A Review of the Environmental Safety of the Cry1F Protein

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INTRODUCTION

This document provides a comprehensive review of the information and data relevant to the environmental risk assessment of Cry1F, a family of proteins encoded by genes isolated from *Bacillus thuringiensis* (Bt), and it presents a summary statement about the environmental safety of these proteins when produced in genetically engineered (GE) cotton (*Gossypium hirsutum*) and maize (*Zea mays*) plants. All sources of information reviewed herein are publicly available and include dossiers presented to regulatory authorities, decision documents prepared by regulatory authorities, product descriptions prepared by product developers, and peer-reviewed literature.

Environmental risk assessments related to the planting of GE crops are conducted on a case-by-case basis, taking into account the biology of the plant, the characteristics of the transgenes and any encoded proteins, the phenotype conferred by the transgenes, the intended uses of the crop, and the nature of the receiving environment into which the plant will be introduced. These assessments, which consider both potential hazards and exposure levels, typically involve comparisons to an untransformed parental line or closely related isolines (Craig, Tepfer, Degrassi, andx` Ripandelli, 2008; OECD, 2007). The goal of these comparisons is the identification of potential risks the GE plant might present beyond those already accepted when similar, non-GE plants are grown in the environment. The consequences of these risks, if any, are then evaluated.

Several regulatory authorities have performed environmental risk assessments on GE crop varieties producing Cry1F. Table 1 shows the current status[1] regulatory approvals for the environmental release of Cry1F cotton event DAS-24236-5 and Cry1F maize events DAS-01507-1 and DAS-06275-8.[2] In some countries a separate regulatory approval may be given when an already approved event is combined with other GE events in a stack (Que *et al.*, 2010). The table shows the date of the earliest approval given for the event.

Table 1. Regulatory approvals for the environmental release of GE cotton and maize varieties containing Cry 1F (as of February 28, 2013).

Country	DAS-24236-5 Cotton	DAS-01507-1 Maize	DAS-06275-8 Maize
Argentina		2005	
Brazil	2009	2008	
Canada		2002	2006
Colombia		2007	
Honduras		2009	
Japan		2002	2008
Paraguay		2012	
United States	2004	2001	2004
Uruguay		2011	

1 Regulations may require periodic renewal of pesticide registrations. For example, the current status of USEPA registrations can be found at http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm.

2 Many other regulatory authorities have also approved Cry1F cotton and maize for food and feed use. Additional information can be found at http://cera-gmc.org/index.php?action=gm_crop_database.

Key words

Cry1F, insecticidal crystalline proteins, binary toxin, *Bacillus thuringiensis*, insect resistance, genetically engineered, environmental risk assessment

ORIGIN AND FUNCTION OF THE Cry1F PROTEIN

Bacillus thuringiensis and the Cry1F Insecticidal Protein

Bacillus 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 (See, for example, Apaydin, Çınar, Turanli, Harsa, and Güneş, 2008; Martínez and Caballero, 2002; Seifinejad, Jouazni, Hosseinzadeh, and Abdmishani, 2008). The species has been studied extensively and used commercially for many years due to its ability to synthesize proteins with pesticidal properties (Hofte and Whiteley, 1989; OECD, 2007; Schnepf *et al.*, 1998; van Frankenhuyzen, 2009). Preparations of natural isolates of *B. thuringiensis* were first used as a commercial insecticide in France in 1938, and *B. thuringiensis* subspecies *kurstaki* has been registered with USEPA since 1961 (USEPA, 1998). Microbial preparations of *B. thuringiensis* are currently approved for use around the world including in Australia, Canada, the European Union, and the United States (APVMA, 2013; DGSANCO, 2013; Health Canada, 2008; Kumar, Sharma, and Malik, 1996; Schnepf *et al.*, 1998). Because these preparations are derived from cultured cells of Bt bacteria, they contain a complex mixture of the pesticidal substances produced by the particular Bt strain used.

Several hundred pesticidal substances have been isolated from Bt cultures (Crickmore *et al.*, 2012), and these substances display tremendous variety in chemical structure, mode of action, and target specificity (Hofte and Whiteley, 1989; OECD, 2007; Pigott and Ellar, 2007; Schnepf *et al.*, 1998; Vachon, Laprade, and Schwartz, 2012; van Frankenhuyzen, 2009). They include antifungal compounds, ß-exotoxins, Cyt (cytolytic) proteins, vegetative insecticidal proteins (Vip), and the ß-endotoxins,[3] a group that includes the insecticidal Cry (crystalline) proteins (Hofte and Whiteley, 1989; OECD, 2007; Schnepf *et al.*, 1998). These substances may interact with each other to influence the toxicity and activity spectrum of individual bacterial preparations (OECD, 2007; Schnepf *et al.*, 1998).[4] The Cry proteins have been studied extensively and used widely in agriculture for their ability to cause cell disruption in the digestive tracts of insect pests, resulting in the interruption of feeding and eventual insect death (Mendelsohn, Kough, Vaituzis, and Matthews, 2003; OECD, 2007).

A report, published in 1991, described the discovery of a novel 133.6 kDa protein, by *B. thuringiensis* subsp. *aizawai*. This protein is highly toxic to the lepidopterans *Heliothis virescens* and *Ostrinia nubilalis* and moderately toxic to *Spodoptera exigua* (Chambers *et al.*, 1991; Gaspers *et al.*, 2011), but not toxic to coleopteran species (Oppert, Ellis, and Babcock, 2010). The protein is heat labile, rapidly digested by pepsin, and not glycosylated (Ladics, Bardina, Cressman, Mattsson, and Sampson, 2006). The protein sequence placed it within the Cry1 group of Bt proteins, but it possessed, at best, only 72% sequence similarity to proteins with the existing subgroups: Cry1A, Cry1B, Cry1C, Cry1D, and Cry1E (Chambers *et al.*, 1991; Crickmore *et al.*, 2012). In addition, the N-terminal region of the new Cry1 protein, which typically encodes the insecticidal component of other Cry1 group proteins, was at most 52% similar to the N-terminal sequences of other Cry1 proteins. It was determined that the protein was not a member of any of the existing subgroups, and a new subgroup, Cry1F, was designated for this protein. To date, 11 proteins have been designated as belonging to the Cry1F subgroup (Crickmore *et al.*, 2012).

Mechanism of Cry1F Insecticidal Activity

Like many other Cry proteins, the mechanism of activity for Cry1F begins with an enzymatic cleavage of the 130 kDa protoxin to release a 65 kDa core toxin (Gao *et al.*, 2006). In this process, a short peptide is cleaved from the N-terminal end and almost half of the peptide from the C-terminal end is removed. These termini are believed to be involved in the formation of crystalline inclusion bodies (Gao *et al.*, 2006) The remaining protein possesses two strongly hydrophobic regions thought to be involved in midgut membrane binding (Chambers *et al.*, 1991; Jurat-Fuentes and Adang, 2001). Once bound, toxin molecules form oligomers, creating pores in the membrane and causing osmotic destabilization and cell death (Jurat-Fuentes and Adang, 2001).

Considerable attention has been devoted to understanding the nature of membrane binding among the Cry1 group of proteins[5] to determine whether multiple toxins share the same binding site—a factor that could affect the development of cross-resistance to Bt proteins in an insect population. For example Cry1F and Cry1A both bind to the same midgut receptor in *Plutella xylostella* (Ballester, Granero, Tabashnik, Malvar, and Ferré, 1999), possibly contributing to observed cross-resistance to the two toxins (González-Cabrera, Herrero, and Ferré, 2001). Similarly, a shared binding site for Cry1A and Cry1F exists in *Heliothis virescens* (Blanco *et al.*, 2008; Jurat-Fuentes and Adang, 2001), in *Trichoplusia ni* (Iracheta, Pereyra-Alférez, Galán-Wong, and Ferré, 2000), and in *Spodoptera frugiperda* (Sena, Hernández-Rodríguez, and Ferré, 2009). However, in some species Cry1F does not interact with the known receptor for Cry1Ac in *Heliothis virescens* (Jurat-Fuentes and Adang, 2001, 2006), and cross-resistance between Cry1F and Cry1A proteins is low in some insect species (Pereira, Lang, Storer, and Siegfried, 2008; Storer *et al.*, 2010). It appears that the presence and nature of shared binding sites for Cry1 proteins differs among insect species (Blanco *et al.*, 2010;

3 Also called thurigiensin (Liu *et al*., 2010; OECD, 2007).

4 The insecticidal activity of Bt bacteria preparations is due to a combination of multiple toxins, as well as qualities of the bacterial spores, which can have an impact on selectivity and host range (Schnepf *et al*., 2005; Tabashnik, 1992). Therefore, the activity spectrum of sprays made from Bt bacterial cultures may differ from the activity spectrum of individual Bt proteins produced by a GE plant (OECD, 2007).

5 Due to greater differences in protein sequence, there is a reduced likelihood of shared binding sites between Cry1F and Cry proteins in other groups (Gouffon, Van Vliet, Van Rie, Jansens, and Jurat-Fuentes, 2011; comparing the binding of Cry1F and Cry2Ae).

Ferré and Rie, 2002; Pereira, Siqueira, Zhuang, Storer, and Siegfried, 2010; Tabashnik *et al.*, 2003).

Further research into the basis for Bt toxin susceptibility indicates that Cry1F binding to the midgut cell membranes may be necessary but not sufficient for an insect to be susceptible to Cry1F (Ballester *et al.*, 1999; Coates *et al.*, 2011). Alternative resistance mechanisms could involve the protoxin activation process, degradation of the active toxin, or some undetermined mechanism (Jurat-Fuentes and Adang, 2006; Eliseu J G Pereira *et al.*, 2010).

Numerous studies have helped determine the activity spectrum of Cry1F. It appears to be toxic to lepidopteran species but not to coleopterans (Balog, Szenasi, Szekeres, and Palinkas, 2011; Oppert et al., 2010). Susceptible species include tobacco budworm (*Heliothis virescens*), beet armyworm (*Spodoptera exigua*), soybean looper (*Pseudoplusia includens*), cotton bollworm (*Helicoverpa zea*), fall armyworm (*Spodoptera frugiperda*), lesser cornstalk borer (*Elasmopalpus lignosellus*), wax moth (*Galleria mellonella*), and European corn borer (*Ostrinia nubilalis*) (Adamczyk and Gore, 2004; Adamczyk *et al.*, 2008; Ballester *et al.*, 1999; Blanco *et al.*, 2008, 2010; Buntin, 2008; Chambers *et al.*, 1991; Hanley, Huang, and Pett, 2003; Iracheta *et al.*, 2000; Siebert, Babcock, *et al.*, 2008; Tindall, Siebert, Leonard, All, and Haile, 2009; USEPA, 2001).

Modifications to the Genes Encoding Cry1F in GE Cotton and Maize

Cotton: The *cry1F* sequence used to produce cotton event DAS-24236-5 was derived from Cry1F from *B. thuringiensis* subsp. *aizawai* strain PS811. The nucleotides encoding the C-terminal portion of Cry1F, normally removed during protoxin activation, were replaced with nucleotides 1811-1917 from *cry1C* and nucleotides 1918-3447 from *cry1Ab*. The amino acids encoded by these nucleotides are removed during protoxin activation. A synthetic version of this sequence was prepared using codons optimal for protein expression in plants (Murray, Lotzer, and Eberle, 1989). These codon changes did not alter the final amino acid sequence of the active Cry1F protein.[6]

Maize: The *cry1F* sequence used to produce maize events DAS-01507-1 and DAS-06275-8 was derived from *cry1F* from *B. thuringiensis* subsp. *aizawai* strain PS811. A synthetic version of this sequence was prepared by deleting the codons encoding the C-terminal 569 amino acids, which would normally be cleaved during protoxin activation. In addition, the sequence employed codons optimal for protein expression in maize plants (Murray *et al.*, 1989). These codon changes did not alter the final amino acid sequence of the active Cry1F protein. A leucine residue was added at position 604 of the protein, creating an *XhoI* restriction site to facilitate gene cloning.[7]

Descriptions of the genetic elements used in the production of Cry1F cotton and maize events are provided in Table 2.

Table 2. Genetic elements used in the production of GE insect-resistant cotton and maize varieties (USDA, 2000, 2003, 2004a)

Genetic Element	DAS-24236-5 Cotton	DAS-01507-1 Maize	DAS-06275-8 Maize
Promoter	Mannopine synthase promoter from *Agrobacterium tumefaciens* strain LBA 4404 pTi15955, including copies of the octopine synthase enhancer from pTiAch5	Ubiquitin promoter (plus intron and 5' untranslated sequence) from *Zea mays*	Ubiquitin promoter (plus intron and 5' untranslated sequence) from *Zea mays*
Gene	Synthetic, plant-optimized, full length version of Cry1F from *B.t.* subsp. *aizawai*. Nucleotides 1-1810 of the coding sequence encode the toxic portion of Cry1Fa2. Nucleotides 1811- 1917 encode a portion of the Cry1C protoxin. Nucleotides 1918-3447 encode a portion of the Cry1Ab protoxin.	Plant-optimized version of truncated Cry1F from *B.t.* subsp. *aizawai*	Maize plant-optimized version of truncated Cry1F from *B.t.* subsp. *aizawai*
Terminator	Terminator from *Agrobacterium tumefaciens* strain LBA 4404 pTi15955	Terminator from *Agrobacterium tumefaciens* strain LBA 4404 pTi15955	Terminator sequence from *Solanum tuberosum* proteinase inhibitor II

Expression of Cry1F in GE Insect-Resistant Cotton and Maize

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 where the transgene 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 under which the plant was growing (Siebert et al., 2009).

6 The DNA sequence used in the original transformation process, which resulted in the isolation of event DAS-24236-5, also contained the *pat* gene, which confers tolerance to glufosinate-ammonium herbicides. For a full discussion of the environmental safety of the PAT protein, please see "A Review of the Environmental Safety of the PAT Protein" (CERA, 2011).

7 The DNA sequence used in the original transformation process, which resulted in the isolation of event DAS-01507-1, also contained the *pat* gene, which confers tolerance to glufosinate-ammonium herbicides. The DNA sequence used in the original transformation process, which resulted in the isolation of event DAS-06275-8, also contained the *bar* gene, which confers tolerance to glufosinate-ammonium herbicides. For a full discussion of the environmental safety of the PAT and BAR proteins, please see "A Review of the Environmental Safety of the PAT Protein" (CERA, 2011).

Data from enzyme-linked immunosorbent assays (ELISA), showing levels of Cry1F protein expression in GE cotton and maize events have been made available in publicly accessible regulatory submissions and decision documents associated with regulatory authorization processes. Samples were collected from several tissue types, and at multiple growth stages, from plants grown in several different locations to produce data representative of the typical range of protein expression. Tables 3 and 4 present the highest reported values of Cry1F expression in GE cotton and maize plants, respectively. Protein expression data may be used to estimate the potential exposure of various organisms in the environment to Cry1F when cotton and maize plants producing Cry1F are cultivated. Currently available protein expression data for Cry1F by cotton event DAS-24236-5 and by maize events DAS-01507-1 and DAS-06275-8 used alone and when stacked with other GE events are presented in Annex I.

Table 3. Highest reported protein concentrations of Cry1F in various plant tissues from GE cotton event DAS-24236-5 (USDA, 2003).

Tissue	Cry1F ng/mg tissue dry weight[1]
Young leaf (3-6 weeks)	16.8
Terminal leaf	20.7
Flower	12.3
Square	9.4
Boll (early)	9.2
Whole plant (seedling)	23.3
Whole plant (pollination)	38.4
Whole plant (defoliation)	37.6
Root (seedling)	2.3
Root (pollination)	0.62
Root (defoliation)	1.7
Pollen	1.1
Nectar	Not Detected
Seed	8.2

1 Results based on fresh tissue weight for pollen, nectar, and seed.

Table 4. Highest reported protein concentrations of Cry1F in various plant tissues from GE maize events DAS-06275-8 and DAS-01507-1 (USDA, 2004a).

Tissue	Growth Stage [1]	Line 6275 ng/mg dry tissue weight	Line 1507 ng/mg dry tissue weight
Leaf	V9	23.8	24
Whole plant	V9	7.87	6.8
Whole plant	R1	9.57	4.7
Pollen	R1	4.6	27.2
Stalk	R1	16.4	10.3
Forage	R4	7.77	3.2
Whole plant	Senescence	3.07	2.4

1 V9 – Collar of 9th leaf is visible.
R1 – Silks emerged, tassel shedding pollen
R4 – Soft dough, most kernels pasty with semi-solids
Maturity – Kernel moisture 25-35%

NON-TARGET ORGANISM TESTING AND IMPACTS OF EXPOSURE TO THE Cry1F PROTEIN

The Cry1F toxin has insecticidal properties against certain lepidopteran insect species when expressed in cotton and maize plants. The toxin targets lepidopteran insect pests, thereby reducing feeding damage (Adamczyk and Gore, 2004; Adamczyk *et al.*, 2008; Buntin, 2008; CFIA, 2002, 2005, 2006; EFSA, 2009a, 2009b; FSANZ, 2003, 2004; JBCH, 2004, 2006a, 2006b, 2006c, 2006d, 2006e, 2009, 2010; PDOA, 2006; Siebert, Babcock, *et al.*, 2008; Siebert, Nolting, *et al.*, 2008; USDA, 2000, 2001, 2003, 2004a, 2004b, 2004c; USEPA, 2001, 2005). Organisms in the environment that are not pests of maize but are directly or indirectly exposed to Cry1F are called non-target organisms (NTOs).

Assessments of impacts to NTOs include the review of data submitted to regulators by the product developer to demonstrate that NTOs exposed to the Cry1F, either directly or indirectly, are not harmed. The NTO risk assessment typically begins with a determination of the organisms that are likely to be directly or indirectly exposed to Cry1F. Particular consideration is often given to NTOs having beneficial environmental functions, such as pollinators or the natural enemies of agricultural pests. Regulatory authorities may give special attention to NTOs that have been designated as threatened or endangered species or species of recognized cultural value. These species, or valid surrogates for these species, are then tested to determine if exposure to Cry1F could cause significant adverse impacts.

Assessments of the potential impacts to NTOs, and the regulatory decisions informed by the assessments, have been grounded in the well-documented and long history of evaluation of chemical insecticidal formulations including microbial formulations of *B. thuringiensis* (Carstens *et al.*, 2012; Romeis *et al.*, 2008, 2013; Sanvido *et al.*, 2012; USEPA, 2007). The "tiered" approach for assessing the impacts

of chemical pesticides on NTOs has been used effectively for many years, and tiered testing has also been determined by scientists and regulators to be appropriate for the assessment of potential impacts of GE crops on NTOs (Duan, Lundgren, Naranjo, and Marvier, 2010; Dutton, Romeis, and Bigler, 2003; EFSA, 2006a; Garcia-Alonso e*t al.*, 2006; Raybould, 2006; Romeis *et al.*, 2008, 2013; USEPA, 2007, 2011). Early tier studies generally involve the exposure of NTOs or surrogate species to high concentrations of the pesticide, under controlled laboratory conditions. These studies identify those species that are significantly affected by the pesticide. Such effects, when found, may require further analysis at a higher tier level. Early tier tests also identify NTOs that are unaffected by the pesticidal protein and for which higher tier testing is therefore unnecessary. Higher level tier testing may also be appropriate when the results of early tier tests are inconclusive. Testing at higher tiers typically involves increasing levels of complexity and increasingly realistic assay conditions (EFSA, 2006a; Garcia-Alonso *et al.*, 2006; Romeis *et al.*, 2008; USEPA, 2007, 2011).

The potential for harm to NTOs from exposure to Cry1F has been considered in risk assessments conducted by several regulatory authorities (CFIA, 2002, 2005, 2006; EFSA, 2009a, 2009b; FSANZ, 2003, 2004; JBCH, 2006c, 2006d, 2006e, 2009, 2010, 2004, 2006a, 2006b; PDOA, 2006; USDA, 2000, 2001, 2003, 2004a, 2004b, 2004c; USEPA, 2001, 2005). Data collected from laboratory and field trials of GE cotton and maize producing Cry1F and submitted to regulators have established that the Cry1F protein is active specifically against the subset of lepidopteran pests which feed on the aboveground parts of cotton and maize plants and are harmless to vertebrate species and other NTOs (CFIA, 2002, 2005, 2006; EFSA, 2009a, 2009b; FSANZ, 2003, 2004; JBCH, 2006c, 2006d, 2006e, 2009, 2010, 2004, 2006a, 2006b; PDOA, 2006; USDA, 2000, 2001, 2003, 2004a, 2004b, 2004c; USEPA, 2001, 2005).

Routes of Environmental Exposure

Direct exposure occurs when NTOs feed on living crop tissues expressing Cry1F or on crop residues, either above or below ground. Indirect exposure results from the predation by one organism on another organism that has had direct exposure to Cry1F (J.-C. Tian *et al.*, 2012). In addition to direct consumption of parts of the GE cotton or maize plant, regulatory authorities may consider other routes of potential indirect exposure to the Cry1F toxin: exposure to the toxin in pollen, exposure to toxin deposited in the soil by decomposing plant material, and exposure to predator species consuming herbivores that have been feeding on the GE maize plants (CFIA, 2002, 2005, 2006; EFSA, 2009a, 2009b; FSANZ, 2003, 2004; JBCH, 2006c, 2006d, 2006e, 2009, 2010, 2004, 2006a, 2006b; PDOA, 2006; USDA, 2000, 2001, 2003, 2004a, 2004b, 2004c; USEPA, 2001, 2005). Regulators may consider protein expression data to determine potential routes and levels of exposure. For example, plant tissues producing little or no Cry1F are unlikely to pose a hazard to NTOs. (See Tables 3 and 4 and Annex I for Cry1F expression level data in the tissues of approved cotton and maize varieties.) Data submitted to regulatory authorities indicate that Cry1F is quickly degraded once released from decomposing plant tissue and is not likely to persist or accumulate in the soil environment (Herman, Wolt, and Halliday, 2002; Shan, Embrey, Herman, and McCormick, 2008; USDA, 2003).

Ecotoxicological Testing of Cry1F on Non-Target Organisms

Ecotoxicological testing of Cry1F on NTOs has been conducted on a variety of well-characterized test organisms that are typically used for ecotoxicological testing of chemical pesticides, and the data from these tests have been evaluated by regulatory authorities in the course of performing risk assessments for the environmental release of GE cotton and maize varieties (Dutton *et al.*, 2003; Raybould, 2007; Romeis *et al.*, 2008; USEPA, 2007; Wolt *et al.*, 2010). Because Cry1F is toxic to several lepidopteran species, regulatory authorities have generally requested data for impacts of Cry1F on non-target lepidopterans, such as the monarch butterfly or other lepidopteran species of local importance (Wolt, Conlan, and Majima, 2005). Regulators may also request impact data on representative pollinator species, *i.e.*, honeybees; representative soil dwelling arthropod species; and non-arthropod, soil-dwelling species, such as earthworms, to demonstrate that there are no significant impacts to these species from exposure to Cry1F. Test organisms have included *Apis mellifera* (honeybee); *Hippodamia convergens* (ladybird beetle) *Chrysoperla carnea* (green lacewing); *Danaus plexippus* (monarch butterfly); *Nasonia vitripennis* (parasitic wasp); *Folsomia candida* (springtail); *Daphnia magna* (crustacean); and *Eisenia foetida* (earthworm). Test organisms were exposed to levels of Cry1F many times higher than the highest exposure levels predicted from the observed tissue concentrations of Cry1F in GE cotton and maize plants (See Tables 3 and 4). None of the test organisms showed a significant response to Cry1F (Hanley e*t al.*, 2003; Hellmich *et al.*, 2001; J.-C. Tian *et al.*, 2012, 2013; X.C. Tian *et al.*, 2005; USDA, 2000, 2003; USEPA, 2001; Wolt *et al.*, 2005) (See Annex II). Additionally, vertebrate toxicological testing and nutritional equivalence testing has been conducted on *Mus musculus* (mouse); *Oncorhynchus mykiss* (rainbow trout); *Gallus domesticus* (chicken); *Rattus norvegicus* (rat); *Sus domestica* (pig); and *Colinus virginianus* (northern bobwhite quail) (Appenzeller, Malley, Mackenzie, Hoban, and Delaney, 2009; Dryzga, Yano, Andrus, and Mattsson, 2007; FSANZ, 2004; MacKenzie *et al.*, 2007; Scheideler, Rice, Smith, Dana, end Sauber, 2008; Stein *et al.*, 2009; USDA, 2000, 2003; USEPA, 2001) (See Annex II).

The results from Tier 1 tests discussed above indicate that no higher tier testing should be necessary from a regulatory standpoint, because no adverse effects were noted;[8] however, studies of the effects of Cry1F on natural populations of NTOs have been performed (Balog *et al.*, 2011; Higgins *et al.*, 2009; USDA, 2004c; USEPA, 2001). These field studies found no significant differences between NTO

8 Conducting field studies is considered case-by-case, based on the level of potential hazard and exposure, and goals may be adjusted as information and experience accumulate (USEPA, 2007).

arthropods in fields where Cry1F maize was grown and fields where a non-GE maize variety was grown.

Regulatory authorities have considered the potential impact of Cry1F on natural populations of NTOs and determined that adverse effects on NTOs are unlikely for several reasons. First, Cry1F has a narrow spectrum of pesticidal activity. Second, Tier I laboratory assays, employing a range of invertebrate species present in cotton and maize agricultural ecosystems, or surrogates for those species, have shown that Cry1F causes no significant observable effects in these species. Third, Tier I studies have demonstrated that Cry1F has no observable effect on representative vertebrate and aquatic species. Fourth, the levels of Cry1F used in these Tier I assays were much higher than those measured in GE cotton and maize tissues growing in the field. Fifth, field studies of maize varieties producing Cry1F show no significant adverse effects on rove beetles, a beneficial, non-target arthropod (Balog *et al.*, 2011). Sixth, when compared to insect control via Cry1F, traditional insect control using chemical pesticides significantly alters species diversity and harms non-target species (Higgins *et al.*, 2009; USDA, 2003).[9] Together, these findings indicate that Cry1F is unlikely to have adverse effects on natural populations of organisms, except for the target lepidopteran crop pests it is meant to control (Balog *et al.*, 2011; CFIA, 2005, 2006; CTNBio, 2008, 2009a; EFSA, 2009a; Higgins *et al.*, 2009; JBCH, 2006e, 2006c, 2006d; PDOA, 2006; J.-C. Tian *et al.*, 2012; USDA, 2001, 2004b, 2004c; USEPA, 2005; Wolt *et al.*, 2005).

ESTABLISHMENT AND PERSISTENCE IN THE ENVIRONMENT OF COTTON AND MAIZE PLANTS EXPRESSING Cry1F

Biology of the Plant Species

The biology of the non-GE plant species in the receiving environment is typically the starting point for environmental risk assessments of GE plants (OECD, 2003, 2007, 2008). Information about the biology of the non-GE plant can be used to assess whether a GE variety of the plant may become weedy, invasive, or otherwise harmful to the environment. It can also provide details on significant interactions between the plant and other organisms that may be important when considering potential harms. By considering the biology of the host plant, a risk assessor can identify potential hazards that may be associated with the expression of the novel protein (*e.g.*, Cry1F) and then be able to assess the likelihood of these hazards. For example, whether the plant is an annual or perennial species or whether the plant is self pollinated or wind pollinated can bear on the assessment of the likelihood of the GE plant establishing and persisting outside of cultivation (EFSA, 2006a; OECD, 1992, 2003, 2007, 2008).

Phenotypic Data

Information about the phenotype of GE plants expressing Cry1F is collected from laboratory, greenhouse, and field trial studies and is presented in regulatory submissions to (1) identify any intentional changes to the phenotype that might impact the environmental safety of the plant and (2) to identify any unintended changes to the biology of the plant that might impact environmental safety. Phenotypic data in regulatory submissions and peer reviewed publications have focused on characteristics of the plant that might contribute to its survival or persistence (*i.e.*, potential weediness), or those that may negatively affect agricultural performance (*e.g.*, disease susceptibility and yield data). The phenotypic observations take into account the desired phenotype resulting from the transgenic trait, in this case insect predation resistance mediated by Cry1F. Some of the collected data are quantitative (*e.g.*, plant height or percent seed germination) while other data are qualitative and observational (*e.g.*, symptoms of disease susceptibility). Statistically significant differences between GE cotton (CTNBio, 2009a; JBCH, 2006b, 2006d; USDA, 2004b) or maize plants (EFSA, 2009a; JBCH, 2004, 2006e; USDA, 2000, 2001, 2004a, 2004c) expressing Cry1F and controls were observed, but these differences were not consistent among the field trial locations and fell within the reported range for non-GE cotton and maize varieties. Collectively, regulators have determined that the phenotypic data do not support the hypothesis that the expression of Cry1F had any unintended impact on the gross morphology or phenotypic characteristics of cotton or maize plants, besides conferring resistance to lepidopteran insect pests.

Weediness in Agricultural Environments

Cotton: Cultivated cotton lacks weedy or aggressive characteristics, and it is not generally considered to be an economically important agricultural weed, although it can grow as a perennial in areas lacking a cold season. Researchers and regulators have evaluated the potential for insect-resistant GE cotton varieties to become weeds, including cotton producing the Cry1F protein, and they have found that there are no characteristics of insect-resistant cotton that would increase its potential to become an agricultural weed, because any volunteer cotton plants would be readily controlled using conventional weed management techniques (Eastick and Hearnden, 2006; Eastick, 2002; USDA, 2003, 2004b; USEPA, 2005).

Maize: Maize is not generally regarded as a weed, possessing few of the characteristics that increase the likelihood of a plant to become a weed, such as seed dormancy, shattering, and competitiveness (Baker, 1965, 1974). There are no data indicating that expression of Cry1F results in altered seed dormancy, over-wintering capacity, or other characteristics that would alter the prevalence of volunteer maize in subsequent growing seasons. Following-season maize volunteers producing Cry1F would not be expected to present any unusual weed management challenges and can be dealt with in the same manner as conventional volunteers of maize (Carpenter *et al.*, 2002; Raybould *et al.*, 2011; USDA, 2000, 2001, 2004a, 2004c; USEPA, 2001, 2005).

9 This study also found similar dynamics for nontarget arthropods in fields of Cry1F maize and non-Bt maize, at both community and individual taxa levels (Higgins *et al.*, 2009).

Weediness in Non-Agricultural Environments

The primary mechanisms by which Cry1F may be introduced into a non-agricultural environment is through the movement of propagules outside of cultivated areas (Lee and Natesan, 2006), and regulators evaluate how such introductions may result in a GE plant becoming weedy or invasive.

Cotton: While all plants may exhibit weedy characteristics under certain conditions, commercial varieties of cotton are not considered to pose a significant weed risk in non-agricultural environments. Selective breeding has resulted in modern cotton varieties' dependence on human intervention, and factors such as water stress and cold severely limit the ability of commercial varieties to survive in non-agricultural environments. Although insect resistance mediated through the Cry1F protein may provide some fitness advantage to an escaped GE cotton plant, researchers and regulators have determined that such an advantage would be insufficient to allow GE cotton expressing Cry1F to persist in a non-agricultural environment (Carpenter *et al.*, 2002; Eastick & Hearnden, 2006; Eastick, 2002; JBCH, 2006b, 2006c, 2006d; USDA, 2003, 2004b; USEPA, 2005).

Maize: As a result of extensive selective breeding, commercial maize varieties are severely restricted in their ability to persist in non-agricultural environments without human intervention, and maize is not considered to be an invasive or aggressive weed outside of agricultural systems (Carpenter *et al.*, 2002). Agronomic data show that Cry1F does not have a significant impact on traits associated with weediness. Although release from natural control factors (including insect herbivores) has been offered as a partial explanation for the success of invasive species (Blumenthal, 2005; Keane and Crawley, 2002; Mack, 1996; Mason, Braun, Warwick, Zhu, and Stewart, 2004), regulatory decisions have determined that it is unlikely that resistance to lepidopteran pests would allow maize producing Cry1F to become invasive in non-agricultural environments (Carpenter *et al.*, 2002; USDA, 2000, 2001, 2004a, 2004c; USEPA, 2001).

Movement of the Transgene to Sexually Compatible Relatives

The movement of transgenes from a GE plant to its wild relatives is pollen mediated, and the production of reproductively viable hybrids depends on several factors: whether the pollen donor is self-pollinated, the physical and temporal proximity of the GE plants to sexually compatible species, pollen mobility and viability, and the presence of appropriate pollinators (Chandler and Dunwell, 2008).

Cotton: The *Gossypium* genome is very complex and is organized into eight diploid species groups and one tetraploid species group, which includes *G. hirsutum.* Crosses within groups can occur, but crosses between groups are rare, and offspring display meiotic abnormalities and infertility, including crosses between *G. hirsutum* and members of the diploid species. Hybridization between *G. hirsutum* and the three wild tetraploid species (*G. mustelinum*, *G. darwinii,* and *G. tomentosum*) as well as crosses with feral populations of *G. barbadense* and *G. hirsutum* can be readily made experimentally and result in fertile offspring. Under the favorable conditions discussed above, spontaneous hybridizations can occur when commercial varieties of *G. hirsutum* are grown near natural populations of tetraploid species (OECD, 2008). However, the frequency of such crosses between transgenic *G. hirsutum* and sexually compatible wild relatives is considered to be no greater than crosses between traditionally bred varieties of *G. hirsutum* and wild species (Carpenter *et al.*, 2002; OECD, 2008; USDA, 2003, 2004b; USEPA, 2005).

Maize: Maize is predominantly wind pollinated and does not have sexually compatible relatives that are considered invasive (Carpenter *et al.*, 2002; OECD, 2003). Maize freely hybridizes with wild teosintes, but gene introgression is thought to be limited (Baltazar, De Jesús Sánchez-Gonzalez, De la Cruz-Larios, and Schoper, 2005; Castillo-Gonzalez and Goodman, 1997; OECD, 2003). Wild teosinte populations are limited to Mexico, Guatemala, and a single population in Nicaragua, and while teosinte is considered a serious weed by some farmers in Mexico, it is used as a forage plant by other farmers, and it is also considered a culturally significant species (González and Corral, 1997; Mondragon-Pichardo and Vibrans, 2005). Crosses between teosinte and GE maize expressing Cry1F are not expected to occur more frequently than those between teosinte and traditionally bred maize varieties (Carpenter *et al.*, 2002; USDA, 2000, 2001, 2003, 2004a; USEPA, 2001).

COMPOSITIONAL ANALYSIS OF COTTON AND MAIZE PLANTS EXPRESSING Cry1F

A compositional analysis is required in many regulatory approval processes for GE plants intended to be used in food or feed. Compositional data can be used to identify unintended changes in the crop due to the presence of the transgene. The analysis typically compares the GE plant to the untransformed parent line or a closely related isoline, and the analytes measured depend on the crop and its intended uses. The analysis may use plants grown in a variety of locations and may include data from multiple growing seasons, because local environmental conditions may impact nutritional composition even in conventionally bred varieties.[10] The goal of the analysis is to verify that the values obtained for the GE plant are within the range observed in traditional varieties grown under comparable conditions.

Seed and forage from Cry1F maize and seed from Cry1F cotton has undergone 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. Some crop plants produce toxins or anti-nutritive compounds, and levels of these compounds are also measured to determine whether

10 In some cases when the GE maize plants contained *cry1F* as well as a gene for herbicide tolerance (*pat*), composition data were collected from plants that had been treated with glufosinate-ammonium, as well as from plants grown in the same location but not sprayed, to determine whether the herbicide had any effect.

the presence of the transgenes has inadvertently resulted in elevated production of these substances. Maize is known to produce the anti-nutritive compounds phytic acid, raffinose, and trypsin inhibitor (OECD, 2003), and cotton produces the toxins gossypol and cyclopropenoid fatty acids (OECD, 2008). Levels of these substances produced by cotton and maize varieties expressing Cry1F were measured and compared with levels in conventional cotton and maize varieties. The data from publicly available sources are summarized in Annex III. All differences noted between the GE cotton event DAS-24236-5 and the comparator varieties were either within the normal range of variation for cotton, or the differences were deemed irrelevant to environmental safety (CFIA, 2005; CTNBio, 2009a; FSANZ, 2004; Health Canada, 2006a; JBCH, 2006b, 2006c; UKACRE, 2004; UKDEFRA, 2005; USDA, 2003; USFDA, 2004a). A similar comparison for maize events DAS-01507-1 and DAS-06275-8 and comparator varieties revealed no differences relevant to environmental safety (CFIA, 2006; COGEM, 2005; CTNBio, 2008, 2009b; EC, 2006; EFSA, 2005, 2006b, 2009a; FSANZ, 2003; Health Canada, 2006b, 2002; Herman et al., 2004; MSPS, 2012; PDOA, 2006; SAGPA, 2008; SFOPH/SFOA, 2001; USDA, 2001, 2004a, 2004c; USFDA, 2001, 2004b).

CONCLUSION

The Cry1F protein produced by insect-resistant GE cotton and maize plants is derived from the common soil bacterium *Bacillus thuringiensis* and is specifically toxic to Lepidoptera. Toxicity testing with a range of representative non-target organisms demonstrated that Cry1F produced no observable effects at concentrations significantly higher than the expected environmental concentrations of Cry1F. Field data suggest that cultivation of GE maize plants expressing Cry1F does not affect the abundance of non-target arthropods. Cry1F in plants can be toxic to non-target Lepidoptera, but regulatory risk assessments for approved products have concluded that the risk is low, due to the lack of exposure to the toxin in the environment, especially when compared to other insect-control practices. The weight of evidence from analyses of phenotypic and compositional data demonstrates that Cry1F expression in approved cotton and maize varieties do not alter the gross physiology of the crop plants and indicates that these plants are not more likely to become weedy or invasive than conventional cotton and maize varieties.

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ANNEX I:

The tables that follow present summary data from applicant dossiers and regulatory decisions 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.

Summary Data for Cotton

Table I.1. Summary of the expression of the novel protein Cry1F in cotton line MXB-13 (FSANZ, 2004)

Cotton Tissue	Mean Protein Expression (ng/mg dry weight)[1]
Young leaf (3-6 weeks)	6.81
Terminal leaf	8.19
Flowers	5.44
Square	4.88
Boll (early)	3.52
Whole plant (seedling)	14.1
Whole plant (pollination)	25.3
Whole plant (defoliation)	22.0
Root (seedling)	0.88
Root (pollination)	0.54
Root (defoliation)	0.51
Pollen	0.06
Nectar	Not detected
Seed	4.13
Cotton Processed Fraction	
Cottonseed	3.1
Kernel	3.9
Hulls	0.16
Toasted meal	Not detected
Refined oil	Not detected

1 Results are reported in ng protein/mg sample dry weight, with fresh weight used for cottonseed, pollen, nectar, and processed products.
2 Calculated concentration is less than the LOQ of the method.

Table I.2. Summary of the expression of Cry1F protein in cotton Event 281-24-236 as determined by ELISA (USDA, 2003)

Tissue	Cry1F ng/mg tissue dry weight[1]		
	Mean[2]	Standard Deviation	Range
Young leaf (3-6 weeks)	6.48	3.3	1.2–16.8
Terminal leaf	7.67	5.3	1.3–20.7
Flower	5.71	2.1	3.0–12.3
Square	5.04	1.8	2.0–9.4
Boll (early)	4.02	2.0	1.2–9.2
Whole plant (seedling)	11.5	4.3	5.5–23.3
Whole plant (pollination)	22.8	7.2	12.1–38.4
Whole plant (defoliation)	21.1	9.9	8.4–37.6
Root (seedling)	0.72	0.6	0.21–2.3
Root (pollination)	0.36	0.1	0.10–0.62
Root (defoliation)	0.61	0.5	0.12–1.7
Pollen	(0.09)[3]	0.3	ND[4]–1.1
Nectar	ND	Not applicable	ND–ND
Seed	5.13	1.2	3.2–8.2

1 Results based on fresh tissue weight for pollen, nectar, and seed.
2 Means are calculated from samples taken across all six locations.
3 Calculated concentration is less than the LOQ of the method.
4 Absorbance value of sample was less than the lowest standard absorbance.

Table I.3. High End Exposure Estimates[1] (HEEE) for expression of Cry1F protein in 281-24-236 (USDA, 2003)

Tissue	HEEE (ng/mg tissue)
Leaf (terminal)	18.1
Whole plant (defoliation)	40.5
Root (defoliation)	1.6
Pollen	0.7
Nectar	<0.05 ng/µL
Seed	7.5

1 HEEE was calculated as the Mean + 1.96* Standard Deviation of expression values reported in Table I.2.

Table I.4. Summary of the expression of Cry1F in Event 281-24-236 cotton processed fractions (USDA, 2003)

Processed Fraction	ng Cry1F/mg tissue fresh weight
Cottonseed	3.3
Kernel	3.0
Hulls	0.22
Toasted meal	Not detected

Summary Data for Maize

Table I.5. Summary of Cry1F protein levels in tissue collected from maize hybrid line 1507.[1] (USDA, 2000)

Tissue	Mean Cry1F (pg/µg total protein)	Standard Deviation	Range
Leaf	110.9	27.2	56.6–148.9
Pollen	135.5	13.5	113.4–168.2
Silk	50.3	16.5	26.8–79.8
Stalk	550.0	104.0	355.9–737.4
Whole plant	1063.8	361.7	303.2–1572.7
Grain	89.8	23.3	71.2–114.8
Senescent whole plant	714.3	95.5	622.2–845.3

1 Values are means across four sites from mean values calculated from the analysis of five individual samples per site for leaf, pollen, silk, stalk, grain, and one pooled sample per site for both whole plant samples.

Table I.6. Summary of expression levels of Cry1F protein, measured in tissues collected from transgenic maize hybrid line 6275H and control near isogenic hybrid CHPH09B/2MW by ELISA. The plant tissues were obtained from field trials conducted in Chile in 2001-2002 (USDA, 2004a).

Hybrid	Tissue	Growth Stage[1]	Mean[2]	Standard Deviation	Range[3]	Number of Samples[4]
6275H	Leaf	V9	16.7	4.60	0–23.8	30/1
CHPH09B/2MW			0	0	0–0	6/6
6275H	Root	V9	6.14	1.87	0–8.14	18/1
CHPH09B/2MW			0	0	0–0	6/6
6275H	Whole Plant	V9	6.22	1.16	4.98–7.87	6/0
CHPH09B/2MW			0	0	0–0	6/6
6275H	Leaf	R1	28.5	5.38	16.5–36.7	30/0
CHPH09B/2MW			0	0	0–0	6/6
6275H	Root	R1	6.60	1.98	3.14–10.9	30/0
CHPH09B/2MW			0	0	0–0	6/6
6275H	Whole Plant	R1	7.16	1.45	5.32–9.57	6/0
CHPH09B/2MW			0	0	0–0	6/6
6275H	Pollen	R1	3.67	0.34	3.09–4.60	30/0
CHPH09B/2MW			0	0	0–0	6/6
6275H	Stalk	R1	11.0	2.67	6.77–16.4	30/0
CHPH09B/2MW			0	0	0–0	6/6
6275H	Leaf	R4	44.8	16.8	35.8–109.2	18/0
CHPH09B/2MW			0	0	0–0	6/6
6275H	Root	R4	5.99	1.89	2.35–9.26	18/0
CHPH09B/2MW			0	0	0–0	6/6
6275H	Forage	R4	6.26	1.09	5.05–7.77	6/0
CHPH09B/2MW			0	0	0–0	6/6
6275H	Grain	Maturity	1.14	0.27	0.62–1.68	30/0
CHPH09B/2MW			0	0	0–0	6/6
6275H	Leaf	Senescence	0.71	1.14	0–3.09	18/10
CHPH09B/2MW			0	0	0–0	6/6
6275H	Root	Senescence	1.97	2.03	0.29–6.91	18/0
CHPH09B/2MW			0	0	0–0	6/6
6275H	Whole Plant	Senescence	2.47	0.41	1.95–3.07	6/0
CHPH09B/2MW			0	0	0–0	6/6

1 **V9 –** Collar of 9th leaf is visible; **R1 –** Silks emerged, tassel shedding pollen; **R4 –** Soft dough, most kernels pasty with semi-solids; **Maturity –** Kernel moisture 25–35%
2 (ng/mg tissue dry weight).
3 ng/mg tissue dry weight).
4 Number of samples is the number of samples analyzed/number of samples less than the LLOQ. A value of zero was assigned to samples below LLOQ for calculation purposes.

Table I.7. Comparison of Cry1F tissue expression[1] in plant parts of transgenic maize lines 6275 and 1507 (USDA, 2004a).

		Line 6275			Line 1507		
Tissue	**Growth Stage[2]**	**Mean**	**Standard Deviation**	**Range**	**Mean**	**Standard Deviation**	**Range**
Leaf	V9	16.7	4.6	0–23.8	12.1	6.2	0–24
Whole plant	V9	6.22	1.16	4.98–7.87	5.2	1.9	2.6–6.8
Whole plant	R1	7.16	1.45	5.32–9.57	3.6	1.1	2.5–4.7
Pollen	R1	3.67	0.34	3.09–4.6	21.9	2.9	16.4–27.2
Stalk	R1	11	2.67	6.77–16.4	5.8	1.7	3.3–10.3
Forage	R4	6.26	1.09	5.05–7.77	1.7	1.1	0–3.2
Whole plant	Senescence	2.47	0.41	1.95–3.07	1.6	0.6	0.9–2.4

1 Means are expressed as ng/mg dry tissue weight.
2 V9 – Collar of 9th leaf is visible.
R1 – Silks emerged, tassel shedding pollen.
R4 – Soft dough, most kernels pasty with semi-solids.
Maturity – Kernel moisture 25–35%.

Table I.8. Expression levels[1] in grain from transgenic maize lines 1507 and 59122 x 1507 x NK603[2] (EFSA, 2009a).

Maize Line	**Mean**	**Range**
1507	1.9	1.3–2.5
59122 x 1507 x NK603	2.1	1.5–3.1

1 Means are expressed as ng/mg dry tissue weight.
2 Event 59122 expresses the Bt toxins Cry34Ab1 and Cry35Ab1. Event NK603 expresses the PAT protein conferring resistance to the herbicide glufosinate.

ANNEX II: SUMMARY OF CRY1F ECOTOXICITY DATA

Table II.1. Summary of Guideline Hazard Tests for effects of Cry1F protein on beneficial and non-target insects (USDA, 2000; USEPA, 2001).

Guideline	**Study Title**	**Results**
OPPTS[1] 885.4380	Acute Dietary Toxicity LD_{50} and/or NOEC – Honeybees	LD_{50} and/or NOEC ≥ 640 ng Cry1F/larvae
OPPTS 885.4340	Non-target Insect – Green Lacewing	LC_{50} > 480 µg Cry1F/g diet
OPPTS 885.4340	Non-target Insect – Parasitic Hymenoptera (*Nasonia vitripennis*)	LC_{50} > 320 µg Cry1F/g diet
OPPTS 885.4340	Non-target Insect – Ladybird Beetle (*Hippodamia convergens*)	LC_{50} > 480 µg Cry1F/g diet
OECD 202	Acute Dietary Toxicity – Daphnia magna	48-hour EC_{50} > 100 mg Cry1F pollen/L
OECD 207	Acute Toxicity – Earthworm	LC_{50} > 2.5 mg Cry1F/kg dry soil
OPP 71-2 OECD 205	Acute Toxicity – Northern Bobwhite Quail	5-day LC_{50} > 100,000 mg Cry1F maize grain/kg diet
OPPTS 885.4340	Chronic Exposure – *Folsomia candida*	LC_{50} and NOEL > 12.5 mg Cry1F/kg soil
OPPTS 885.4340	Non-target Insect – Monarch Butterfly (*Danaus plexippus*)	LC_{50} > 10,000 ng/mL

1 US EPA Office of Prevention, Pesticides, and Toxic Substances Test Guideline numbers.

Table II.2. Summary of Guideline Hazard Tests for Effect of Cry1F Protein (USDA, 2003).

Guideline	Study Title	Protein Source	Results
OECD 401	Acute Toxicity – Mouse	Microbe-derived Cry1F protein	LD_{50}>600 mg Cry1F/kg
OPP B, 71-2	Acute Dietary Toxicity – Northern Bobwhite Quail	Cotton meal prepared from 3006-210/281-24-236 cottonseed	8-day LC_{50}>100,000 µg meal/kg diet
OECD 207	Acute Toxicity – Earthworm	Microbe-derived Cry1F, alone or in combination with microbe-derived Cry1Ac protein	14-day LC_{50}>247 mg Cry1F/kg soil 762 x EEC in soil
OECD proposed	Chronic Toxicity – Collembola	Microbe-derived Cry1F, alone or in combination with microbe-derived Cry1Ac protein	LC_{50}>702 µg Cry1F/kg 2167 x EEC in soil
OECD 202	Acute Dietary Toxicity – *Daphnia magna*	Combination of microbe-derived Cry1F and Cry1Ac proteins	48-hour EC_{50}>510 µg Cry1F/L 395 x EEC in water
OECD 203	Acute Dietary Toxicity – Rainbow Trout	Cotton meal prepared from 3006-210/281-24-236 cottonseed	8-day LC_{50}>0.209 mg/kg diet 162 x EEC in water
OPPTS 885.4380	Acute Dietary Toxicity LD_{50} – Honeybees	Combination of microbe-derived Cry1F and Cry1Ac proteins	LC_{50}>1.98 µg Cry1F/g diet 2.8 x high-end expression in pollen
OPPTS 885.4340	Non-target Insect – Green Lacewing	Combination of microbe-derived Cry1F and Cry1Ac proteins	LC_{50}≥5.2µg Cry1F/g of diet 7 x high-end expression in pollen 104 x high-end expression in nectar
OPPTS 885.4340	Non-target Insect – Parasitic Hymenoptera	Microbe-derived Cry1F, alone or in combination with microbe-derived Cry1Ac protein	LC_{50}>5.2µg Cry1F/mL 7 x high-end expression in pollen 104 x high-end expression in nectar
OPPTS 885.4340	Non-target Insect – Ladybird Beetle	Microbe-derived Cry1F, alone or in combination with microbe-derived Cry1Ac protein	LC_{50}>300 µg Cry1F/mL 428 x high-end expression in pollen

Table II.3. Potential toxicity of Cry1F (FSANZ, 2004).

Guideline	Study Title	Test Material/Control	Results
OECD 401	Acute Oral Toxicity Limit – Mouse 5 male and five female CD-1 mice 2000 mg/kg body weight (600 mg Cry1F/kg bw) administered by 2 gavage doses, 1 hour apart	*Pseudomonas fluorescens*-derived Cry1F protein/0.5% methylcellulose	LD_{50}>600 mg Cry1F/kg body weight
OECD 401	Acute Oral Toxicity Limit – Mouse 5 male and five female CD-1 mice 5000 mg/kg body weight (375 mg Cry1F/kg bw and 350 mg Cry1Ac/kg bw) administered by 3 gavage doses, 1 hour apart	50:50 mixture of Cry1F (15% pure) and Cry1Ac (14% pure) derived from *Pseudomonas fluorescens*/0.5% methylcellulose	LD_{50}>375 mg Cry1F/kg body weight and LD_{50}>350 mg Cry1Ac/kg bodyweight

ANNEX III: SUMMARY OF COMPOSITIONAL ANALYSES OF GE PLANTS EXPRESSING CRY1F, INCLUDING ANALYSES OF TOXINS AND ANTI-NUTRIENTS

Summary Data for Cotton

Proximates

Table III.1. Summary of expression levels of Cry34Ab1 protein measured in tissues collected from corn hybrid 59122 (event DAS-59122-7) (USDA, 2004).

Component[1]	MXB-13[2]	Control	Paired t-test P Value	Dunnet Adjusted P Value	Literature Range[3]
Ash	**3.9** [3.5–4.1] 0.21	**4.0** [3.7–4.4] 0.28	0.238	0.489	4.1–4.9
Fat	**22.9** [20.9–23.7] 1.02	**22.6** [21.4–24.3] 1.15	0.657	0.941	16.1–26.7
Moisture	**3.5** [2.6–5.6] 1.09	**3.3** [2.5–4.2] 0.65	0.659	0.943	5.4–15.9
Protein	**27.9** [26.4–29.0] 0.95	**27.6** [26.1–29.3] 1.19	0.717	0.966	12–32
Carbohydrates	**45.4** [43.5–47.2] 1.34	**45.8** [42.1–48.1] 2.09	0.691	0.956	42.8–47.8
Calories (Kcalories/100 gm)	**499** [489–505] 5.32	**497** [491–504] 4.93	0.552	0.875	479–508
Crude Fibre	**15.9** [14.7–17.0] 0.79	**17.6** [16.6–18.6] 0.69	0.003	0.009	17.2
Acid Detergent Fibre	**25.2** [23.9–26.4] 0.96	**25.2** [23.1–27.2] 0.96	0.989	1.0	26
Neutral Detergent Fibre	**34.1** [30.7–36.9] 2.35	**35.9** [32.8–38.5] 1.92	0.316	0.613	37

1 All values (mean and range) expressed as % dry weight.
2 Values shown are the mean (bold), the range (in brackets), and the standard deviation.
3 Combined literature range.

Table III.2. Proximate analysis of cottonseed processed fractions (FSANZ, 2004).[1]

Component	Kernels		
	MXB-13	Control	Literature Range
Moisture	6.9	7.6	Not applicable
Component	**Hulls**		
	MXB-13	**Control**	**Literature Range**
Ash	2.8	3.0	2.39–3.97
Fat	2.0	3.0	1.0–3.3
Moisture	10.6	10.3	8.5–12.3
Protein	6.2	7.1	4.0–6.9
Carbohydrates	89.0	86.8	Not applicable
Calories (Kcalories/100 gm)	399	403	Not applicable
Component	**Toasted Meal**		
	MXB-13	**Control**	**Literature Range**
Ash	6.7	6.0	4.6–9.8
Fat	2.0	4.6	0.6–4.7
Moisture	9.2	2.2	9–13.3
Protein	51.3	47.2	43.0–52.4
Carbohydrates	40.0	42.1	Not applicable
Calories (Kcalories/100 gm)	383	399	Not applicable
Crude fibre	9.3	12.4	8.4–15.3
Acid detergent fibre	14.1	18.5	12.2–23.9
Neutral detergent fibre	20.2	24.2	15.8–32.4
Component	**Refined Oil**		
	MXB-13	**Control**	**Literature Range**
Fat	100.1	100.2	Not applicable
Moisture	<0.1	<0.1	Not applicable
Protein	<0.1	<0.1	Not applicable

1 All values are expressed % dry weight except for the refined oil, which is % fresh weight (FSANZ, 2004).

Table III.3. Summary of the mineral analysis of MXB-13 and control cottonseed from 6 sites (FSANZ, 2004).

Component (mg/100 gm dry weight)	MXB-13[1]	Control	Paired t-test P Value	Dunnet Adjusted P Value	Literature Range[2]
Calcium	**160** [140–190] 18.25	**151** [129–185] 20.89	0.076	0.178	108–210
Copper	**0.93** [0.79–1.11] 0.11	**0.91** [0.83–1.03] 0.08	0.829	0.992	0.4–1.19
Iron	**5.59** [4.76–6.67] 0.71	**6.17** [4.95–7.65] 1.00	0.099	0.227	3.79–15.1
Magnesium	**417** [370–450] 35.14	**421** [377–461] 31.68	0.799	0.988	305–460
Manganese	**1.51** [1.35–1.66] 0.14	**1.42** [1.27–1.68] 0.15	0.149	0.328	1.0–2.0
Molybdenum	**<0.2** [<0.2] -	**<0.2** [<0.2] -	-	-	0.1–0.4
Phosphorus	**687** [590–769] 61.39	**699** [579–869] 107.72	0.763	0.980	447–750
Potassium	**1219** [1109–1324] 70.87	**1237** [1065–1371] 102.26	0.406	0.731	990–1280
Sodium	**26.5** [<10–40] 19.16	**15.6** [<10–24] 7.25	-	-	3–38
Zinc	**4.43** [4.09–4.82] 0.31	**4.23** [3.61–5.38] 0.62	0.247	0.502	2.49–4.2
Sulphur	**275** [226–315] 35.26	**276** [248–293] 16.65	0.857	0.996	144–260

1 Values shown are the mean (bold), the range (in brackets), and the standard deviation.
2 Combined literature range.

Table III.4. Mineral analysis of cottonseed processed fractions (FSANZ, 2004).

Component	Hulls		
	MXB-13	Control	Literature Range
Calcium	150	146	100–250
Copper	0.36	0.33	0.3–1.3
Iron	2.14	2.97	1.8–13.1
Magnesium	183	181	120–230
Manganese	1.70	1.49	1.2–2.2
Molybdenum	<0.2	<0.2	0–0.15
Phosphorus	96	113	50–260
Potassium	1208	1215	870–1240
Sodium	12.9	16.1	5–20
Zinc	1.30	1.23	0.6–2.2
Sulphur	59	54	30–100
Component	**Toasted Meal**		
	MXB-13	**Control**	**Literature Range**
Calcium	203	191	160–360
Copper	1.74	1.41	0.7–2.2
Iron	9.98	11.35	7.5–22.8
Magnesium	718	628	440–820
Manganese	2.05	1.89	1.4–2.5
Molybdenum	<0.2	<0.2	0.13–0.51
Phosphorus	1388	1155	860–1540
Potassium	1696	1534	1280–1980
Sodium	<10	15.2	4–330
Zinc	8.07	7.10	4.9–8.3
Sulphur	506	443	280–500

Table III.5. Summary of the fatty acid analysis of MXB-13 and control cottonseed from 6 sites (FSANZ, 2004).

Fatty Acids (% dry weight)	MXB-131	Control	Paired t-test P value	Dunnet Adjusted P value	Literature Range2
8:0 Caprylic	<0.0200	<0.0200			
10:0 Capric	<0.0200	<0.0200			
12:0 Lauric	<0.0200	<0.0200			
14:0 Myristic	**0.198** [0.163–0.224] 0.03	**0.185** [0.165–0.208] 0.02	0.192	0.408	0.22–0.36
14:1 Myristoleic	<0.0200	<0.0200			
15:0 Pentadecanoic	<0.0200	<0.0200			0.11–0.20
15:1 Pentadecenoic	<0.0200	<0.0200			
16:0 Palmitic	**5.11** [4.86–5.38] 0.22	**5.03** [4.59–5.36] 0.31	0.621	0.922	8.31–9.31
16:1 Palmitoleic	**0.117** [0.106–0.125] 0.01	**0.113** [0.098–0.128] 0.01	0.389	0.709	0.16–0.24
17:0 Heptadecanoic	<0.0200	<0.0200			0.04–0.07
17:1 Heptadecenoic	<0.0200	<0.0200			
18:0 Stearic	**0.595** [0.549–0.643] 0.05	**0.563** [0.531–0.58] 0.02	0.036	0.088	0.78–1.09
18:1 Oleic	**3.66** [3.35–3.85] 0.23	**3.51** [3.13–3.89] 0.28	0.227	0.469	4.96–5.36
18:2 Linoleic	**11.6** [9.49–12.8] 1.14	**11.7** [10–12.9] 1.27	0.889	0.998	15.5–16.7
18:3 Gamma Linolenic	<0.0200	<0.0200			
18:3 Linolenic	**0.0900** [0.0813–0.0966 0.01	**0.0888** [0.079–0.101] 0.01	0.742	0.974	0.04–0.10
20:0 Arachidic	**0.0668** [0.0596–0.0724] 0.01	**0.0638** [0.0563–0.0677] 0.01	0.298	0.584	0.09–0.10
20:1 Eicosenoic	<0.0200	<0.0200			
20:2 Eicosadienoic	<0.0200	<0.0200			
20:3 Eicosatrienoic	<0.0200	<0.0200			
20:4 Arachidonic	<0.0200	<0.0200			
22:0 Behenic	**0.0361** [0.0337–0.0398] 0.00	**0.0354** [0.0324–0.0423] 0.00	0.608	0.914	0.04–0.06

1 Values shown are the mean (bold), the range (in brackets), and the standard deviation.
2 Literature ranges from Berberich *et al.,* 1996.

Table III.6. Summary of the amino acid analysis of MXB-13 and control cottonseed from 6 sites (FSANZ, 2004).

Amino Acids (% dry weight)	MXB-13[1]	Control	Paired t-test P value	Dunnet Adjusted P value	Literature Range[2]
Aspartic acid	**2.60** (2.46–2.79) 0.12	**2.51** (2.37–2.69) 0.13	0.399	0.725	2.03–2.62
Threonine	**0.787** (0.743–0.95) 0.08	**0.766** (0.704–0.832) 0.05	0.622	0.924	0.65–0.92
Serine	**1.27** (1.21–1.33) 0.04	**1.22** (1.15–1.29) 0.06	0.300	0.590	0.90–1.25
Glutamic acid	**5.49** (5.36–5.86) 0.19	**5.41** (5.04–5.92) 0.35	0.749	0.977	4.74–5.28
Proline	**1.04** (0.992–1.131) 0.05	**1.03** (0.968–1.142) 0.07	0.829	0.993	0.72–1.14
Glycine	**1.15** (1.09–1.24) 0.05	**1.12** (1.04–1.19) 0.06	0.569	0.889	0.88–1.17
Alanine	**1.08** (1.03–1.18) 0.05	**1.05** (0.98–1.13) 0.06	0.508	0.840	0.83–1.11
Cysteine	**0.423** (0.387–0.457) 0.02	**0.404** (0.360–0.435) 0.03	0.264	0.533	0.43–0.79
Valine	**1.23** (1.14–1.30) 0.07	**1.19** (1.10–1.35) 0.10	0.562	0.885	0.99–1.22
Methionine	**0.391** (0.347–0.434) 0.03	**0.378** (0.331–0.407) 0.03	0.408	0.733	0.30–0.42
Isoleucine	**0.888** (0.827–0.939) 0.04	**0.867** (0.811–0.961) 0.06	0.614	0.919	0.69–0.88
Leucine	**1.60** (1.53–1.73) 0.07	**1.56** (1.46–1.68) 0.08	0.536	0.864	1.27–1.61
Tyrosine	**0.718** (0.665–0.784) 0.04	**0.691** (0.638–0.754) 0.04	0.437	0.769	0.65–0.79
Phenylalanine	**1.44** (1.35–1.53) 0.06	**1.40** (1.30–1.53) 0.08	0.619	0.922	1.16–1.44
Histidine	**0.734** (0.633–0.790) 0.06	**0.684** (0.638–0.728) 0.04	0.189	0.403	0.60–0.73
Lysine	**1.16** (1.07–1.23) 0.07	**1.08** (0.97–1.18) 0.08	0.113	0.258	0.90–1.22
Arginine	**3.08** (2.88–3.4) 0.22	**2.91** (2.73–3.05) 0.13	0.307	0.600	2.52–3.02
Tryptophan	**0.275** (0.247–0.296) 0.02	**0.258** (0.24–0.266) 0.01	0.074	0.174	0.23–0.32

1 Values shown are the mean (bold), the range (in brackets), and the standard deviation.
2 Literature range from Berberich *et al.*, 1996.

Table III.7. Amino acid analysis of cotton seed meal (FSANZ, 2004).

Component (mg/100 g dry weight)	Meal		
	MXB-13	Control	Literature Range[1]
Aspartic acid	4.70	4.15	3.72–4.27
Threonine	1.65	1.32	1.46–1.61
Serine	2.27	1.84	1.91-2.15
Glutamic acid	9.58	8.59	8.40–10.2
Proline	1.91	1.63	1.42–1.69
Glycine	2.15	1.88	1.80–2.12
Alanine	2.04	1.77	1.62–1.86
Cysteine	0.795	0.723	0.64–0.84
Valine	2.28	2.11	1.66–2.10
Methionine	0.760	0.683	0.58–0.79
Isoleucine	1.65	1.50	1.17–1.61
Leucine	3.02	2.65	2.45–2.63
Tyrosine	1.39	1.12	0.94–1.24
Phenylalanine	2.79	2.41	2.19–2.44
Histidine	1.51	1.31	1.21–1.51
Lysine	2.26	2.01	1.56–1.97
Arginine	5.86	5.00	4.35–5.03
Tryptophan	0.548	0.468	0.49–0.60

1 Combined literature range.

Toxins and anti-nutrients

Table III.8. Tocopherol analysis of refined cottonseed oil (FSANZ, 2004).

Component (mg/kg)	MXB-13	Control	Literature Range[1]
Alpha Tocopherol	515	548	136–674
Beta Tocopherol	<60.0	<60.0	Not detected–29
Gamma Tocopherol	372	372	138–746
Delta Tocopherol	<60.0	<60.0	Not detected–75

1 Combined literature range.

Table III.9. Tocopherol analysis of cottonseed oil of Event 281-24-236 and non-transgenic control (USDA, 2003).

Component (mg/kg)	MXB-13	Control	Literature Range[1]
Alpha Tocopherol	501	549	320
Beta Tocopherol	<60.0	<60.0	Not detected
Gamma Tocopherol	374	344	313
Delta Tocopherol	<60.0	<60.0	Not detected

1 Combined literature range.

Table III.10. Summary of Gossypol and Cyclopropenoid fatty acids in cottonseed (FSANZ, 2004).

Component	MXB-13[1]	Control	Paired t-test P value	Dunnet Adjusted P value	Literature Range[2]
Gossypol (% dry weight)	**0.791** [0.623–0.876] 0.09	**0.870** [0.715–1.034] 0.11	0.137	0.304	0.39–1.7
Sterculic acid (% of fatty acids)	**0.292** [0.26–0.325] 0.03	**0.321** [0.252–0.361] 0.04	0.020	0.050	0.48–0.70
Malvalic acid (% of fatty acids)	**0.344** [0.313–0.42] 0.04	**0.397** [0.33–0.463] 0.06	0.022	0.056	0.22–0.45
Dihydrosterculic acid (% of fatty acids)	**0.209** [0.187–0.243] 0.02	**0.220** [0.183–0.259] 0.03	0.167	0.361	0.29–0.50

1 Values shown are the mean (bold), the range (in brackets), and the standard deviation.
2 Cottonseed Oil, 1990.

Table III.11. Summary of anti-nutrient analysis of cottonseed of Event 281-24-236 and non-transgenic control line (USDA, 2003).

Component	MXB-13	Control	Literature Range[2]
Gossypol (% dry weight)	0.793	0.841	0.71–1.24
Sterculic acid (% of fatty acids)	0.303	0.311	0.13–0.66
Malvalic acid (% of fatty acids)	0.384	0.340	0.17–0.61
Dihydrosterculic acid (% of fatty acids)	0.225	0.213	0.11–0.22

1 OECD Draft Consensus Document, 2002.

Table III.12. Anti-nutrient analysis of cottonweed processed products of Event 281-24-236 and non-transgenic control (USDA, 2003).

Component	Sample	MXB-13	Control	Literature Range[1]
Total Gossypol (% dry weight)	Kernel	1.04	0.976	Not available
	Toasted Meal	1.07	0.907	0.93–1.43
	Refined Oil	<0.002	<0.002	0.09
Free Gossypol (% dry weight)	Kernel	0.884	0.839	Not available
	Toasted Meal	0.051	0.044	0.02–0.07
	Refined Oil	<0.002	<0.002	Not detectable
Sterculic acid (% of fatty acids)	Refined Oil	0.228	0.217	0.58
Malvalic acid (% of fatty acids)	Refined Oil	0.262	0.272	0.41
Dihydrosterculic acid (% of fatty acids)	Refined Oil	0.222	0.212	0.22

1 OECD Draft Consensus Document, 2002.

Table III.13. Polyphenol and gossypol analysis of cotton leaves and squares of Event 281-24-236 and non-transgenic control (USDA, 2003).

Genotype	Tissue	Site	Total Polyphenols (%)	Total Gossypol (%)
281-24-236	Terminal Leaf	NC	1.51	0.031
Control	Terminal Leaf	NC	1.53	0.031
281-24-236	Terminal Leaf	TXT1	0.95	0.025
Control	Terminal Leaf	TXT1	0.56	<0.020
281-24-236	Square	NC	0.69	0.069
Control	Square	NC	0.74	0.081
281-24-236	Square	TXT1	0.69	0.095
Control	Square	TXT1	0.68	0.094

Summary Data for Maize

Table III.14. Means and P values (across all sites) for the proximate analysis of grain from corn line 1507 and a control corn hybrid from samples collected in 1998/1999 field trials in Chile (FSANZ, 2003).

Analyte	1507	Control	P Value	Literature Range[1]
Fat	3.83	3.94	0.046	3.1–5.7
Protein	11.20	11.32	0.611	6.0–12
ADF	3.55	3.68	0.250	3.0–4.3
NDF	10.47	10.08	0.315	8.3–11.9
Ash	1.51	1.50	0.335	1.1–3.9
Carbohydrates[2]	83.45	83.23	0.352	63.3–89.7

1 Watson, 1982 and 1987.
2 Carbohydrates are calculated as the percentage of dry weight = 100% - % protein - % ash.

Table III.15. Proximate analysis of grain across all sites (FSANZ, 2003).

Analyte (% dry weight)	1507 Unsprayed Mean±Standard Error	1507 Sprayed[1] Mean±Standard Error	Control Mean±Standard Error	Literature Range[2]
Fat	4.21±0.12	4.41±0.14	4.41±0.12	3.1–5.7
Protein	11.73±0.24	12.04±0.28	10.98±0.24	6.0–12
ADF	2.37±0.17	2.52±0.18	2.29±0.17	3.0–4.3
NDF	10.16±0.30	10.54±0.35	10.13±0.30	8.3–11.9
Carbohydrates[3]	82.46±0.57	81.97±0.25	83.00±0.28	63.3–89.7
Ash	1.60±0.04	1.67±0.05	1.56±0.04	1.1–3.9

1 Plants were sprayed with glufosinate-ammonium herbicide.
2 Watson, 1982 and 1987.
3 Carbohydrates are calculated as the percentage of dry weight = 100% - % protein - % ash.

Table III.16. Summary of proximates analysis in grain for transgenic maize hybrid line 6275H and control near isogenic hybrid CHPH09B/2MW. Samples were obtained from field trials conducted in Chile in 2001-2002. Values are averages across the six locations (USDA, 2004a).

Analyte[1]	Mean[2] for 6275H	Mean[3] for CHPH09B/2MW	Standard Error of the Mean	Literature Range[4]
Fat	4.62	4.80	0.15	1.2–18.8*
Protein	9.88	9.66	0.13	8–14*
Fiber (crude)	2.0	2.2	0.06	2.0–5.5*
ADF	2.7	3.5	0.11	3.0–4.3*
NDF	10.0	10.7	0.45	8.3–11.9
Ash	1.16	1.16	0.04	1.1–3.9
Carbohydrates[5]	84.3	84.4	0.23	78.4–89.8

1 Percent of dry weight.
2 Least square means.
3 Least square means.
4 Watson, 1987.
5 Carbohydrates are calculated as the percentage of dry weight = 100% - % protein - % ash.
* Watson, 1982.

Table III.17. Summary of proximates analysis in forage for transgenic maize hybrid line 6275H and control near isogenic hybrid CHPH09B/2MW. Samples were obtained from field trials conducted in Chile in 2001-2002. Values are averages across the six locations (USDA, 2004a).

Analyte[1]	Mean[2] for 6275H	Mean[3] for CHPH09B/2MW	Standard Error of the Mean	Literature Range[4]
Fat	1.95	2.78	0.08	0.7–6.7
Protein	6.85	6.96	0.13	3.5–15.9
Fiber (crude)	23.9	23.3	0.33	19–42
ADF	30.3	28.7	0.59	30 (mean)
NDF	49.3	49.3	0.54	51 (mean)
Ash	5.00	4.76	0.04	1.3–10.5
Carbohydrates[5]	86.1	85.5	0.20	66.9–94.5

1 Percent of dry weight.
2 Least square means.
3 Least square means.
4 Watson, 1982.
5 Carbohydrates are calculated as the percentage of dry weight = 100% - % protein - % ash.

Nutrients

Table III.18. Amino acid composition of grain from transgenic corn line 1507 and a non-transgenic control (FSANZ, 2003).

Amino Acid[1]	1507	Control	P Value	Literature Range
Glycine	0.39	0.40	0.150	0.26–0.47[2] 0.24–0.41[3]
Threonine	0.40	0.41	0.302	0.29–0.39 0.21–0.37
Valine	0.51	0.52	0.902	0.21–0.52 0.25–0.67
Isoleucine	0.40	0.40	0.952	0.26–0.40 0.19–0.39
Leucine	1.42	1.43	0.880	0.78–1.52 0.43–1.35
Phenyalanine	0.56	0.57	0.479	0.29–0.57 0.04–0.54
Histidine	0.29	0.30	0.822	0.20–0.28 0.21–0.32
Lysine	0.32	0.32	0.522	0.20–0.38 0.19–0.36
Arginine	0.44	0.45	0.672	0.29–0.59 0.28–0.55
Cysteine	0.21	0.23	<0.0001	0.12–0.16 0.13–0.27
Methionine	0.19	0.20	0.020	0.10–0.21 0.12–0.26
Tryptophan	0.08	0.08	0.065	0.05–0.12 0.05–0.10
Serine	0.54	0.55	0.390	0.42–0.55 0.25–0.46
Alanine	0.84	0.85	0.727	0.64–0.99 0.37–0.81
Glutamic Acid	2.14	2.18	0.472	1.24–1.96 0.89–2.02
Proline	1.01	1.03	0.679	0.66–1.03 0.43–1.01
Aspartic Acid	0.77	0.81	0.102	0.58–0.72 0.37–0.80
Tyrosine	0.20	0.20	0.954	0.29–0.47 0.17–0.31

1 Values are means expressed as a percentage on a dry weight basis.
2 Watson, 1982.
3 Data from analyses of 22 commercial hybrids.

Table III.19. Amino acid composition of grain from transgenic corn line 1507 and a non-transgenic control.(FSANZ, 2003).

Amino Acid[1]	1507 Unsprayed Mean±Standard Error	1507 Sprayed[2] Mean±Standard Error	Control Mean±Standard Error	Literature Range
Glycine	0.41±0.0090	0.42±0.0102	0.38±0.0090	0.26–0.47[2] 0.24–0.41[3]
Threonine	0.41±0.0080	0.41±0.0094	0.37±0.0080	0.29–0.39 0.21–0.37
Valine	0.51±0.0106	0.52±0.0125	0.47±0.0106	0.21–0.52 0.25–0.67
Isoleucine	0.41±0.0098	0.41±0.0116	0.36±0.0098	0.26–0.40 0.19–0.39
Leucine	1.38±0.03	1.41±0.04	1.23±0.04	0.78–1.52 0.43–1.35
Phenyalanine	0.55±0.018	0.56±0.014	0.49±0.012	0.29–0.57 0.04–0.54
Histidine	0.31±0.0065	0.32±0.0076	0.29±0.0065	0.20–0.28 0.21–0.32
Lysine	0.32±0.008	0.33±0.009	0.31±0.008	0.20–0.38 0.19–0.36
Arginine	0.47±0.012	0.48±0.014	0.44±0.012	0.29–0.59 0.28–0.55
Cysteine	0.22±0.004	0.23±0.005	0.22±0.004	0.12–0.16 0.13–0.27
Methionine	0.20±0.0034	0.21±0.0041	0.20±0.0035	0.10–0.21 0.12–0.26
Tryptophan	0.10±0.0035	0.10±0.0037	0.09±0.0035	0.05–0.12 0.05–0.10
Serine	0.55±0.012	0.56±0.014	0.50±0.012	0.42–0.55 0.25–0.46
Alanine	0.83±0.018	0.85±0.014	0.74±0.018	0.64–0.99 0.37–0.81
Glutamic Acid	2.12±0.050	2.18±0.060	1.90±0.050	1.24–1.96 0.89–2.02
Proline	1.00±0.0212	1.04±0.0258	0.92±0.0217	0.66–1.03 0.43–1.01
Aspartic Acid	0.79±0.0157	0.81±0.0186	0.71±0.0157	0.58–0.72 0.37–0.80
Tyrosine	0.21±0.0048	0.21±0.0057	0.19±0.0048	0.29–0.47 0.17–0.31

1 Values are means expressed as a percentage on a dry weight basis.
2 Plants were sprayed with glufosinate-ammonium herbicide.
3 Watson, 1982.
4 Data from analyses of 22 commercial hybrids.

Table III.20. Summary analysis of anti-nutrients from transgenic maize hybrid line 6275H and control near isogenic hybrid CHPH09B/2MW. Samples were obtained from field trials conducted in Chile in 2001-2002. Values are means[1] across the six locations (USDA, 2004a).

Analyte[2]	6275H	CHPH09B/2MW	Standard Error	Literature Range
Phytic Acid	0.561	0.536	0.02	0.45–1.0[3]
Trypsin Inhibitor (TI units/g)	1.82	2.07	0.07	Not Reported

1 Least square means.
2 Percent of dry weight.
3 OECD Draft Consensus Document, 2002.