A Review of the Food and Feed Safety of the Cry2Ab Protein

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

July 24, 2026

INTRODUCTION

This document provides a comprehensive review of information and data relevant to the assessment of the protein Cry2Ab for food and feed safety. 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 assessments 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 Cry2Ab protein and not on GE crops containing the protein, not all the safety assessment elements in the Codex Plant Guideline are relevant. The topics covered in this monograph are "Origin and Function of Cry2Ab" (including its mechanism of action on targeted species), "Expression of Cry2Ab in Insect-Resistant GE Plants" (including the expression levels of Cry2Ab in various parts of the crops), and "Food and Feed Safety of the Cry2Ab Protein" (including information on toxicology assessment and allergenicity assessment). 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 been consistently considered in food safety assessments in all countries, a discus-

KEYWORDS

Cry2Ab, *Bacillus thuringiensis* (Bt), insect resistance, genetically engineered (GE), food and feed safety assessment

DOI: 10.67498/afsi-a44b

sion on the composition profile of the GE crops expressing the Cry2Ab protein is also included as part of this review.

Global Food Safety Approvals of Cry2Ab Expressing Crops

To date, 60 GE events in which the Cry2Ab protein is expressed have been produced in three crops (cotton, maize, and soybean) and approved in multiple countries. Table 1, 2 and 3 show the details of regulatory approvals[1] for food and feed in these crops, according to ISAAA's GM Approval Database (ISAAA 2025). To date, food and/or feed safety approvals of these crops have been issued in 28 countries and the European Union (EU), representing 60 transformation events (including 'stacked' events) (maize – 46 events, cotton – 11 events, and soybean – 3 events). In total, there are 364 regulatory approvals for food safety in these countries which include 30 approvals in soybean, 72 in cotton and 262 in maize. 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].

Among the approved lines of maize (Table 1), event MON89034 (YieldGard™ VT Pro™) is the only single transformation event[3] with Cry2Ab protein expression approved for food and feed, (or for cultivation) in any country. MON89034 has been approved for food and feed safety in 19 countries and the European Union between 2007 and 2019, with one more recent approval (at the time of this writing) in Switzerland in 2024. Another 45 'stacked event' maize lines developed through conventional breeding to combine the Cry2Ab insect resistance in MON89034 with other GE traits have received food safety approvals in at least one of 17 countries[4] and the European Union. Twelve additional MON89034-derived stacked event maize lines (not included in the table) have received food safety approval only in the European Union. The European Union has issued as many as 26 food safety approvals for maize lines expressing Cry2Ab, and Japan has issued more than 20 food safety approvals as have Colombia and Taiwan. The earliest of these MON89034 stacked event approvals was 2008 in Japan, and a number have been issued since 2020.

Table 1: Cry2Ab maize events with food safety approvals by multiple countries (x = food, or food and feed; o = feed only). Approvals after 2020 are highlighted.

Crop	Event Name*	Argentina	Brazil	Canada	Colombia	European Union	Iran	Japan	Mexico	Nigeria	Paraguay	Philippines	Singapore	South Africa	South Korea	Switzerland	Taiwan	Thailand	Turkey	USA	Uruguay
Zea mays L. (Maize)	MON89034**	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	o	x	
	4114 × MON89034 × MON87411 × DAS40278				x	x		x						o	x		x				
	MON89034 × MON88017***	x	x		x	x	o	x	x		x	x	x	x	x		x	x	o		x
	MON89034 × NK603	x	x		x	x	o	x	x	x		x		x	x		x	x	o		
	MON89034 × TC1507 × MON88017 x 59122	x	x		x	x		x	x			x		x	x		x				
	MON89034 × TC1507 × NK603	x	x		x	x	o	x	x			x		x	x		x				x
	MON89034 × TC1507 × NK603 × MIR162 × DAS40278	x	x		o	x		x	x		x				x		x				
	MON89034 × TC1507 × MON88017 x 59122 × DAS40278				x			x	x						x		x				
	MON89034 × TC1507 × NK603 × DAS40278	x	x	x	x	x	o	x	x					x	x		x				
	MON89034 × TC1507 × NK603 × MIR162	x	x	x	o				x		x				x		x				
	MON89034 × TC1507 x 59122					x						x									
	MON89034 × GA21	x	x																		
	MON87427 × MON89034 × MIR162 × MON87419 × NK603				o			x					x		x						

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. Those feed only approvals have been indicated in Tables 1 and 2.

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.

Crop	Event Name*	Argentina	Brazil	Canada	Colombia	European Union	Iran	Japan	Mexico	Nigeria	Paraguay	Philippines	Singapore	South Africa	South Korea	Switzerland	Taiwan	Thailand	Turkey	USA	Uruguay
	MON87427 × MON89034 × MON810 × MIR162 × MON87411 × MON87419	x			o			x				x	x				x				
Zea mays L. (Maize)	MON87427 × MON87460 × MON89034 × TC1507 × MON87411 x 59122				o			x	x						x		x				
	MON87427 × MON89034 × MIR162 × MON87411	x	x			x		x	x				x		x	x	x				
	MON87427 × MON89034 × MIR162 × NK603	x	x		x	x		x	x		x	x	x		x	x	x	x			
	MON87427 × MON89034 × MON88017	x			x	x		x	x				x		x		x				
	MON87427 × MON89034 × NK603	x			x	x		x	x				x		x		x	x			
	MON87427 × MON89034 × TC1507 × MON87411 x 59122				x			x	x			x	x		x		x				
	MON87427 × MON89034 × TC1507 × MON87411 x 59122 × DAS40278		x	x	x			x	x												
	MON87427 × MON89034 × TC1507 × MON88017 x 59122	x						x	x			x			x	x	x				
	MON87427 × MON89034 × MON87419 × NK603				x																
	MON87427 × MON89034 × TC1507 × MON87411 x 59122 × MON87419							x													
	MON89034 × MON87460								x				x								
	MON87460 × MON89034 × MON88017				x			x	x			x	x		x		x	x			
	MON87460 × MON89034 × NK603				x	x		x	x			x	x		x		x	x			
	Bt11 × MON89034 × GA21	x																			
	Bt11 × MIR162 × MIR604 × MON89034 x 5307 × GA21			x		x		x					x		x		x	x			
	Bt11 × MIR162 × MON89034	x	x												x		x				
	Bt11 × MIR162 × MON89034 × GA21	x	x		x		o		x		x			x	x		x	x			
	MIR162 × MON89034	x	x		x				x						x		x				
	MIR162 × MON89034 × GA21		x																		

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

**MON89034 also has food approvals in Australia, China, Indonesia, Malaysia, New Zealand, Russia and Vietnam. This is the only Cry2Ab maize event approved for food in these countries.

***MON89034 × MON88017 also has food approval in Honduras. This is the only Cry2Ab event with food approval in this country.

In addition to the approvals for maize expressing Cry2Ab, one single transformation event in cotton, MON15985 (Bollgard II™ Cotton), has been issued food and/or feed approvals in 19 countries and the European Union, and 16 of those countries have also approved at least one of nine cotton breeding stacks derived from crosses with MON15985 (Table 2). The earliest Cry2Ab cotton food approvals, as well as approvals for cultivation, were in 2002 in Australia and the USA. One soybean single transformation event, MON87751, has been issued food safety approvals in 19 countries (Table 3) between 2015 and 2024, and one soybean breeding stack derived from crossing with MON87751 has been approved for food safety in as many as 15 countries and the European Union. This breeding stack was issued an approval in the European Union in 2022, without an approval for event MON87751. Brazil also issued an approval for a second soybean stack in 2024.

Regulatory submissions, safety assessments, and decisions for many of these GE events expressing the Cry2Ab 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 2: Cry2Ab expressing cotton events with food safety approvals. Approvals after 2020 are highlighted.

Crop	Event Name*	Argentina	Australia	Brazil	Burkina Faso	Canada	Colombia	European Union	India	Japan	Kenya	Malaysia	Mexico	New Zealand	Nigeria	Paraguay	Philippines	Singapore	South Africa	South Korea	Switzerland	Taiwan	USA	Vietnam
Gossypium hirsutum L. (Cotton)	MON15985		x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x		x	x	o
	MON15985 × MON1445					x		x		x			x	x			x			x				
	MON88913 × MON15985	x		x			x			x			x	x		x	x			x		x		
	LLCotton25 × MON15985									x			x	x						x		x		
	COT102 × MON15985		x							x			x											
	COT102 × MON15985 × MON88913		x	x						x										x		x		
	COT102 × MON15985 × MON88913 × MON88701		x	x			x			x			x							x		x		
	GHB614 × LLCotton25 × MON15985							x		x			x							x	x	x		
	MON88701 × MON88913 × MON15985									x			x							x		x		
	MON88702 × MON15985 × COT102 × MON88701 × MON88913						x													x		x		
	MON15947			x																		x		

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

Table 3: Cry2Ab expressing soybean events with food safety approvals. Approvals after 2020 are highlighted.

Crop	Event Name*	Argentina	Australia	Brazil	Canada	Colombia	European Union	Ghana	Indonesia	Japan	Malaysia	Mexico	New Zealand	Paraguay	Philippines	Singapore	South Korea	Switzerland	Taiwan	USA	Vietnam
Glycine max L. (Soybean)	MON87751	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x
	MON87751 × MON87701 × MON87708 × MON89788	x		x	x	x	x			x		x		x	x		x	x	x		
	MON87701 × MON87751 × MON89788 × MON94313 × MON94637			x																	

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

ORIGIN AND FUNCTION OF CRY2AB

Bacillus thuringiensis and the Cry2Ab 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 Cry2Ab among other Cry proteins) has been registered with US EPA since 1961 (US EPA, 1998). Microbial preparations of *B.*

thuringiensis are currently approved for use around the world including in Africa, Australia, Brazil, Canada, the European Union, and the United States (US EPA, 1998; Health Canada, 2008; 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 (Cry2A, Cry2B, etc.), and proteins within those secondary groups with at least 95% similarity were placed into tertiary groups (Cry2Aa, Cry2Ab, etc.). A fourth level of grouping divided proteins within the tertiary groups that shared more than 95% sequence identity (Cry2Aa1, Cry2Aa2, 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 Cry2 share at least 45% amino acid sequence similarity, those designated as Cry2A are at least 76% similar, and Cry2Ab proteins are at least 95% identical in amino acid sequence. The Cry2Ab protein shares up to 94% sequence identity with other Cry2A proteins (including Cry2Aa, Cry2Ac, and other Cry2A proteins) (Crickmore *et al.* 2021).

Since its initial discovery, genes encoding Cry2Ab proteins have been identified in Bt strains found throughout the world (Jain *et al.*, 2006; Jouzani *et al.*, 2008; Saadaoui *et al.*, 2010; Saleem & Shakoori, 2010; Alvarez & del Valle Loto, 2012). However, the peptide corresponding to *cry2Ab* is typically not produced by these strains, and the native *cry2Ab* is therefore considered to be a pseudo- or cryptic gene (Hodgman *et al.*, 1993). Lack of expression of the native *cry2Ab* is due to the absence of a functional promoter, and it was also determined that a molecular chaperone necessary for the formation of Cry2Ab crystals is missing from the bacterial strains bearing the cryptic *cry2Ab* (Crickmore *et al.*, 1994; Boucias & Pendland, 1998). Expression of Cry2Ab was accomplished in heterologous systems by replacing the regulatory sequences upstream of *cry2Ab* (Widner & Whiteley, 1989, 1990; Dankocsik *et al.*, 1990; Hodgman *et al.*, 1993; Crickmore *et al.*, 1994).

Mechanism of Cry2Ab 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 Cry2Ab Gene and Cry2Ab Protein in GE Plants

There are two main types of modifications to the *cry* genes from Bt that are common for its 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, also termed codon optimization, are primarily used to increase the translation of the gene by modifying codon usage to align with plant preferred codons (Murray *et al.*, 1989). Because the DNA of plants and bacteria differ in nucleotide content (Perlak *et al.*, 1991; Sanahuja *et al.*, 2011), to increase the expression of the Cry2Ab protein in maize to a level sufficient for pest control, the coding sequence has been modified for optimized codon usage (Widner & Whiteley, 1990; Donovan, 1991; EFSA, 2008; FSANZ, 2008). Other strategies include the use of strong promoters, inclusion of plant introns in the expression construct (Sanahuja *et al.*, 2011), and targeting expression of *cry* genes to the chloroplast (McBride *et al.*, 1995; Sanahuja *et al.*, 2011). Examples in MON89034 maize are the use of the first intron from the maize heat shock protein 70 gene (Hsp70) in the *cry2Ab* cassette and signaling the production of the Cry2Ab protein by fusing the coding sequence to a chloroplast transit peptide region of maize ribulose 1,5-biphosphate carboxylase small subunit and its first intron (EFSA, 2008). A similar strategy has also been used in cotton and soybean.

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). These 'truncated' proteins expressed in plants consist of the shorter "activated" form of the Cry protein following protease digestion in the insect midgut (Perlak *et al.*, 1991). In most Bt crops expressing Cry1 proteins, such as Cry1Ab, truncations of the protein rather than the full-length protein from Bt have been submitted for regulatory approvals (OECD, 2007). However, the shorter full-length protoxin expressed by the *cry2Ab* gene, which is already close in size to the 'activated' form of the protein, has not typically been truncated in approved GE crops (OECD, 2007).

All approved GE plants expressing the Cry2Ab protein have been developed using modifications to the nucleotide sequence of *cry2Ab* genes as described above. These modifications do not alter the structure or function of the toxic core 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 Cry2Ab proteins in Bt crops do not raise safety concerns (See Annex 1).

EXPRESSION OF CRY2AB 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 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 (Brune *et al.*, 2021). Each transformation event therefore exhibits a different expression profile.

Data on the level of expression of Cry2Ab 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 Cry2Ab 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 Cry2Ab 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 differed between studies, but typically an enzyme-linked immunosorbent assay (ELISA) or Western blot analysis is used to quantify the amount of Cry2Ab protein present in a sample.

Some examples of Cry2Ab expression data are provided in Annex 2. 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 Cry2Ab 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 to humans is often different from those consumed by animals.

For example, Cry2Ab protein levels expressed in eight different tissues in MON87751 soybean event as presented in Table I.7 in Annex 2 were used along with estimates of soybean consumption to assess exposure to Cry2Ab protein for the general population and non-nursing infants in the US. Based on the levels of expression of Cry2Ab on a fresh weight basis, it was estimated that intake for the overall US population would be 0.000962 mg/kg body weight/day and 0.012355 mg/kg body weight/day for non-nursing infants. The margins of exposure (MOEs) for acute dietary intake of Cry2Ab were estimated to be 2,300,000 times for the general population and 180,000 times for non-nursing infants. These very large MOEs indicate that there is no meaningful risk to human health from dietary exposure to Cry2Ab protein produced by MON87751. For livestock including lactating dairy cows, poultry, and swine, calculations were made considering the daily dietary intake of protein. Based on the calculations, consumption of MON87751 seeds in feeds would result in livestock consuming less than 0.003% (g/g) of the total protein as Cry2Ab. These numbers indicate that adverse health effects in livestock consuming MON87751 are unlikely.

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, 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 Cry2Ab proteins have included an exposure assessment. In every case, for every crop and event approved, the assessment has concluded that the levels of Cry2Ab 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, 2025). 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 Cry2Ab protein (US EPA, 2015; 2010; 2003).

FOOD AND FEED SAFETY OF THE CRY2AB 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, 2003b; Codex, 2009). 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 function or mode of action and 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 Cry2Ab 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. For example, a bioinformatic analysis for the Cry2Ab protein in soybean event MON87751 is described in the safety assessment report by FSANZ (FSANZ 2016). That analysis searched for the Cry2Ab protein sequence from the event in GenBank protein database (PRT_2013) and a subset of sequences derived from PRT_2013 was compiled to contain only toxin proteins (TOX_2013). The search of the PRT_2013 database indicated there was no biologically relevant structural similarity between Cry2Ab and any proteins of concern. The TOX_2013 database search showed that no alignments with Cry2Ab generated an E-score[5] of ≤1e-5. Various regulatory authorities have concluded that Cry2Ab does not share structural similarities with protein toxins to humans or livestock animals (See Annex 1).

Acute Toxicity Studies on the Cry2Ab Protein

Acute toxicity studies have been required by regulatory agencies for assessing food and feed safety of Cry2Ab derived from GE crops. Acute oral toxicology studies with Cry2Ab have been conducted in support of the maize event MON89034. Results indicate that Cry2Ab caused no adverse effects in mice, with No Observed Adverse Effect Levels for Cry2Ab at 2198 mg/kg body weight (bw), the highest doses tested (US EPA, 2010). The Cry2Ab protein from soybean event MON87751 is functionally equivalent to the protein used in acute toxicity assays, originally conducted in support of MON89034, and the data are therefore applicable to MON87751. In a study on the Cry2Ab protein submitted for soybean MON87751, no adverse effects were observed from a mouse 28-day toxicity study at the highest dose tested at 1000 mg/kg bw per day (EFSA, 2018). In all cases, regulators have concluded that the Cry2Ab protein is toxic to lepidopteran insects but non-toxic even in high doses to humans and animals (See Annex 1).

Allergenicity of the Cry2Ab 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 Cry2Ab 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 of the 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 Cry2Ab have 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 Cry2Ab in maize (FSANZ, 2008) and soybean (EFSA, 2018) or the Food Allergy Research and Resource Program (FARRP) allergen database found in AllergenOnline (Goodman *et al.*, 2016) used for the assessment of Cry2Ab in maize (US EPA, 2010). The analysis typically includes searches for overall similarity between the Cry2Ab 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 Cry2Ab protein (not smaller than 8 amino acids) sharing identity with known allergens (US EPA, 2010; USDA APHIS, 2014; EFSA, 2018). The lack of sequence homology based on these analyses indicates that the Cry2Ab protein is not a known allergen and not likely to be cross-reactive to known allergens. Numerous bioinformatic analyses of Cry2Ab in safety assessments of GE crops have not identified any significant similarities to known allergens (See Annex 1).

Digestibility Studies on Cry2Ab 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). For example, the resistance of Cry2Ab protein to degra-

5. Comparisons between highly homologous proteins yield E-values approaching zero, indicating the very low probability that such matches would occur by chance. A larger E-value indicates a lower degree of similarity. Typically, alignments between two sequences will need to have an E-score of 1e-5 (1×10-5) or smaller to be considered to have significant homology

dation by pepsin was investigated in solutions at pH ~ 1.2 in two independent studies followed by an analysis of protein integrity by SDS-PAGE gel electrophoresis and by Western blotting (EFSA, 2018). No intact Cry2Ab protein was observed after 30 seconds of incubation. Low-molecular-weight fragments of ~ 4–5 kDa observed in samples of Cry2Ab completely disappeared after 2 min of incubation. Western blots showed that none of these fragments were immunologically reactive to a polyclonal antibody against Cry2Ab (EFSA, 2018). The digestibility evaluated for safety of Cry2Ab protein in GE crops has indicated that Cry2Ab 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 Cry2Ab 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, and 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 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 (Taylor *et al.*, 2007; Li *et al.*, 2008; Singhal *et al.*, 2011; Weber *et al.*, 2011), often due to the mandatory request for such studies for all single events in the EU (Garcia-Alonso *et al.*, 2025).

Though 90-day feeding studies are generally not required for regulatory approval, there are peer-reviewed studies investigating sub-chronic effects of feeding GE crop-derived food that contains Cry2Ab protein. Bollgard II cotton event 15985 expresses two proteins, Cry1Ac and Cry2Ab. Compositional analyses (see the following section on compositional analysis) were conducted to measure key components and results showed that the cottonseed and cottonseed oil from the GE cotton event were comparable in composition to those of the conventional control cotton line and other commercial varieties (Hamilton *et al.*, 2004). To strengthen the findings from composition analyses, feeding studies were conducted with dairy cows, catfish, and quail. Results from these studies showed the compositional and nutritional equivalence of Bollgard II cotton with conventional cotton varieties (Hamilton *et al.*, 2004).

Liaqat *et al.* (2023) conducted a 90-day risk assessment study on Wistar rats fed with GE maize event CEMB-413 (no information on regulatory approval of this event could be found in existing literature) expressing binary insect-resistance genes (*cry1Ac* and *cry2Ab*) at low (30%) and high (50%) dose. The study concluded that the GE diet had no adverse effects on parameters associated with the animal's health. A similar 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 with 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). Cry2Ab protein is expressed in both events. Studies such as these on whole foods from crops expressing Cry2Ab protein, and similar studies with crops expressing other Cry proteins, support the conclusion that whole food feeding studies indicate no adverse effect with food or feed from GE crops expressing Cry2Ab protein (See Annex 1).

COMPOSITIONAL ANALYSIS OF GE CROPS EXPRESSING CRY2AB 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 part of the hazard identification step and 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 environ-

mental 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 changes are within the range observed in traditional varieties (AFSI 2025; Bajaj *et al.*, 2026).

In the submissions for food safety approvals of crops expressing Cry2Ab, 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 or environmental safety (Hamilton *et al.*, 2004; Drury *et al.*, 2008; Singhal *et al.*, 2011; and See Annex 1). Brief summaries of the compositional profile in Cry2Ab-expressing events for maize, cotton, and soybean are provided in this section.

Maize

Seed and forage from Cry2Ab maize have been subjected to proximate analysis to determine levels of crude protein, crude fat, fiber, moisture, and ash. In addition, levels of select minerals, fatty acids, amino acids, and antioxidants have been determined, and the anti-nutritive compounds of phytic acid, raffinose, and trypsin inhibitor have been measured (OECD, 2002). In line with OECD recommendation (OECD, 2002), nine components were analyzed in forage, and 68 components were analyzed in grain from maize varieties expressing Cry2Ab, in comparison to conventional maize varieties. The results of compositional analysis showed that the forage and kernels of maize MON89034 are compositionally equivalent to those of non-GE control and other reference varieties except for the presence of the novel proteins (See Annex 1). Any noted differences were small, and mean values and ranges of values for the compounds studied were within the range of natural variation. Considering the field trial design, biological variation, and the level of the analytes studied, no statistically significant differences have been found to be biologically relevant in Cry2Ab-expressing maize events.

Cotton

Compositional analysis of cotton event MON15985 involved evaluation of 48 different components important for food and feed uses in cottonseed. The levels of these components were assessed and compared to the parental control (DP50B), as well as other commercial cotton varieties. Field trials were conducted at eight U.S. locations across six states (Alabama, Arizona, Louisiana, Mississippi, South Carolina, and Texas). Proximates (ash, calories, carbohydrate, fat, fiber, moisture, and protein), amino acids, fatty acids, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), gossypol, cyclopropenoid fatty acids and aflatoxin content were measured in cottonseed. Additionally, compositional analyses of cottonseed oil (bleached and deodorized) and meal (toasted) from a subset of samples were conducted. Among the 48 comparisons across the trials, differences were noted in six instances between the GE cotton event MON15985 and the parental line. All these differences were found to be within the normal range of variation for commercial cotton. Statistically different means were not observed at all locations, demonstrating the impact of environmental conditions on variability. These noted differences in composition were considered to be not relevant to food and feed safety of Cry2Ab-expressing MON15985 cotton (See Annex 1).

Soybean

Comparisons were done among forage and seed collected from soybean event MON87751, the conventional non-GE counterpart, and other soybean reference varieties across eight field sites in the United States in 2012, in accordance with the principles and analytes outlined in the OECD consensus document for soybean (OECD, 2012). A total of 66 components were measured in forage and seed derived from this transgenic event. Of these, 50 components were assessed statistically and compared with control in addition to 19 reference varieties of soybean. 14 components were excluded from the analyses as more than 50% of the observations were found to be below the limit of quantitation. Eight of the components analyzed showed a significant difference between MON87751 and the conventional non-GE counterpart. However, these differences were within the range of the other conventional soybean varieties and were not considered biologically meaningful for nutrition or food and feed safety. MON87751 soybean is compositionally equivalent to commercially cultivated varieties of soybean (See Annex 1).

SAFETY ASSESSMENT OF STACKED EVENTS

The global trend in approvals of GE maize, as demonstrated for the *cry2Ab* gene in Table 1, particularly for Bt insect resistance and herbicide tolerance (HT), 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 U.S., the adoption rates for stacked varieties of maize and cotton with one or more Bt and HT traits have increased steadily since 2000, with more than 80 percent of U.S. 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 molecular characterization, 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 29 stacked products with the *cry2Ab* gene (Scientific Opinions available online in the EFSA Journal). EFSA concluded from these assessments that stacked events expressing Cry2Ab (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 Cry2Ab 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 trait products generally considering breeding stacks as safe for cultivation 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 Cry2Ab protein expressed in insect-resistant GE plants is sourced from the common soil bacterium Bt and is toxic to Lepidoptera. Three GE crops (cotton, maize, and soybean) expressing Cry2Ab 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 Cry2Ab 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 Cry2Ab, including concentrations far exceeding expected levels in food derived from Cry2Ab expressing GE plants. Bt is not a known source of allergens and Cry2Ab 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 Cry2Ab 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 Cry2Ab in GE crops does not pose a food or feed safety concern.

ANNEX 1: REGULATORY DOCUMENTS OF SOME CROP EVENTS EXPRESSING THE CRY2AB PROTEIN APPROVED FOR FOOD SAFETY

Crop/Event	Title	Reference
Maize MON89034	Request for the determination of non-regulated status for MON89034. Washington, DC.	USDA APHIS 2006
	United States Department of Agriculture. Finding of no significant impact: Petition for non-regulated status for corn event MON89034 (APHIS 06-298-01p). Washington, DC.	USDA APHIS 2008
	Biotechnology Consultation Note to the File, BNF No. 000107: *Bacillus thuringiensis* Cry1A.105/Cry2Ab2; MON89034. Washington, DC.	US FDA 2007
	Decision document DD2008-74: Determination of the safety of Monsanto Canada Inc.'s corn (*Zea mays* L.) event MON89034. Canadian Food Inspection Agency. Ottawa, Canada.	CFIA 2008
	Application (Reference EFSA-GMO-NL-2007-37) for the placing on the market of the insect-resistant genetically modified maize MON89034, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 909: 1-30.	EFSA 2008
	Final assessment report, Application A595, Food derived from insect-protected corn line MON89034. Food Standards Australia New Zealand. Canberra, Australia.	FSANZ 2008
	Biopesticides registration action document: *Bacillus thuringiensis* Cry1A.105 and Cry2Ab2 insecticidal proteins and the genetic material necessary for their production in corn [PC Codes 006515 (Cry2Ab2), 006514 (Cry1A.105)]. U.S. Environmental Protection Agency, Office of Pesticide Programs, Biopesticides and Pollution Prevention Division, Washington, D.C.	US EPA 2010
	Parecer Técnico No 3045/2011. Comissão Técnica Nacional de Biossegurança. Brasilia, Brazil. MON89034	CTNBio 2011
Cotton MON15985	Petition for determination of regulatory status for Bollgard II cotton event 15985. Washington, DC.	USDA APHIS 2000
	Biopesticides Registration Action Document, a summary of the risk assessments and regulatory decisions for each registered PIP active ingredient. *Bacillus thuringiensis* Cry2Ab2 protein and its genetic material necessary for its production in cotton.	US EPA 2003
	Biotechnology Consultation Note to the file, BNF No. 000074: Insect-protected Bollgard II cotton line 15985. Washington, DC.	US FDA 2002
	Final assessment report, Application A436, Oil and linters derived from insect-protected cotton containing event 15985. Food Standards Australia New Zealand. Canberra, Australia.	FSANZ 2002
	Parecer Técnico No. 8038/2022. Comissão Técnica Nacional de Biossegurança. Brasilia, Brazil. MON15947	CTNBio 2022
	Novel food information -- Food biotechnology, insect-protected cotton event 15985. Ottawa, Canada.	Health Canada 2003
	Decision document DD2003-45: Determination of the safety of Monsanto's insect resistant Bollgard II™ Cotton (*Gossypium hirsutum* L.). Canadian Food Inspection Agency. Ottawa, Canada.	CFIA 2004
	Risk assessment and risk management plan for DIR 066/2006. Office of the Gene Technology Regulator. Canberra, Australia.	OGTR 2006
	Parecer Técnico No. 1832/2009. Comissão Técnica Nacional de Biossegurança. Brasilia, Brazil. (Cotton) MON15985 (Bollgard® II)	CTNBio 2009
	EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2014. Scientific Opinion on applications (EFSA-GMO-UK-2008-57 and EFSA-GMO-RX-MON15985) for the placing on the market of insect-resistant genetically modified cotton MON15985 for food and feed uses, import and processing, and for the renewal of authorization of existing products produced from cotton MON15985, both under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 12(7): 3770, 42 pp.	EFSA 2014
Soybean MON87751	Request for an extension of determination of nonregulated status for lepidopteran-protected soybean MON87751 (APHIS 13-337-01p (Extension of 09-082-01p). Washington DC.	USDA APHIS 2014
	Biopesticides Registration Action Document, a summary of the risk assessments and regulatory decisions for each registered PIP active ingredient. Plant-Incorporated Protectants Bacillus Thuringiensis Cry1A.105 and Cry2Ab2 Proteins and the Genetic Material (Vector PV-GMIR13196) Necessary For Their Production in MON87751 (OECD Unique ID. MON-87751-7) Soybean [PC Code 006614 and PC Code 006527].	US EPA 2015
	Biotechnology Consultation Note to the File BNF No. 0001444. MON87751 insect-resistant soybean. Washington, DC.	US FDA 2015
	Safety Assessment Report, Application A1110, Food derived from insect-protected soybean line MON87751. Food Standards Australia New Zealand. Canberra, Australia.	FSANZ 2016
	EFSA Panel on Genetically Modified Organisms (GMO, Assessment of genetically modified soybean MON87751 for food and feed uses under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2014-121).	EFSA 2018

ANNEX 2: SUMMARY OF CRY2AB PROTEIN EXPRESSION DATA

The tables that follow present summary data from regulatory submission and decision documents. Whenever possible, the data and accompanying statistics are presented as they appeared in the cited document to facilitate cross-referencing. Additional information on data collection and sampling methodologies can be found in the referenced sources.

Table I.1: Summary of Cry2Ab protein levels in tissues from MON89034 (USDA APHIS, 2006; FSANZ, 2008).

Tissue Type	Growth State	Cry2Ab Mean (SD) [Range], n = 15	
		µg/g fresh wt.	µg/g dry wt.
Young leaf	2 – 4 Leaves	29 (6.8) [19 – 43]	180 (59) [94 – 270]
Pollen	Silking	0.34 (0.084) [0.21 – 0.47]	0.64 (0.091) [0.49 – 0.79]
Silk	Silking	8.2 (3.6) [3.3 – 16]	71 (35) [33 – 160]
Forage	Early dent	12 (4.0) [6.5 – 18]	38 (14) [15 – 55]
Forage root	Early dent	4.1 (1.4) [2.2 – 6.5]	21 (5.9) [14 – 33]
Grain	Maturity	1.1 (0.31) [0.67 – 1.8]	1.3 (0.36) [0.77 – 2.1]
Stover	After harvest	22 (3.6) [17 – 29]	62 (15) [46 – 97]
Senescent root	After harvest	5.3 (2.0) [2.4 – 9.1]	26 (8.8) [13 – 43]

Table I.2: Cry2Ab Protein Expression Levels in Tissues From Mon89034 (Mg/G Dry Wt.) (EFSA, 2008).

Year/Location	Grain	Forage	Pollen	Forage root	Stover
2004/Argentina	0.95	45	0.56	31	44
2005/USA	1.3	38	0.64	21	62

Table I.3: Cry2Ab protein expression levels in tissues from MON89034 (µg/g dry wt.) (CFIA, 2008).

Tissue	Cry2Ab
Leaves	130 – 180
Root	21 – 58
Whole Plant	38 – 130
Grain	1.3
Pollen	0.64

Table I.4: Cry2Ab Protein Expression Levels in Over Season Tissues of MON89034 (USDA APHIS, 2006).

Days After Planting (DAP)

Tissue		21 – 29		28 – 43		41 – 53		56 – 68		100 – 120		130 – 160	
		µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW
Leaf	Mean (SD)	180 (59)	29 (6.8)	170 (34)	32 (5.3)	130 (34)	29 (5.4)	160 (44)	37 (12)	N/A	N/A	N/A	N/A
	Range	94 – 270	19 – 43	110 – 230	23 – 44	85 – 200	23 – 41	48 – 210	11 – 56	N/A	N/A	N/A	N/A
Whole Plant	Mean (SD)	130 (51)	13 (4.6)	79 (18)	7.5 (1.8)	40 (9.9)	4.2 (0.94)	39 (16)	5.9 (2.6)	38 (14)	12 (4.0)	62 (15)	22 (3.6)
	Range	52 – 230	5.2 – 21	45 – 110	4.0 – 9.7	22 – 61	2.4 – 5.8	5.0 – 67	0.7 – 11	15 – 55	6.5 – 18	46 – 97	17 - 29
Root	Mean (SD)	56 (17)	6.4 (1.6)	58 (18)	7.6 (4.2)	35 (17)	5.0 (2.7)	26 (7.7)	4.2 (1.2)	21 (5.9)	4.1 (1.4)	26 (8.8)	5.3 (2.0)
	Range	33 – 100	4.4 – 10	25 – 86	2.5 – 15	17 – 74	2.2 – 12	15 – 45	3.2 – 7.6	14 – 33	2.2 – 6.5	13 – 43	2.4 – 9.1

Table I.5: Mean levels of Cry2Ab protein in cotton event 15985, the parental control DP50B, and the non-transgenic control DP50 (USDA APHIS, 2000; FSANZ, 2002; US EPA, 2003; CFIA, 2004; OGTR, 2006).

Cotton Line		Cry2Ab (µg/g fresh weight)
Leaf[1]		
	15985	23.8 ± 6.3[2]
		(10.1 – 33.3)[3]
	DP50B	< 2.65
	DP50	< 2.65
Seed[4]		
	15985	43.2 ± 5.7
		(31.8 – 50.7)
	DP50B	< 2.31
	DP50	< 2.31
Whole plant[5]		
	15985	8.80 ± 1.20
		(7.28 – 10.46)
	DP50B	< 1.24
	DP50	< 1.24
Pollen[5]		
	15985	< 0.25
	DP50B	< 0.25
	DP50	< 0.25

[1]Leaf tissue n = up to 6 plants/plot from each site, 8 sites, taken at 4 – 6 weeks post planting.
[2]Mean and standard deviation were calculated from samples, one from each of 8 field sites except for tissues collected from a single site.
[3]Range is the minimum and maximum value from samples across sites.
[4]Bulk seed samples were collected for each line from each plot.
[5]The sample of whole plant and pollen was up to 16 plants per line.

Table I.6: Levels[1] of Cry2Ab Protein Detected in Leaf Samples Of Transgenic Cotton Line 15985 and a Control Line at Various Sampling Dates During the 1998 And 1999 Growing Seasons (USDA APHIS, 2000; US EPA, 2003; OGTR, 2006).

Days After Planting	15985		DP50B	
	1998	1999	1998	1999
28	21 ± 4.9	7.1 ± 1.6	Not Detected	Not Detected
55	40.1 ± 6.5	14.3 ± 5.3	Not Detected	Not Detected
85	19.7 ± 2.7	17.0 ± 1.2	Not Detected	Not Detected
108	16.7 ± 0.6	11.9 ± 2.9	Not Detected	Not Detected

[1] Mean (µg/g fresh weight) ± standard deviation.

Table I.7: Levels of Cry2Ab Protein in Tissues Harvested at Eight Different Growth Stages From MON87751 Soybean Parts (Averaged Across 5 Sites Except for Pollen) (FSANZ, 2016).

Tissue Type[1]	Development Stage[1]	Mean (SD) Range (µg/g dw)
Leaf	V3 – V4	24 (5.9) 17 – 37
Leaf	V5 – V7	26 (3.1) 20 – 33
Leaf	R2 – R3	32 (5.2) 25 – 43
Leaf	R6	24 (2.7) 18 – 29
Root	R6	15 (2.7) 11 – 22
Forage[2]	R6	14 (2.2) 11 – 18
Seed	R8	4.0 (0.77) 2.6 – 5.1
Pollen/Anther[3]	R2	< LOQ[4]

[1] For information on soybean growth stages see [e.g. Iowa State University]
[2] Forage is the above ground plant parts used for animal feed.
[3] Flowers from which pollen and anther tissue was collected were grown only at one site in Champaign, Illinois
[4] LOQ=limit of quantitation

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

Alvarez, A. and del Valle Loto, F. (2012). Characterization and biological activity of *Bacillus thuringiensis* isolates that are potentially useful in insect pest control. In: Lameed, G. A. (Ed.) Biodiversity Enrichment In a Diverse World. INTECH. https://doi.org/10.5772/48362

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]

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., 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., 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

Boucias, D.G. and Pendland, J.C. (1998). *Bacillus thuringiensis*: Producer of potent insecticidal toxins. In: Boucias, D.G. and Pendland, J.C. (Eds.) Principles of Insect Pathology. Norwell, MA: Kluwer Academic. https://doi.org/10.1007/978-1-4615-4915-4_7

Bravo, A., Pacheco, S., Gomez, I., Soberón, M. (2023). Mode of action of *Bacillus thuringiensis* cry pesticidal proteins. Advances in insect physiology (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). Cartagena Protocol on Biosafety Annex III: Risk Assessment. https://bch.cbd.int/protocol/text/article.shtml?a=cpb-43

CFIA. (2004). Decision document DD2003-45: Determination of the safety of Monsanto's insect resistant Bollgard II™ Cotton (*Gossypium hirsutum* L.). https://inspection.canada.ca/en/plant-varieties/plants-novel-traits/approved-under-review/decision-documents/dd2003-45?wbdisable=true

CFIA. (2008). Decision document DD2008-74: Determination of the safety of Monsanto Canada Inc.'s corn (*Zea mays* L.) event MON89034. https://inspection.canada.ca/en/plant-varieties/plants-novel-traits/approved-under-review/decision-documents/dd2008-74

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. https://www.fao.org/4/a1554e/a1554e00.htm

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

Crickmore, N., Wheeler, V. C., and Ellar, D. J. (1994). Use of an operon fusion to induce expression and crystallisation of a *Bacillus thuringiensis* δ-endotoxin encoded by a cryptic gene. Molecular and General Genetics MGG, 242(3), 365–368. https://doi.org/10.1007/bf00280428

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

CTNBio. (2009). Parecer Técnico No. 1832/2009 [Technical Opinion No. 1832/2009]. Comissão Técnica Nacional de Biossegurança. http://ctnbio.mctic.gov.br/liberacao-comercial#/liberacao-comercial/consultar-processo (Plantas - Algodão/Cotton - MON15985)

CTNBio. (2011). Parecer Técnico No. 3045/2011 [Technical Opinion No. 3045/2011]. Comissão Técnica Nacional de Biossegurança. http://ctnbio.mctic.gov.br/liberacao-comercial#/liberacao-comercial/consultar-processo (Plantas - Milho/Maize)

CTNBio. (2022). Parecer Técnico No. 8038/2022 [Technical Opinion No. 8038/2022]. Comissão Técnica Nacional de Biossegurança. https://www.fao.org/fileadmin/user_upload/gmfp/docs/commercial%20realease%208038-2022.pdf

Dankocsik, C., Donovan, W. P., and Jany, C. S. (1990). Activation of a cryptic crystal protein gene of *Bacillus thuringiensis* subspecies *kurstaki* by gene fusion and determination of the crystal protein insecticidal specificity. Molecular Microbiology, 4(12), 2087–2094. https://doi.org/10.1111/j.1365-2958.1990.tb00569.x

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, S71-S97. 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

Donovan, W.P. (1991). CryIIB crystal protein gene from *Bacillus thuringiensis*. Patent 5,073,632, U.S. Patent Office. https://patents.google.com/patent/US5073632A/en

Drury, S. M., Reynolds, T. L., Ridley, W. P., Bogdanova, N., Riordan, S., Nemeth, M. A., Sorbet, R., Trujillo, W. A. and Breeze, M. L. (2008). Composition of forage and grain from second generation insect-protected corn MON89034 is equivalent to that of conventional corn (*Zea mays* L.). Journal of Agricultural and Food Chemistry 56: 4623–4630. https://doi.org/10.1021/jf800011u

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). (2008). Application (Reference EFSA-GMO-NL-2007-37) for the placing on the market of the insect-resistant genetically modified maize MON89034, for food and feed uses, import and processing under regulation (EC) no 1829/2003 from monsanto. EFSA Journal, 6(12), 909. https://doi.org/10.2903/j.efsa.2008.909

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). (2014). Scientific opinion on applications (EFSA-GMO-UK-2008-57 and EFSA-GMO-RX-MON15985) for the placing on the market of insect-resistant genetically modified cotton MON15985 for food and feed uses, import and processing, and for the renewal of authorisation. EFSA Journal, 12(7), 3770. https://doi.org/10.2903/j.efsa.2014.3770

European Food Safety Authority (EFSA) Panel on Genetically Modified Organisms (GMO), Naegeli, H., Birch, A. N., Casacuberta, J., De Schrijver, A., Gralak, M. A., Jones, H., Manachini, B., Messéan, A., Nielsen, E. E., Nogué, F., Robaglia, C., Rostoks, N., Sweet, J., Tebbe, C., Visioli, F., Wal, J., Álvarez, F., Ardizzone, M., ... Paraskevopoulos, K. (2018). Assessment of genetically modified soybean MON87751 for food and feed uses under regulation (EC) no 1829/2003 (application EFSA-GMO-NL-2014-121). EFSA Journal, 16(8). https://doi.org/10.2903/j.efsa.2018.5346

European Union 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. (2002). Final assessment report, Application A436, Oil and linters derived from insect-protected cotton containing event 15985. Food Standards Australia New Zealand. https://www.foodstandards.gov.au/sites/default/files/food-standards-code/applications/Documents/ACF65C.pdf

FSANZ. (2008). Final assessment report, Application A595, Food derived from insect-protected corn line MON89034. Food Standards Australia New Zealand. https://www.foodstandards.gov.au/sites/default/files/food-standards-code/applications/Documents/A595_DAR_MON89034_Corn_FINAL.pdf

FSANZ. (2016). Safety Assessment Report, Application A1110, Food derived from insect-protected soybean line MON87751. Food Standards Australia New Zealand. Canberra, Australia. https://www.foodstandards.gov.au/sites/default/files/food-standards-code/applications/Documents/A1110-CFS-SD1.pdf

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

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. http://dx.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

Hamilton, K. A., Pyla, P. D., Breeze, M., Olson, T., Li, M., Robinson, E., Gallagher, S. P., Sorbet, R., and Chen, Y. (2004). Bollgard II cotton: Compositional analysis and feeding studies of cottonseed from insect-protected cotton (*Gossypium hirsutum*L.) producing the Cry1Ac and Cry2Ab2 proteins. Journal of Agricultural and Food Chemistry, 52(23), 6969–6976. https://doi.org/10.1021/jf030727h

Health Canada. (2003). Novel food information -- Food biotechnology, insect-protected cotton event 15985. https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products/novel-food-information-insect-protected-cotton-event-15985.html

Health Canada. (2008). *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

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

Hodgman, T. C., Ziniu, Y., Shen, J., and Ellar, D. J. (1993). Identification of a cryptic gene associated with an insertion sequence not previously identified in *Bacillus thuringiensis*. FEMS Microbiology Letters, 114(1), 23–29. https://doi.org/10.1111/j.1574-6968.1993.tb06545.x

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). International Service for Acquisition of Agri-biotech Applications GM Approval Database. 2025. https://www.isaaa.org/gmapprovaldatabase Accessed December 6, 2025.

Jain, D., Udayasuriyan, V., Arulselvi, P. I., Dev, S. S. and Sangeetha, P. (2006). Cloning, characterization, and expression of a new *cry2Ab* gene from *Bacillus thuringiensis* strain 14-1. Applied Biochemistry and Biotechnology 128: 185–194. https://doi.org/10.1385/ABAB:128:3:185

Jouzani, G. S., Abad, A. P., Seifinejad, A., Marzban, R., Kariman, K., and Maleki, B. (2008). Distribution and diversity of Dipteran-specific cry and cyt genes in native *Bacillus thuringiensis* strains obtained from different ecosystems of Iran. Journal of Industrial Microbiology and Biotechnology, 35(2), 83–94. https://doi.org/10.1007/s10295-007-0269-6

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_al_2010.pdf

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

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

Li, M. H., Hartnell, G. F., Robinson, E. H., Kronenberg, J. M., Healy, C. E., Oberle, D. F. and Hoberg, J. R. (2008). Evaluation of cottonseed meal derived from genetically modified cotton as feed ingredients for channel catfish, Ictalurus punctatus. Aquaculture Nutrition 14: 490–498. https://doi.org/10.1111/j.1365-2095.2007.00554.x

Liaqat, A., Salisu, I. B., Bakhsh, A., Ali, Q., Imran, A., Ali, M. A., Farooq, A. M., Rao, A. Q., and Shahid, A. A. (2023). A sub-chronic feeding study of dual toxin insect-resistant transgenic maize (CEMB-413) on Wistar rats. PLOS ONE, 18(8), e0285090. https://doi.org/10.1371/journal.pone.0285090

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

Martínez, 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

Murray, E. E., Lotzer, J. and Eberle, M. (1989). Codon usage in plant genes. Nucleic Acids Research 17: 12–18. https://doi.org/10.1093/nar/17.2.477

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. (2002). Consensus document on compositional considerations for new varieties of maize (*Zea mays*): Key food and feed nutrients, anti-nutrients, and secondary plant metabolites. Series on the Safety of Novel Foods and Feeds No. 6. 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. (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. (2012). Revised consensus document on compositional considerations for new varieties of soybean [Glycine max (L.) Merr.]: Key food and feed nutrients, anti-nutrients, toxicants and allergens. Series on the Safety of Novel Foods and Feeds No. 25. 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

OGTR. (2006). Risk assessment and risk management plan for DIR 066/2006. Office of the Gene Technology Regulator. https://www.ogtr.gov.au/sites/default/files/2021-06/dir066-2006-full_risk_assessment_and_risk_management_plan.pdf

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 D. A.Fischhoff. (1991). Modification of the coding sequence enhances plant expression of insect control protein genes. Proceedings of the National Academy of Sciences U. S. A. 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

Saadaoui, I., Al-Thani, R., Al-Saadi, F., Hassan, N. B., Abdelkefi-Mesrati, L., Schultz, P., Rouis, S., and Jaoua, S. (2010). Characterization of tunisian *Bacillus thuringiensis* strains with abundance of *kurstaki* subspecies harbouring insecticidal activities against the lepidopteran insect *Ephestia kuehniella*. Current Microbiology, 61(6), 541–548. https://doi.org/10.1007/s00284-010-9650-1

Saleem, F. and A. R. Shakoori. (2010). Characterization of cry2A-type gene(s) from Pakistani isolates of *Bacillus thuringiensis* toxic to Lepidopteran and Dipteran insects. Pakistan Journal of Zoology 42: 181–193. https://www.researchgate.net/publication/261726542_Characterization_of_cry2A-type_Genes_from_Pakistani_Isolates_of_Bacillus_thuringiensis_Toxic_to_Lepidopteran_and_Dipteran_Insects

Sanahuja, G., R. Banakar, R. M. Twyman, T. Capell, and P. Christou, "*Bacillus thuringiensis*: a century of research, development and commercial applications," Plant Biotechnol. J., vol. 9, no. 3, pp. 283–300, Apr. (2011). 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: 775–806. https://doi.org/10.1128/mmbr.62.3.775-806.1998

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

Singhal, K. K., Tyagi, A. K., Rajput, Y. S., Singh, M., Kaur, H., Perez, T., and Hartnell, G. F. (2011). Feed intake, milk production and composition of crossbred cows fed with insect-protected bollgard II® cottonseed containing Cry1Ac and Cry2Ab proteins. Animal, 5(11), 1769–1773. https://doi.org/10.1017/s1751731111000899

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

Taylor, M., Lucas, D., Nemeth, M., Davis, S., and Hartnell, G. (2007). Comparison of broiler performance and carcass parameters when fed diets containing combined trait insect-protected and glyphosate-tolerant corn (MON89034 × NK603), control, or conventional reference corn. Poultry Science, 86(9), 1988–1994. https://doi.org/10.1093/ps/86.9.1988

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

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. (2003). *Bacillus thuringiensis* Cry2Ab2 protein and its genetic material necessary for its production in cotton. https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/decision_PC-006487_4-Mar-03.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). Biopesticides registration action document: *Bacillus thuringiensis* Cry1A.105 and Cry2Ab2 insecticidal proteins and the genetic material necessary for their production in corn [PC Codes 006515 (Cry2Ab2), 006514 (Cry1A.105)]. U.S. Environmental Protection Agency, Office of Pesticide Programs, Biopesticides and Pollution Prevention Division, Washington, D.C. https://archive.epa.gov/pesticides/biopesticides/web/pdf/brad_006514-006515.pdf (MON89034)

US EPA. (2015). Biopesticides Registration Action Document, a summary of the risk assessments and regulatory decisions for each registered PIP active ingredient. Plant-Incorporated Protectants Bacillus Thuringiensis Cry1A.105 and Cry2Ab2 Proteins and the Genetic Material (Vector PV-GMIR13196) Necessary For Their Production in MON87751 (OECD Unique ID. MON-87751-7) Soybean [PC Code 006614 and PC Code 006527]. https://downloads.regulations.gov/EPA-HQ-OPP-2014-0456-0008/content.pdf

US EPA. (2025). 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 FDA. (2002). Biotechnology Consultation Note to the file, BNF No. 000074: Insect-protected Bollgard II cotton line 15985. https://www.hfpappexternal.fda.gov/scripts/fdcc/index.cfm?set=NewPlantVarietyConsultations&id=MON-15985-7

US FDA. (2007). Biotechnology Consultation Note to the File, BNF No. 000107: *Bacillus thuringiensis* Cry1A.105/Cry2Ab2; MON89034. https://www.hfpappexternal.fda.gov/scripts/fdcc/index.cfm?set=NewPlantVarietyConsultations&id=MON-89034-3

US FDA. (2015). Biotechnology Consultation Note to the File BNF No. 0001444, MON87751 insect-resistant soybean. https://www.hfpappexternal.fda.gov/scripts/fdcc/index.cfm?set=NewPlantVarietyConsultations&id=MON-87751-7

USDA APHIS. (2000). Petition for determination of regulatory status for Bollgard II cotton event 15985. https://www.aphis.usda.gov/sites/default/files/00_34201p.pdf

USDA APHIS. (2006). Request for the determination of non-regulated status for MON89034. https://www.aphis.usda.gov/sites/default/files/06_29801p.pdf

USDA APHIS. (2008). United States Department of Agriculture. Finding of no significant impact: Petition for non-regulated status for corn event MON89034 (APHIS 06-298-01p). https://www.aphis.usda.gov/sites/default/files/06_29801p.pdf

USDA APHIS. (2014). Request for an extension of determination of nonregulated status for lepidopteran-protected soybean MON87751 (APHIS 13-337-01p (Extension of 09-082-01p). https://www.aphis.usda.gov/sites/default/files/13_33701p.pdf

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 GAIN Report CH2024-0009. New & Renewed Biosafety Certificates Issued - January (2024). January 26, 2024. https://fas.usda.gov/data/china-new-and-renewed-biosafety-certificates-issued-january-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

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

Weber, B. M., Nuttelman, B. L., Griffin, W. A., Erickson, G. and Klopfenstein, T. (2011). Feedlot cattle performance when fed silage and grain from second-generation insect protected corn, parental line or reference hybrids. Nebraska Beef Cattle Reports 76–77. https://digitalcommons.unl.edu/animalscinbcr/642

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

Widner, W. R., and Whiteley, H. R. (1989). Two highly related insecticidal crystal proteins of *Bacillus thuringiensis* subsp. *kurstaki* possess different host range specificities. Journal of Bacteriology, 171(2), 965–974. https://doi.org/10.1128/jb.171.2.965-974.1989

Widner, W. R., and Whiteley, H. R. (1990). Location of the dipteran specificity region in a lepidopteran-dipteran crystal protein from *Bacillus thuringiensis*. Journal of Bacteriology, 172(6), 2826–2832. https://doi.org/10.1128/jb.172.6.2826-2832.1990

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

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