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SCIENTIFIC OPINION

# Assessment of genetically modified cotton T304-40 × GHB119 × COT102 (application EFSA-GMO-BE-2018-155)

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts.

## Abstract

Genetically modified cotton T304-40 × GHB119 × COT102 was developed by crossing to combine three single events: T304-40, GHB119 and COT102. The three-event stack cotton expresses Cry1Ab, Cry2Ae, Vip3Aa19 and PAT/bar to confer herbicide tolerance and insect resistance. Furthermore, event COT102 expresses the antimicrobial APH4 protein used during its molecular development. The GMO Panel previously assessed the three single cotton events and did not identify safety concerns. Since then, no new data on the single cotton events were identified that would require modification of the original conclusions on their safety. The molecular characterisation, comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological, allergenicity and nutritional assessment does not indicate interactions which would give rise to new food and feed safety and nutritional concerns. The GMO Panel concludes that three-event stack cotton, as described in this application, is as safe as its non-GM comparator and non-GM cotton varieties tested, and no post-market monitoring of food/feed is considered necessary. In the case of release of processed cotton T304-40 × GHB119 × COT102 or accidental spillage of viable GM cotton seeds, this would not raise environmental safety concerns. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of cotton T304-40 × GHB119 × COT102. The GMO Panel concludes that three-event stack cotton is as safe as its non-GM comparator and the tested non-GM cotton varieties with respect to potential effects on human and animal health and the environment.

**KEYWORDS**

COT102, cotton (*Gossypium hirsutum*, *Gossypium barbadense*), genetic engineering, GHB119, GM, import and processing, T304-40

# CONTENTS

## SUMMARY

Following the submission of application EFSA-GMO-BE-2018-155 under Regulation (EC) No 1829/2003 from BASF Agricultural Solutions (referred to hereafter as 'the applicant'), the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') was asked to deliver a scientific opinion on the safety of genetically modified (GM) herbicide tolerant and insect resistant cotton (*Gossypium hirsutum and Gossypium barbadense*) T304-40 × GHB119 × COT102 (referred to hereafter as 'three-event stack cotton'). The scope of application EFSA-GMO-BE-2018-155 is for import, processing, and food and feed uses within the European Union (EU) of the three-event stack cotton, and does not include cultivation in the EU. The three-event stack cotton was produced by crossing to combine three single cotton events: T304-40 expressing Cry1Ab to confer resistance to lepidopteran pests and PAT/bar to confer tolerance to glufosinate-ammonium-based herbicides, GHB119 expressing Cry2Ae to confer resistance to lepidopteran pests and PAT/bar to confer tolerance to glufosinate-ammonium-based herbicides, and COT102 expressing Vip3Aa19 to confer resistance to lepidopteran pests. There is also expression of APH4 which was used as selectable marker (hygromycin resistance) used during the molecular development of this event.

The GMO Panel evaluated the three-event stack cotton with reference to the scope and appropriate principles described in its applicable guidelines for the risk assessment of GM plants and the post-market environmental monitoring (PMEM). The GMO Panel considered the information submitted in application EFSA-GMO-BE-2018-155, additional information provided by the applicant during the risk assessment, the scientific comments submitted by the Member States and the relevant scientific literature. For application EFSA-GMO-BE-2018-155, previous assessments of the three single events (T304-40, GHB119 and COT102), provided a basis for the assessment of the three-event stack cotton. No safety concerns were identified by the GMO Panel in their previous assessments. No safety issue concerning the three single cotton events was identified by the updated bioinformatic analyses, nor reported by the applicant since the publication of the previous GMO Panel scientific opinions. Therefore, the GMO Panel considers that its previous conclusions on the safety of the single cotton events remain valid.

For the three-event stack cotton, the risk assessment included the molecular characterisation of the inserted DNA and analysis of protein expression. An evaluation of the comparative analysis of agronomic, phenotypic and compositional characteristics was carried out, and the safety of the newly expressed proteins and the whole food and feed were evaluated with respect to potential toxicity, allergenicity and nutritional characteristics. Environmental impacts and a PMEM plan provided by the applicant were also evaluated. The molecular characterisation data establish that the events T304-40, GHB119 and COT102 combined in the three-event stack cotton have retained their integrity. Protein expression analysis showed that the levels of the newly expressed proteins are similar in the three-event stack cotton and in the single events.

The selection of test materials, the field trial sites and the associated management practices and the agronomic-phenotypic characterisation as an indicator of the overall field trial quality was considered by the GMO Panel with the conclusion that the field trials are appropriate to support the comparative analysis. The comparative analysis of agronomic and phenotypic characteristics and seeds composition identified no differences between cotton T304-40 × GHB119 × COT102 and the non-GM comparator (referred to hereafter as comparator) that required further assessment except for the changes in length uniformity and percentage (%) of fruit retention, which are further assessed in Section 3.4.4.1. These changes raised no concern when further assessed for food/feed safety and environmental impact. The molecular characterisation, the comparative analysis and the outcome of the toxicological, allergenicity and nutritional assessment indicate that the combination of the single cotton events and of the newly expressed proteins in the three-event stack cotton does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that cotton T304-40 × GHB119 × COT102, is as safe as the comparator and the selected commercial non-GM cotton reference varieties (referred to hereafter as non-GM reference varieties). Considering the combination of the single events and their potential interactions, the outcome of the comparative analysis, and the routes and levels of exposure, the GMO Panel concludes that cotton T304-40 × GHB119 × COT102 would not raise safety concerns in the case of the release of GM cotton material, including viable seeds, into the environment.

The GMO Panel considers that post-market monitoring (PMM) of cotton T304-40 × GHB119 × COT102 is not necessary. The PMEM plan submitted and the suggested reporting intervals are in line with the intended uses of the three-event stack cotton.

Based on the relevant publications identified through the literature searches, the GMO Panel does not identify any safety issue pertaining to the intended uses of cotton T304-40 × GHB119 × COT102.

Considering the close genetic relationship and extensive bidirectional introgression between *G. hirsutum* and *G. barbadense*, along with the fact that the inserted traits, driven by well-characterised regulatory elements ensuring stable expression, are not expected to affect species-specific metabolic pathways, the GMO panel considers that the information collected in application EFSA-GMO-BE-2018-155 allows, for this specific application, to conclude on the safety of cotton T304-40 × GHB119 × COT102 in *G. hirsutum* and *G. barbadense*.

The GMO Panel concludes that cotton T304-40 × GHB119 × COT102, as described in this application, is as safe as its comparator and the selected non-GM reference varieties with respect to potential effects on human and animal health and the environment. However, in accordance with the previous assessment of the single event COT102, the GMO Panel considers that the risk assessment may need to be updated in case products containing hygromycin B or other substrates of the APH4 enzyme obtain future market approval in the EU.

# 1 | INTRODUCTION

The scope of the application EFSA-GMO-BE-2018-155 is for food and feed uses, import and processing of the genetically modified (GM) herbicide tolerant and insect resistant cotton T304-40×GHB119×COT102.

To obtain cotton stack T304-40×GHB119×COT102, the three single events in *Gossypium hirsutum* L. were combined by conventional crosses; however, the scope of applications EFSA-GMO-BE-2018-155 also covers *G. barbadense*. *G. hirsutum* and *G. barbadense* are genetically close cotton species which have been sympatric over the last several millennia leading to pervasive genome-wide bidirectional introgression (Yuan et al., 2021). In addition, intentional interspecific hybridisation has occurred many times in both directions, with the goal of producing commercial varieties that possess the traits of both *G. hirsutum* and *G. barbadense* species (Jareczek et al., 2023).

## 1.1 | Background

On 12th October 2018, the European Food Safety Authority (EFSA) received from the Belgian Competent Authority application EFSA-GMO-BE-2018-155 for authorisation of cotton T304-40×GHB119×COT102 (Unique Identifier BCS-GHØØ4-7×BCS-GHØØ5-8×SYN-IR1Ø2–7), submitted by BASF Agricultural Solutions (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003.[1] Following receipt of application EFSA-GMO-BE-2018-155, EFSA informed EU Member States and the European Commission (EC), and made the application available to them. Simultaneously, EFSA published a summary of the application.[2]

EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003 and Regulation (EU) No 503/2013,[3] with the EFSA guidance documents, and, asked the applicant to supplement the initial application, when needed. On 8th July 2020, EFSA declared the application valid.

From validity date, EFSA and the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application EFSA-GMO-BE-2018-155. This time limit was extended whenever EFSA and/or GMO Panel requested supplementary information to the applicant. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicant during the risk assessment was made available to the EU Member States and European Commission (for further details, see the section 'Documentation', below). In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC.[4] The EU Member States had 3 months to make their opinion known on application EFSA-GMO-BE-2018-155 as of date of validity.

## 1.2 | Terms of Reference as provided by the requestor

According to Articles 6 and 18 of Regulation (EC) No 1829/2003, EFSA and its GMO Panel were requested to carry out a scientific risk assessment of cotton T304-40×GHB119×COT102 in the context of its scope as defined in application EFSA-GMO-BE-2018-155.

According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the EFSA overall opinion in accordance with Articles 6(5) and 18(5). In addition to the present scientific opinion, EFSA was also asked to report on the particulars listed under Articles 6(5) and 18(5) of Regulation (EC) No 1829/2003, but not to give an opinion on them because they pertain to risk management.[5]

# 2 | DATA AND METHODOLOGIES

## 2.1 | Data

The GMO Panel based its scientific assessment of three-event stack cotton on the valid application EFSA-GMO-BE-2018-155, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by EU Member States and relevant peer-reviewed scientific publications. As part of this comprehensive information package, the GMO Panel received additional unpublished studies submitted by the applicant in order to comply with the specific provisions of Regulation (EU) No 503/2013. A list of these additional unpublished studies is provided in Appendix A.

[1]Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.

[2]Available online: https://open.efsa.europa.eu/questions/EFSA-Q-2018-00809?search=EFSA-Q-2018-00809.

[3]Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L157, 8.6.2013, p. 1–48.

[4]Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.

[5]These particulars are available online at: https://open.efsa.europa.eu/study-inventory/EFSA-Q-2018-00809.

## 2.2 | Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 1829/2003, the applicable guidelines (i.e. EFSA GMO Panel, 2010a, 2011a, 2011b, 2015) and explanatory notes and statements (i.e. EFSA, 2010, 2017a, 2017b, 2019a, 2019b; EFSA GMO Panel, 2010b) for the risk assessment of GM plants.

For this application, in the context of the contracts OC/EFSA/GMO/2018/04 and OC/EFSA/GMO/2021/06 the contractors performed preparatory work for the evaluation of the applicant's literature search and bioinformatics analyses on cotton T304-40 × GHB119 × COT102.

# 3 | ASSESSMENT

## 3.1 | Introduction

Cotton T304-40 × GHB119 × COT102 was produced by crossing to combine three single cotton events: T304-40 expressing Cry1Ab and PAT/bar conferring resistance to lepidopteron pests and tolerance to glufosinate-ammonium-based containing herbicides, GHB119 expressing PAT/bar and Cry2Ae conferring tolerance to glufosinate-ammonium-based containing herbicides and resistance to lepidopteran pests, and COT102 expressing APH4 and Vip3Aa19 conferring resistance to hygromycin B (used as a selectable marker during its molecular development) and lepidopteran pests.

All three single events were assessed previously (Table 1) and no safety concerns for human and animal health or environmental safety were identified.

**TABLE 1** Single cotton events previously assessed by the GMO Panel and publication date of the corresponding EFSA scientific opinions.

| Events | Application or mandate | Reference |
|---|---|---|
| T304-40 | EFSA-GMO-NL-2011-97 | EFSA GMO Panel (2013) |
| | GMFF-2024-23010 | EFSA GMO Panel (2025) |
| GHB119 | EFSA-GMO-NL-2011-96 | EFSA GMO Panel (2016) |
| COT102 | EFSA-GMO-DE-2017-141 | EFSA GMO Panel (2023a, 2023b) |

## 3.2 | Updated information on single events[6]

Since publication of the scientific opinions on the single cotton events by the GMO Panel (Table 1), no safety issue concerning the three single events has been reported by the applicant.

Updated bioinformatic analyses of the junction regions for cotton events T304-40, GHB119 and COT102, using up-to-date sequence databases and methodology specified in EFSA guidance (EFSA GMO Panel, 2011a), confirmed that no known endogenous genes were disrupted by any of the inserts.

Updated bioinformatic analyses of the amino acid sequence of the newly expressed PAT/bar, Cry1Ab, Cry2Ae, VIP3Aa19 and APH4 proteins confirmed previous results indicating no significant similarities to known toxins and allergens. Updated bioinformatic analyses of the newly created open reading frames (ORFs) within the inserts or spanning the junctions between the insert and the flanking regions for events T304-40, GHB119 and COT102 indicated that the production of new peptides showing significant similarities to toxins or allergens for any of the events in cotton T304-40 × GHB119 × COT102 is highly unlikely, which confirmed previous analyses (Table 1).

In order to update the bioinformatic analyses to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis with microbial DNA for cotton events T304-40, GHB119 and COT102. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.4.4.2.

Based on the above information, the GMO Panel considers that its previous conclusions on the safety of the single cotton events remain valid. In accordance with the previous assessment of the single event COT102, the GMO Panel considers that the risk assessment of cotton T304-40 × GHB119 × COT102 may need to be updated in case products containing hygromycin B, or other substrates of the APH4 enzyme, for therapeutic, prophylactic or any other medical uses in humans or animals obtain future market approval in the EU.

[6]Additional information: 23/2/2024, 12/9/2024, 3/9/2025.

## 3.3 | Systematic literature review[7]

The GMO Panel assessed the applicant's literature searches on cotton T304-40 × GHB119 × COT102, which include a scoping review, according to the guidelines given in EFSA (2010, 2019b).

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application EFSA-GMO-BE-2018-155. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for cotton T304-40 × GHB119 × COT102 at present.

The GMO Panel considered the overall quality of the performed literature searches acceptable. The literature searches identified 32 relevant publications on cotton T304-40 × GHB119 × COT102 from online bibliographic databases. The relevant publications are listed in Appendix B.

None of the relevant publications identified through the literature searches reported information pointing to safety issues associated with cotton T304-40 × GHB119 × COT102 relevant to the scope of this application.

## 3.4 | Risk assessment of the three-event stack cotton T304-40 × GHB119 × COT102

### 3.4.1 | Molecular characterisation[8]

In line with the requirements laid down by Regulation (EU) 503/2013, the possible impact of the combination of the events on the integrity of the events, the expression levels of the newly expressed proteins or the biological functions conferred by the individual inserts are considered below.

#### 3.4.1.1 | *Genetic elements and biological function of the inserts*

Cotton events T304-40, GHB119 and COT102 were combined by conventional crossing to produce the three-event stack cotton T304-40 × GHB119 × COT102. The structure of the inserts introduced into the three-event stack cotton is described in detail in the respective EFSA scientific opinions (Table 1) and no new genetic modifications were involved. Genetic elements in the expression cassettes of the single events are summarised in Table 2.

Intended effects of the inserts in cotton T304-40 × GHB119 × COT102 are summarised in Table 3.

Based on the known biological function of the newly expressed proteins (Table 3), the only foreseen interactions identified by the GMO panel at the biological level are between the Cry proteins or between the Vip3Aa19 and the Cry proteins in susceptible insects, which will be dealt with in Sections 3.4.4. Furthermore, the potential for a functional interaction between the newly expressed proteins with impact on the safety of cotton T304-40 × GHB119 × COT102 for humans and animals is addressed in Section 3.4.3.1.1.

**TABLE 2** Genetic elements in the expression cassettes of the events stacked in cotton T304-40 × GHB119 × COT102.

| Event | Promoter | 5′ UTR | Transit peptide | Coding region | Terminator |
|---|---|---|---|---|---|
| T304-40 | Ps7s7 (*Subterranean clover stunt virus* genome segment 7) | Tapetum-specific E1 (5′ *e1* from *Oryza sativa*) | – | *cry1Ab* (*Bacillus thuringiensis* subsp. *berliner* 1715) | 3′ NADP-me1 (*Flaveria bidentis*) |
| | 35S (*Cauliflower mosaic virus* P35S3) | – | – | *bar* (*Streptomyces hygroscopicus*) | 3′ nos (*Agrobacterium tumefaciens*) |
| GHB119 | 35S (*Cauliflower mosaic virus*) | 5′ cab22L (*Petunia hybrida*) | TPssuAt (*ats1A* gene of *A. thaliana*) | *cry2Ae* (*Bacillus thuringiensis* subsp. *dakota* 1715) | 3′ 35S (*Cauliflower mosaic virus*) |
| | PCsVMX XYZ (*Cassava Vein Mosaic Virus*) | – | – | *bar* (*Streptomyces hygroscopicus* strain ATCC21705) | 3′ nos (*A. tumefaciens*) |
| COT102 | Ubq3 (*A. thaliana*) | – | – | *aph4* (*Escherichia coli*) | NOS (*A. tumefaciens*) |
| | Act2 (*A. thaliana*) | – | – | *vip3Aa19** (*B. thuringiensis* strain AB88) | NOS (*A. tumefaciens*) |

–, when no element was specifically introduced to optimise expression.

*Codon optimised.

[7]Additional information: 29/5/2024, 31/7/2024, 12/9/2024, 30/9/2025.

[8]Dossier: Part II – Section 1.2; additional information: 14/7/2023, 7/11/2023, 12/4/2024, 12/9/2024, 18/4/2025, 3/9/2025.

**TABLE 3** Characteristics and intended effects of the events stacked in cotton T304-40×GHB119×COT102.

| Event | Protein | Donor organism and biological function | Intended effects in GM plant |
|---|---|---|---|
| T304-40 | Cry1Ab | Based on genes from *Bacillus thuringiensis*, subsp. *berliner* 1715, Cry1Ab confers resistance to insect pests of the lepidopteran family; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998). | Cotton T304-40 expresses a chimeric, truncated cry1Ab gene. Cry1Ab is a chimeric protein toxic to certain lepidopteran larvae feeding on cotton. |
| | PAT/bar | Based on the bar gene from *Streptomyces hygroscopicus*, Phosphinothricin-acetyltransferase (PAT) enzyme confers resistance to the antibiotic bialaphos (Eckes et al., 1989) | Cotton T304-40 expresses the PAT/bar protein, which acetylates L-glufosinate-ammonium and thereby confers tolerance to glufosinate ammonium-based herbicides. |
| GHB119 | Cry2Ae | Based on genes from *Bacillus thuringiensis* subsp. *dakota* 1715, Cry1Ae confers resistance to insect pests of the lepidopteran family; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998). | Cotton GHB119 expresses a cry2Ae gene. Cry2Ae is a chimeric protein toxic to certain lepidopteran larvae feeding on cotton. |
| | PAT/bar | Based on the bar gene from *Streptomyces hygroscopicus* strain ATCC21705, Phosphinothricin-acetyl-transferase (PAT) enzyme confers resistance to the antibiotic bialaphos (Eckes et al., 1989). | Cotton GHB119 expresses the PAT/bar protein, which acetylates L-glufosinate-ammonium and thereby confers tolerance to glufosinate-ammonium-based herbicides |
| COT102 | APH4 | Based on gene *aph4* from *E. coli* strain K-12, phosphotransferase enzyme which catalyses the phosphorylation of hygromycin and some related aminoglycosides (Waldron, 1997). | Cotton COT102 expresses the APH4 protein, which phosphorylates hygromycin. The expression of APH4 allows growth and thereby enables selection of the transformed cells in the presence of hygromycin. |
| | Vip3Aa19 | Based on a gene from *B. thuringiensis* strain AB88 (Estruch et al., 1996). In addition to Cry proteins, *B. thuringiensis* also produces insecticidal proteins during its vegetative growth stage. These are referred to as vegetative insecticidal proteins (Vip) (Fang et al., 2007). | Cotton COT102 expresses a modified version of the *B. thuringiensis vip3Aa1* gene, and encodes Vip3Aa19, a protein toxic to certain lepidopteran larvae feeding on cotton. |

#### 3.4.1.2 | *Integrity of the events in the three-event stack*

The genetic stability of the inserted DNA over multiple generations in the single cotton events T304-40, GHB119 and COT102 was previously demonstrated (Table 1 and Section 3.2) by Southern analyses and by PCR followed by DNA analysis demonstrating that the sequences of the events (inserts and their flanking regions) in the three-event cotton stack are identical to those already assessed (Table 1 and Section 3.2), thus confirming that the integrity of these events was maintained in the three-event stack cotton.

#### 3.4.1.3 | *Information on the expression of the insert*

Protein levels of Cry1Ab, Cry2Ae, PAT/bar, APH4 and Vip3Aa19 were analysed by an enzyme-linked immunosorbent assay (ELISA) in material harvested in field trials across four locations in Argentina and Brazil during the 2015/2016 and 2016/2017 growing seasons, respectively. Samples analysed included leaves (BBCH 14–16, BBCH 51–55, BBCH 60–67), roots (BBCH 14–16), pre-candle square (BBCH 60–67), immature boll (BBCH 60–67), pollen (BBCH 60–69), whole plant (BBCH 60–67) and fuzzy seeds (BBCH 97) from plants treated and not treated with glufosinate-ammonium-based herbicide. Since seeds and pollen are the main raw commodities used for food and feed purposes, their protein levels are summarised in Appendix C.

In order to assess the changes in protein expression levels which may result from interactions between the events, protein levels were determined for the three-event stack and the corresponding single events in different parts of the plant.

The levels of all the newly expressed proteins in the three-event stack cotton and the corresponding singles were comparable in all tissues, except for the higher PAT/bar protein level in the stack, which was expected because of the combination of events T304-40 and GHB119, both producing PAT/bar protein in the three-event stack cotton (Appendix C). Therefore, there is no indication of an interaction that may impact on the levels of the newly expressed proteins in this stack.

#### 3.4.1.4 | *Conclusion on molecular characterisation*

The molecular data establish that the events stacked in cotton T304-40×GHB119×COT102 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the three-event stack and in the single events, except for the higher PAT/bar protein level in the stack, which was expected because of the combination of events T304-40 and GHB119, both producing PAT/bar protein in the three-event stack cotton.

Based on the known biological function (Table 3) of the newly expressed proteins, the only foreseen interactions at the biological level are between the Cry proteins or between the Vip3Aa19 and the Cry proteins, which will be dealt with in Section 3.4.4.

## 3.4.2 | Comparative analysis[9]

### 3.4.2.1 | *Overview of studies conducted for the comparative analysis*

Application EFSA-GMO-BE-2018-155 presents data on agronomic and phenotypic characteristics, as well as seed composition of three-event stack cotton T304-40 × GHB119 × COT102 (Table 4).

**TABLE 4** Main comparative analysis studies to characterise the three-event stack cotton provided in the application EFSA-GMO-BE-2018-155.

| Study focus | Study details | Comparator | Non-GM reference varieties |
|---|---|---|---|
| Agronomic and phenotypic analysis | Field study, Brazil in 2016/2017 and Argentina in 2015/2016, 12 sites[a] | FM993 | 6[b] |
| Compositional analysis | Field study, Brazil in 2016/2017 and Argentina in 2015/2016, 8 sites[a] | | |

[a]Two field trials were located in Chaco Argentina and one in Santa Fé, Argentina; two field trials in Mato Grosso, Brazil, one field trial in each of Bahia, Goiàs and one in Minas Gerais. Three additional field trials used only for the agronomic and phenotypic analysis were located in Bahia (one field trial) and São Paulo (two field trials). An additional field trial present in Santiago del Estero, Argentina was partially excluded from the statistical analysis due to herbicide drift occurred on 28 January 2016; the quality of this field trial site was compromised, thus only the endpoints collected before the occurrence of the herbicide drift were included in the statistical analysis.
[b]The non-GM cotton were ST457, FM910, FM966, FMT701, Guazuncho 2 INTA and La Banda 300 INTA.

### 3.4.2.2 | *Experimental field trial design and statistical analysis*

At each field trial site, the following materials were grown in a randomised complete block design with four replicates: the three-event stack cotton not exposed to the intended herbicide (not treated), the three-event stack cotton exposed to the intended herbicide (treated), the comparator FM993 and three commercial non-GM cotton reference varieties (hereafter, 'non-GM reference varieties').

The agronomic, phenotypic and compositional data were analysed as specified by EFSA GMO Panel (2010a, 2011b). This includes, for each of the two treatments of the three-event stack cotton, the application of a difference test (between the GM cotton and the non-GM comparator) and an equivalence test (between the GM cotton and the set of non-GM commercial reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[10]

### 3.4.2.3 | *Suitability of selected test materials*

#### 3.4.2.3.1 | *Selection of the test materials*

To obtain the three-event stack cotton, the single events T304-40, GHB119 and COT102 were transferred and stabilised in the genetic background of the non-GM cotton FM993 variety. The comparator used in the field trials is the non-GM cotton variety FM993, which has a similar genetic background as cotton T304-40 × GHB119 × COT102 (as documented by the pedigree and by the additional information). The GMO panel considered this to be a suitable comparator. The three-event stack cotton and its comparator, were considered appropriate for growing in environments across Argentina and Brazil where the comparative field trials were conducted.

Commercial non-GM reference varieties were selected by the applicant and, at each selected site, three reference varieties were tested (Table 4). On the basis of the provided information, the GMO Panel considers the selected non-GM reference varieties acceptable for the comparative assessment.

#### 3.4.2.3.2 | *Seed production and quality*

Seeds of the three-event stack cotton and its comparator used in the 2015/2016 and 2016/2017 field trials were produced in Guanica in Puerto Rico, USA, in 2015 from plants free of quarantine pests, harvested and stored under similar conditions, before being sown in the field trial sites. The seed lots were verified for their identity by event specific PCR. The germination capacity of the GM three-event stack cotton was compared with that of its comparator (in line with the AOSA rules for testing seeds AOSA (2009)). The results indicated similar seed germination.

The GMO Panel considers that the starting seed used as test material in the agronomic, phenotypic and compositional studies was of suitable quality.

[9]Dossier: Part II – Section 1.3; additional information: 14/7/2023, 7/11/2023, 12/4/2024, 29/4/2024.
[10]In detail, the four outcomes are: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence).

#### 3.4.2.3.3 | *Conclusion on suitability*

The GMO Panel is of the opinion that the three-event stack cotton, its comparator and the non-GM cotton reference varieties were properly selected and are of sufficient quality. Therefore, the test materials are considered acceptable for the comparative analysis.

### 3.4.2.4 | *Representativeness of the receiving environments*

#### 3.4.2.4.1 | *Selection of field trial sites*

The selected field trials sites were located in commercial cotton-growing regions of Brazil and Argentina. The soil and climatic characteristics of the selected fields[11] correspond to optimal, near optimal and sub optimal conditions for cotton cultivation (Sys et al., 1993).

The selected sites, including the subset chosen for the compositional analysis, reflect commercial cotton-growing regions in which the test materials are likely to be grown.

#### 3.4.2.4.2 | *Meteorological conditions*

Daily maximum and minimum mean temperatures and sum of precipitations were provided for each site. Some exceptional weather conditions were reported at two of the selected sites.[12] However, due to the lack of major impacts on plant growth, the GMO Panel considers that these exceptional weather conditions did not invalidate the selection of the field trial sites for the comparative analysis.

#### 3.4.2.4.3 | *Management practices*

The field trials included plots containing three-event stack cotton, plots with the comparator and plots with non-GM cotton reference varieties, mostly managed according to local agricultural practices. In addition, the field trials included plots containing three-event stack cotton managed following the same agricultural practices, and exposed to the intended herbicide. The glufosinate-ammonium-based herbicide was applied at the BBCH[13] 13–16 and at the BBCH 32–55 growth stages. Despite not considered a normal agricultural practice, thinning was applied at all field trial sites, except for one located in Mato Grosso, to achieve a more homogeneous plant density across plots. The GMO Panel considers that management practices including sowing, harvesting and application of plant protection product were acceptable for the selected receiving environments.

#### 3.4.2.4.4 | *Conclusion on representativeness*

The GMO Panel concludes that the geographical locations, soil and climatic characteristics, meteorological conditions and most of the management practices are typical for receiving environments where the tested materials could be grown.

### 3.4.2.5 | *Agronomic and phenotypic analysis*

Thirty-nine agronomic and phenotypic endpoints were collected from the field trials (Table 4). Of those, six endpoints (including information on biotic and abiotic stressors) were measured on a categorical scale and were analysed with the Cochran–Mantel–Haenszel (CMH) test.[14] An additional categorical endpoint, lepidoptera larvae/visual inspection, was not analysed with formal statistical methods because of lack of variability in the data.

The remaining 32 endpoints[15] were analysed as described in Section 3.4.2.2, with the following outcome:

[11]Soil types of the field trials were sand, loamy sand, sandy clay loam, sandy clay, silt loam, loam and clay; soil organic matter ranged from 1.4% to 5.2%; pH ranged from 5.3 to 7.1; average temperatures and sum of precipitations during the crop growing season ranged respectively from 22.9°C to 27.1°C and from 534 mm to 2725 mm.

[12]Continuous precipitations at the initial growing season were registered at each of the two field trials located in Mato Grosso, Brazil.

[13]BBCH scale describes phenological stages (Meier, 2001).

[14]These included plant lodging, boll type and rating of biotic and abiotic stressors; stressor ratings were evaluated at four different stages (BBCH 12–14, 52–59, 61–67 and 80–87).

[15]Initial stand count, final stand count, % ground cover, days to flowering, heat units to flowering, % open bolls, days to maturity, heat units to maturity, plant height, height to node ratio, first fruit branch, number of fruiting branches per plant, number of vegetative branches per plant, number of fruiting branches bolls per plant, number of vegetative bolls per plant, number of bolls per plant, number of potential fruiting sites, % fruit retention, % harvestable fruiting branch bolls, fuzzy seeds yield, lint yield, seed cotton weight per boll, lint weight per boll, fuzzy seeds weight per boll, % lint, 100 fuzzy seed weight, fuzzy seeds per boll and fibre properties (micronaire index, strength, upper half mean length index, length uniformity and elongation).

- For the three-event stack cotton (not treated), the test of difference identified statistically significant differences with the comparator for 13 endpoints.[16] All these endpoints fell under equivalence category I or II except for length uniformity, which fell under equivalence category IV.[17]
- For the three-event stack cotton (treated), the test of difference identified statistically significant differences with the comparator for 14 endpoints.[18] All these endpoints fell under equivalence category I or II except for length uniformity, which fell under equivalence category IV, % fruit retention, which was not categorised for equivalence because the variability among the reference varieties was estimated to be 0.[19]

The CMH test identified statistically significant differences between the three-event stack cotton and the comparator for abiotic and biotic stressor ratings and lodged plant ratings; however, the ratings were low for all the test materials.

For length uniformity and % fruit retention, statistically significant differences were identified between the three-event stack cotton and the comparator and equivalence could not be established. Length uniformity is an endpoint related to the latest developmental stages of cotton plants and (taken alone) is not an indicator of the quality of the field trials.

The GMO Panel also noticed that the differences were small in magnitude for both endpoints. For these reasons, the GMO Panel considered that these materials are appropriate for the comparative analysis.

#### 3.4.2.6 | *Compositional analysis*

Fuzzy seeds of three-event stack cotton harvested from eight sites (Table 4) were analysed for 70 constituents, including those recommended by OECD (OECD, 2009). The statistical analysis as described in Section 3.4.2.2 was not applied to 21 constituents[20] because their concentration in at least one third of the samples were below the limit of quantification.

The statistical analysis was applied to 49 constituents[21]; a summary of the outcome of the test of difference and the test of equivalence is presented in Table 5:

- For three-event stack cotton not treated with the intended herbicide, statistically significant differences with the comparator were found for 18 endpoints. All these endpoints fell under equivalence category I or II. The equivalence test could not be done for methionine because of the lack of variation among the non-GM reference varieties.
- For three-event stack cotton treated with the intended herbicide, statistically significant differences with the comparator were found for 20 endpoints. All these endpoints fell under equivalence category I or II. The equivalence test could not be done for methionine because of the lack of variation among the non-GM reference varieties.

**TABLE 5** Outcome of the comparative compositional analysis in fuzzy seeds for three-event stack cotton. The table shows the number of endpoints in each category.

| | | Test of difference[a] | | | |
|---|---|---|---|---|---|
| | | Not treated[b] | | Treated[b] | |
| | | Not different | Significantly different | Not different | Significantly different |
| **Test of equivalence**[c] | Category I/II | 30 | 18[d] | 28 | 20[d] |
| | Category III/IV | – | – | – | – |
| | Not categorised | 1[e] | – | 1[e] | – |
| | Total endpoints | 49 | | 49 | |

(Continues)

[16]Heat units to flowering, fuzzy seeds weight per boll, seed cotton weight per boll, % lint, 100 fuzzy seeds weight, fuzzy seeds yield, lint yield, lint weight per boll and the following fibre endpoints: lint upper half mean length, index strength, micronaire index, elongation and length uniformity.

[17]For length uniformity, the estimated mean values (%) were 83.7 (comparator), 81.5 (GM cotton not treated), 81.6 (GM cotton treated) and 83.8 (reference varieties); the equivalence limits were (82.5, 85.1).

[18]Days to maturity, plant height, height to node ratio, fuzzy seeds weight per boll, seed cotton weight per boll, number of fuzzy seeds per boll, 100 fuzzy seeds weight, lint weight per boll, % fruit retention and the following fibre endpoints: upper half mean length, index strength, micronaire index, elongation and length uniformity.

[19]For length uniformity, the estimated mean values (%) were 83.7 (comparator), 81.5 (GM cotton not treated), 81.6 (GM cotton treated) and 83.8 (reference varieties); the equivalence limits were (82.5, 85.1). For % fruit retention, the estimated mean values were 58.5 (comparator), 59.7 (GM cotton not treated), 61.8 (GM cotton treated) and 62.3 (reference varieties).

[20]Sodium, caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), γ-linolenic acid (C18:3), octadecatetraenoic acid (C18:4), eicosenoic acid (C20:1), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4), eicosapentaenoic acid (C20:5), erucic acid (C22:1), n3-docosapentaenoic acid (C22:5), n6-docosapentaenoic acid (C22:5), docosahexaenoic acid (C22:6), lignoceric acid (C24:0).

[21]Moisture, crude protein, crude fat, ash, total carbohydrates (by calculation), acid detergent fibre, neutral detergent fibre, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), behenic acid (C22:0), total gossypol, free gossypol, dihydrosterculic acid, malvalic acid, sterculic acid, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, zinc, α-tocopherol.

**TABLE 5** (Continued)

[a]Comparison between three-event stack cotton and its comparator.

[b]Treated/not treated with the intended herbicide.

[c]Four different outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.

[d]Endpoints with significant differences between three-event stack cotton and its comparator falling in equivalence category I-II. Not treated only: crude fat, linolenic acid (C18:3); treated only: arginine, glutamic acid, histidine, total gossypol; both treated and not treated: total carbohydrates (by calculation), crude protein, acid detergent fibre, neutral detergent fibre, total dietary fibre, myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), copper, phosphorus, dihydrosterculic acid, malvalic acid, sterculic acid.

[e]Endpoint not categorised for equivalence and without significant differences between the three-event stack cotton and its comparator: methionine.

The GMO Panel assessed all the significant differences between the three-event stack cotton and the comparator, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. No endpoints with outcomes under category III/IV were identified.

#### 3.4.2.7 | *Conclusion on comparative analysis*

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic-phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis.

Considering the natural variability observed for the set of non-GM reference varieties, the GMO Panel concludes that:

- None of the differences identified in agronomic and phenotypic characteristics tested between three-event stack cotton and the comparator needs further assessment regarding potential environmental impact except for length uniformity and % fruit retention, which are further assessed in Section 3.4.4.1.
- None of the differences identified in seed composition between the three-event stack cotton and the comparator needs further assessment regarding food and feed safety.

### 3.4.3 | Food/feed safety assessment[22]

The three single events included in this stack cotton application have been previously assessed, and no safety concerns were identified by the EFSA GMO Panel (Table 1). The three-event stack cotton will undergo existing production processes used for conventional cotton. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of the three-event stack cotton into food and feed products is not expected to result in products differing from those of conventional cotton varieties currently in the EU market.[23]

In accordance with Regulation (EU) No 503/2013 for the risk assessment of genetically modified food or feed containing stacked events, the GMO Panel evaluated the potential for interactions resulting from the combination of the single transformation events impacting on toxicity (Section 3.4.3.1), allergenicity (Section 3.4.3.2) and nutritional assessment (Section 3.4.3.3) as described below.

#### 3.4.3.1 | *Toxicology*

##### 3.4.3.1.1 | *Testing of newly expressed proteins*

Five proteins (Cry1Ab, Cry2Ae, PAT/bar, APH4 and Vip3Aa19) are newly expressed in the three-event stack cotton (Section 3.4.1). The GMO Panel has previously assessed these proteins in the context of the single events (Table 1), and no safety concerns were identified for humans, farmed and companion animals. The GMO Panel is not aware of any new information that would change its previous conclusions on the safety of these proteins. The potential for a functional interaction among the proteins newly expressed in three-event stack cotton has been assessed by the applicant and evaluated by the GMO Panel with regard to human and animal health.

**TABLE 6** Intended effects and mode of action of the newly expressed proteins (NEPs) in cotton T304-40×GHB119×COT102.

| Protein | Intended effect and mode of action in GM plant |
|---|---|
| Cry1Ab, Cry2Ae | The two insecticidal proteins Cry1Ab and Cry2Ae are delta-endotoxins acting through cellular receptors found in lepidopteran species. It is reported that the gastrointestinal tract of mammals, including humans, lacks receptors with high specific affinity to Cry proteins (Hammond et al., 2013; Jurat-Fuentes & Crickmore, 2017; Koch et al., 2015). |

[22]Dossier: Part II – Sections 1.4, 1.5, 1.6, 2; additional information: 12/4/2024.

[23]On-going assessment of novel food applications (https://food.ec.europa.eu/food-safety/novel-food/authorisations/summary-applications-and-notifications_en).

**TABLE 6** (Continued)

| Protein | Intended effect and mode of action in GM plant |
|---|---|
| Vip3Aa19 | The Vip3Aa19 protein is secreted by *B. thuringiensis* during its vegetative phase acting in lepidopterous species via a mechanism similar to that of Cry proteins (Bel et al., 2017; Chakroun et al., 2016). |
| PAT/bar | The PAT/bar protein confers tolerance to glufosinate-ammonium-based herbicides acting by acetylation of glufosinate-ammonium. |
| APH4 | The APH4 protein catalyses the highly specific phosphorylation of the 4-hydroxyl group of hygromycin B, inactivating its antibiotic activity. The APH4 protein is used as a selection marker in the molecular development of the GM plant and shows no agriculturally relevant property in the cultivated plant. |

The two enzymatic proteins catalyse distinct biochemical reactions, acting on unrelated substrates and are not expected to interact, while the insecticidal proteins Cry1Ab, Cry2Ae and Vip3Aa19 act through cellular receptors found in target insect species (Table 6). On the basis of the known biological function of the individual newly expressed proteins, there is no expectation for possible interactions relevant to the food and feed safety of this three-event stack cotton (Table 3).

During the assessment of the COT102 event, the GMO Panel sought advice from the European Medicines Agency (EMA) regarding the uses of hygromycin B in humans and animals in the European Union (EFSA GMO Panel, 2023a). EMA confirmed[24] that there were no products containing hygromycin B authorised for therapeutic, prophylactic or any other medical uses in humans or animals in the EU Member States and there were no central authorisations for human or veterinary use for medicinal products that contain hygromycin B.

The GMO Panel concludes that there are no safety concerns for human and animal health related to the newly expressed proteins Cry1Ab, Cry2Ae, PAT/bar, APH4 and Vip3Aa19 and their combination in the three-event stack cotton.

#### 3.4.3.1.2 | *Testing of new constituents other than proteins*

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no new constituents other than the newly expressed proteins have been identified in seed from the three-event stack cotton. Therefore, no further food/feed safety assessment of components other than the newly expressed proteins is required.

#### 3.4.3.1.3 | *Information on altered levels of food and feed constituents*

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, none of the differences identified between the three-event stack cotton and its non-GM comparator in seed composition require further assessment (Section 3.4.2.7).

#### 3.4.3.1.4 | *Testing of the whole genetically modified food and feed*

The GMO Panel considers animal studies with food/feed derived from the three-event stack cotton unnecessary based on the outcome of molecular characterisation, comparative analysis and toxicological assessment (EFSA GMO Panel, 2011a). There were no concerns regarding the stability and expression of the inserts or interaction between the three events, and no toxicological concerns regarding the composition of the three-event stack cotton were identified (Sections 3.4.1, 3.4.2.6 and 3.4.3.1).

The GMO Panel had previously concluded that the 90-day feeding studies in rodents on whole food/feed from cotton T304-40, GHB119 and COT102 are in line with Regulation (EU) No 503/2013 and do not show adverse effects related to diets incorporating the respective single events (EFSA GMO Panel, 2018, 2023a).

### 3.4.3.2 | *Allergenicity*

The strategies to assess the potential risk of allergenicity focus: (i) on the source of the recombinant protein; (ii) on the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons; and (iii) on whether the transformation may have altered the allergenic properties of the modified plant. Furthermore, the assessment also takes into account potential adjuvant properties of the newly expressed proteins, which is defined as the ability to enhance an allergic reaction.

#### 3.4.3.2.1 | *Assessment of allergenicity of the newly expressed proteins*

The GMO Panel has previously evaluated the safety of Cry1Ab, Cry2Ae, PAT/bar, APH4 and Vip3Aa19 proteins individually, and no evidence of allergenicity was identified in the context of the single event applications assessed (Table 1). No new information on allergenicity of the proteins newly expressed in this three-event stack cotton that might change the

[24]Correspondence between EFSA and EMA is available online https://open.efsa.europa.eu/study-inventory/EFSA-Q-2017-00271.

previous conclusions of the GMO Panel has become available. Based on the current knowledge, and as there is no evidence of allergenicity of the newly expressed proteins, there are no expected concerns of allergenicity as a consequence of their simultaneous presence in this three-event stack cotton.

The GMO Panel has previously evaluated the safety of the newly expressed proteins, and no evidence of adjuvanticity was identified in the context of the applications assessed (Table 1). This aspect has been discussed in detail by EFSA (EFSA, 2018; Parenti et al., 2019). To date, there is no evidence for adjuvanticity in the GMOs assessed by the Panel. This three-event stack cotton has similar levels of the individual Bt proteins as those in the respective single cotton events (see Section 3.4.1). The GMO Panel did not find indications that the Bt proteins at the levels expressed in this three-event stack cotton might be adjuvants able to enhance an allergic reaction.

The applicant also provided information on the safety of the Cry1Ab, Cry2Ae, PAT/bar, APH4 and Vip3Aa19 proteins regarding their potential to cause a celiac disease response. For such assessment, the applicant followed the principles described in the EFSA GMO Panel guidance document (EFSA GMO Panel, 2017). No indications of safety concern were identified by the GMO Panel as the relevant peptides containing the motif fail to mimic gluten sequences.

The GMO Panel considers that there are no indications that the Cry1Ab, Cry2Ae, PAT/bar, APH4 and/or Vip3Aa19 proteins in this three-event stack cotton may be allergenic.

#### 3.4.3.2.2 | *Assessment of allergenicity of the whole GM plant*

The GMO Panel regularly reviews the available publications on food allergy to cotton. However, cotton is not considered a common allergenic food[25] (OECD, 2009). Therefore, the GMO Panel does not request experimental data to analyse the allergen repertoire of GM cotton.

In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (see Sections 3.4.1, 3.4.2 and 3.4.3), the GMO Panel found no indications of a potentially increased allergenicity of food and feed derived from this three-event stack cotton with respect to that derived from the non-GM comparator and the non-GM reference varieties tested.

### 3.4.3.3 | *Dietary exposure assessment to new constituents*

In line with Regulation (EU) No 503/2013 the applicant provided dietary exposure estimates to Cry1Ab, Cry2Ae, PAT, APH4 and Vip3Aa19 proteins newly expressed in the three-event stack cotton. Dietary exposure was estimated based on protein expression levels reported in this application for the three-event stack cotton treated with the intended herbicide, the current available consumption data and feed practices, the foods and feeds currently available in the market and the described processing conditions.

For the purpose of estimating dietary exposure, the levels of newly expressed proteins in the three-event stack cotton seeds and pollen were derived from field trials (four replicates from four locations) in Argentina and Brazil conducted during the 2015/2016 and 2016/2017 growing seasons, respectively (see Section 3.4.1.3). Table 7 presents the protein expression levels used to estimate both human and animal dietary exposure.

**TABLE 7** Mean values ($n = 16$, µg/g dry weight and µg/g fresh weight) for newly expressed proteins in seeds and pollen from three-event stack cotton grains treated with the intended herbicide.[a]

| | Tissue/developmental stage | |
|---|---|---|
| **Protein** | **Fuzzy seeds (µg/g dry weight)** | **Pollen (µg/g fresh weight)[b]** |
| Cry1Ab | 3.53 | 0.81 |
| Cry2Ae | 36.80 | 1.41 |
| PAT/bar | 218.44 | 5.79 |
| Vip3Aa19 | 3.77 | 0.88 |
| APH4 | < LOQ[c] | 3.53 |

[a]Intended herbicide: glufosinate-ammonium.

[b]Concentrations values in pollen were adjusted to 6% moisture content before using them to estimate dietary exposure to the different newly expressed proteins via the consumption of pollen supplements.

[c]APH4 values were reported below the limit of quantification in fuzzy seeds (1.0 µg/g dw) and pollen (1.2 µg/g fw).

[d]For APH4 in pollen, $n = 9$.

[25]Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

#### 3.4.3.3.1 | *Human dietary exposure*

The applicant considered the dietary exposure to Cry1Ab, Cry2Ae, PAT/bar, APH4 and Vip3Aa19 newly expressed proteins as negligible in the European population. The GMO Panel identified different cottonseed-derived products such as flour and oil as well as by-products (cottonseed linters[26]) used for human consumption, with the refined bleached deodorised (RBD) oil being currently the most relevant. The GMO Panel confirmed that no consumption data of cottonseed, cottonseed oil or other cottonseed-derived products were available in the EFSA Comprehensive European Food Consumption Database.[27]

Cottonseed oil might be consumed as an ingredient in the production of a wide variety of food products such as dressings, mayonnaise, fine bakery wares, chocolate spreads and chips. However, considering that RBD cottonseed oil and cottonseed linters are free from proteins, no dietary exposure to Cry1Ab, Cry2Ae, PAT, APH4 and Vip3Aa19 proteins is expected from the consumption of these products derived from the three-event stack cotton. Dietary exposure to the newly expressed proteins cannot be excluded via the consumption of cottonseed flour, although as indicated above, this product seems not to be consumed (or in very small amounts) in Europe at present.

An ad hoc dietary exposure scenario was considered by the GMO Panel for consumers of pollen supplements under the assumption that these supplements might be made of pollen from the three-event stack cotton. Consumption data on pollen supplements are available for few consumers across seven different European countries.[28] The low number of consumers available adds uncertainty to the exposure estimations which should be carefully interpreted, and only allows the estimation of dietary exposure for average consumers. Expression levels in pollen as described in Table 7 were used to derive concentrations in pollen supplements considering around 6% moisture content in these products. The highest mean acute dietary exposure would be between 0.75 µg/kg bw per day for Cry1Ab and 5.4 µg/kg bw per day for PAT, in the elderly population. Similarly, the highest mean chronic dietary exposure in consumers of pollen supplements would be between 0.50 µg/kg bw per day for Cry1Ab and 3.6 µg/kg bw per day for PAT, also in the elderly population.

#### 3.4.3.3.2 | *Animal dietary exposure*

Dietary exposure to Cry1Ab, Cry2Ae, PAT and Vip3Aa19 proteins in the three-event stack cotton was estimated by the applicant across different animal species as below described, assuming the consumption of cotton products commonly entering the feed supply chain (i.e. undelinted seeds and meal). The GMO Panel observed that the applicant had not provided an estimate of dietary exposure to APH4 in the three-event stack cotton because all samples were below LOQ, and therefore the GMO Panel covered this aspect within this opinion.

A conservative scenario with 100% replacement of conventional cotton products by the three-event stack cotton products was considered.

Mean levels (dry weight) of the newly expressed proteins in undelinted seeds from three-event stack event cotton treated with the intended herbicide used for animal dietary exposure are listed in Table 7.

All seed samples analysed in the three-event stack cotton for the presence of APH4 protein were below the limit of quantification (LOQ = 1.00 µg/g dry weight); for the purpose of estimating daily dietary intake (DDE), the determined values of LOQ were used as substitutes to compute an average concentration of APH4 in seed to be used for the exposure calculations.[29]

Mean levels of Cry1Ab, Cry2Ae, PAT/bar, Vip3Aa19 and APH4 proteins in cotton meal were calculated to be respectively 1.75-fold than in seed, based on factors that take into account the protein content in these feed materials relative to cotton seed, and assuming that no protein is lost during their production/processing (EFSA GMO Panel, 2023b).

The applicant estimated dietary exposure to Cry1Ab, Cry2Ae, PAT/bar, Vip3Aa19 and APH4 proteins via the consumption of undelinted seeds in dairy cow, dairy sheep and dairy goat and cottonseed meal in dairy cow, beef cattle, dairy sheep, dairy goat, rabbit, fattening pig, lactating sow, broiler, laying hens, turkey and horse.

Estimations were based on default values for animal body weight, daily feed intake and inclusion rates (percentage) of cottonseed meal and undelinted seeds in rations, as provided for the EU by EFSA GMO Panel (2023b).

Estimated dietary exposure in the concerned animals is reported in Appendix D.

#### 3.4.3.4 | *Nutritional assessment of endogenous constituents*

The intended traits of this three-event stack cotton are herbicide tolerance and insect resistance, with no intention to alter nutritional parameters. Comparison of the composition of this three-event stack cotton with the non-GM comparator and the tested non-GM reference varieties did not identify differences that would require further safety assessment. From

[26]Short cellulose fibres left on the seed after the staple cotton is removed by ginning.

[27]https://www.efsa.europa.eu/en/applications/gmo/tools. EFSA consumption database: version 1.0 (updated March 2022).

[28]https://www.efsa.europa.eu/en/data-report/food-consumption-data. Latest update: 18 December 2024.

[29]Left-censored data were treated by the substitution method (WHO, 2009), and as also indicated in the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010b). For all samples reported as < LOD or as < LOQ, LB and UB values were derived by assigning a value of zero and LOD/LOQ, respectively. For the dietary exposure estimations, only the UB scenario (worst-case) was used; uncertainties linked to this approach will be considered should the exposure estimations be used in the safety assessment of the NEPs. This approach is also mentioned in the corresponding documents on animal and human dietary exposure (EFSA, 2019a; EFSA GMO Panel, 2023b).

these data, the GMO Panel concludes that this three-event stack cotton is nutritionally equivalent to the non-GM comparator and the tested non-GM reference varieties used.

#### 3.4.3.5 | *Conclusions on the food/feed safety assessment*

The newly expressed proteins Cry1Ab, Cry2Ae, PAT/bar, APH4 and Vip3Aa19 in the three-event stack cotton do not raise safety concerns for human and animal health. No interactions between the newly expressed proteins relevant for food and feed safety were identified, and no overall toxicological concerns on the three-event stack cotton were identified. Similarly, the GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvanticity related to the presence of the newly expressed proteins in this three-event stack cotton, or regarding its overall allergenicity. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of cotton T304-40 × GHB119 × COT102 does not represent any nutritional concern, in the context of the scope of this application. It should be noted that for the overall assessment of this three-stack cotton, the GMO Panel also evaluated the additional unpublished studies, as listed in Appendix A and the relevant scientific publications listed in Appendix B.

### 3.4.4 | Environmental risk assessment[30]

Considering the scope of this application, which excludes cultivation, the environmental risk assessment (ERA) mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed with GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the deliberate or accidental release of GM material into the environment, including spillage of viable three-event stack cotton seeds during transportation and/or processing (EFSA GMO Panel, 2010a).

#### 3.4.4.1 | *Persistence and invasiveness of the GM plant*

In Southern Europe, *G. herbaceum*, *G. barbadense* and *G. hirsutum* have been grown since the 19th century and led to transient or locally naturalised cotton plants in the same area (Celesti-Grapow et al., 2010; Davis, 1967; Sarno et al., 1993; Tutin et al., 1992). However, survival of cottonseeds outside cultivation areas in Europe is limited due to the absence of a seed dormancy phase. Even if seeds from spillage germinate, the resulting cotton plants are unlikely to survive due to factors such as cold climatic conditions, the susceptibility to diseases and their low competitiveness (Eastick & Hearnden, 2006). For example, after the end of cotton cultivation in Italy in 1950s, no feral cotton was reported in southern Italy, except in some restricted areas (Celesti-Grapow et al., 2010; Sarno et al., 1993). Also, in other cotton-growing regions, such as in Australia, surveys showed that feral GM cotton established infrequently along transportation routes and mostly as transient populations (Addison et al., 2007). Field observations indicate that cottonseed may survive and overwinter in some EU regions, resulting in volunteers in subsequent crops (e.g. Charles et al., 2013). However, cotton volunteers have been shown to rarely yield as well as newly planted seeds due to seedling diseases and early emergence in cool conditions. Thus, the establishment and survival of feral and volunteer cotton plants in the EU is currently limited and transient.

It is unlikely that the intended traits of the three-event stack cotton and the observed differences in length uniformity and percentage of fruit retention (see Section 3.4.2.5) will provide a selective advantage to cotton plants, except when they are exposed to glufosinate-ammonium-based herbicides or infested by insect pests that are susceptible to the Cry1Ab, Cry2Ae and/or Vip3Aa19 proteins.

The GMO Panel considers that the fitness advantage provided by the intended traits and the observed differences in length uniformity and percentage of fruit retention will not allow the GM plant to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits will not affect the overall persistence and invasiveness of the GM plant.

The results of an additional study provided by the applicant on seed germination (Appendix A) provided no evidence that three-event stack cotton T304-40 × GHB119 × COT102 has a higher risk of persistence and invasiveness than its comparator.

The GMO Panel concludes that it is very unlikely that the three-event stack cotton will differ from conventional cotton varieties in its ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable three-event stack cotton seeds.

#### 3.4.4.2 | *Potential for gene transfer*

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer (HGT) of DNA, or through vertical gene flow via cross-pollination from feral plants originating from spilled grains.

[30]Dossier: Part II – Section 5; additional information: 3/9/2025.

*Plant-to-microorganism gene transfer*

The probability and potential adverse effects of HGT of the recombinant DNA have been assessed in previous GMO Panel Scientific Opinions for the single events (Table 1). This assessment included consideration of homology-based recombination processes, as well as non-homologous end joining and microhomology-mediated end joining. Possible fitness advantages that the bacteria in the receiving environments would gain from acquiring recombinant DNA were considered. No concern as a result of an unlikely, but theoretically possible, HGT of the recombinant genes to bacteria in the gut of domesticated animals and humans fed GM material or in other receiving environments was identified.

The applicant submitted an updated bioinformatic analysis for each of the single events to assess the possibility for HGT by homologous recombination. This information confirms the assessments provided in the context of previous Scientific Opinions of the single events (EFSA GMO Panel, 2018, 2023a).

Synergistic effects of the recombinant genes, for instance due to combinations of recombinogenic sequences, which would cause an increase in the likelihood for HGT or a selective advantage were not identified.

Therefore, the GMO Panel concludes that the unlikely, but theoretically possible, horizontal transfer of recombinant genes from this three-event stack cotton to bacteria does not raise any environmental safety concern.

*Plant-to-plant gene transfer*

The potential for occasional feral cotton T304-40 × GHB119 × COT102 plants originating from seed import spills to transfer recombinant DNA to sexually compatible plants and the environmental consequences of this transfer were considered.

For plant-to-plant gene transfer to occur, imported GM cotton seeds need to germinate and develop into plants in areas containing sympatric wild relatives and/or cultivated cotton with synchronous flowering and environmental conditions favouring cross-pollination.

Cotton is an annual predominantly self-pollinating crop, although cross-pollination can occur at low frequencies in the presence of insect pollinators (such as wild bees, honeybees, bumblebees) (OECD, 2008). For cotton, no wild relatives have been reported in Europe; therefore, any vertical gene transfer is limited to *G. hirsutum*, *G. barbadense* and *G. herbaceum* cotton plants. However, gene transfer to *G. herbaceum* is considered unlikely due to the difference in ploidy level.

The potential of spilled cotton seeds to establish, grow and produce pollen is extremely low and transient (see Section 3.4.4.1). Therefore, the likelihood/frequency of cross-pollination between occasional feral GM cotton plants resulting from seed spillage, and weedy or cultivated *Gossypium* plants is extremely low. Even if cross-pollination would occur, the GMO Panel is of the opinion that the likelihood of environmental effects as a consequence of the spread of genes from occasional feral GM cotton plants in Europe will not differ from that of conventional cotton varieties for the reasons given in Section 3.4.4.1.

### 3.4.4.3 | *Interactions of the GM plant with target organisms*

Taking the scope of application EFSA-GMO-BE-2018-155 into account (no cultivation), potential interactions of occasional feral three-event stack cotton plants arising from seed import spills with the target organisms are not considered a relevant issue.

### 3.4.4.4 | *Interactions of the GM plant with non-target organisms*

The environmental risk assessment considers potential effects of the GM plant on populations of non-target organisms, defined as all those species directly or indirectly exposed to the GM plant and which are not targets of the newly expressed metabolite(s) it expresses. The GMO Panel evaluated the potential hazards of the NEPs and considered that the environmental exposure of non-target organisms to spilled GM cotton material or occasional feral GM cotton plants arising from spilled cotton T304-40 × GHB119 × COT102 seeds will be limited. Additionally, ingested proteins are typically degraded before entering the environment through faecal material of animals fed with GM cotton (Harmon & Swanson, 2020; Miner-Williams et al., 2014; Mok & Urschel, 2020; Santos-Hernandez et al., 2018; van Bruchem et al., 1985), and the data provided for the assessment of protein stability in the context of the assessment of the single events and also for this application (Section 3.4.3.1.1 and Appendix A) supports that also the NEPs will be degraded. As compared to non-GM cotton, the GMO Panel considers that potential interactions of cotton T304-40 × GHB119 × COT102 with non-target organisms do not raise any additional environmental safety concern. Interactions that may occur between the insecticidal proteins Cry1Ab, Cry2Ae and Vip3Aa19 will not alter this conclusion.

### 3.4.4.5 | *Interactions with biogeochemical cycles*

Biogeochemical cycles encompass the microbiologically mediated movement, transformation and storage of carbon, nitrogen and other compounds that are considered here for the receiving environments. The GMO Panel evaluated the potential hazards of the NEPs and considered that the environmental exposure to spilled GM cotton material or occasional feral GM cotton plants arising from spilled cotton T304-40 × GHB119 × COT102 seeds will be limited, whereas exposure to manure and faeces of animals fed with cotton T304-40 × GHB119 × COT102 material is expected to be higher. However,

ingested proteins are typically degraded before entering the environment through faecal material of animals fed with GM cotton (Harmon & Swanson, 2020; Miner-Williams et al., 2014; Mok & Urschel, 2020; Santos-Hernandez et al., 2018; van Bruchem et al., 1985), and the data provided for the assessment of protein stability in the context of the assessment of the single events and also for this application (Section 3.4.3.1.1 and Appendix A) supports that also the NEPs will be degraded. As compared to non-GM cotton, the GMO Panel considers that potential interactions of cotton T304-40 × GHB119 × COT102 with biogeochemical cycles do not raise any environmental safety concern.

#### 3.4.4.6 | *Conclusion of the environmental risk assessment*

The GMO Panel concludes that it is unlikely that three-event stack cotton T304-40 × GHB119 × COT102 would differ from conventional cotton varieties in its ability to persist under European environmental conditions. Taking into account the scope of application EFSA-GMO-BE-2018-155, interactions of occasional feral three-event stack cotton plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from three-event stack cotton to bacteria does not indicate a safety concern. Therefore, considering the introduced traits, the outcome of the agronomic and phenotypic analysis, and the routes and levels of exposure, the GMO Panel concludes that three-event stack cotton would not raise safety concerns in the event of release of processed GM cotton T304-40 × GHB119 × COT102 or the accidental spillage of viable GM cotton seeds into the environment.

## 3.5 | Post-market monitoring[31]

### 3.5.1 | Post-market monitoring of GM food/feed

The GMO Panel concludes that three-event stack cotton T304-40 × GHB119 × COT102, as described in this application, does not raise any nutritional concern and is as safe as the comparator and the non-GM reference varieties tested (Section 3.4.3). Therefore, the GMO Panel does not see the necessity for post-market monitoring of food and feed from the three-event stack cotton, as described in this application.

### 3.5.2 | Post-market environmental monitoring

The objectives of a post-market environmental monitoring (PMEM) plan, according to Annex VII of Directive 2001/18/EC, are: (i) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the ERA are correct; and (ii) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the ERA.

Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific rationale of the PMEM plan provided by the applicant (EFSA GMO Panel, 2011b).

As the ERA did not identify potential adverse environmental effects from three-event stack cotton T304-40 × GHB119 × COT102, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for three-event stack cotton T304-40 × GHB119 × COT102 includes: (1) the description of a monitoring approach involving operators (federations involved in import and processing), reporting to the applicant, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a co-ordinating system established by CropLife Europe for the collection of information recorded by the various operators; and (3) the review of relevant scientific publications retrieved from literature searches (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis for the duration of the authorisation period.

The GMO Panel considers that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of the three-event stack cotton and agrees with the proposed reporting intervals.

### 3.5.3 | Conclusions on post-market monitoring

No post-market monitoring of food and feed is necessary. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of the three-event stack cotton.

[31]Dossier: Part II – Section 6; additional information: 23/2/2024.

### 3.6 | Cotton species covered by the scope of the application[32]

*G. hirsutum* and G. barbadense are genetically close cotton species which have been sympatric over the last several millennia leading to pervasive genome-wide bidirectional introgression (Yuan et al., 2021). In addition, intentional interspecific hybridisation has occurred many times in both directions, with the goal of producing commercial varieties that possess the traits of both *G. hirsutum* and *G. barbadense* species (Jareczek et al., 2023). The events present in cotton T304-40 × GHB119 × COT102 are not predicted to interact with metabolic pathways controlling any traits that differ between *G. hirsutum* and *G. barbadense*. Moreover, the combined events contain promoters and terminators widely used for constitutive expression of proteins (Lepetit et al., 1992; Kummari et al., 2020; de Paes Melo et al., 2021; Brooks et al., 2023; Villao-Uzho et al., 2023) and are expected to lead to similar expression of the newly expressed proteins in both species. Based on these observations, the GMO panel considers that the information collected in application EFSA-GMO-BE-2018-155 allows, for this specific application, to conclude on the safety of cotton T304-40 × GHB119 × COT102 in *G. hirsutum* and *G. barbadense*.

## 4 | OVERALL CONCLUSIONS

The GMO Panel was asked to carry out a scientific assessment of cotton T304-40 × GHB119 × COT102 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

No new information was identified on the three single cotton events (T304-40, GHB119, COT102) that would lead to a modification of the original conclusions on their safety.

The molecular characterisation, the comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological, allergenicity and nutritional assessment indicate that the combination of the single cotton events and of the newly expressed proteins in the three-event stack cotton does not give rise to food/feed safety and nutritional concerns. The GMO Panel concludes that the three-event stack cotton, as described in this application, does not raise any nutritional concern and is as safe as its comparator and the selected non-GM reference varieties.

The GMO Panel concludes that additional environmental effects as compared to conventional cotton resulting from the release of the three-event stack cotton into the environment are unlikely.

Based on the relevant publications identified through the literature searches, the GMO Panel did not identify any safety issues pertaining to the intended uses of cotton T304-40 × GHB119 × COT102.

In addition, the GMO Panel considered the additional unpublished studies listed in Appendix A, which did not raise any concern for human and animal health and the environment. Given the absence of safety and nutritional concerns for foods and feeds from the three-event stack cotton, the GMO Panel considers that PMM of these products is not necessary. The PMEM plan and reporting intervals are in line with the intended uses of the three-event stack cotton. In conclusion, the GMO Panel considers that cotton T304-40 × GHB119 × COT102, as described in this application, is as safe as the comparator and the selected non-GM reference varieties with respect to potential effects on human and animal health and the environment.

Considering the close genetic relationship and extensive bidirectional introgression between *G. hirsutum* and *G. barbadense*, along with the fact that the inserted traits, driven by well-characterised regulatory elements ensuring stable expression, are not expected to affect species-specific metabolic pathways, the GMO panel considers that the information collected in application EFSA GMO BE 2018 155 allows, for this specific application, to conclude on the safety of cotton T304-40 × GHB119 × COT102 in *G. hirsutum* and *G. barbadense*.

The GMO Panel considers that the risk assessment may need to be updated in case products containing hygromycin B or other substrates of the APH4 enzyme obtain future market approval in the EU.

## 5 | DOCUMENTATION AS PROVIDED TO EFSA (IF APPROPRIATE)

- Letter from Competent Authority of Belgium received on 12 October 2018 concerning a request for authorisation of placing on the market of genetically modified cotton T304-40 × GHB119 × COT102, submitted in accordance with Regulation (EC) No1829/2003 by BASF Agricultural Solutions Seed US LLC (EFSA Ref. EFSA-GMO-BE-2018-147; EFSA-Q-2018-00809).
- The application was made valid on 8 July 2020.
- Risk assessment stopped on 8 July 2020 due to "single event" principle for COT102.
- Risk assessment resumed on 10 May 2023.
- Additional information (2) was requested on 26 May 2023.
- Additional information (2) was received on 14 July 2023.
- Additional information (3) was requested on 8 August 2023.
- Additional information (3) was received on 7 November 2023.
- Additional information (4) was requested on 12 December 2023.

[32]Additional information: 12/9/2024, 20/5/2025.

- Additional information (4) was received on 23 February 2024 partial; 12 April 2024 partial; 29 April 2024 partial; 12 September 2024 complete.
- Additional information (5) was requested on 3 April 2024.
- Additional information (5) was received on 29 May 2024 partial; 31 July 2024 complete.
- Additional information (6) was requested on 18 July 2024.
- Additional information (6) was received on 18 April 2025.
- Additional information (7) was requested on 19 August 2024.
- Additional information (7) was received on 12 September 2024.
- Additional information (8) was requested on 5 September 2024.
- Additional information (8) was received on 12 September 2024.
- Additional information (9) was requested on 20 March 2025.
- Additional information (9) was received on 20 May 2025.
- Additional information (10) was requested on 16 July 2025.
- Additional information (10) was received on 3 September 2025.
- Additional information (11) was requested on 23 September 2025.
- Additional information (11) was received on 30 September 2025.

## ABBREVIATIONS

| | |
|---|---|
| bw | body weight |
| dw | dry weight |
| ERA | environmental risk assessment |
| ELISA | enzyme-linked immunosorbent assay |
| fw | fresh weight |
| GM | genetically modified |
| GMO | genetically modified organisms |
| HGT | horizontal gene transfer |
| HR | homologous recombination |
| NEPs | newly expressed proteins |
| ORFs | open reading frames |
| PCR | polymerase chain reaction |
| PMEM | post-market environmental monitoring plan |
| PMM | post-market monitoring |

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## REQUESTOR

Competent Authority of Belgium

## QUESTION NUMBER

EFSA-Q-2018-00809



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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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# APPENDIX A

## Additional studies

List of additional studies performed by or on behalf of the applicant with regard to the evaluation of the safety of cotton T304-40 × GHB119 × COT102 for humans, animal or the environment.

| Study identification | Title |
|---|---|
| **EBWIS016** | Algodão T304-40 × GHB119 × COT102 - Avaliação da Artropodofauna em áreas cultivadas no Brasil na safra 2016/2017. April 25, 2018. 523 pages. BASF Doc ID 2020/2029924 |
| **M-508050-01-1** | Cry2Ae protein – Acute toxicity study by oral gavage in mice. January 19, 2015. 44 pages. BASF DocID 2020/2016555 |
| **M-591413-01-1** | T304-40 × GHB119 × COT102 Cotton – Seed Germination Potential, 2016. Final Report Study Number 16-RSWIS055 |
| **M-085589-02-1**[a] | PAT/bar protein: Heat stability study |
| **M-208793-04-1**[a] | PAT/bar protein: in vitro digestibility study in human simulated intestinal fluid |
| **M-217195-04-1**[a] | PAT/bar protein: in vitro digestibility study in human simulated gastric fluid |
| **M-353455-01-1**[a] | Cry1Ab protein – In vitro digestibility study in human simulated gastric fluid |
| **M-411947-01-1**[a] | Analysis of the heat stability of the Cry2Ae protein |
| **M-429308-01-1**[a] | Analysis of the heat stability of the Cry1Ab protein |
| **M-461494-01-1**[a,b,c] | Recombinant PAT/bar protein: Acute toxicity by oral gavage in female mice |
| **M-475319-01-1**[a,b,c] | PAT/bar protein – Acute toxicity by oral gavage in mice |
| **M-554703-01-1**[c] | The effect of temperature on PAT/bar as assessed by the PAT quantitative activity assay |
| **M-557508-01-1**[c] | The effect of temperature on PAT/bar as assessed by ELISA |

[a]Study previously assessed in EFSA GMO Panel ….. (AP122).
[b]Study previously assessed in EFSA GMO Panel ….. (RX004).
[c]Study previously assessed in EFSA GMO Panel ….. (RX010).

## APPENDIX B

### List of relevant publications identified by the applicant through literature searches (January 2008 to June 2025)

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**Literature search dossier (18-TXWIS008)**

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**Literature search dossier (18-TXWIS008)**

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# APPENDIX C

## Protein expression data

**TABLE C.1** Mean, standard deviation and range of protein levels in fuzzy seeds (μg/g dry weight and μg/g of fresh weight) and pollen (μg/g of fresh weight) from cotton T304-40 × GHB119 × COT102, T304-40, GHB119 (treated) and from cotton T304-40 × GHB119 × COT102 and COT102 (not treated), from field trials performed across four locations in Argentina and Brazil during the 2015/2016 and 2016/2017, respectively ($n = 16$).[a]

| Protein | Event(s) | Fuzzy seeds (μg/g dry weight) (dw) | Fuzzy seeds (μg/g fresh weight) (fw) | Pollen (μg/g fresh weight) (fw) |
|---|---|---|---|---|
| **PAT/bar** | **T304-40 × GHB119 × COT102** | 218.44 ± 49.80 (135.93–315.69) | 195.56 ± 48.06 (120.37–288.21) | 5.79 ± 4.61 (1.53–16.66) |
| | **GHB119** | 116.13 ± 26.27 (85.78–167.62) | 104.08 ± 24.97 (75.33–152.75) | 2.72 ± 1.82 (0.29–7.88) |
| | **T304-40** | 150.31 ± 45.69 (88.63–221.83) | 133.98 ± 42.49 (77.41–201.47) | 4.51[a] ± 4.40 (< LLOQ[b]–17.69) |
| **Cry1Ab** | **T304-40 × GHB119 × COT102** | 3.53 ± 0.77 (2.16–4.94) | 3.16 ± 0.72 (1.91–4.47) | 0.81 ± 0.44 (0.31–1.86) |
| | **T304-40** | 3.32 ± 0.82 (1.46–4.28) | 2.95 ± 0.75 (1.26–3.89) | 0.82 ± 0.37 (0.33–1.89) |
| **Cry2Ae** | **T304-40 × GHB119 × COT102** | 36.80 ± 10.07 (23.29–69.55) | 32.86 ± 9.05 (20.56–61.41) | 1.41 ± 1.72 (0.08–6.80) |
| | **GHB119** | 38.55 ± 13.01 (24.53–65.84) | 34.55 ± 11.94 (21.50–60.01) | 0.68 ± 0.49 (0.10–1.69) |
| **Vip3Aa19** | **T304-40 × GHB119 × COT102*** | 3.97 ± 1.28 (2.84–6.91) | 3.52 ± 1.06 (2.59–6.01) | 0.63 ± 0.41 (0.28–1.70) |
| | **COT102*** | 4.14 ± 1.22 (3.03–7.14) | 3.68 ± 1.00 (2.70–6.16) | 0.68 ± 0.46 (0.28–1.82) |
| **APH4** | **T304-40 × GHB119 × COT102*** | ND[b] < LLOQ[b] | ND[b] < LLOQ[b] | 2.17[a] ± 0.65 (< LLOQ[b]–3.03) |
| | **COT102*** | ND[b] < LLOQ[b] | ND[b] < LLOQ[b] | 2.42[a] ± 0.77 (< LLOQ[b]–3.74) |

*Not treated.

[a]Number of samples is $n = 16$ except for: PAT $n = 15$ in pollen for T304-40; APH4 $n = 10$ in pollen for T304-40 × GHB119 × COT102 and COT102.

[b]ND: not determined for concentration (LLOQ = 0.15 μg/g fw for pollen in PAT; LLOQ = 1.00 μg/g dw for fuzzy seeds in APH4 and LLOQ = 1.20 μg/g fw for pollen in APH4).

# APPENDIX D

## Animal dietary exposure

**TABLE D.1** Dietary exposure to Cry1Ab protein in farmed and companion animals, based on the consumption of cotton undelinted seeds.

| | Body weight | Total daily intake | Inclusion rates | Daily dietary exposure | |
|---|---|---|---|---|---|
| Animal species | kg | kg/animal | % | μg/kg | mg/kg |
| Dairy cow | 650 | 25 | 10 | 13.58 | 0.01 |
| Dairy Sheep | 80 | 2.8 | 25 | 30.89 | 0.03 |
| Dairy Goat | 60 | 3.4 | 20 | 40.01 | 0.04 |

**TABLE D.2** Dietary exposure to Cry1Ab protein in farmed and companion animals, based on the consumption of cottonseed meal.

| | Body weight | Total daily intake | Inclusion rates | Daily dietary exposure | |
|---|---|---|---|---|---|
| Animal species | kg | kg/animal | % | μg/kg | mg/kg |
| Dairy cow | 650 | 25 | 5 | 11.88 | 0.01 |
| Beef cattle | 500 | 12 | 5 | 7.42 | 0.01 |
| Dairy Sheep | 80 | 2.8 | 20 | 43.26 | 0.04 |
| Dairy Goat | 60 | 3.4 | 15.5 | 54.28 | 0.05 |
| Rabbit | 2 | 0.15 | 43 | 199.31 | 0.20 |
| Fattening pig | 100 | 3 | 5 | 9.27 | 0.01 |
| Lactating sow | 200 | 6 | 10 | 18.54 | 0.02 |
| Broiler | 2 | 0.158 | 5 | 24.41 | 0.02 |
| Laying hens | 1.9 | 0.13 | 5 | 21.14 | 0.02 |
| Turkey | 7 | 0.5 | 10 | 44.14 | 0.04 |
| Horse | 450 | 9 | 10 | 12.36 | 0.01 |

**TABLE D.3** Dietary exposure to Cry2Ae protein in farmed and companion animals, based on the consumption of cotton undelinted seeds.

| | Body weight | Total daily intake | Inclusion rates | Daily dietary exposure | |
|---|---|---|---|---|---|
| Animal species | kg | kg/animal | % | μg/kg | mg/kg |
| Dairy cow | 650 | 25 | 10 | 141.54 | 0.14 |
| Dairy Sheep | 80 | 2.8 | 25 | 322.00 | 0.32 |
| Dairy Goat | 60 | 3.4 | 20 | 417.07 | 0.42 |

**TABLE D.4** Dietary exposure to Cry2Ae protein in selected animals, based on the consumption of cottonseed meal.

| | Body weight | Total daily intake | Inclusion rates | Daily dietary exposure | |
|---|---|---|---|---|---|
| Animal species | kg | kg/animal | % | μg/kg | mg/kg |
| Dairy cow | 650 | 25 | 5 | 123.85 | 0.12 |
| Beef cattle | 500 | 12 | 5 | 77.28 | 0.08 |
| Dairy Sheep | 80 | 2.8 | 20 | 450.80 | 0.45 |
| Dairy Goat | 60 | 3.4 | 15.5 | 565.65 | 0.57 |
| Rabbit | 2 | 0.15 | 43 | 2076.90 | 2.08 |
| Fattening pig | 100 | 3 | 5 | 96.60 | 0.10 |
| Lactating sow | 200 | 6 | 10 | 193.20 | 0.19 |
| Broiler | 2 | 0.158 | 5 | 254.38 | 0.25 |
| Laying hens | 1.9 | 0.13 | 5 | 220.32 | 0.22 |
| Turkey | 7 | 0.5 | 10 | 460.00 | 0.46 |
| Horse | 450 | 9 | 10 | 128.80 | 0.13 |

**TABLE D.5** Dietary exposure to PAT/bar protein in farmed and companion animals, based on the consumption of cotton undelinted seeds.

| | Body weight | Total daily intake | Inclusion rates | Daily dietary exposure | |
|---|---|---|---|---|---|
| **Animal species** | **kg** | **kg/animal** | **%** | **µg/kg** | **mg/kg** |
| Dairy cow | 650 | 25 | 10 | 840.15 | 0.84 |
| Dairy Sheep | 80 | 2.8 | 25 | 1911.35 | 1.91 |
| Dairy Goat | 60 | 3.4 | 20 | 2475.65 | 2.48 |

**TABLE D.6** Dietary exposure to PAT/bar protein in farmed and companion animals, based on the consumption of cottonseed meal.

| | Body weight | Total daily intake | Inclusion rates | Daily dietary exposure | |
|---|---|---|---|---|---|
| **Animal species** | **kg** | **kg/animal** | **%** | **µg/kg** | **mg/kg** |
| Dairy cow | 650 | 25 | 5 | 735.13 | 0.74 |
| Beef cattle | 500 | 12 | 5 | 458.72 | 0.46 |
| Dairy Sheep | 80 | 2.8 | 20 | 2675.89 | 2.68 |
| Dairy Goat | 60 | 3.4 | 15.5 | 3357.60 | 3.36 |
| Rabbit | 2 | 0.15 | 43 | 12328.21 | 12.33 |
| Fattening pig | 100 | 3 | 5 | 573.41 | 0.57 |
| Lactating sow | 200 | 6 | 10 | 1146.81 | 1.15 |
| Broiler | 2 | 0.158 | 5 | 1509.97 | 1.51 |
| Laying hens | 1.9 | 0.13 | 5 | 1307.77 | 1.31 |
| Turkey | 7 | 0.5 | 10 | 2730.50 | 2.73 |
| Horse | 450 | 9 | 10 | 764.54 | 0.76 |

**TABLE D.7** Dietary exposure to Vip3Aa19 protein in farmed and companion animals, based on the consumption of cotton undelinted seeds.

| | Body weight | Total daily intake | Inclusion rates | Daily dietary exposure | |
|---|---|---|---|---|---|
| **Animal species** | **kg** | **kg/animal** | **%** | **µg/kg** | **mg/kg** |
| Dairy cow | 650 | 25 | 10 | 14.50 | 0.01 |
| Dairy Sheep | 80 | 2.8 | 25 | 32.99 | 0.03 |
| Dairy Goat | 60 | 3.4 | 20 | 42.73 | 0.04 |

**TABLE D.8** Dietary exposure to Vip3Aa19 protein in farmed and companion animals, based on the consumption of cottonseed meal.

| | Body weight | Total daily intake | Inclusion rates | Daily dietary exposure | |
|---|---|---|---|---|---|
| **Animal species** | **kg** | **kg/animal** | **%** | **µg/kg** | **mg/kg** |
| Dairy cow | 650 | 25 | 5 | 12.69 | 0.01 |
| Beef cattle | 500 | 12 | 5 | 7.92 | 0.01 |
| Dairy Sheep | 80 | 2.8 | 20 | 46.20 | 0.05 |
| Dairy Goat | 60 | 3.4 | 15.5 | 57.97 | 0.06 |
| Rabbit | 2 | 0.15 | 43 | 212.85 | 0.21 |
| Fattening pig | 100 | 3 | 5 | 9.90 | 0.01 |
| Lactating sow | 200 | 6 | 10 | 19.80 | 0.02 |
| Broiler | 2 | 0.158 | 5 | 26.07 | 0.03 |
| Laying hens | 1.9 | 0.13 | 5 | 22.58 | 0.02 |
| Turkey | 7 | 0.5 | 10 | 47.14 | 0.05 |
| Horse | 450 | 9 | 10 | 13.20 | 0.01 |

**TABLE D.9** Dietary exposure to APH4 protein in farmed and companion animals, based on the consumption of cotton undelinted seeds.

| | Body weight | Total daily intake | Inclusion rates | Daily dietary exposure | |
|---|---|---|---|---|---|
| **Animal species** | **kg** | **kg/animal** | **%** | **µg/kg** | **mg/kg** |
| Dairy cow | 650 | 25 | 10 | 0.01 | 0.00001 |
| Dairy Sheep | 80 | 2.8 | 25 | 0.03 | 0.00003 |
| Dairy Goat | 60 | 3.4 | 20 | 0.04 | 0.00004 |

**TABLE D.10** Dietary exposure to APH4 protein in farmed and companion animals, based on the consumption of cottonseed meal.

| | Body weight | Total daily intake | Inclusion rates | Daily dietary exposure | |
|---|---|---|---|---|---|
| **Animal species** | **kg** | **kg/animal** | **%** | **µg/kg** | **mg/kg** |
| Dairy cow | 650 | 25 | 5 | 0.02 | 0.00002 |
| Beef cattle | 500 | 12 | 5 | 0.01 | 0.00001 |
| Dairy Sheep | 80 | 2.8 | 20 | 0.08 | 0.00008 |
| Dairy Goat | 60 | 3.4 | 15.5 | 0.09 | 0.00009 |
| Rabbit | 2 | 0.15 | 43 | 0.35 | 0.00035 |
| Fattening pig | 100 | 3 | 5 | 0.02 | 0.00002 |
| Lactating sow | 200 | 6 | 10 | 0.03 | 0.00003 |
| Broiler | 2 | 0.158 | 5 | 0.04 | 0.00004 |
| Laying hens | 1.9 | 0.13 | 5 | 0.04 | 0.00004 |
| Turkey | 7 | 0.5 | 10 | 0.08 | 0.00008 |
| Horse | 450 | 9 | 10 | 0.02 | 0.00002 |