*

In field trials in 2007, significant differences were found in different compounds like

> *"myristic acid, palmitoleic acid and α-tocopherol, as well as decreased levels of palmitic acid".*

Considering the incomplete data regarding phenotypic changes in MON 15985, the different levels of several compounds such as the increased levels of myristic acid, or the decreased levels of palmitic acid observed in both field trials in 1999 and 2007, should have been assessed in more detail.

Food/feed safety assessment

Effects of processing

Data presented by the applicant was considered as not acceptable, yet instead of asking for valid data, the GMO panel simply states

> *"that the effect of processing on cotton MON 15985 is not expected to be different from the effect on conventional cotton varieties".*

Subchronic toxicity

The applicant provided a 90-day study in rats. Cotton MON 15985 was included in levels of 2% and 5% and six groups were fed conventional cottonseed (5% inclusion level). According to EFSA:

> *"Several significant differences were observed between the test and the control group in haematology, clinical chemistry and urine analyses. These differences were not dose related, occurred at only one time point and in one sex and/or fell within the range of reference groups. No significant differences in absolute and relative organ weights were observed. Macroscopic examination and histopathology of selected tissues and organs revealed no test-substance-related changes.*

Testbiotech believes that the inclusion levels used by Monsanto are too low for a toxicological study.

With regard to the possible toxicity of cotton MON 15985, EFSA correctly points to the fact that Bt toxin Cry1Ac enhances immune reactions (Vázquez-Padrón et al., 1999 and 2000) and is used as an adjuvant in medicine. Cry1Ac is also a Bt toxin, known for its synergistic effects with other Bt toxins (Sharma et al., 2010). As cotton MON15985 is a stacked event producing two different Cry toxins, these specific characteristics should have been investigated in much more detail. EFSA simply states that, as in their previous risk assessments, they could not detect any adjuvant effects. However, since no clear guidance is given on how to assess these effects and no systematic testing has been performed, the opinions of EFSA have to be regarded as arbitrary and inconclusive.

Nutritional assessment

According to EFSA:

> *"The nutritional similarity of cotton MON 15985 to commercial non-GM cotton varieties, indicated by compositional data, was corroborated by a study with MON 15985 in catfish and a number of published feeding studies with this cotton in dairy cattle (Castillo et al., 2004), chickens (Mandal et al., 2004) and quails (Hamilton et al., 2004)."*

Apart from an analysis of the catfish study, EFSA abstains from scrutinising the data presented by the applicant.

Environmental risk assessment

Comments from some Member States' experts have highlighted that some plant species in Europe can cross with cotton and cotton is grown in several regions. As spillage from cotton seeds is likely to occur, concerns that transgenes might be distributed in the environment were raised by experts from EU Member States, such as Spain, where cotton is grown commercially. 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 has occurred. Thus the risk for contamination and uncontrolled spread of the transgenes seems to be much more relevant than EFSA assumes.

Testbiotech agrees with the comments of several Member States that spillage, persistence and invasiveness are relevant risks in certain countries. No viable seed should be imported into countries or regions where cotton plants can survive and spread into the environment, such as Italy, Greece and Spain.

Further, cotton MON 531 includes two bacterial antibiotic resistance genes and other sequences of bacterial origin, which may allow double homologous recombination to plasmid sequences present in the environment.

EFSA states:

> *"AAD protein was not detected in any of the samples analysed since the aadA gene is under the control of a prokaryotic promoter."*

Regarding the presence of antibiotic resistance genes in cotton 15985, Testbiotech supports the comments of several Member States' experts who gave their opinion on the application and pointed to the fact that antibiotic resistance genes should not be used anymore. The aadA-gene belongs to group II of antibiotic resistance genes, which should be restricted to field trial purposes and should not be present in genetically modified plants to be placed on the market.

Other

As a legal dossier compiled by Professor Ludwig Kraemer shows, EU regulations require the monitoring of effects on health at the stage of consumption. Directive 2001/18 and Regulation

1829/2003 both require that potential adverse effects on human health from genetically modified plants are monitored during use and consumption stage. Therefore, EFSA's 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.

Conclusion

In the light of the substantial lack of data and major gaps in the risk assessment highlighted, no market authorisation can be given.

References

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