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Assessment of genetically engineered cotton COT102 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2017-141) by Syngenta

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Introduction

Cotton COT102 expresses Vip3Aa19 (derived from the native Vip3Aa1 protein found in Bacillus thuringiensis strain AB88). The Vip3Aa19 protein has insecticidal activity against several lepidopteran species, including cotton bollworm and fall armyworm. Cotton COT102 also expresses the APH4 protein, which is used as an antibiotic resistance marker gene (ARMG), thus inactivating the antibiotic activity of hygromycin B.

1. Systematic literature review

A literature review as requested in Regulation (EU) No 503/2013 was provided by the applicant. It shows that despite the transgenic cotton being developed in 2007, there is no independent research data available in regard to health or environmental risks. The Vip3Aa protein is also produced in other transgenic plants (such as maize MIR162), and it would, therefore, have been necessary to widen the systematic review to include other events with similar gene constructs.

2. Molecular characterisation

The genetic engineering process for this event resulted in several open reading frames, which were investigated only in regard to proteins with known toxicity or allergenicity. Other potential biologically active gene products were not taken into account. Thus, uncertainties remain in relation to unintended biologically active substances arising from the process of genetic engineering and the newly introduced gene constructs.

Gene expression under stress conditions

There is a great deal of evidence that genetic background, soil and climate conditions all substantially impact Bt toxin 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 newly expressed proteins 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, as it is known that the genetic background of the varieties can influence the level of gene expression.

Expression of the additional proteins was, nevertheless, only measured under field conditions in four locations in the US for one year (2018), with no exceptional weather conditions being reported. It is evident that these conditions are not representative of all growing conditions, e. g. in 2023, when the temperatures were much higher due to ongoing climate change. Therefore, new data on gene expression need to be made available. Data should also cover a much broader range of defined biotic and abiotic stressors to demonstrate stability in gene expression under sufficiently realistic conditions.

Consequently, the GE cotton plants tested in field trials do not sufficiently represent the products intended for import. The data presented by the applicant are insufficient to conclude on the impact environmental factors may have on gene expression, as laid down in EU Regulation 503/2013.

3. Comparative assessment of plant composition as well as the agronomic and phenotypic characteristics

Implementing Regulation 503/2013 requests:
"*The different sites selected for the field trials shall reflect the different meteorological and agronomic conditions under which the crop is to be grown; the choice shall be explicitly justified. The choice of non-genetically modified reference varieties shall be appropriate for the chosen sites and shall be justified explicitly.*"

The data presented by Syngenta do not meet the requirements of Implementing Regulation 503/2013: (1) the field trials were not conducted in all relevant regions where the GE cotton will be cultivated, and no specific weather conditions were taken into account; (2) the field trials did not take all relevant agricultural management practices into account; (3) not all relevant genetic backgrounds were taken into account.

Data on environmental factors and stress conditions - and their impact on plant composition and phenotype
Field trials to assess plant composition as well as the agronomic and phenotypic characteristics of the GE cotton, were only conducted in the US for one year (2018) at 10 sites, with no exceptional weather conditions being reported apart from a thunderstorm. It is evident that these weather conditions are not representative of all growing conditions, for example, in 2023, when temperatures were much higher due to ongoing climate change. Therefore, new data have to be made available on gene expression, covering a much broader range of defined biotic and abiotic stressors to demonstrate stability in gene expression under sufficiently realistic conditions.

In order to assess changes in plant composition and phenotypic characteristics, the plants should have been grown in various environmental conditions and exposed to well-defined environmental stress conditions. Furthermore, it should also should have been noted that the vip3 gene appears to have the potential to induce unexpected metabolic changes, as observed in maize. These effects are influenced by environmental conditions (patent EP 3632202).

No experiments were requested to show to which extent specific environmental conditions influence

plant composition and agronomic characteristics. Hence, no data were made available, as laid down in Implementing regulation 503/2013, to assess whether the expected environmental conditions where the plants are likely to be cultivated will influence the expression of the studied endpoints.

Assessment of plant composition as well as the phenotypic and agronomic characteristics
For plant composition: statistical analysis was applied to a total of 44 constituents in seeds. Significant differences with the non-GE comparator were found for 12 endpoints.

For agronomic characteristics: the statistical analysis was applied to only 9 endpoints, with 4 criteria showing significant differences.

Given the above findings on the impact of environmental factors and genetic backgrounds as well as the number of significant findings, EFSA should have requested more data: data on agronomic and phenotypic endpoints should be generated from a wider range of clearly defined stress factors, including all relevant agricultural practices and genetic backgrounds. It should have been noted that the vip3 gene appears to induce pleiotropic effects, causing unexpected metabolic changes (at least in maize), with these effects being influenced by environmental conditions (patent EP 3632202).

Therefore, a more detailed analysis should have been carried out to investigate changes in plant composition and phenotype, and also to investigate potential unintended changes in metabolic pathways and the emergence of unintended biologically active gene products.

In summary, the GE cotton plants tested in the field trials do not sufficiently represent the products intended for import. Due to the absence of independent data on this cotton, we strongly recommend establishing a system with independent controls to repeat the trials, and also to double check the data on plant composition and agronomic characteristics.

4. Toxicity

Implementing Regulation 503/2013 requests:
"*Toxicological assessment shall be performed in order to:*
(a) demonstrate that the intended effect(s) of the genetic modification has no adverse effects on human and animal health;
(b) demonstrate that unintended effect(s) of the genetic modification(s) identified or assumed to have occurred based on the preceding comparative molecular, compositional or phenotypic analyses, have no adverse effects on human and animal health;"
"*In accordance with the requirements of Articles 4 and 16 of Regulation (EC) No 1829/2003, the applicant shall ensure that the final risk characterisation clearly demonstrates that:*
(a) the genetically modified food and feed has no adverse effects on human and animal health;"

A 90-day feeding study was provided by the applicant, with only a 3% and a 10% cotton seed content in the diet. The material was not tested for contamination with other GE traits and/ or pesticides. Therefore, the data appear to be insufficient to conclude on food safety.

Negative impacts of Bt toxins on human and animal health cannot be excluded *a priori*. Bt toxins have several modes of action. The biological characteristics of the Bt toxins produced in the plants are altered and not identical to their natural templates. There is very little data available in regard to the exact mode of action of Vip3Aa19, thus adding to uncertainty in regard to food and feed safety.

Feedings studies with the isolated proteins were performed. However, these feeding studies suffer from a lack of realistic conditions, e. g. the combination of the proteins with proteinase inhibitors (PI) produced by the plants, which can strongly enhance the toxicity of Bt toxins: it is likely that PIs delay the degradation of Bt proteins, and thus also enhance their toxicity. Monsanto showed in the 1990s that maize, cotton and soybeans produce protease inhibitors (PIs), which considerably enhance the toxicity of Bt proteins in plants. In the presence of PIs, Bt toxin will degrade much more slowly than in isolation. This results in a much higher toxicity of the Bt toxin (if it is taken up together with the plant tissue) compared to the isolated toxin (MacIntosh et al., 1990; Zhao et al., 1999; Zhang et al., 2000; Gujar et al., 2004; Zhu et al., 2007; Pardo-López et al., 2009; Ma et al., 2013; Mesén-Porras et al., 2020). The described effects indicate, for example, a 20-fold higher toxicity of Bt proteins if produced in the plants and taken up with PIs (MacIntosh et al., 1990). Differences in toxicity between toxins produced in isolation compared to those produced by the plants are also described for Vip3A efficacy in transgenic plants (Khan et al., 2020).

Therefore, any risk assessment that does not take synergistic effects caused by the combination of plant material or other stressors with the Bt toxins into account, is not reliable and systematically underestimates the risks. This finding is also highly relevant for the assessment of allergenicity and other immune responses as well as for any potential impact on the intestinal microbiome.

In conclusion, the EFSA opinion on the application for authorisation of the transgenic cotton cannot be said to fulfill the requirements for assessment in regard to toxicity and allergenicity.

5. Environmental risk assessment

According to EFSA (2023a), in Europe, gene flow from feral COT102 plants (after spillage) could occur to *G. hirsutum*, *G. barbadense* and *G. herbaceum* cotton plants (OECD, 2008; see also Montes et al., 2017). However, EFSA assumes the potential effects to be minimal and transient. Furthermore, EFSA assumes that the likelihood of environmental effects as a consequence of the spread of genes from occasional feral GE cotton plants in Europe, is no different to that of conventional cotton varieties.

There was no request for empirical data in regard to hybrid offspring with other cotton plants. Therefore, the EFSA opinion is not sufficiently backed by science: the characteristics of potential hybrids and next generations need to be investigated, and cannot simply be predicted from the data of the original event. It is well known that there can be next generation effects, including interference from the genetic background, that cannot be predicted from the assessment of the original event (Bauer-Panskus et al., 2020). Special attention should be paid to Vázquez-Barrios et al., (2021), who showed unexpected effects in hybrids between transgenic cotton and wild populations, thus indicating invasive characteristics. It is important to note that these hybrids appear

to have occurred from gene flow of transgenic cotton, without commercial cultivation or experimental releases being involved.

EFSA should have requested data from the applicant to show that no adverse effects can occur through gene flow to other cotton plants. In the absence of such data, the risk assessment cannot be regarded as valid. Consequently, the EFSA environmental risk assessment is not acceptable.

Furthermore, empirical data should have also been requested in regard to effects caused by the transfer of antibiotic resistance genes to bacteria in the soil and the gut. There is some likelihood of the gene sequence inherited by the cotton being taken up by bacteria (see the comments made by experts from member states, EFSA 2023b), so that the effects on the bacterial community may go beyond its potential effects on resistance to specific antibiotics. Therefore, the EFSA assessment is inadequate.

It should, furthermore, not be overlooked that EU GMO regulation includes a request to generally avoid the usage of antibiotic marker genes in GE plants. Commission Implementing Regulation (EC) 503/13 states that “the applicant shall endeavour to minimise the presence of inserted nucleic acid(s) sequences not essential to achieve the desired trait” and that “the applicant shall therefore aim to develop GMOs
without the use of antibiotic resistance marker genes” (see also comments made by experts from member states). Therefore, EFSA should have given much more weight to this issue.

6. Others

Monitoring
If approval for import is granted, the applicant must ensure the development of post-market monitoring (PMM) to collect reliable information regarding the detection of any indications showing that (adverse) effects on health may be related to the consumption of GM food or feed. Thus, the monitoring report should at very least contain detailed information on: i) actual volumes of the GE products imported into the EU; ii) the ports and silos where shipments of the GE products were unloaded; iii) the processing plants where the GE products was transferred to; iv) the amount of the GE products used on farms for feed and v) transport routes of the GE products. Environmental monitoring should be run in regions where viable material of the GE products, such as seeds, are transported, stored, packaged, processed or used for food/feed. If there are losses or spread of viable seeds, all receiving environments need to be monitored. Furthermore, environmental exposure through organic waste material, by-products, sewage or faeces containing GE products during or after the production process should be part of the monitoring procedure (see also comments made by experts from member states, EFSA, 2023b).

General aspects
A Testbiotech report published in 2021 (Testbiotech, 2021) shows how the European Food Safety Authority (EFSA) intentionally sets aside crucial issues. This careless approach exemplifies the overall decrease in general food safety standards that has been ongoing since the introduction of GE plants. At the same time, the number of events authorised for import has steadily increased.

The Testbiotech report published in 2021 provides evidence that the genetic engineering of food plants has layers of complexity that go far beyond what can be assessed by current standards of risk assessment. The safety of the plants is claimed on the basis of approval processes that only consider risks that are easiest to assess. It shows that, since the introduction of the first transgenic plants into the food chain, uncertainties in regard to safety have been steadily increasing, and that risks may have accumulated unnoticed. Overall, the safety of food products has been decreasing while, at the same time, EFSA and the Commission were unable to present sufficiently robust criteria and methods to significantly improve health safety. Instead, specific areas of risk assessment have been intentionally ignored from the beginning.

In light of these findings, the Commission should try to avoid 'rubber stamping' all applications for the import of GE plants, and thus reduce the overall number of products entering the market. They must also ensure that all these products undergo much more thorough risk assessment.

In addition, the Commission should recognise that the lack of independent risk research data for most genetically engineered plants with pending marketing applications is a problem, including in the present case. Independent risk research, as laid down in Directive 2001/18, is completely absent.

In response, the Commission and member states should start an initiative to introduce comprehensive, systematic and independent research that follows the perspective of the precautionary principle, and the protection of health and the environment. It is time to end this leap into the dark and organise research that serves the interests of the public as well as the protection of health and the environment.

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