A Review of the Food and Feed Safety of the Cry1Ab Protein

Agriculture & Food Systems Institute
1900 L Street NW, Suite 302, Washington, DC 20036 USA

July 24, 2026

INTRODUCTION

This document provides an updated comprehensive review of information and data relevant to the assessment of the protein Cry1Ab for food and feed safety. The original version of this review was published in 2016 (AFSI, 2016). All sources of information cited in this document are publicly available and include dossiers presented to regulatory authorities, decision summaries prepared by regulatory authorities, peer reviewed literature, and product summaries prepared by product developers. These documents reflect a common approach to safety assessment based on comparison of the genetically engineered (GE) plant to the non-GE plant and the safety of the non-GE plant as an accepted level of safety (OECD, 1986; NRC, 1989; CBD, 2000; Codex, 2003a, 2003b; EFSA, 2006, 2011).

The Codex Alimentarius Guidance CAC/GL 45-2003 (Codex Plant Guideline) covers safety assessment of foods derived from GE plants and provides a framework for conducting food safety assessment on GE plants (Codex, 2003a, 2003b). Safety assessments related to the use of GE plants in food and feed are conducted on a case-by-case basis, taking into account the following factors:

- The biology of the unmodified plant;
- The traditional uses of the unmodified plant in food and feed;
- The intended uses of the GE plant in food and feed;
- The nature of the transgene, the donor organism, and the protein it produces;
- The phenotype conferred by the transgene;
- Compositional analyses of key components including metabolites;
- The presence of known toxins, allergens, and anti-nutritional substances;
- Toxicologic and allergenic properties of the expressed protein;
- Feeding studies for GE plant that is intended to confer nutritional improvement;
- The potential impact of food and feed processing on safety.

Since this monograph is on the safety of the Cry1Ab protein and not on GE crops containing the protein, not all the safety assessment elements in the Codex Plant Guideline are relevant. The three topics covered in this monograph are “Origin and Function of Cry1Ab” (including its mechanism of action on targeted species), “Expression of Cry1Ab in Insect Resistant GE Plants” (including the expression levels of Cry1Ab in various parts of the crops), and “Food and Feed Implications of the Cry1Ab Protein” (including information on toxicology and allergenicity assessments). Compositional analysis, which is a key component of the Codex Plant Guideline and is commonly part of the submission for food safety approvals, is mainly used to identify unintended changes in the nutritional profile of the food crop due to the insertion of the gene (Codex, 2003b) and is not directly relevant to risks associated with the protein. However, because compositional analysis has

KEYWORDS

Cry1Ab, *Bacillus thuringiensis* (Bt), insect resistance, genetically engineered (GE), food and feed safety assessment

DOI: 10.67498/afsi-3q95

been consistently considered in food safety assessments in all countries, a discussion of the composition profile of the GE crops expressing the Cry1Ab protein is also included as part of this review.

Global Food Safety Approvals of Cry1Ab Expressing Crops

At the first writing of this review (AFSI, 2016), three GE crops (maize, cotton, and rice) in which the Cry1Ab protein is expressed for insect resistance had been approved for food safety in at least one of 18 countries and the European Union (EU), representing 42 transformation events. As of the writing of this current version of the review, almost ten years later in 2025, food safety approvals for three additional crops expressing the Cry1Ab protein (cowpea, eucalyptus, and sugarcane) have been issued. Table 1-5 show the details of regulatory approvals[1] for food and feed in these crops, according to ISAAA's GM Approval Database (ISAAA, 2025).

Approvals for food safety of these six crops have been issued in more than 30 countries and the European Union, representing 80 transformation events (including 'stacked' events): Maize - 57 events, Cotton - 16 events, Rice - 3 events, Sugarcane - 2 events, Cowpea - 1 event, Eucalyptus - 1 event. In total, there have been 427 regulatory approvals for food safety; 372 of these are in maize, 45 in cotton, and 10 in other crops. Food safety approvals are issued more often than approvals for cultivation, primarily to establish food safety of the GE crops by importing countries where they are intended for food and/or feed, not to be grown[2].

Maize is the most approved of these crops. Eight maize single transformation events[3] have been approved for food safety globally over 30 years, seven before 2010 (Table 1) and one other, DBN9936, approved after 2010 (Tables 2). Event Bt176 (NaturGard KnockOut™, Maximizer™) was the earliest maize event expressing Cry1Ab to receive food safety approvals and has been approved in eleven countries and the European Union between 1995–2003 with one more recent approval in 2018 in Zambia. Two other maize single transformation events expressing Cry1Ab, event Bt11 (Agrisure™ CB/LL) and event MON810 (YieldGard™, MaizeGard™), have received food safety approvals in 25 and 24 countries, respectively, and the European Union. Many of these approvals for Cry1Ab maize were issued before 2010 (Table 1), and twelve countries have granted approvals since 2010 (Table 2). The most recent approvals (at the time of this writing in December 2025) have been in Ghana (in 2024) for Bt11 and in Indonesia (in 2022) and Thailand (in 2022) for MON810.

Beyond these maize single transformation events with Cry1Ab that have received food safety approvals, there have been many more food safety approvals for maize 'stacked events' developed through conventional breeding to combine Cry1Ab insect resistance with other GE traits. Twenty-one stacked event maize lines derived from crossing MON810 with other GE maize have been approved for food safety in at least one of 16 countries[4] and the European Union (Table 3). Another 28 breeding stacks derived from crossing with Bt11 have been approved in 18 countries and the European Union (Table 4). Around half of these food safety approvals for these lines with Cry1Ab derived from conventional breeding have been issued in the last ten years, with a number of approvals by some countries in the last five years.

Table 1: Cry1Ab expressing maize single transformation events with food safety approvals up to 2010.

Crop	Event Name*	Argentina	Australia	Brazil	Canada	China	Colombia	EU	Japan	Mexico	New Zealand	Philippines	Russia	South Africa	South Korea	Switzerland	Taiwan	Uruguay	USA
Zea mays (Maize)	Bt176	x	x		x	x		x	x		x	x		x	x	x			x
	Bt11	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	MON810	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x
	Bt10														x				
	MON801																		x
	MON802				x														x
	MON809				x														x

OECD Unique Identifiers for the event names can be found in the ISAAA GM Crop Approval Database.

1. 'Regulatory approval' should not be interpreted as an indication that the product is in commercial production. There are many examples of products that were assessed for safety and granted regulatory approval but were never commercialized, or if they were, have been subsequently discontinued.

2. Some countries issue a separate approval for food and feed. In a few cases, countries have approved events for feed only, not for food. This review only accounts for approvals for 'food' or 'food and feed'; Feed-only approvals are not included in Tables 1-5.

3. A' single-transformation event' refers to the specific, one-time insertion of foreign DNA into a plant genome, creating a distinct GE plant. A 'single transformation event' can have one or multiple ('stacked') genes inserted, different than 'stacked' events developed through conventional breeding. In this document, 'stacked events' refer to events with multiple genes combined through conventional breeding ('breeding stacks').

4. Some countries, including the US, Canada, Australia/New Zealand, consider breeding stacks with approved single events as safe for food based on the existing approvals of the transformation events from which the stacks are derived and therefore approvals of breeding stacks may not be recorded in these countries.

Table 2: Cry1Ab expressing maize single transformation events with food safety approvals since 2010. Approvals after 2020 are highlighted.

Crop	Event Name*	China	Ghana	Indonesia	Kenya	Malaysia	Nigeria	Paraguay	Singapore	Thailand	USA	Vietnam	Zambia
Zea mays (Maize)	Bt176												x
	Bt11		x	x		x	x	x	x	x		x	
	MON810			x	x	x	x	x	x	x		x	
	DBN9936	x									x		

**OECD Unique Identifiers for the event names can be found in the ISAAA GM Crop Approval Database.*

Table 3: Cry1Ab expressing maize event MON810 breeding stacks with food safety approvals. Approvals after 2020 are highlighted.

Crop	Event Name*	Argentina	Brazil	Colombia	EU	Japan	Mexico	Nigeria	Paraguay	Philippines	Singapore	South Africa	South Korea	Switzerland	Taiwan	Uruguay
Zea mays (maize)	4114 × MON810 × MIR604				x											
	4114 × MON810 × NK603				x											
	DP4114 × MON810 × MIR604 × NK603			x	x	x	x						x			
	GA21 × MON810				x	x				x		x	x			
	LY038 × MON810	x				x	x									
	MON810 × MIR162	x	x													
	MON810 × MIR162 × NK603	x														
	MON810 × MON88017			x	x	x	x			x	x	x	x	x	x	
	MON863 × MON810				x	x	x			x		x	x		x	
	MON863 × MON810 × NK603				x	x	x	x		x		x	x		x	
	MON87427 × MON89034 × MON810 × MIR162 × MON87411 × MON87419	x		x		x				x	x				x	
	NK603 × MON810**	x	x	x	x	x	x			x	x	x	x	x	x	x
	NK603 × MON810 x 4114 × MIR604			x		x	x						x		x	
	T25 × MON810			x		x										
	TC1507 × 59122 × MON810 × NK603			x	x	x	x								x	
	TC1507 × 59122 × MON810 × MIR604 × NK603			x	x	x	x			x			x		x	
	TC1507 × MON810***	x	x	x			x		x	x		x	x		x	
	TC1507 × MON810 × MIR162	x	x	x		x	x						x		x	
	TC1507 × MON810 × MIR162 × NK603	x	x	x	x	x	x			x			x		x	
	TC1507 × MON810 × NK603	x	x	x	x	x	x		x	x		x			x	x
	TC1507 × MON810 × NK603 × MIR604			x		x							x			

**OECD Unique Identifiers for the event names can be found in the ISAAA GM Crop Approval Database.*
***Pakistan has also approved NK603 × MON810. This is the only stack with MON810 with food safety approval in this country.*
****Iran has approved TC1507 × MON810**. This is the only stack with MON810 with food safety approval in this country.*

Table 4: Cry1Ab expressing maize event Bt11 breeding stacks with food safety approvals. Approvals after 2020 are highlighted.

Crop	Event Name*	Argentina	Brazil	Colombia	EU	Japan	Mexico	Paraguay	Philippines	Singapore	South Africa	South Korea	Switzerland	Taiwan	Thailand	Uruguay
Zea mays (Maize)	3272 × Bt11 × MIR604 × GA21	x	x	x		x	x		x	x	x	x		x	x	
	3272 × Bt11 x 59122 × MIR604 × TC1507 × GA21					x										
	3272 × Bt11 × MIR162 × GA21	x						x				x		x		
	3272 × Bt11 × MIR162 × MIR604 × TC1507 x 5307 × GA21					x						x		x		
	3272 × Bt11 × MIR604 × TC1507 x 5307 × GA21			x		x	x		x	x		x		x		
	5307 × MIR604 × Bt11 × TC1507 × GA21			x		x	x			x	x	x		x		
	5307 × MIR604 × Bt11 × TC1507 × GA21 × MIR162	x	x	x		x	x			x	x	x		x		
	Bt11 x 5307		x													
	Bt11 x 59122 × MIR604 × TC1507 × GA21			x	x	x	x		x	x	x	x		x		
	Bt11 × DP4114 × MIR162 × MZIR098 × NK603											x				
	Bt11 × GA21**	x	x	x	x	x	x	x	x	x	x	x		x	x	x
	Bt11 × MIR162	x	x	x	x	x	x		x	x	x	x		x	x	
	Bt11 × MIR162 × NK603	x	x					x								
	Bt11 × MIR162 × GA21	x	x	x	x	x	x	x	x	x	x	x		x	x	x
	Bt11 × MIR162 × MIR604		x		x											
	Bt11 × MIR162 × TC1507 × GA21	x		x		x	x		x	x	x	x	x	x		
	Bt11 × MIR162 × MIR604 × GA21	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
	Bt11 × MIR162 × MIR604 × MON89034 x 5307 × GA21***				x	x				x		x		x	x	
	Bt11 × MIR162 × MIR604 × TC1507		x													
	Bt11 × MIR162 × MON89034	x	x									x		x		
	Bt11 × MIR162 × MON89034 × GA21	x	x	x			x	x			x	x		x	x	
	Bt11 × MIR162 × TC1507 × GA21	x														
	Bt11 × MIR604		x		x	x	x		x	x	x	x		x	x	
	Bt11 × MIR604 × GA21			x	x	x	x		x	x	x	x		x	x	
	Bt11 × MON89034		x													
	Bt11 × MON89034 × GA21	x														
	Bt11 × TC1507	x	x													
	Bt11 × TC1507 × GA21	x		x	x		x		x	x		x		x		

**OECD Unique Identifiers for the event names can be found in the ISAAA GM Crop Approval Database.*
*** China, Indonesia, and Vietnam have also approved Bt11 × GA21. This is the only stack with Bt11 with food safety approval in these three countries.*
****Canada has also approved Bt11 × MIR162 × MIR604 × MON89034 x 5307 × GA21. This is the only stack with Bt11 with food safety approval in this country.*

There have also been approvals for food safety for Cry1Ab expressing cotton, cowpea, eucalyptus, rice, and sugarcane in some countries (Table 5). Cotton event COT67B was the earliest Cry1Ab expressing cotton with food safety approvals in six countries between 2009–2012. Cotton event T304-40 was approved in 14 countries, including three approvals, in Singapore, South Korea, and Switzerland, since 2020. Seven cotton breeding stacks with T304-40 have also received food safety approval in at least one of eleven countries. Two rice events expressing Cry1Ab protein were approved for food safety in China, and one other Cry1Ab rice event has recently been approved for food safety in Iran. Two sugarcane events expressing Cry1Ab were approved for food safety in Brazil in 2017, and one was also approved in Canada and the US in 2018. Brazil also issued a food safety approval for one eucalyptus event and one eucalyptus breeding stack with Cry1Ab in 2024. Two countries in Africa, Nigeria and Ghana, have recently issued approvals for food safety for one cowpea event expressing the *cry1Ab* gene for resistance to the cowpea pod borer insect pest.

Regulatory submissions, safety assessments, and decisions for many of these GE events expressing the Cry1Ab protein are available from different regulatory agencies around the world. A number of those documents are publicly available and are listed in Annex 1. Where regulatory decisions or outcomes of regulatory risk assessments are referred to throughout this monograph, examples of these decisions or assessments can be found in the documents in Annex 1.

Table 5: Cry1Ab expressing cotton, cowpea, eucalyptus, rice, sugarcane with food safety approvals. Approvals after 2020 are highlighted.

Crop	Event Name*	Argentina	Australia	Brazil	Canada	China	Colombia	EU	Ghana	Iran	Japan	Malaysia	Mexico	New Zealand	Nigeria	Philippines	Singapore	South Korea	Switzerland	Taiwan	USA
Gossypium hirsutum (Cotton)	COT67B (IR67B)		x		x						x		x	x							x
	T304-40		x		x	x	x	x			x	x		x		x	x	x	x	x	x
	GHB614 × T304-40						x														
	GHB614 × T304-40 × GHB119			x			x	x			x		x					x	x	x	
	T304-40 × GHB119			x													x	x			
	T304-40 × GHB119 × COT102			x			x										x				
	GHB614 × T304-40 × GHB119 × COT102	x	x	x																	
	GHB811 × T304-40 × GHB119 × COT102	x		x			x														
	GHB811 × T304-40 × GHB119 × COT102 × MON88701						x				x							x		x	
Vigna unguiculata (Cowpea)	*AAT709A								x						x						
Eucalyptus sp. (Eucalyptus)	1521K059			x																	
	955S024 x 1521K059 × H421			x																	
Oryza sativa (Rice)	Huahui-1 (TT51-1)					x															
	Shanyou63					x															
	*Tarom molail + cry1Ab									x											
Saccharum sp. (Sugarcane)	CTB141175/01-A (CTC175-A)			x	x																x
	CTC20BT			x																	

**OECD Unique Identifiers for the event names can be found in the ISAAA GM Crop Approval Database.*

ORIGIN AND FUNCTION OF CRY1AB

Bacillus thuringiensis and the Cry1Ab Insecticidal Protein

Insect resistant GE crops adopted at high rates have predominantly been developed using genes from the bacterium *Bacillus thuringiensis* (Bt) (Bravo *et al.*, 2023; Gassmann & Reisig, 2023). *B. thuringiensis* is a rod-shaped, gram-positive bacterium capable of forming long-lived endospores. It is often referred to as a soil bacterium, although it is ubiquitous in the environment (Martínez & Caballero, 2002; Apaydin *et al.*, 2008; Seifinejad *et al.*, 2008). *B. thuringiensis* has been studied extensively and used commercially for many years due to its ability to synthesize proteins with pesticidal properties (Höfte & Whiteley, 1989; Cannon, 1996; Schnepf *et al.*, 1998; OECD, 2007; van Frankenhuyzen, 2009). The species produces diverse insecticidal proteins with distinct structures and modes of action (Crickmore *et al.*, 2021; Liu *et al.*, 2021; Bravo *et al.*, 2023). Of these, the Cry insecticidal proteins, so named because they are the primary component of the protein parasporal crystals that are characteristic of spore formation in *B. thuringiensis*, have been used most commonly (Höfte & Whiteley, 1989; Kumar *et al.*, 1996; Schnepf *et al.*, 1998; OECD, 2007; Liu *et al.*, 2021; Bravo *et al.*, 2023).

Preparations of natural isolates of *B. thuringiensis* were first used as a commercial insecticide in France in 1938, and *B. thuringiensis* subspecies *kurstaki* (which produces Cry1Ab among other Cry proteins) has been registered with US EPA since 1961 (US EPA, 1998). Microbial preparations of *B. thuringiensis* are approved for use around the world including in Africa, Australia, Brazil, Canada, the European Union, and the United States (US EPA, 1998; Health Canada, 2008b; Kabaluk *et al.*, 2010; do Nascimento *et al.*, 2022; APVMA, 2023; Ragasruthi *et al.*, 2024; EU Pesticides Database, 2025). Bt preparations are the most widely used microbial insecticides in agriculture and forestry and considered a low-risk option to control Lepidoptera, Diptera and Coleoptera insects, due to their lack of polluting residues and high specificity to target insect species (Adang *et al.*, 2014; Liu *et al.*, 2021; Ragasruthi *et al.*, 2024).

There have been changes to the nomenclature assigned to the insecticidal proteins from *B. thuringiensis* and from other bacteria over the years (Crickmore *et al.*, 2021; Bravo *et al.*, 2023). A systematic nomenclature for identifying and differentiating Cry proteins proposed in 1989 initially grouped them into four classes I, II, III, and IV based on their toxicity to particular orders of insects (Höfte & Whiteley, 1989; OECD, 2007). CryI proteins were those toxic to Lepidoptera, CryII proteins were those toxic to Lepidoptera and Diptera, CryIII proteins were toxic to Coleoptera and CryIV proteins were those toxic to Diptera. As technology for studying the proteins advanced, it became apparent that proteins with shared sequence homology had different pesticidal specificities, and the proteins were subsequently reclassified not by pesticidal specificities but according to amino acid sequence similarity (Crickmore *et al.*, 1998). Proteins sharing not more than 45% amino acid sequence similarity were divided into primary groups (Cry1, Cry2, etc.). Within those primary groups, proteins with up to 78% sequence similarity were placed into secondary groups (Cry1A, Cry1B, etc.), and proteins within those secondary groups with at least 95% similarity were placed into tertiary groups (Cry1Aa, Cry1Ab, etc.). A fourth level of grouping divided proteins within the tertiary groups that shared more than 95% sequence identity (Cry1Aa1, Cry1Aa2, etc.) (Crickmore *et al.*, 1998).

This four-level naming system within the Cry proteins has been modified only slightly in recent years, based mainly on structural similarities (Crickmore *et al.*, 2021). The Cry proteins are now clearly designated as separate from other bacterial derived-insecticidal proteins (e.g., Cyt, Vip, SIP) based on structural differences, with the defining structure for the Cry proteins being the classic three-domain structure (Crickmore *et al.*, 2021). To date, according to this system based on amino acid sequence identity, there are 755 different Cry proteins described and classified in at least 57 groups and 166 subgroups (Crickmore *et al.*, 2021; Bravo *et al.*, 2023). The proteins designated as Cry1 share at least 45% amino acid sequence similarity, those designated as Cry1A are at least 76% similar, and Cry1Ab proteins are at least 95% identical in amino acid sequence. The Cry1Ab protein shares up to 94% sequence identity with other Cry1A proteins (including Cry1Aa and Cry1Ac).

Mechanism of Cry1Ab Insecticidal Activity

The classic model for Cry protein mode of action follows a series of steps (Vachon *et al.*, 2012; Pardo-López *et al.*, 2013; Adang *et al.*, 2014; Bravo *et al.*, 2023). The crystal is produced by the Bt bacterium during sporulation and ingested by the insect. The protein in the crystal, in the form of a 'protoxin', is solubilized in the insect midgut, and the solubilized protoxin is activated by midgut proteases. Two different types of Cry protoxins have been described based on their size, short (~70–73 kDa) protoxins characteristic of the Cry2 and Cry3 proteins and long (~120–130 kDa) protoxins characteristic of the Cry1 proteins (OECD, 2007). After activation (cleavage) by host gut proteases, all Cry protoxins yield a ~55–60 kDa three-domain 'activated' (toxic) core (de Maagd *et al.*, 2001; OECD, 2007). Cry1 proteins are cleaved at both the C-terminal and N-terminal regions of the protoxin, while cleavage only occurs at the N-terminal region of the shorter Cry2 and Cry3 protoxin, although precise cleavage sites are not clear (Liu *et al.*, 2020).

This 'activated' form of the protein is believed to interact with specific receptors on the surface of midgut epithelial cells, allowing them to insert into the membrane through the formation of transmembrane pores. These pores lead to increased permeability, osmotic imbalance, and disruption of the membrane. This damage to the cells of the midgut epithelial tissues ultimately leads to the death of the insect. Although there are other models for the insecticidal mechanism of the Cry proteins, this classic 'pore formation' model is the most widely accepted (Bravo *et al.*, 2023). It has also

been proposed that the binding to receptors of the active toxin in the insect midgut stimulates an intracellular signaling pathway that leads to necrotic cell death with a minimal role of pore formation, although there is more evidence supporting the importance of pore formation than this 'signaling pathway' theory proposes (Soberón *et al.*, 2009; Vachon *et al.*, 2012; Adang *et al.*, 2014; Khorramnejad *et al.*, 2020; Bravo *et al.*, 2023).

A distinguishing characteristic of the Cry proteins is the three-domain structure that forms the 'active' protein central to the insecticidal mode of action (Crickmore *et al.*, 2021; Bravo *et al.*, 2023). The N-terminal domain I is involved in toxin oligomerization, membrane insertion and pore formation; domain II is involved in binding to Cry receptors and is considered an important specificity domain; domain III binds to larval gut proteins and is also involved in stabilization of the three-domain toxic core against protease action (Bravo *et al.*, 2023). Cry proteins show high levels of specificity for their target organism, relying on the specific interaction with the different midgut membrane proteins of the insect that are the Cry receptors (Pardo-López *et al.*, 2013; Adang *et al.*, 2014). While a great deal is understood about the Cry protein mode of action and the different receptors, the details by which solubilization, binding, oligomerization, pore formation, and membrane insertion occur and their role in the interaction of the active toxin with the host receptor in determining the specificity of the Cry proteins to target organisms remain significant topics of study (Jurat-Fuentes & Crickmore, 2017; Bravo *et al.*, 2023).

Modifications to the Cry1Ab Gene and Cry1Ab Protein in GE Plants

There are two main types of modifications to the *cry* genes from Bt that are common for use in GE plants (Sanahuja *et al.*, 2011; Koch *et al.*, 2015). The first type, typical for any bacterial gene expressed in GE plants, involves modifications to the nucleotide sequence. These modifications are primarily used to increase the translation of the gene by modifying codon usage to align with plant-preferred codons. Because the DNA of plants and bacteria differ in nucleotide content (Perlak *et al.*, 1991; Koziel *et al.*, 1993; Sanahuja *et al.*, 2011), to increase the expression of the Cry protein in the plant to a level sufficient for pest control, the sequences used for GE plants have been modified from that in the bacteria to increase the GC content and frequency of ATTTA sequences (Perlak *et al.*, 1991, Koziel *et al.*, 1993, Sanahuja *et al.*, 2011). The use of strong promoters and inclusion of plant introns in the expression construct (Sanahuja *et al.*, 2011), or other enhancers such as the 5' untranslated region of the small subunit of the RUBISCO gene (SSU UTR5') can further increase expression. Directing the *cry* gene expression into the chloroplast genome of the plant also significantly increases *cry* gene expression (McBride *et al.*, 1995; Sanahuja *et al.*, 2011).

The second type of modification involves modifications to the amino acid sequence of the resulting protein through deletion of sequences outside of the sequence of the core protein responsible for insecticidal activity (Perlak *et al.*, 1991). In most GE crops expressing the Cry1Ab protein, truncations of the Bt protein have been utilized. The Cry protein expressed in these plants consist of the "activated" form of the Cry protein that still requires binding to a specific receptor or receptors in the insect midgut and retains the species specificity of the full-length protein (Perlak *et al.*, 1991). Research on the Cry1 proteins suggests that the active toxin and the full-length protoxin may both have a toxic effect on target insects, but via different pathways, raising prospects for future genetic engineering with these Cry proteins using the amino acid sequence of the full-length protoxin to produce novel insect resistant varieties (Gómez *et al.*, 2014; Tabashnik *et al.*, 2015).

Most approved GE plants expressing the Cry1Ab protein have been developed using these types of modifications to the *cry1Ab* gene and Cry1Ab protein. These modifications do not alter the structure or function of the protein to the degree that it affects the safety of these proteins, and the history of safe use of the 'native' cry proteins as a pesticide applied to crops can still be applied to the assessment of these modified proteins (Koch *et al.*, 2015). For these reasons, regulatory authorities have concluded in their safety assessments that these modifications to the Cry1Ab proteins in Bt crops do not raise safety concerns (See Annex 1).

EXPRESSION OF CRY1AB IN INSECT RESISTANT GE PLANTS

Food and feed safety assessments of Bt crops typically take into consideration the concentration levels of the Cry protein in various parts of the GE plants because these levels, together with consumption information, can be used to estimate the human and animal exposure to the protein for the safety assessment (Delaney *et al.*, 2008; Brune *et al*., 2021). Transgene expression levels in a GE plant can be influenced by several factors related to the genetic transformation process, including the types of promoter and terminator sequences employed, as well as the chromosomal location of the transgene that has been incorporated into the genome. Expression levels may also be influenced by the type of tissue sampled, the age of the plant at the time the sample was taken, and the environmental conditions during plant growth (Siebert *et al.*, 2009; Brune *et al.*, 2021). Each transformation event therefore exhibits a different expression profile.

Data on the level of expression of Cry1Ab in GE plants that have obtained regulatory approvals are available in publicly accessible regulatory submissions and decision documents (See Annex 1). Typically, one or more samples of plant tissue are taken at a field trial site and pooled for analysis. The amount of Cry1Ab is normally determined on a dry weight basis then calculated to provide values relative to the total fresh weight of the sample and represented in a ratio (e.g., micrograms of Cry1Ab protein per gram of fresh weight). Samples are usually collected from several tissue types and at multiple growth stages providing data from plants over time and from multiple locations. The exposure assessment may

also consider the effects of processing on levels of the Cry protein. In most cases the data are presented as a mean value (normally a mean of means as values were averaged within a field trial and across trials as well) and a range (normally also a range of means representing the average expression at a trial site, although this may vary depending on the individual example). In other data sets, means are provided with the standard deviation or the standard error of means. Tissue types and collection methods may differ between studies, but all use an enzyme-linked immunosorbent assay (ELISA) or Western blot analysis to quantify the amount of Cry1Ab protein present in a sample.

Some examples of Cry1Ab protein expression data and references are presented in Table 6. Variations in methodology for sample collection make direct statistical cross-comparisons of the data inappropriate, but the weight of evidence from regulatory submissions suggests that Cry1Ab is expressed at very low levels relative to the total protein synthesized by the plant. For food and feed safety assessment, the level of protein in the tissue, once measured, can then be used to calculate the exposure to humans and animals taking into consideration the dietary exposure, i.e., the amount of crop consumed as a percentage of the diet and the parts and proportions of crops consumed, if this is known (Delaney *et al.*, 2008). These patterns can vary depending on the population likely to be exposed, and dietary exposure for humans is often different from those consumed by animals.

Table 6: Examples of expression data used for assessment of Cry1Ab in some GE events.

Crop/Event	Year –Trial Locations	Tissue	Cry1Ab Levels* (ug/g fresh weight)	
Maize Bt11 (X4334-CBR)	1998 US**	Leaf	4.3 ± 0.66	FSANZ 2000b
		Kernel	1.5 ± 0.21	
		Husk	1.1 ± 0.26	
		Stalk	0.71 ± 0.11	
Maize Bt11 (X4734-CBR)	1998 US**	Leaf	5.05 ± 0.35	
		Kernel	1.30 ± 0.28	
		Husk	0.84 ± 0.18	
		Stalk	0.55 ± 0.06	
Maize MON810	1994 US	Young leaf	(7.93-10.34)	EFSA 2009b
		Whole plant	(3.65-4.65)	
		Harvested grain	(0.19-0.39)	
	1995 France and Italy	Young leaf tissue	(7.59-9.39)	
		Forage tissue	(4.21-9.23)	
		Harvested grain	(0.42-0.69)	
Cotton T304-40	2007 USA	Seed	0.562 (0.527–1.57)	EFSA 2013
	2008 Spain	Seed	2.63 (1.77–3.63)	
Cowpea AAT709A	2014 Nigeria	Leaf	5.1 (1.9-8.1)	CSIR-SARI 2020
		Flower	4.5 (3.2-5.2)	
		Pods	3.7 (2.9-4.0)	
		Green Cotyledons	2.2 (1.95-3.0)	
		Dry Seed	2.5 (2.3-2.8)	
	2014 Greenhouse	Pollen	0.12 (0.06-0.18)	
		Anther Wall	1.1 (0.98-1.2)	
		Roots	0.25 (0.18-0.33)	

** Values represent the means (if reported) with the standard error or with the lowest and highest individual values shown in parentheses (as reported).*
*** FSANZ 2000b describes plants 'grown in two locations' and refers to an unpublished study by Schramm and Warnick (1998) for the analysis conducted in the USA, but the location where the plants were grown is not specified.*

As an example of a Cry1Ab exposure assessment, the assessment of AAT709A cowpea recently approved in Nigeria and Ghana considered the level of expression in the cowpea plant (shown in Table 6) along with estimates of consumption levels to determine dietary exposure to humans and animals (CSIR-SARI, 2020). For humans, the assessment focused on Cry1Ab in the dried grain as the main part of the crop used for food. Using the estimated highest per capita utilization of cowpea grain and the average adult body weight in Nigeria, the maximum daily cowpea intake was calculated and the daily dietary exposure of 2.35ug/kg body weight was estimated conservatively. This is orders of magnitudes lower than the highest dosage tested (up to 4000 mg/kg bodyweight) in mice with no acute toxicity (CSIR-SARI, 2020). (See the discussion on toxicity testing.) For other cowpea products used as food, including green leaves, immature pods, and green seeds, the level of Cry1Ab was measured and used for the exposure assessment, but consumption patterns were not available to calculate dietary exposure. For animals, dietary exposure was calculated for forage, hay, and seed based on consumption patterns in livestock, including cattle, sheep, swine, and for seed only in poultry. The highest Cry1Ab daily dietary exposure estimate was 1.76 mg/kg for lambs feeding on forage from AAT709A cowpea (CSIR-SARI, 2020).

The mechanism of insecticidal activity, specificity to the target pests, and the history of safe use of the Cry proteins, and other information such as bioinformatics and toxicity testing (see below), provide a meaningful weight of evidence for the safety of these proteins, i.e., low risk due to negligible hazard for humans and animals, even without a detailed exposure assessment (Koch *et al.*, 2015). However, because the Cry genes have a 'toxic' mode-of-action (toward the insect pest of the crop), safety assessments of Cry1Ab proteins have included an exposure assessment. In every case, for every crop and event approved, the assessment has concluded that the levels of Cry1Ab detected in the crop do not raise any food or feed safety concerns (See Annex 1).

US EPA grants registrations for the sale and use of pesticides, including plants genetically engineered to produce a pesticidal substance (termed plant-incorporated-protectants (PIPs) by the US EPA). As part of the registration process, US EPA issues tolerances or tolerance exemptions for residues of pesticidal substances on food and/or feed (Mendelsohn *et al.*, 2003). These tolerance exemptions are issued, for any pesticide, when the EPA determines based on toxicity and exposure data that there is no maximum residue limit necessary to establish "reasonable certainty of no harm" (US EPA, 2025a). The dietary exposure assessment is a key consideration in establishing a tolerance limit or exemption. EPA has issued tolerance exemptions for all registered GE crops expressing the Cry1Ab protein (US EPA, 2008, 2010, 2012, 2025b).

FOOD AND FEED SAFETY OF THE CRY1AB PROTEIN

General Considerations in Assessing Food and Feed Safety of GE Crops

In assessing food safety for GE crops, comparative assessment is a key concept, although it is not a safety assessment in and of itself. This concept is used to identify relevant differences between the new food and its conventional counterpart. It helps to identify potential safety and nutritional issues and therefore is widely accepted as the most appropriate strategy for safety assessment of GE foods (Codex, 2003a, 2003b). Regulatory agencies around the world regulate GE crops for food and/or feed use based on safety assessment of the specific GE crop products. Although countries follow the same Codex Plant Guideline, the data requirements for regulatory approvals may not be the same in all countries/regions (Waters *et al.*, 2021).

According to the Codex Plant Guideline (Codex, 2003b), when assessing potential toxicity of an expressed protein in GE crops, the following aspects should be considered: history of safe use of the protein in food or feed, primary sequence similarity between the protein and known protein toxins and anti-nutrients, stability to heat or processing and to enzymatic degradation, and oral toxicity studies in cases where the protein present in the food is not similar to proteins that have previously been consumed safely in food. In addition, allergenicity of the protein should be assessed. These factors, along with the function or mode of action, and the expression levels and potential dietary exposure, as described above, and the outcome of the compositional analysis, are considered as part of a "weight-of-evidence" approach in identifying the hazard associated with the newly expressed protein (Codex, 2003b; Delaney *et al.*, 2008; EFSA, 2011).

Toxicological Studies on the Cry1Ab Protein

Toxicity Prediction Based on Bioinformatics

To assess the safety of GE crops, one important consideration is possible protein structural similarities of the introduced proteins to known protein toxins (Delaney *et al.*, 2008; Brune *et al.*, 2021). In regulatory submissions, several different databases have been utilized for a bioinformatic analysis to compare the amino acid sequence of the introduced protein to that of known toxins, including TOXIN6, GenBank, RefSeq, Uniprot Swissprot, PIR (Protein Information Resource), PRF (Protein Research Foundation) and PDB (Protein Data Bank) or other protein toxin databases. As an example, a bioinformatic analysis for the Cry1Ab protein in eucalyptus event 1521K059 is described in Avisar *et al.*, 2024. That analysis searched for the Cry1Ab protein sequence from the event in NCBI nr and UniProt databases and screened the search outcomes for the terms 'toxic', 'toxin, 'anti-nutrition', 'agglutinin', 'trypsin inhibitor' and 'protease inhibitor' and employed the Toxic Exposome Database (T3DB) to identify any homology to known toxins. No similarities with known toxins were found in the searches. Regulatory authorities have assessed

the bioinformatic analysis for protein toxicity and concluded that Cry1Ab does not share structural similarities with protein toxins to humans or livestock animals (See Annex 1).

Acute Toxicity Studies on Cry1Ab Protein

Acute toxicity studies have been required by regulatory agencies for assessing food and feed safety of Cry1Ab derived from GE crops. The studies have mainly been acute oral toxicity tests in rodents exposed to the protein for up to 14 days at a high dose. Delaney *et al.* (2008) summarized results of acute mouse toxicology studies with various proteins in GE crops (based on personal communication with developers), including Cry1Ab at a dose of >4000 mg/kg body weight, and concluded that no tested transgenic protein in GE crops has demonstrated adverse effects in acute toxicity studies. An unpublished report by Naylor (1992) on an acute oral toxicity study conducted with Cry1Ab on mice was submitted in support of the 2010 EPA registration extension for the Cry1Ab protein in maize events MON810 and Bt11 (US EPA, 2010). The 1992 study showed no acute toxicity at a dose of >4000 mg/kg body weight. This same 1992 study was considered almost 30 years later for the approval of Cry1Ab cowpea event AAT709A (CSIR-SARI, 2020). Regulators have concluded in approvals for food and feed safety that the Cry1Ab protein is not toxic even in high doses to humans or animals (See Annex 1).

Allergenicity of Cry1Ab Protein

Another consideration for the safety of GE crops is the risk of introducing new allergens through the expression of new proteins in the plant. Here the primary focus is on the allergenicity of the Cry1Ab protein, not that of the whole plant. As with toxicity assessment, the assessment of allergenicity for a protein follows a weight-of-evidence approach by taking into account all information obtained, since none of the commonly used experimental methods can provide confirmative evidence on allergenicity (Codex, 2003b; EFSA, 2006, 2011; Herman *et al.*, 2006; Codex, 2009). Allergenicity assessments of newly expressed proteins in GE crops typically consider the source of the gene, the similarity of the amino acid sequence of the protein of interest to that of known allergens, the stability of the protein to digestibility by pepsin in an *in vitro* digestibility assay, and if it were ever necessary, *in vitro* human sera testing or clinical testing (Codex, 2003b; Goodman *et al.*, 2005; Delaney *et al.*, 2008). Studies on structural similarity to known allergens and protein digestibility studies are routinely considered in the assessment of potential allergenicity of newly expressed proteins in GE crops (See Annex 1).

Allergenicity Prediction Based on Bioinformatics

The primary amino acid sequence of the Cry proteins in GE crops, including Cry1Ab, has repeatedly been compared to the amino acid sequence of known allergens using bioinformatic analysis (Randhawa *et al.*, 2011). These analyses compare the protein produced in the plant to allergens in databases, such as the COMPARE allergen database (van Ree *et al.*, 2021) used for the allergenicity assessment of Cry1Ab in eucalyptus (Avisar *et al.*, 2024) or the Food Allergy Research and Resource Program (FARRP) allergen database found in AllergenOnline (Goodman *et al.*, 2016) used for the assessment of Cry1Ab in cowpea (CSIR-SARI, 2020). The analysis typically includes searches for overall similarity between the Cry1Ab protein and known allergens, sequence identity with known allergens above 35% in a given 80-amino acid length of sequence, and small regions of the Cry1Ab protein (not smaller than 8 amino acids) sharing identity with known allergens (EFSA, 2009a,b; CSIR-SARI, 2020; Avisar *et al.*, 2024). The lack of sequence homology based on these analyses indicates that the Cry1Ab protein is not a known allergen and not likely to be cross-reactive to known allergens. Numerous bioinformatic analyses of Cry1Ab in safety assessments of GE crops have not identified any significant similarities to known allergens (See Annex 1).

Digestibility Studies on Cry1Ab Protein

Rapid digestibility studies are also used to test for allergenicity, as well as toxicity, of newly expressed proteins in GE plants, usually through a simulated (mammalian) gastric fluid assay (Delaney *et al.*, 2008). Rapidly digestible proteins, like most food proteins, are unlikely to result in allergic or toxic reactions. Digestibility studies are an expected test for safety of newly expressed proteins in GE crops, although some studies have concluded that stability to digestion and allergenicity are not related (Fu *et al.*, 2002; Herman *et al.*, 2007; Bøgh & Madsen, 2016). Allergenicity and toxicity assessment based on history of safe use, mode of action and bioinformatics, without digestibility studies, may be sufficient to demonstrate safety of newly expressed proteins in GE crops (Brune *et al.*, 2021).

A standard protocol for an *in vitro* pepsin digestion assay is typically used to assess digestibility in safety assessments of newly expressed proteins (Thomas *et al.*, 2004, Delaney *et al.*, 2008). An unpublished report by Ream (1994) evaluating the digestibility of Cry1Ab in simulated gastric fluid (SGF) containing pepsin was submitted in support of the 2010 EPA registration extension for the Cry1Ab protein in MON810 and Bt11 (US EPA, 2010). The 1994 study showed rapid degradation of the Cry1Ab protein, supporting the conclusion that Cry1Ab expressed in transgenic plants will be readily digested in the human and animal gastric systems (CSIR-SARI, 2020). This same 1994 study was considered almost 30 years later in the weight-of-evidence approach for assessment of allergenicity and toxicity in the approval of Cry1Ab cowpea event 709A (CSIR-SARI, 2020). As expected, a test for Cry1Ab protein susceptibility to digestion in SGF containing pepsin in support of the safety assessment for eucalyptus (Avisar *et al.*, 2024) demonstrated rapid digestion of the protein. The digestibility evaluated for safety of Cry1Ab protein in GE crops has indicated that Cry1Ab is rapidly digested like most food proteins and is unlikely to result in allergic or toxic reactions (See Annex 1).

Feeding Studies on Food and Feed From GE Crops Expressing the Cry1Ab Protein

When the weight-of-evidence in the food and feed safety assessment of newly expressed proteins in GE crops based on all of the above safety considerations indicates no potential adverse effect on human or animal health, whole food studies should not be necessary. Animal feeding studies should not be conducted when there is not a testable risk hypothesis (Delaney *et al.*, 2008; Devos *et al.*, 2016; Waters *et al.*, 2021). Feeding studies that aim to evaluate potential adverse effects of a whole food are inherently difficult to design, and the subsequent data interpretation is also difficult (Bartholomaeus *et al.*, 2013). It is worth noting that according to a review (Zdziarski *et al.*, 2014) on feeding studies with GE crops using rats, many feeding studies either lack methodological details, methodological consistency, or defined criteria for outcomes that would be considered toxicologically or pathologically significant, making generalization difficult. However, such studies are periodically associated with food and feed safety review for GE plants often due to the mandatory request for such studies for all single events in the EU (Garcia-Alonso *et al.*, 2025).

In association with EU regulatory approvals for MON810 and Bt11, the EFSA GMO Panel evaluated toxicity data prepared by the applicants from the peer-reviewed literature (EFSA, 2009a,b). This included whole food animal feeding studies which were reviewed for animal feed safety. The impacts of diets containing GE events in studies conducted on rodents, or occasionally chickens, were analyzed (including general health indicators such as growth, organ development, blood biochemical parameters and histopathological changes) and regulatory reviews indicate that no significant safety issues were identified (EFSA, 2009a,b). The EFSA GMO Panel also noted 'the absence of adverse effects due to dietary exposure to maize MON810 has been obtained in 90-day feeding studies in rats supplied diets containing maize with stacked GE maize events, in which one of the parents was MON810 (EFSA, 2009b). Similarly, results submitted by the applicant for T304-40 cotton indicated no adverse effects from a 13-week feeding study with T304-40 seed meal fed to rats and a 42-day study with T304-40 toasted seed meal fed to chickens (EFSA, 2013).

Although 90-day rat feeding studies are not typically required for regulatory approval of GE crops without a testable hypothesis, there are several peer-reviewed studies investigating subchronic effects of feeding GE crops derived food or feed that contain Cry1Ab protein. For example, in a 90-day study in Wistar rats fed with GE rice expressing Cry1Ab protein at a rate of 0.54 mg Bt toxin per kg body weight, no biologically relevant effects were found (Schrøder *et al.*, 2007). A project funded by the European Commission found that in two 90-day feeding trials with two different GE maize MON810 varieties, the MON810 maize at a level of up to 33 % in the diet did not induce adverse effects in Wistar Han RCC rats (Zeljenková *et al.*, 2014). A 90-day feeding study on calf fed with diet containing MON810 maize did not find any performance issue among the animals, in terms of growth rate and meat quality, or transfer of transgenic DNA to calf tissue (Furgał-Dierżuk *et al.*, 2014). In a four-generation study (Halle & Flachowsky, 2014) in which hens were fed with Bt maize containing Cry1Ab, no significant findings were identified after studying feed intake, growth, laying or breeding performance.

A 90-day feeding study was done on Wistar Han RCC rats using grain from LP007-1, a new maize variety developed by Longping Biotechnology (Hainan) Co., Ltd., expressing modified *cry1Ab*, *cry2Ab*, *vip3Aa* and *cp4-epsps* genes for insect-resistance and herbicide-tolerance traits (Zhou *et al.*, 2023). The rats were fed maize grains from LP007-1 at 25% and 50% concentrations by mass. No biologically relevant differences were observed in rats fed with maize LP007-1 compared to rats fed a non-GE control maize with respect to a variety of parameters. The results of this study provided evidence that LP007-1 maize did not exhibit toxicity (Zhou *et al.*, 2023). While event LP007-1 has not been authorized by any regulatory agency, another event developed by the same company, LP026-2 expressing *cry2Ab*, *cry1Fa*, *cry1Ab* and *epsps* (Ag) genes, has been issued a biosafety certificate for nationwide cultivation and processing, by the Ministry of Agriculture and Rural Affairs, People's Republic of China (USDA GAIN Report CH2024-0009). Cry1Ab protein is expressed in both events. These and other studies (Gu *et al.*, 2014; Guo *et al.*, 2014; Swiatkiewicz *et al*., 2014; Wang *et al.*, 2014; Furgał-Dierżuk *et al.*, 2015; Tufarelli *et al.*, 2015; Zhu *et al.*, 2015) support the conclusion that whole food feeding studies indicate no adverse effect with food or feed from GE crops expressing Cry1Ab protein.

COMPOSITIONAL ANALYSIS OF GE PLANTS EXPRESSING CRY1AB PROTEIN

A compositional analysis is required in most regulatory approval processes for GE plants intended to be used in food or feed (WHO, 1995; FAO/WHO, 1996; Codex, 2003a, 2003b; EFSA, 2006, 2011). Compositional data is primarily used to establish whether the genetic modification has resulted in changes in key components that could lead to adverse effects on human or animal health (Codex, 2003a, 2003b). This is the hazard identification step and is part of the weight of evidence approach used to identify potential unintended changes that could lead to adverse effects. The results of the compositional analysis may be taken as an indication that the GE crop is 'as safe as' the non-GE crop if no biologically relevant changes are identified, but it is not necessary to establish safety of the protein. The analysis does not usually test a plausible risk hypothesis related to the protein, as is best practice for risk assessment of GE crops (Delaney *et al.*, 2008; Brune *et al.*, 2021; Waters *et al.*, 2021).

Composition data has been produced for all single-transformation events that have been developed and evaluated in accordance with the Codex framework. The analysis typically compares the GE plant to the untransformed parent line or a

closely related isoline, and the analytes measured depend on the crop and its intended uses. The analysis may use plants grown in a variety of locations and may include data from multiple growing seasons, because local environmental conditions may impact nutritional composition even in conventionally bred varieties. The goal of the analysis is to verify that the genetic modification has not resulted in significant changes in values of key analytes compared with the comparator and if significant changes are observed, to establish whether these are within the range observed in traditional varieties (AFSI, 2025; Bajaj *et al.*, 2026).

In the submissions for food safety approvals of crops expressing Cry1Ab, any differences noted between the GE plant and the comparator were within the normal range of variation, and the comparisons revealed no differences relevant to food safety (See Annex 1). Brief summaries of the compositional profile in Cry1Ab-expressing events for maize, cotton, cowpea, eucalyptus, and sugarcane are provided in this section.

Maize

Compositional analysis on maize event MON810 grain samples harvested from six trial sites in the U.S. in 1994 compared data for protein, fat, ash, carbohydrates, calories, moisture, amino acids, fatty acids, calcium and phosphorus with the control line MON818 and the reported literature ranges for commercial hybrids. Additionally, components of forage from MON810 grown in three trial sites in France were also analyzed using validated methods. Comparative compositional analyses showed that both grain and forage from MON810 were similar in composition to commercial corn grain. Based on this information, US FDA concluded that the composition of grain and forage from Cry1Ab-expressing MON810 did not raise safety or nutritional concerns (US FDA, 1996).

A more recent example is that of DBN9336 maize, which expresses two traits, insect resistance and herbicide tolerance, due to the presence of the Cry1Ab protein and EPSP (5-enolpyruvate shikimate-3-phosphate) synthase, respectively. As expected, these traits did not alter the levels of key nutrients, anti-nutrients, secondary metabolites, or toxicants in engineered maize. The recommended key components per OECD were analyzed in seeds of DBN9336 and their levels were compared with the non-GE control line, DBN318, grown in confined field trials. Most components were similar between the two lines and were within the ranges reported in the scientific literature, although it was noted by the developer that some components, such as Vitamin E, Vitamin B1, methionine, and phenylalanine from different locations were outside of literature-reported ranges. However, the differences were relatively small and unlikely to have any biological relevance, and the developer concluded that Cry1Ab-expressing DBN9936 maize was compositionally and nutritionally comparable to conventional corn varieties. After consultation with US FDA, the agency did not have any concerns about safety, nutrition, and regulatory compliance of food and feed derived from this maize event (US FDA, 2021).

Cotton

The composition data obtained from COT67B and control Coker 312 cotton plants grown in 2004 at four different locations in the US indicated that the GE event was similar in composition to the control (Health Canada, 2008a). Forty-one analytes were measured in cottonseed including proximates (moisture, protein, fat, carbohydrate, acid detergent fibre, neutral detergent fiber and total dietary fiber), minerals (calcium and phosphorus), fatty acids, vitamin E, amino acids, and anti-nutrients (free and total gossypol), and cyclopropenoid fatty acids (sterculic, malvalic, and dihydrosterculic acid). Six analytes (calcium, fatty acids – palmitic, stearic, oleic, cycloprenoid fatty acid – dihydrosterculic acid, and vitamin E) showed a statistically significant difference between COT67B and control. However, levels of these six analytes were within the published range for the respective analyte in cottonseed and these differences between the Cry1Ab-expressing COT67B and the control were not considered biologically relevant (Health Canada, 2008a). Additionally, a study to examine the levels of antinutrients (gossypol and cyclopropenoid fatty acids) in processed fractions, refined oil and cottonseed meal was also conducted and the levels of antinutrients in COT67B processed fractions were similar to those of the control (Health Canada, 2008a).

Cowpea

The compositional analysis of 709A cowpea event expressing Cry1Ab protein involved pairwise comparisons of major nutrient components between samples of whole grain, leaves, and fodder collected from event 709A and control cowpea (CSIR-SARI, 2020). These samples were collected from plants grown at four different locations in Ghana and Nigeria, representing typical cowpea growing conditions in West Africa. Concentrations of key minerals and phytic acid were determined in samples of whole grain. Across the four locations in Ghana and Nigeria, no statistically significant differences in concentrations of proximates (protein, fat, ash, and carbohydrates), moisture, calories, minerals, or phytic acid between grain samples collected from event 709A and control cowpea were found. Similarly, there were no significant differences in concentrations of proximates, moisture, and calories between 709A and control cowpea leaf and fodder samples when analyzed using the combined-sites model. Based on the composition data on whole grain, leaves, and fodder from 709A cowpea, authorities in Nigeria and Ghana concluded that GE cowpea grain, leaves, and fodder were compositionally equivalent to these same products from non-transgenic control cowpea. The studies accompanying the dossier showed that there were no increases in antinutritional factors such as phytate and oligosaccharides in GE cowpea (CSIR-SARI, 2020).

Eucalyptus

Transgenic eucalyptus event 1521K059, approved by CTNBio for commercial use in Brazil in 2023, expresses Cry1Ab, Cry2Aa, and Cry1Bb. Composition studies of leaves were conducted, comparing the GE event 1521K059 with its respective conventional clone, as well as comparing it to other conventional clones used as commercial references. The levels of total proteins, ether extract, carbohydrates, fixed mineral residue, energy value, minerals, fibers, and moisture were quantified. The results confirmed that eucalyptus 1521K059 does not differ from conventional eucalyptus in its composition, except for the presence and expression of the *cry2Aa*, *cry1Ab*, and *cry1Bb* genes (CTNBio, 2023). CTNBio concluded that the biosafety data for event 1521K059 meet the relevant standards for ensuring the biosafety of human and animal health and of the environment.

Sugarcane

A safety and nutritional assessment by Health Canada concluded that raw and refined sugar from GE sugarcane event CTC175-A expressing Cry1Ab protein is not materially different in composition from raw and refined sugar currently on the market (Health Canada, 2017). Compositional parameters recommended in the OECD consensus document on compositional considerations for sugarcane were evaluated (OECD, 2011; Health Canada, 2017). Proximates and sucrose were measured, as well as neutral detergent fiber and acid detergent fiber due to relevance to the transformed event. Data from six field trials in Brazil over two growing seasons were compared with the control cultivar (isoline CTC 20) and four reference varieties (CTC 20 null, CTC9001, CTC4 and CTC15). No statistically significant differences were observed in analytical results between CTC175-A and the control or the reference varieties, except for crude fiber which raised no safety concerns (Health Canada, 2017). US FDA also concluded that raw or refined sugar from GE CTC175-A sugarcane does not raise issues that would require further evaluation by FDA (US FDA, 2018).

SAFETY ASSESSMENT OF STACKED EVENTS

The global trend in approvals of GE maize, as demonstrated for the *cry1Ab* gene in Tables 1-4, particularly for Bt insect resistance and herbicide tolerance, has clearly been toward events with stacked traits, i.e., those with more than one gene introduced typically by conventional breeding with two or more GE plant varieties of the same species (EFSA, 2007; Goodwin *et al.*, 2021). This is also the trend for other crops including cotton and soybean. In the US, the adoption rates for stacked varieties of maize and cotton with one or more Bt and herbicide tolerant traits have increased steadily since 2000, with more than 80 percent of US acreage of these two crops planted with stacked seeds in 2025 (USDA ERS, 2025).

Most regulatory authorities, including in the US, Canada, and Australia/New Zealand, do not require additional safety assessments for stacked GE events produced by conventional breeding ('breeding stacks') where all single events have been previously assessed (US EPA, 2009; Sul *et al.*, 2021). In contrast, the European Union mandates safety assessments for all breeding stacks even when all singles have been assessed and determined to be as safe as the conventional counterparts (EFSA, 2007, 2011; Garcia-Alonso *et al.*, 2025). Besides the safety data on the single transformation GE events used to produce the stacked events through crossing, data on possible changes and potential adverse effects as a result of interactions between the introduced genetic modifications are taken into account for food and feed safety assessments of stacked events (EFSA, 2006; De Schrijver *et al.*, 2007). These reviews consider data on the stacked event including intactness and stability of the trait, expression levels of the protein(s), comparative analysis of agronomic, phenotypic, and compositional characteristics, and the outcome of toxicological, allergenicity and nutritional assessment.

The European Food Safety Authority has issued opinions based on extensive reviews for as many as 22 stacked maize products and one stacked cotton with the *cry1Ab* gene. (Scientific Opinions available in the EFSA Journal). EFSA concluded from these assessments that stacked events expressing Cry1Ab (as for other Bt proteins) did not add extra food or feed risk via interactions between the expressed gene products since the expressed proteins are non-toxic to humans and animals and the expression levels are too low to trigger synergistic, antagonistic, or other combined effects. Similar conclusions have been reached on stacked events expressing Cry1Ab in other countries based mainly on information and data generated for the safety assessment of the single events. As experience has increased with the assessment and adoption of GE crops, many countries have developed simplified procedures for regulation of stacked events, generally considering breeding stacks as safe for food and feed based on the existing approvals of the transformation events from which the stacks are derived (Kok *et al.*, 2014; Goodwin *et al.*, 2021; Sul *et al.*, 2021).

CONCLUSION

The Cry1Ab protein expressed in insect-resistant GE plants is derived from the common soil bacterium Bt and is specifically toxic to Lepidoptera. Six GE crops (cotton, cowpea, eucalyptus, maize, rice, sugarcane) expressing Cry1Ab protein have been approved for food and subject to food safety assessment in more than 30 countries. Bioinformatic analyses in publicly available regulatory submissions and peer reviewed literature demonstrate that Cry1Ab does not share sequence or structural characteristics with known human or animal toxins. Toxicity studies submitted with regulatory dossiers did not identify any toxic effect in humans or animals at any tested concentration of Cry1Ab, including concentrations far exceeding expected levels in food derived from Cry1Ab expressing GE plants. Bt is not a known source of allergens and Cry1Ab protein does not share sequence homology with known allergens. It is rapidly degraded by simulated

gastric fluid. There has been no evidence of an added risk to human or animal health due to interaction effects between the Cry1Ab protein and other newly expressed products, including other Cry proteins, combined in approved 'stacked events', beyond the safety assessment of the single transformation events used to breed for the stacked events. To date, regulators have consistently concluded based on a weight-of-evidence approach that Cry1Ab in GE crops does not pose a food or feed safety concern.

ANNEX 1: REGULATORY DOCUMENTS OF SOME CROP EVENTS EXPRESSING THE CRY1AB PROTEIN APPROVED FOR FOOD SAFETY

Crop/Event	Title	Reference
Maize Bt176	Plant Pesticide Bacillus Thuringiensis CryIA(b) Delta-Endotoxin and the Genetic Material Necessary for its Production (Plasmid Vector pCIB4431) in Corn	US EPA 1995
	Biotechnology Consultation Note to the File BNF No. 000024. Corn transformation event 176	US FDA 1995
	Novel food information: Insect Resistant Corn, 176	Health Canada 1995
	Decision Document DD96-09: Determination of Environmental Safety of Event 176 Bt Corn (*Zea mays* L.) Developed by Ciba Seeds and Mycogen Corporation.	CFIA 1996a
	Final Risk Analysis Report. Application A385. Food derived from insect-protected Bt-176 corn.	FSANZ 2000a
	Approved Type 1 Use Regulation. Maize resistant to Lepidoptera and tolerant to glufosinate herbicide (Modified cry1Ab, bar, *Zea mays* subsp. mays (L.) Iltis) (Event176, OECD UI:SYN-EV176-9).	J-BCH 2007a
Maize Bt11	Decision Document DD96-12: Determination of Environmental Safety of Northrup King Seeds' European Corn Borer (ECB) Resistant Corn (*Zea mays* L.)	CFIA 1996b
	Biotechnology Consultation Note to the File BNF No. 000017. Transformation event Bt11 corn.	US FDA 1996a
	Novel food information: Insect Resistant and Herbicide Tolerant Corn, BT11	Health Canada 1996
	Draft Risk Assessment Report Application A386 Food produced from insect protected, herbicide tolerant Bt-11 corn line.	FSANZ 2000b
	Approved Type 1 Use Regulation. Maize resistant to Lepidoptera and tolerant to glufosinate herbicide (Modified cry1Ab, pat, *Zea mays* subsp. mays (L.) Iltis) (Bt11, OECD UI: SYN-BTØ11-1). 2007.	J-BCH 2007b
	Technical Opinion No 1255-2008. Commercial release of genetically modified insect-resistant corn event Bt11	CTNBio 2008
	Scientific opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-RX-Bt11) for renewal of the authorisation of existing products produced from insect-resistant genetically modified maize Bt11, under Regulation (EC) No 1829/2003 from Syngenta.	EFSA 2009a
	Cry1Ab and Cry1F *Bacillus thuringiensis* (Bt) Corn Plant-Incorporated Protectants.	US EPA 2010
	Assessment of genetically modified maize Bt11 for renewal authorisation under Regulation (EC) No 1829/2003 (application EFSA-GMO-RX-016)" EFSA Journal 19(1)	EFSA 2021
Maize MON810	Biotechnology Consultation Note to the File BNF No. 000034: Corn MON810 MON-ØØ81Ø-6.	US FDA 1996
	DD1997-19: Determination of the Safety of Monsanto Canada Inc.'s YieldgardTM Insect Resistant Corn (*Zea mays* L.) Line MON810.	CFIA 1997
	Novel food information: Insect Resistant Corn MON810.	Health Canada 1997
	Final Risk Assessment Report Application A346 Food produced from insect protected corn line MON810.	FSANZ 2000c
	Approved Type 1 Use Regulation. Lepidoptera resistant maize (cry1Ab, *Zea mays* L.) (MON810, OECD UI:MON-ØØ81Ø-6)	J-BCH 2004
	Technical Opinion No 1100-2007. Commercial release of genetically modified insect-resistant corn event MON810	CTNBio 2007
	Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMO-RX-MON810) for the renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON810; (2) feed consisting of and/or containing maize MON810, including the use of seed for cultivation; and of (3) food and feed additives, and feed materials produced from maize MON810, all under Regulation (EC) No 1829/2003 from Monsanto.	EFSA 2009b
	Cry1Ab and Cry1F *Bacillus thuringiensis* (Bt) Corn Plant-Incorporated Protectants.	US EPA 2010
Cotton COT67B	Petition for determination of nonregulated status event COT67B.	USDA APHIS 2007
	Final Assessment Report. Application A615. Food Derived from Insect-Protected Cotton Line COT67B.	FSANZ 2008
	Novel food information: Insect Resistant Cotton. 2008a.	Health Canada 2008a
	BRAD *Bacillus thuringiensis* modified Cry1Ab (SYN-IR67B-1) and Vip3Aa19 (SYN-IR102-7) insecticidal proteins and the genetic material necessary for their production in COT102 X COT67B cotton	US EPA 2008
	Biotechnology Consultation Note to the File BNF No. 000112. Cotton transformation event COT67B	US FDA 2009
	Approved Type 1 Use Regulation. Cotton resistant to Lepidoptera (Modified cry1Ab, *Gossypium hirsutum* L.) (COT67B, OECD UI: SYN-IR67B-1)	J-BCH 2012
	Decision Document DD2011-83 Determination of the Safety of Syngenta Seeds Canada Inc.'s Cotton (*Gossypium hirsutum*) Event COT67B	CFIA 2017

Crop/Event	Title	Reference
Cotton T304-40	Petition for determination of nonregulated status for Insect resistant and glufosinate ammonium-tolerant cotton (T304-40).	USDA APHIS 2008
	Application A1028. Food derived from insect-protected and herbicide- tolerant cotton line T304-40. Assessment Report.	FSANZ 2009a
	Application A1028 – food derived from insect protected and herbicide tolerant cotton line T304-40. Supporting Document.	FSANZ 2009b
	Decision Document DD2011-87: Determination of the Safety of Bayer CropScience Inc.'s TwinLink (T304-40 X GHB119) cotton (*Gossypium hirsutum*) and Cotton Events T304-40 and GHB119	CFIA 2011
	Novel Food Information – Cotton event T304-40.	Health Canada 2011
	Biotechnology Consultation - Note to the File Biotechnology Notification File BNF No. 000118. Cotton T304-40 and GHB119 BCS-GHØØ4-7 × BCS-GHØØ5-8	US FDA 2011
	Final Cry1Ab-Cry2Ae-TwinLink BRAD 01-26-12	US EPA 2012
	Approved Type 1 Use Regulation. Cotton tolerant to glufosinate herbicide and resistant to Lepidoptera (modified bar, modified cry1Ab, *Gossypium hirsutum* L.) (T304-40, OECD UI: BCS-GHØØ4-7).," 2013.	J-BCH 2013
	EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2013. Scientific Opinion on application EFSA-GMO-NL-2011-97 for the placing on the market of insect-resistant and herbicide-tolerant genetically modified cotton T304-40 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience	EFSA 2013
Cowpea AAT709A	Decision Document for a permit for the Commercial release of Pod Borer - Resistant Cowpea (PBR – Cowpea)-event AAT709A, genetically modified for lepidopteran insect pest (Maruca vitrata) resistance, issued to Institute for Agricultural Research (IAR), Zaria.	NBMA-Nigeria 2019
	Insect Resistant Cowpea Event 709A. Regulatory Dossier Number AAT/DPS-20VUIR-GH.	CSIR-SARI 2020
	Review of Risk Assessment on an Application from the Council for Scientific and Industrial Research (CSIR), Savanna Agricultural Research Institute (SARI) for Commercial use of Cowpea (*Vigna unguiculata* L. Walp.) Genetically Modified for Resistance to Maruca pod borer (Maruca vitrata) Event AAT-709AA-4 (hereafter referred to as 709A cowpea) in Ghana.	NBA-Ghana 2022
Eucalyptus 1521K059	Technical Opinion No.8393/2023. Commercial release of genetically modified eucalyptus 1521K059 and its derivatives	CTNBio 2023
Sugarcane CTC175-A	Biotechnology consultation note to the file, BNF No. 000159: Sugar from Sugarcane CTC175-A.	US FDA 2018
	Technical Opinion No 5483/2017. Commercial release of genetically modified sugarcane for insect resistance	CTNBio 2017

REFERENCES

Adang, M. J., Crickmore, N., and Jurat-Fuentes, J. L. (2014). Diversity of *Bacillus thuringiensis* crystal toxins and mechanism of action. Advances in insect physiology (pp. 39–87). Elsevier. https://doi.org/10.1016/b978-0-12-800197-4.00002-6

Agriculture & Food Systems Institute (AFSI). (2016). A review of the food and feed safety of the Cry1Ab protein. https://foodsystems.org/publication/a-review-of-the-food-and-feed-safety-of-the-cry1ab-protein/

Agriculture & Food Systems Institute (AFSI). (2025). Crop Composition Database, Version 10.1, Agriculture & Food Systems Institute www.cropcomposition.org

Apaydin, Ö., Çınar, Ç., Turanli, F., Harsa, Ş., and Güneş, H. (2008). Identification and bioactivity of native strains of *Bacillus thuringiensis* from grain-related habitats in turkey. Biological Control, 45(1), 21–28. https://doi.org/10.1016/j.biocontrol.2008.01.011

APVMA Record of Approved Active Constituents. (2023). Public Chemical Registration Information System - PUBCRIS. Australian Pesticides and Veterinary Medicines Authority. https://portal.apvma.gov.au/pubcris [Content last reviewed Oct 19, 2023. Accessed 26-Jan-2026]

Avisar, D., Manoeli, A., dos Santos, A. A., Porto, A. C. D. M., Rocha, C. D. S., Zauza, E., Gonzalez, E. R., Soliman, E., Gonsalves, J. M. W., Bombonato, L., Galan, M. P., Domingues, M. M., Candelaria, M. C., Mafia, R., Graça, R. N., Azulay, S., Livne, S., Dias, T. B., Drezza, T. R., ... Pinheiro, A. C. (2024). Genetically engineered eucalyptus expressing pesticidal proteins from *Bacillus thuringiensis* for insect resistance: A risk assessment evaluation perspective. Frontiers in Bioengineering and Biotechnology, 12, 1322985. https://doi.org/10.3389/fbioe.2024.1322985

Bajaj, B., Brune, P., Fast, B., Barthet,V.J., Bedair, M., Edwards, A., Gillikin, N., Helm, J., Ma, E., Raychaudhuri, A., Roberts, D., Rodriguez, D., Scaife, A., and Oh, S. (2026). Report on Agriculture & Food Systems Institute's Crop Composition Database Version 10.1: An open-access resource for high-quality composition data, Journal of Food Composition and Analysis, Volume 151, March 2026, 108981. https://doi.org/10.1016/j.jfca.2026.108981

Bartholomaeus, A., Parrott, W., Bondy, G., and Walker, K., and on behalf of the ILSI International Food Biotechnology Committee Task Force on the Use of Mammalian Toxicology Studies in the Safety Assessment of GM Foods (2013). The use of whole food animal studies in the safety assessment of genetically modified crops: Limitations and recommendations. Critical Reviews in Toxicology, 43(sup2), 1–24. https://doi.org/10.3109/10408444.2013.842955

Bøgh, K. L. and Madsen, C. B. (2016). Food allergens: is there a correlation between stability to digestion and allergenicity? Critical Reviews in Food Science and Nutrition, 56(9), 1545–1567. https://doi.org/10.1080/10408398.2013.779569

Bravo, A., Pacheco, S., Gómez, I., and Soberón, M. (2023). Mode of action of *Bacillus thuringiensis* Cry pesticidal proteins. In Advances in insect physiology (Vol. 64, pp. 55–92). Elsevier. https://doi.org/10.1016/bs.aiip.2023.09.003

Brune, P., Chakravarthy, S., Graser, G., Mathesius, C. A., McClain, S., S. Petrick, J., Sauve-Ciencewicki, A., Schafer, B., Silvanovich, A., Brink, K., Burgin, K., Bushey, D., L. Cheever, M., Edrington, T., Fu, H., Habex, V., Herman, R. A., Islamovic, E., Lipscomb, E. A., ... Goodwin, L. (2021). Core and supplementary studies to assess the safety of genetically modified (GM) plants used for food and feed. Journal of Regulatory Science, 9(1), 45–60. https://doi.org/10.21423/jrs-v09i1brune

Cannon, R. J. C. (1996). *Bacillus thuringiensis* use in agriculture: a molecular perspective. Biological Reviews 71: 561–636. https://doi.org/10.1111/j.1469-185X.1996.tb01285.x

CBD. (2000). The Cartagena Protocol on Biosafety, 2000. https://bch.cbd.int/protocol/text/

CFIA. (1996b). Decision Document DD96-12: Determination of Environmental Safety of Northrup King Seeds' European Corn Borer (ECB) Resistant Corn (*Zea mays* L.), 1996. https://www.inspection.gc.ca/plants/plants-with-novel-traits/approved-under-review/decision-documents/dd1996-12/eng/1303862914603/1303863058362(Bt11)

CFIA. (1997). DD1997-19: Determination of the Safety of Monsanto Canada Inc.'s YieldgardTM Insect Resistant Corn (*Zea mays* L.) Line MON810., 1997. https://www.inspection.gc.ca/plants/plants-with-novel-traits/approved-under-review/decision-documents/dd1997-19/eng/1303947308350/1303947444023(MON810)

CFIA. (2011). Decision Document DD2011-87: Determination of the Safety of Bayer CropScience Inc.'s TwinLink (T304-40 X GHB119) cotton (*Gossypium hirsutum*) and Cotton Events T304-40 and GHB119. https://inspection.canada.ca/en/plant-varieties/plants-novel-traits/approved-under-review/decision-documents/dd2011-87(T304-40)

CFIA. (2017). Decision Document DD2011-83 Determination of the Safety of Syngenta Seeds Canada Inc.'s Cotton (*Gossypium hirsutum*) Event COT67B. https://inspection.canada.ca/en/plant-varieties/plants-novel-traits/approved-under-review/decision-documents/dd2011-83(COT67B)

Codex. (2003a). Principles for the risk analysis of foods derived through modern biotechnology. Codex Alimentarius Commission (Codex). https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B44-2003%252FCXG_044e.pdf

Codex. (2003b). Guideline for the conduct of food safety assessment of foods derived from recombinant DNA plants. Codex Alimentarius Commission (Codex). https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B45-2003%252FCXG_045e.pdf

Codex. (2009). Foods derived from modern biotechnology, 2009. https://www.fao.org/4/a1554e/a1554e00.pdf

Crickmore, N., Zeigler, D. R., Feitelson, J., Schnepf, E., Van Rie, J., Lereclus, D., Baum, J. A. and Dean, D. H. (1998). Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. Microbiology and Molecular Biology Reviews 62: 807–813. https://doi.org/10.1128/mmbr.62.3.807-813.1998

Crickmore, N., Berry, C., Panneerselvam, S., Mishra, R., Connor, T. R., and Bonning, B. C. (2021). A structure-based nomenclature for *Bacillus thuringiensis* and other bacteria-derived pesticidal proteins. Journal of Invertebrate Pathology, 186, 107438. https://doi.org/10.1016/j.jip.2020.107438

CSIR-SARI. (2020). Council for Science and Industrial Research (CSIR)- Savanna Agricultural Research Institute. 2020. Insect Resistant Cowpea Event 709A. Regulatory Dossier Number AAT/DPS-20VUIR-GH. https://nba.gov.gh/environmental-release-publicnotice/#:~:text=In%20consonance%20with%20Biosafety%20Act,date%20of%20the%20first%20notification. [Accessed 19JAN2026].

CTNBio. (2007). Technical Opinion No 1100-2007. Commercial release of genetically modified insect-resistant corn event MON810. http://ctnbio.mctic.gov.br/en/liberacao-comercial/-/document_library_display/SqhWdohU4BvU/view/1462561#/liberacao-comercial/consultar-processo (MON810).

CTNBio. (2008). Technical Opinion No 1255-2008. Commercial release of genetically modified insect-resistant corn event Bt11. http://ctnbio.mctic.gov.br/en/liberacao-comercial/-/document_library_display/#/liberacao-comercial/consultar-processo (Bt11).

CTNBio. (2017). Technical Opinion No 5483/2017. Commercial release of genetically modified sugarcane for insect resistance https://ctnbio.mctic.gov.br/documents/566529/2256882/Parecer+T%C3%A9cnico+n%C2%BA%205483+-+2017/8d60c9de-687f-4cf8-90d2-a1957bca52b-1?version=1.0 [Accessed 21-Jan-2026]. (CTC175-A).

CTNBio. (2023). Technical Opinion No.8393/2023. Commercial release of genetically modified eucalyptus 1521K059 and its derivatives https://ctnbio.mctic.gov.br/documents/566529/2313088/Parecer+T%C3%A9cnico+8393_2023/65bd7130-e9ef-49e2-be70-8ec97468c03a?version=1.0 [Accessed 21-Jan-2026]. (1521K059).

de Bortoli, C. P., Kanno, R. H., Candelaria, M. C., Salante Ferreira, É. A., dos Santos Rocha, L., Ferraz Gomes, L. M., dos Santos, A. A., da Silva Rocha, C., Avisar, D., Jurat-Fuentes, J. L., and Gonzalez, E. R. (2025). Pyramiding of cry genes in high-dose transgenic eucalyptus provides control of Thyrinteina arnobia (Lepidoptera: Geometridae) and reduces the risk of resistance. GM Crops & Food, 17(1), 2604373. https://doi.org/10.1080/21645698.2025.2604373

de Maagd, R. A., Bravo, A., and Crickmore, N. (2001). How *Bacillus thuringiensis* has evolved specific toxins to colonize the insect world. Trends in Genetics, 17(4), 193-199. https://doi.org/10.1016/s0168-9525(01)02237-5

De Schrijver, A., Devos, Y., Van den Bulcke, M., Cadot, P., De Loose, M., Reheul, D., and Sneyers, M. (2007). Risk assessment of GM stacked events obtained from crosses between GM events. Trends in Food Science & Technology, 18(2), 101–109. https://doi.org/10.1016/j.tifs.2006.09.002

Delaney, B., Astwood, J. D., Cunny, H., Conn, R. E., Herouet-Guicheney, C., MacIntosh, S., Meyer, L. S., Privalle, L., Gao, Y., Mattsson, J., and Levine, M. (2008). Evaluation of protein safety in the context of agricultural biotechnology. Food and Chemical Toxicology, 46(SUPPL. 2):872–897. https://doi.org/10.1016/j.fct.2008.01.045

Devos, Y., Naegeli, H., Perry, J. N., and Waigmann, E. (2016). 90-day rodent feeding studies on whole GM food/feed. EMBO reports, 17(7), 942–945. https://doi.org/10.15252/embr.201642739

do Nascimento, J., Cristina Goncalves, K., Pinto Dias, N., Luiz de Oliveira, J., Bravo, A., and Antonio Polanczyk, R. (2022). Adoption of *Bacillus thuringiensis* -based biopesticides in agricultural systems and new approaches to improve their use in brazil. Biological Control, 165, 104792. https://doi.org/10.1016/j.biocontrol.2021.104792

European Food Safety Authority (EFSA). (2006). Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. EFSA Journal, 4(99), 1–100. https://doi.org/10.2903/j.efsa.2006.99

European Food Safety Authority (EFSA). (2007). Guidance document for the risk assessment of genetically modified plants containing stacked transformation events by the scientific panel on genetically modified organisms (GMO). EFSA Journal, 5(7). https://doi.org/10.2903/j.efsa.2007.512

European Food Safety Authority (EFSA). (2009a). Opinion on application reference EFSA-GMO-RX-Bt11 for renewal of the authorisation of existing products produced from insect-resistant genetically modified maize Bt11, under regulation (EC) no 1829/2003 from syngenta. EFSA Journal, 7(2), 977. https://doi.org/10.2903/j.efsa.2009.977

European Food Safety Authority (EFSA). (2009b). Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMO-RX-MON810) for the renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON810; (2) feed consisting of and/or containing maize MON810, including the use of seed for cultivation; and of (3) food and feed additives, and feed materials produced from maize MON810, all under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 1149: 1–85. https://www.efsa.europa.eu/en/efsajournal/pub/1149 (MON810)

European Food Safety Authority (EFSA) Panel on Genetically Modified Organisms (GMO). (2011). Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal, 9(5). https://doi.org/10.2903/j.efsa.2011.2150

European Food Safety Authority (EFSA) Panel on Genetically Modified Organisms (GMO). (2013). Scientific opinion on application EFSA-GMO-NL-2011-97 for the placing on the market of insect-resistant and herbicide-tolerant genetically modified cotton T304-40 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience AG. EFSA Journal, 11(6), 3251. https://doi.org/10.2903/j.efsa.2013.3251

European Food Safety Authority (EFSA) Panel on Genetically Modified Organisms (GMO), Naegeli, H., Bresson, J., Dalmay, T., Dewhurst, I. C., Epstein, M. M., Firbank, L. G., Guerche, P., Hejatko, J., Moreno, F. J., Mullins, E., Nogué, F., Rostoks, N., Sánchez Serrano, J. J., Savoini, G., Veromann, E., Veronesi, F., Álvarez, F., Ardizzone, M., and Raffaello, T. (2021). Assessment of genetically modified maize Bt11 for renewal authorisation under regulation (EC) no 1829/2003 (application EFSA-GMO-RX-016). EFSA Journal, 19(1). https://doi.org/10.2903/j.efsa.2021.6347

EU Pesticides Database (v3.3) Managed by Directorate General for Health & Food Safety. Accessed on December 4 (2025). https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances

FAO/WHO. (1996). Biotechnology and food safety. Report of a Joint FAO/WHO Consultation. Food and Agriculture Organization (FAO)/World Health Organization (WHO), Food and Nutrition Paper 61. https://wellcomecollection.org/works/fn9yjrer

FSANZ. (ANZFA) (2000a). Final Risk Analysis Report. Application A385. Food derived from insect-protected Bt-176 corn. Available: https://www.foodstandards.gov.au/sites/default/files/food-standards-code/applications/Documents/A385IR.pdf [Accessed 21-Jan-2026] (Bt176)

FSANZ. (ANZFA) (2000b). Draft Risk Assessment Report Application A386 Food produced from insect protected, herbicide tolerant Bt-11 corn line., 2000. https://www.foodstandards.gov.au/sites/default/files/food-standards-code/applications/Documents/A386%20FA.pdf (Bt11)

FSANZ. (ANZFA) (2000c). Final Risk Assessment Report Application A346 Food produced from insect protected corn line MON810., 2000. https://www.foodstandards.gov.au/sites/default/files/food-standards-code/applications/Documents/Application%20A346%20Draft%20IR.pdf (MON810)

FSANZ. (2008). Final Assessment Report. Application A615. Food Derived from Insect-Protected Cotton Line COT67B., 2008. https://www.food-standards.gov.au/sites/default/files/2023-11/FAR_A615_GM_Cotton.pdf (COT67B)

Fu, T., Abbott, U. R., and Hatzos, C. (2002). Digestibility of food allergens and nonallergenic proteins in simulated gastric fluid and simulated intestinal FluidA comparative study. Journal of Agricultural and Food Chemistry, 50(24), 7154–7160. https://doi.org/10.1021/jf020599h

Furgał-Dierżuk, I., Strzetelski, J., Kwiatek, K., Twardowska, M., Mazur, M., Sieradzki, Z., Kozaczyński, W., and Reichert, M. (2014). The effect of genetically modified maize (MON 810) and soyabean meal (Roundup Ready) on rearing performance and transfer of transgenic DNA to calf tissues. Journal of Animal and Feed Sciences, 23(1), 13–22. https://doi.org/10.22358/jafs/65711/2014

Furgał-Dierżuk, I., Strzetelski, J., Twardowska, M., Kwiatek, K., and Mazur, M. (2015). The effect of genetically modified feeds on productivity, milk composition, serum metabolite profiles and transfer of tDNA into milk of cows. Journal of Animal and Feed Sciences, 24(1), 19–30. https://doi.org/10.22358/jafs/65649/2015

Garcia-Alonso M., Alcalde E., Criel I., Novillo C., Kostolaniova P., and Podevin N. (2025). Challenges and opportunities in the regulatory landscape of genetically modified crops in the European Union. Frontiers in Bioengineering and Biotechnology, 13:1709118. https://doi.org/10.3389/fbioe.2025.1709118

Gassmann, A. J., and Reisig, D. D. (2023). Management of insect pests with Bt crops in the United States. Annual Review of Entomology, 68(1), 31–49. https://doi.org/10.1146/annurev-ento-120220-105502

Goodman, R. E., Hefle, S. L., Taylor, S. L., and van Ree R. (2005). Assessing Genetically Modified Crops to Minimize the Risk of Increased Food Allergy: A Review. International Archives of Allergy and Immunology 137(2): 153. https://doi.org/10.1159/000086314

Goodman, R. E., Ebisawa, M., Ferreira, F., Sampson, H. A., van Ree, R., Vieths, S., Baumert, J. L., Bohle, B., Lalithambika, S., Wise, J., and Taylor, S. L. (2016). AllergenOnline: A peer-reviewed, curated allergen database to assess novel food proteins for potential cross-reactivity. Molecular Nutrition & Food Research, 60(5), 1183–1198. https://doi.org/10.1002/mnfr.201500769

Goodwin, L., Hunst, P., Burzio, L., Rowe, L., Money, S., and Chakravarthy, S. (2021). Stacked trait products are as safe as non-genetically modified (GM) products developed by conventional breeding practices. Journal of Regulatory Science, 9(1), 22–25. https://doi.org/10.21423/jrs-v09i1goodwin

Gómez, I., Sánchez, J., Muñoz-Garay, C., Matus, V., Gill, S. S., Soberón, M., and Bravo, A. (2014). *Bacillus thuringiensis* Cry1A toxins are versatile proteins with multiple modes of action: Two distinct pre-pores are involved in toxicity. Biochemical Journal, 459(2), 383–396. https://doi.org/10.1042/bj20131408

Gu, J., Bakke, A. M., Valen, E. C., Lein, I., and Krogdahl, Å. (2014). Bt-maize (MON810) and non-GM soybean meal in diets for Atlantic salmon (Salmo salar L.) juveniles – impact on survival, growth performance, development, digestive function, and transcriptional expression of intestinal immune and stress responses. PLOS ONE, 9(6), e99932. https://doi.org/10.1371/journal.pone.0099932

Guo, Q., Wang, S., Wang, Y., Wei, L., Zhu, H., Zhu, L., Shang, J., Li, Y., and Wang, J. (2014). Embryotoxicity of transgenic rice TT51 and Cry1Ab Bt insecticidal toxin in rat post implantation whole embryo culture. Journal of Food and Nutrition Research, 2(3), 115–121. https://doi.org/10.12691/jfnr-2-3-4

Halle, I., and Flachowsky, G. (2014). A four-generation feeding study with genetically modified (Bt) maize in laying hens. Journal of Animal and Feed Sciences, 23(1), 58–63. https://doi.org/10.22358/jafs/65717/2014

Health Canada. (1995). Insect Resistant Corn, 176, 1995. https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products/insect-resistant-corn-176.html [Accessed 21-Jan-2026] (Bt176).

Health Canada. (1996). Insect Resistant and Herbicide Tolerant Corn, BT11, 1996. https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products/insect-resistant-herbicide-tolerant-corn-bt11.html [Accessed 21-Jan-2026](Bt11)

Health Canada. (2008a). Novel Food Information - Insect Resistant Cotton COT67B. https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products/novel-food-information-insect-resistant-cotton-cot67b.html

Health Canada. (2008b). *Bacillus thuringiensis*: Re-Evaluation Decision Document. Ottawa, Canada. https://pesticidetruths.com/wp-content/uploads/2013/06/Health-Canada-Bacillus-thuringiensis-2008-05-06-Re-Evaluation-Decision-Document-RVD2008-18.pdf

Health Canada. (2011). Novel Food Information – Cotton event T304-40. https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products/novel-food-information-cotton-event-t304-40.html

Health Canada. (2017). Novel Food Information - Sugarcane CTC175-A. https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products/novel-food-information-sugarcane-ctc175-a.html

Herman, R. A., Storer, N. P., and Gao, Y. (2006). Digestion Assays in Allergenicity Assessment of Transgenic Proteins. Environmental Health Perspectives 114(8): 1154–1157. https://doi.org/10.1289/ehp.8803

Herman, R. A., Woolhiser, M. M., Ladics, G. S., Korjagin, V. A., Schafer, B. W., Storer, N. P., Green, S. B., and Kan, L. (2007). Stability of a set of allergens and non-allergens in simulated gastric fluid. International Journal of Food Sciences and Nutrition, 58(2), 125–141. https://doi.org/10.1080/09637480601149640

Höfte, H. and Whiteley, H. R. (1989). Insecticidal crystal proteins of *Bacillus thuringiensis*. Microbiological Reviews 53: 242–255. https://doi.org/10.1128/mr.53.2.242-255.1989

ISAAA. (2025). ISAAA's GM approval database. https://www.isaaa.org/gmapprovaldatabase (accessed December 5, 2025).

J-BCH. (2004). Approved Type 1 Use Regulation. Lepidoptera resistant maize (*cry1Ab*, *Zea mays* L.) (MON810, OECD UI:MON-ØØ81Ø-6), 2004. https://www.biodic.go.jp/bch/download/en_lmo/MON810enRi.pdf(MON810)

J-BCH. (2007a). Approved Type 1 Use Regulation. Maize resistant to Lepidoptera and tolerant to glufosinate herbicide (Modified *cry1Ab*, *bar*, *Zea mays subsp. mays* (L.) Iltis) (Event176, OECD UI: SYN-EV176-9), 2007. https://www.biodic.go.jp/bch/english/lmo_2007.html (Bt176)

J-BCH. (2012). Approved Type 1 Use Regulation. Cotton resistant to Lepidoptera (Modified *cry1Ab*, *Gossypium hirsutum* L.) (COT67B, OECD UI: SYN-IR67B-1), 2012. https://www.biodic.go.jp/bch/download/en_lmo/H24.9.4.5-2.pdf (COT67B)

J-BCH. (2013). Approved Type 1 Use Regulation. Cotton tolerant to glufosinate herbicide and resistant to Lepidoptera (modified *bar*, modified *cry1Ab*, *Gossypium hirsutum* L.) (T304-40, OECD UI: BCS-GHØØ4-7)., 2013. https://www.biodic.go.jp/bch/download/en_lmo/H25.9.17_English2-2.pdf (T304-40)

Jurat-Fuentes, J. L., and Crickmore, N. (2017). Specificity determinants for cry insecticidal proteins: Insights from their mode of action. Journal of Invertebrate Pathology, 142, 5–10. https://doi.org/10.1016/j.jip.2016.07.018

Kabaluk, J. T., Svircev, A. M., Goettel, M. S., and Woo, S. G. (2010). The use and regulation of microbial pesticides in representative jurisdictions worldwide. IOBC Global, page 99. https://www.iobc-global.org/download/Microbial_Regulation_Book_Kabaluk_et_a

Khorramnejad, A., Domínguez-Arrizabalaga, M., Caballero, P., Escriche, B., and Bel, Y. (2020). Study of the *Bacillus thuringiensis* Cry1Ia protein oligomerization promoted by midgut brush border membrane vesicles of lepidopteran and coleopteran insects, or cultured insect cells. Toxins, 12(2), 133. https://doi.org/10.3390/toxins12020133

Koch, M. S., Ward, J. M., Levine, S. L., Baum, J. A., Vicini, J. L., and Hammond, B. G. (2015). The food and environmental safety of Bt crops. Frontiers in Plant Science, 06. https://doi.org/10.3389/fpls.2015.00283

Kok, E. J., Pedersen, J., Onori, R., Sowa, S., Schauzu, M., De Schrijver, A., and Teeri, T. H. (2014). Plants with stacked genetically modified events: To assess or not to assess?. Trends in Biotechnology, 32(2), 70–73. https://doi.org/10.1016/j.tibtech.2013.12.001

Koziel, M. G., Beland, G. L., Bowman, C., Carozzi, N. B., Crenshaw, R., Crossland, L., Dawson, J., Desai, N., Hill, M., Kadwell, S., Launis, K., Lewis, K., Maddox, D., McPherson, K., Meghji, M. R., Merlin, E., Rhodes, R., Warren, G. W., Wright, M., and Evola, S. V. (1993). Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis* . Nature Biotechnology, 11(2), 194–200. https://doi.org/10.1038/nbt0293-194

Kumar, A. P., Sharma, R. P., and Malik, V. S. (1996). The insecticidal proteins of *Bacillus thuringiensis*. Advances in Applied Microbiology 42: 1–43. https://doi.org/10.1016/S0065-2164(08)70371-X

Liu, L., Li, Z., Luo, X., Zhang, X., Chou, S., Wang, J., and He, J. (2021). Which is stronger? a continuing battle between cry toxins and insects. Frontiers in Microbiology, 12, 665101. https://doi.org/10.3389/fmicb.2021.665101

Liu, S., Wang, S., Wu, S., Wu, Y., and Yang, Y. (2020). Proteolysis activation of Cry1Ac and Cry2Ab protoxins by larval midgut juice proteases from Helicoverpa armigera. PLOS ONE, 15(1), e0228159. https://doi.org/10.1371/journal.pone.0228159

Liu, X., Liu, S., Bai, S., He, K., Zhang, Y., Dong, H., Zhang, T., and Wang, Z. (2024). Toxicity of cry- and Vip3Aa-Class proteins and their interactions against Spodoptera frugiperda (Lepidoptera: Noctuidae). Toxins, 16(4), 193. https://doi.org/10.3390/toxins16040193

Martinez, C., and Caballero, P. (2002). Contents of cry genes and insecticidal toxicity of *Bacillus thuringiensis* strains from terrestrial and aquatic habitats. Journal of Applied Microbiology, 92(4), 745–752. https://doi.org/10.1046/j.1365-2672.2002.01579.x

McBride, K. E., Svab, Z., Schaaf, D. J., Hogan, P. S., Stalker, D. M., and Maliga, P. (1995). Amplification of a chimeric *Bacillus* gene in chloroplasts leads to an extraordinary level of an insecticidal protein in tobacco. Nature Biotechnology, 13(4), 362–365. https://doi.org/10.1038/nbt0495-362

Mendelsohn, M., Kough, J., Vaituzis, Z. and Matthews, K. (2003). Are Bt crops safe? Nature Biotechnology 21: 1003–1009. https://doi.org/10.1038/nbt0903-1003

NBA-Ghana. (2022). Review of Risk Assessment on an Application from the Council for Scientific and Industrial Research (CSIR), Savanna Agricultural Research Institute (SARI) for Commercial use of Cowpea (*Vigna unguiculata* L. Walp.) Genetically Modified for Resistance to Maruca pod borer (Maruca vitrata) Event AAT-709AA-4 (hereafter referred to as 709A cowpea) in Ghana. https://s3.amazonaws.com/km.documents.attachments/9294/bc7c/7eea3ea1f0688cb639a7b36e [Accessed 26-Jan-2026)

NBMA-Nigeria. (2019). Decision Document for a permit for the Commercial release of Pod Borer - Resistant Cowpea (PBR – Cowpea)-event AAT709A, genetically modified for lepidopteran insect pest (Maruca vitrata) resistance, issued to Institute for Agricultural Research (IAR), Zaria. https://s3.amazonaws.com/km.documents.attachments/3e6e/ea14/d29e2e51fa60b3d17a4b339e

NRC. (1989). Field Testing Genetically Modified Organisms: Framework for Decisions. National Academy of Sciences, National Research Council (NRC) committee on Scientific Evaluation of the Introduction of Genetically Modified Microorganisms and Plants into the Environment. Washington (DC): National Academy Press, 1989. https://doi.org/10.17226/1431

OECD. (1986). Recombinant DNA safety considerations. Organisation for Economic Co-operation and Development. https://archive.org/details/recombinantdnasa0000orga

OECD. (2007). Consensus document on safety information on transgenic plants expressing *Bacillus thuringiensis*-derived insect control proteins. Organisation for Economic Co-operation and Development. https://www.oecd.org/en/topics/sub-issues/biosafety-novel-food-and-feed-safety/consensus-documents-work-on-harmonisation-of-regulatory-oversight-in-biotechnology.html

OECD. (2011). Consensus document on compositional considerations for new varieties of sugarcane (Saccharum ssp. hybrids): Key food and nutrients, anti-nutrients and toxicants. Organisation for Economic Co-operation and Development. https://www.oecd.org/en/topics/sub-issues/biosafety-novel-food-and-feed-safety/consensus-documents-work-on-harmonisation-of-regulatory-oversight-in-biotechnology.html

Pardo-López, L., Soberón, M. and Bravo, A. (2013). *Bacillus thuringiensis* insecticidal three-domain Cry toxins: mode of action, insect resistance and consequences for crop protection. FEMS Microbiology Reviews 37: 3–22. https://doi.org/10.1111/j.1574-6976.2012.00341.x

Perlak, F. J., Fuchs, R. L., Dean, D. A., McPherson, S. L., and Fischhoff, D. A. (1991). Modification of the coding sequence enhances plant expression of insect control protein genes. Proceedings of the National Academy of Sciences of the United States of America, 88(8), 3324–3328. https://doi.org/10.1073/pnas.88.8.3324

Ragasruthi, M., Balakrishnan, N., Murugan, M., Swarnakumari, N., Harish, S., and Sharmila, D. J. S. (2024). *Bacillus thuringiensis* (Bt)-based biopesticide: Navigating success, challenges, and future horizons in sustainable pest control. Science of The Total Environment, 954, 176594. https://doi.org/10.1016/j.scitotenv.2024.176594

Randhawa, G. J., Singh, M., and Grover, M. (2011). Bioinformatic analysis for allergenicity assessment of *Bacillus thuringiensis* cry proteins expressed in insect-resistant food crops. Food and Chemical Toxicology, 49(2), 356–362. https://doi.org/10.1016/j.fct.2010.11.008

Sanahuja, G., Banakar, R., Twyman, R. M., Capell, T., and Christou, P. (2011). *Bacillus thuringiensis*: a century of research, development and commercial applications. Plant Biotechnology Journal, 9(3), 283–300. https://doi.org/10.1111/j.1467-7652.2011.00595.x

Schnepf, H. E., Crickmore, N., Van Rie, J., Lereclus, D., Baum, J. A., Feitelson, J., Zeigler, D. R., and Dean, D. H. (1998). *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiology and Molecular Biology Reviews, 62(3), 775–806. https://doi.org/10.1128/mmbr.62.3.775-806.1998

Schnepf, H. E., Lee, S., Dojillo, J., Burmeister, P., Fencil, K., Morera, L., Nygaard, L., Narva, K. E., and Wolt, J. D. (2005). Characterization of Cry34/Cry35 binary insecticidal proteins from diverse *Bacillus thuringiensis* strain collections. Applied and Environmental Microbiology, 71(4), 1765–1774. https://doi.org/10.1128/AEM.71.4.1765-1774.2005

Schrøder, M., Poulsen, M., Wilcks, A., Kroghsbo, S., Miller, A., Frenzel, T., Danier, J., Rychlik, M., Emami, K., Gatehouse, A., Shu, Q., Engel, K.-H., Altosaar, I., and Knudsen, I. (2007). A 90-day safety study of genetically modified rice expressing Cry1Ab protein (*Bacillus thuringiensis* toxin) in Wistar rats. Food and Chemical Toxicology, 45(3), 339–349. https://doi.org/10.1016/j.fct.2006.09.001

Seifinejad, A., Jouzani, G. R. S., Hosseinzadeh, A., and Abdmishani, C. (2008). Characterization of lepidoptera-active cry and vip genes in iranian *Bacillus thuringiensis* strain collection. Biological Control, 44(2), 216–226. https://doi.org/10.1016/j.biocontrol.2007.09.010

Siebert, M. W., Patterson, T. G., Gilles, G. J., Nolting, S. P., Leonard, B. R., Van Duyn, J. W., Lassiter, R. B., Braxton, L. B. and Van Duyn, J. W. (2009). Quantification of Cry1Ac and Cry1F *Bacillus thuringiensis* insecticidal proteins in selected transgenic cotton plant tissue types. Journal of Economic Entomology 102: 1301–1308. https://doi.org/10.1603/029.102.0357

Soberón, M., Gill, S. S., and Bravo, A. (2009). Signaling versus punching hole: How do *Bacillus thuringiensis* toxins kill insect midgut cells?. Cellular and Molecular Life Sciences, 66(8), 1337–1349. https://doi.org/10.1007/s00018-008-8330-9

Sul, S., Suh, S., Park, S., and Kim, H. (2021). Regulatory policy on genetically modified breeding stack in key countries and the current status in korea. Food Science and Biotechnology, 30(13), 1627–1634. https://doi.org/10.1007/s10068-021-01004-9

Swiatkiewicz, S., Swiatkiewicz, M., Arczewska-Wlosek, A., and Jozefiak, D. (2014). Genetically modified feeds and their effect on the metabolic parameters of food-producing animals: A review of recent studies. Animal Feed Science and Technology, 198, 1–19. https://doi.org/10.1016/j.anifeedsci.2014.09.009

Tabashnik, B. E., Zhang, M., Fabrick, J. A., Wu, Y., Gao, M., Huang, F., Wei, J., Zhang, J., Yelich, A., Unnithan, G. C., Bravo, A., Soberón, M., Carrière, Y., and Li, X. (2015). Dual mode of action of Bt proteins: Protoxin efficacy against resistant insects. Scientific Reports, 5(1), 15107. https://doi.org/10.1038/srep15107

Thomas, K., Aalbers, M., Bannon, G. A., Bartels, M., Dearman, R. J., Esdaile, D. J., Fu, T. J., Glatt, C. M., Hadfield, N., Hatzos, C., Hefle, S. L., Heylings, J. R., Goodman, R. E., Henry, B., Herouet, C., Holsapple, M., Ladics, G. S., Landry, T. D., MacIntosh, S. C., ... Zawodny, J. (2004). A multi-laboratory evaluation of a common *in vitro* pepsin digestion assay protocol used in assessing the safety of novel proteins. Regulatory Toxicology and Pharmacology, 39(2), 87–98. https://doi.org/10.1016/j.yrtph.2003.11.003

Tufarelli, V., Selvaggi, M., Dario, C., and Laudadio, V. (2015). Genetically modified feeds in poultry diet: Safety, performance, and product quality. Critical Reviews in Food Science and Nutrition, 55(4), 562–569. https://doi.org/10.1080/10408398.2012.667017

US EPA. (1995). Plant Pesticide *Bacillus thuringiensis* CryIA(b) Delta-Endotoxin and the Genetic Material Necessary for its Production (Plasmid Vector pCIB4431) in Corn. Federal Register / Vol. 60, No. 158 / Wednesday, August 16, 1995 / Rules and Regulations. https://www.govinfo.gov/content/pkg/FR-1995-08-16/pdf/95-20014.pdf (Bt176)

US EPA. (1998). Reregistration eligibility decision (RED): *Bacillus thuringiensis* (EPA 738-R-98-004). U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, D.C. https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/red_PC-006400_30-Mar-98.pdf

US EPA. (2008). BRAD *Bacillus thuringiensis* modified Cry1Ab (SYN-IR67B-1) and Vip3Aa19 (SYN-IR102-7) insecticidal proteins and the genetic material necessary for their production in COT102 X COT67B cotton. https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/decision_PC-006529_12-Aug-08.pdf

US EPA. (2009). SAP Minutes No. 2009-04. A set of scientific issues considered by the Environmental Protection Agency regarding: the data required to register plant incorporated protectants. https://archive.epa.gov/scipoly/sap/meetings/web/pdf/022526finalreport.pdf

US EPA. (2010). Cry1Ab and Cry1F *Bacillus thuringiensis* (Bt) Corn Plant-Incorporated Protectants., 2010. https://www3.epa.gov/pesticides/chem_search/reg_actions/pip/cry1f-cry1ab-brad.pdf (Bt11, MON810).

US EPA. (2012). Final Cry1Ab-Cry2Ae-TwinLink BRAD 01-26-12, https://www.regulations.gov/document/EPA-HQ-OPP-2011-0988-0008 [Accessed 21-Jan-2026] (T304-40)

US EPA. (2025a). Setting tolerances for pesticide residues https://www.epa.gov/pesticide-tolerances/setting-tolerances-pesticide-residues-foods [Last updated on June 26, 2025; Accessed 22-Jan-2026]

US EPA. (2025b). Statement on Bt10. https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra/statement-bt10 [Last updated on March 26, 2025; Accessed 26-Jan-2026]

US FDA. (1995). Biotechnology Consultation Note to the File BNF No. 000024. Corn transformation event 176. 1995. https://wayback.archive-it.org/7993/20171031095230/https://www.fda.gov/Food/IngredientsPackagingLabeling/GEPlants/Submissions/ucm161142.htm (Bt176)

US FDA. (1996). Biotechnology Consultation Note to the File BNF No. 000034. https://wayback.archive-it.org/7993/20171031094423/https://www.fda.gov/Food/IngredientsPackagingLabeling/GEPlants/Submissions/ucm161154.htm (MON809 and MON810)

US FDA. (2009). Biotechnology Consultation Note to the File BNF No. 000112. Cotton transformation event COT67B 1999 https://wayback.archive-it.org/7993/20171031090254/https://www.fda.gov/Food/IngredientsPackagingLabeling/GEPlants/Submissions/ucm181933.htm [Accessed 21-JAN-2026] (COT67B)

US FDA. (2011). Biotechnology Consultation - Note to the File Biotechnology Notification File BNF No. 000118. Cotton T304-40 and GHB119 BCS-GHØØ4-7 × BCS-GHØØ5-8 https://wayback.archive-it.org/7993/20171031091353/https://www.fda.gov/Food/IngredientsPackagingLabeling/GEPlants/Submissions/ucm271378.htm (T304-40)

US FDA. (2018). Biotechnology consultation note to the file, BNF No. 000159: Sugar from Sugarcane CTC175-A. https://hfpappexternal.fda.gov/scripts/fdcc/index.cfm?set=NewPlantVarietyConsultations&id=CTC175-A&sort=Date_FDA_Letter_Completion&order=DESC&startrow=1&type=basic&search=sugarcane (CTC175A)

US FDA. (2021). Biotechnology Consultation – CFSAN and CVM Note to File BNF 000166: Corn DBN9936 DBN-Ø9936-2 https://hfpappexternal.fda.gov/scripts/fdcc/index.cfm?set=NewPlantVarietyConsultations&id=DBN-O9936-2&sort=Date_FDA_Letter_Completion&order=-DESC&startrow=1&type=basic&search=corn (DBN9936)

USDA Economic Research Service (ERS). (2025). Recent Trends in GE Adoption. https://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-united-states/recent-trends-in-ge-adoption [Accessed Dec. 22, 2025].

USDA APHIS. (2007). Petition for determination of nonregulated status event COT67B. https://www.aphis.usda.gov/sites/default/files/07_10801p.pdf (COT67B)

USDA APHIS. (2008). Petition for determination of nonregulated status for Insect resistant and glufosinate ammonium-tolerant cotton (T304-40). https://www.aphis.usda.gov/sites/default/files/08_34001p.pdf

USDA GAIN Report CH2024-0009. New & Renewed Biosafety Certificates Issued - January (2024). January 26, 2024.

Vachon, V., Laprade, R., and Schwartz, J. (2012). Current models of the mode of action of *Bacillus thuringiensis* insecticidal crystal proteins: A critical review. Journal of Invertebrate Pathology, 111(1), 1–12. https://doi.org/10.1016/j.jip.2012.05.001

van Frankenhuyzen, K. (2009). Insecticidal activity of *Bacillus thuringiensis* crystal proteins. Journal of Invertebrate Pathology, 101(1), 1–16. https://doi.org/10.1016/j.jip.2009.02.009

van Ree, R., Sapiter Ballerda, D., Berin, M. C., Beuf, L., Chang, A., Gadermaier, G., Guevera, P. A., Hoffmann-Sommergruber, K., Islamovic, E., Koski, L., Kough, J., Ladics, G. S., McClain, S., McKillop, K. A., Mitchell-Ryan, S., Narrod, C. A., Pereira Mouriès, L., Pettit, S., Poulsen, L. K., ... Bowman, C. (2021). The COMPARE database: A public resource for allergen identification, adapted for continuous improvement. Frontiers in Allergy, 2, 700533. https://doi.org/10.3389/falgy.2021.700533

Wang, E. H., Yu, Z., Hu, J., Jia, X. D., and Xu, H. B. (2014). A two-generation reproduction study with transgenic Bt rice TT51 in Wistar rats. Food and Chemical Toxicology, 65, 312–320. https://doi.org/10.1016/j.fct.2013.11.045

Waters, S., Ramos, A., Hendrickson Culler, A., Hunst, P., Zeph, L., Gast, R., Mahadeo, D., Sottosanto, J., Huber, S., Shan, G., Chakravarthy, S., and Goodwin, L. (2021). Recommendations for science-based safety assessment of genetically modified (GM) plants for food and feed uses. Journal of Regulatory Science, 9(1), 16–21. https://doi.org/10.21423/jrs-v09i1waters

WHO. (1995). Application of the Principles of Substantial Equivalence to the Safety Evaluation of Foods or Food Components from Plants Derived by Modern Biotechnology. A Report of a WHO Workshop. World Health Organisation (WHO). https://iris.who.int/handle/10665/58909

Zdziarski, I., Edwards, J., Carman, J. A. and Haynes, J. I. (2014). GM crops and the rat digestive tract: A critical review. Environment International 73: 423–433. https://doi.org/10.1016/j.envint.2014.08.018

Zeljenková, D., Ambrušová, K., Bartušová, M., Kebis, A., Kovrižnych, J., Krivošíková, Z., Kuricová, M., Líšková, A., Rollerová, E., Spustová, V., Szabová, E., Tulinská, J., Wimmerová, S., Levkut, M., Révajová, V., Ševčíková, Z., Schmidt, K., Schmidtke, J., La Paz, J. L., ... Steinberg, P. (2014). Ninety-day oral toxicity studies on two genetically modified maize MON810 varieties in Wistar Han RCC rats (EU 7th framework programme project GRACE). Archives of Toxicology, 88(12), 2289–2314. https://doi.org/10.1007/s00204-014-1374-8

Zhou, Q., Li, S., Zhao, M., Liu, Y., He, N., Zhou, X., Zhou, D., and Qian, Z. (2023). A 90-day feeding study of genetically modified maize LP007-1 in Wistar Han RCC rats. Food and Chemical Toxicology, 180, 114026. https://doi.org/10.1016/j.fct.2023.114026

Zhu, H., Chen, Y., Li, Y., Wang, J., Ding, J., Chen, X., and Peng, Y. (2015). A 90 day safety assessment of genetically modified rice expressing Cry1Ab/1Ac protein using an aquatic animal model. Journal of Agricultural and Food Chemistry, 63(14), 3627–3633. https://doi.org/10.1021/jf5055547