A Review of the Environmental Safety of the Cry3Bb1 Protein

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March 25, 2014

INTRODUCTION

This document provides a comprehensive review of the information and data relevant to the environmental risk assessment of Cry3Bb1, a protein encoded by a gene isolated from *Bacillus thuringiensis* (Bt), and it presents a summary statement about the environmental safety of this protein when produced in genetically engineered (GE) 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 (ERAs) related to the planting of GE crops are conducted on a case-by-case basis and consider both potential hazards and exposure levels. ERAs may consider 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. Assessments typically involve comparisons to an untransformed parental line or a closely related isoline. The goal of these comparisons is the identification of potential risks the GE plant might present beyond those already deemed acceptable when similar, non-GE plants are grown in the environment. The consequences of these risks, if any, are then evaluated (OECD, 2007; Craig *et al.*, 2008).

Several regulatory authorities have performed environmental risk assessments on GE maize varieties producing Cry3Bb1 (CERA, 2014). Table I shows the current status[1] of regulatory approvals for the environmental release of maize varieties containing Cry3Bb1 events MON863 or MON88017. 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; Storer *et al.*, 2012). The table shows the date of the earliest approval given for the event.

Table 1. Regulatory approvals for the environmental release of GE maize varieties containing Cry3Bb1 (CERA, 2014)(as of January 30, 2014)

Country	MON863	MON88017
Argentina		2010
Canada	2003	2006
Japan	2004	2006
United States	2003	2005

ORIGIN AND FUNCTION OF THE Cry3Bb1 PROTEIN

Bacillus thuringiensis and the Cry3Bb1 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 *et al.*, 2008; Martínez and Caballero, 2002; Seifinejad *et al.*, 2008). The species has been studied extensively and used commercially for many years due to its ability to synthesize proteins that possess selective pesticidal properties (Hofte and Whiteley, 1989; Cannon, 1996; Schnepf *et al.*, 1998; OECD, 2007; van Frankenhuyzen, 2009; Sanahuja *et al.*, 2011). Preparations of natural isolates of *B. thuring-*

Key words

Cry3Bb1, insecticidal crystalline proteins, *Bacillus thuringiensis*, insect resistance, genetically engineered, environmental risk assessment

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[1] Some countries' regulations may require periodic renewal of GE crop registrations. For example, the current status of USEPA registrations can be found at http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm.

iensis were first used as a commercial insecticide in France in 1938 (Sanahuja *et al.*, 2011), 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 (Kumar *et al.*, 1996; Schnepf *et al.*, 1998; USEPA, 1998; Baum *et al.*, 1999; Health Canada, 2008; Sanchis and Bourguet, 2008; APVMA, 2013; DGSANCO, 2013).

Several hundred pesticidal substances have been isolated from Bt cultures (Cannon, 1996; Prieto-Samsónov *et al.*, 1997; Crickmore *et al.*, 2012), and these substances display tremendous variety in chemical structure, mode of action, and target specificity (Hofte and Whiteley, 1989; Boucias and Pendland, 1998; Schnepf *et al.*, 1998; OECD, 2007; Pigott and Ellar, 2007; van Frankenhuyzen, 2009; Vachon *et al.*, 2012). Insecticidal preparations derived from cultured cells of Bt bacteria may contain a complex mixture of the pesticidal substances produced by the particular Bt strain used (Tabashnik, 1992; Schnepf *et al.*, 2005; Sanahuja *et al.*, 2011). They include antifungal compounds, ß-exotoxin,[2] vegetative insecticidal proteins (Vip), the Cyt (cytolytic) proteins, and the δ-endotoxins, a group that includes the insecticidal Cry (crystalline) proteins (Hofte and Whiteley, 1989; Schnepf *et al.*, 1998; OECD, 2007; Pardo-López *et al.*, 2013). These substances may interact with each other to influence the toxicity and activity spectrum of individual bacterial preparations (Schnepf e*t al.*, 1998; OECD, 2007). Therefore, the activity spectrum of sprays made from Bt bacterial cultures may be much broader when compared to the activity spectrum of individual Bt proteins produced by a GE plant (OECD, 2007). The Cry proteins have been studied extensively and used widely in agriculture as environmentally safe pesticides that control a broad range of economically significant insect pests (Gill *et al.*, 1992; Cannon, 1996; Prieto-Samsónov *et al.*, 1997; Evans, 2002; Mendelsohn *et al.*, 2003; Gómez *et al.*, 2007; OECD, 2007; Pardo-López *et al.*, 2013).

In 1991 a new strain of *Bacillus thuringiensis* subsp. *kumamotoensis*, designated EG4691, was discovered that produced a crystalline protein related to CryIIIA. This protein had insecticidal activity against two significant agricultural insect pests: the Colorado potato beetle (*Leptinotarsa decemlineata*) and the southern corn rootworm (*Diabrotica undecimpunctata howardi*), both coleopterans. However it was not toxic to *Musca domestica*, a dipteran, nor to the lepidopteran species *Heliothis virescens*, *Plutella xylostella*, or *Trichoplusia ni* (Rupar *et al.*, 1991). The protein was later found to be toxic to larvae of *D. virgifera virgifera*, the western corn rootworm (English *et al.*, 2000; Siegfried *et al.*, 2005). Larval stages of *Diabrotica* sp. are generally more sensitive to the toxin than the adult stages (Al-Deeb and Wilde, 2005; Meissle *et al.*, 2011). This strain of Bt was developed as a microbial pesticide spray and has been sold commercially[3] since 1995 to control Colorado potato beetle and other coleopteran insect pests (USDA, 2001; Vaughn *et al.*, 2005).

Subsequently, the gene that encoded this protein, named *cryIIIB2*, was isolated from a 95 MDa plasmid in strain EG4961 and sequenced. The gene consisted of 652 codons, and it encoded a 74 kDa protein, originally designated CryIIIB2 (Rupar *et al.*, 1991; Donovan *et al.*, 1992; USDA, 2001). After revisions to the nomenclature of Bt proteins, CryIIIB, was renamed Cry3Bb1 and the associated gene *cry3Bb1* (Crickmore *et al.*, 1998). Cry3Bb1 shares approximately 85% sequence homology with Cry3A (Galitsky *et al.*, 2001).

Mechanism of Cry3Bb1 Insecticidal Activity

The mode of action for Cry3Bb1 is similar to that of other Cry toxins: once consumed by the target insect, the toxin dissolves and is activated by midgut proteases (Kaiser-Alexnat, 2009; Kaiser-Alexnat *et al.*, 2009), resulting in a 70 kDa protein that binds to specific membrane receptors in the brush border membrane of susceptible insects (Donovan *et al.*, 1992; Kaiser-Alexnat *et al.*, 2009). After specific interactions with the receptor, which may be modulated by cadherin (Park *et al.*, 2009; Sayed *et al.*, 2010), the toxin is thought to insert itself into the membrane and cause the formation of pores, resulting in ionic disequilibrium and cell lysis (Gill *et al.*, 1992; Prieto-Samsónov *et al.*, 1997; Gómez *et al.*, 2007; Gassmann *et al.*, 2011, 2012; Höss *et al.*, 2013). An α-helical domain in the Cry3Bb1 is thought to be responsible for the formation of the ion channels in the midgut cell membranes (Galitsky *et al.*, 2001). The mutation of specific Cry3Bb1 amino acids adjacent to or within loop regions that separate the helices results in a protein that binds more effectively to the midgut brush border membrane.[4] This enhanced binding contributes to increased toxicity to *Diabrotica* sp. (English *et al.*, 2000), and to date, two transgenic maize lines, MON863 and MON88017, have been developed using an enhanced Cry3Bb1.

Modifications to the genes encoding Cry3Bb1 in GE maize

MON863: The *cry3Bb1* sequence used to create maize event MON863 was modified from that of the wild-type *cry3Bb1* gene. A restriction site, added to facilitate the cloning of the gene, resulted in the addition of an alanine residue at position two of the amino acid sequence of Cry3Bb1 (USDA, 2001; USEPA, 2003). In addition, six amino acids in the wild-type sequence were substituted with different amino acids to enhance the insecticidal activity of the protein (English *et al.*, 2000; FSANZ, 2003; USEPA, 2003). The modified sequence, which consists of 653 amino acids, is 98.9% homologous to the wild type sequence (USDA, 2001). The specific amino acids changes are listed in Table 2. The protein is not glycosylated post-translation (CFIA, 2003, 2006; FSANZ, 2003, 2006).

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[2] Also called thuringiensin (OECD, 2007; Liu *et al*., 2010).

[3] Raven Biological Insecticide, Ecogen, Inc., Langhorne, PA.

[4] For example, substitutions at amino acid positions 311, 313, and 317 in a surface-exposed loop of the wild type Cry3Bb1 protein alter the hydrophobicity of the region and increase insecticidal activity (English *et al*., 2000).

Table 2. Amino acid substitutions in wild-type Cry3Bb1 used in the production of MON863 (USDA, 2001).

Wild-type Cry3Bb1	Position in Sequence	MON863 Cry3Bb1
--	2	Alanine
Aspartic acid	166	Glycine
Histidine	232	Arginine
Serine	312	Leucine
Asparagine	314	Threonine
Glutamic acid	318	Lysine
Glutamine	349	Arginine

MON88017: The amino acid sequences of MON863 and MON88017 are 99.8% homologous, differing by only one amino acid: the amino acid residue at position 166 in MON88017 is the same as in the wild-type sequence, aspartic acid, rather than glycine, as in MON863 (USDA, 2004). The Cry3Bb1 produced by MON88017 is not glycosylated (USDA, 2004).

Descriptions of the genetic elements used in the production of Cry3Bb1 maize events MON863 and MON88017 are provided in Table 3.

Table 3. Genetic elements used in the production of MON863 and MON88017 maize events (USDA, 2001, 2004; USFDA, 2001, 2005; FSANZ, 2003, 2006; JBCH, 2006a; b, 2008a; b, 2009a; b; EFSA, 2004, 2009a; JBCH, 2004a; b; c; d; COGEM, 2005a; b; Siegfried *et al*., 2005; Vaughn *et al*., 2005)

MON863	
Genetic Element	**Function**
4-AS1	Root-enhanced promoter containing four tandem copies of AS1 and a single portion of the 35S promoter of Cauliflower Mosaic Virus CaMV
wtCAB	5' untranslated leader of the wheat chlorophyll a/b-binding protein
ract1 intron	Intron from the rice actin gene
cry3Bb1	Coding sequence for a synthetic variant of Cry3Bb1 protein from *Bacillus thuringiensis* subsp. *kumamotoensis*
tahsp 17 3'	3' nontranslated region of the coding sequence for wheat heat shock protein 17.3 which ends transcription and directs polyadenylation
MON88017	
p-e35S	Promoter with duplicated enhancer region from CaMV
wtCAB	5' untranslated leader of the wheat chlorophyll a/b-binding protein
ract1 intron	Intron from the rice actin gene
cry3Bb1	Coding sequence for a synthetic variant of Cry3Bb1 protein from *Bacillus thuringiensis* subsp. *kumamotoensis*
tahsp 17 3'	3' nontranslated region of the coding sequence for wheat heat shock protein 17.3 which ends transcription and directs polyadenylation

Expression of Cry3Bb1 in GE Insect-Resistant 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 (See, e.g., Siebert *et al*., 2009).

Data from enzyme-linked immunosorbent assays, showing levels of Cry3Bb1 protein expression in GE maize events, have been made publicly accessible via regulatory dossiers 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 Cry3Bb1 expression. Protein expression data may be used to estimate the potential exposure of various organisms in the environment to Cry3Bb1 when maize plants producing Cry3Bb1 are cultivated. Currently available protein expression data for Cry3Bb1 by maize events MON863 and MON88017, used alone and when stacked with other GE events, are presented in Annex I.[5]

NON-TARGET ORGANISM TESTING AND IMPACTS OF EXPOSURE TO THE Cry3Bb1 PROTEIN

Range of Non-Target Organisms Potentially Impacted by Cry3Bb1

When expressed in maize plants, the Cry3Bb1 toxin has insecticidal properties against certain coleopteran rootworm species (*Diabrotica* sp.), which, in their larval form, would otherwise cause feeding damage to the crop (English *et al*., 2000; Rice, 2003, 2004; Hibbard *et al*., 2005; Siegfried *et al*., 2005; Vaughn *et al*., 2005; Clark *et al*., 2006; Nowatski *et al*., 2006; Prasifka *et al*., 2013). Organisms in the environment that are not pests of maize but may be directly or indirectly exposed to Cry3Bb1 expressed in transgenic maize plants are called non-target organisms (NTOs). The assessment of impacts to NTOs involves the review of data submitted to regulators by the product developer to demonstrate that NTOs exposed to Cry3Bb1, either directly or indirectly, are not harmed significantly.

The NTO risk assessment typically begins with a determination of the organisms that are likely to be directly or indirectly exposed to Cry3Bb1. Particular consideration is often given to NTOs having beneficial environmental functions, such as pollinators, decomposers, or the natural enemies of agricultural pests. Regulatory authorities may also give special attention to NTOs that have been designated as threatened or endangered species or have recognized cultural value, such as the Monarch butterfly. These species, or valid surro-

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[5] Throughout the remainder of this monograph, the proteins produced by events MON863 and MON88017 will be referred to collectively as "Cry3Bb1."

gates for these species, are then tested to determine whether exposure to Cry3Bb1 could cause significant adverse impacts (Romeis *et al.*, 2008, 2013; Knecht *et al.*, 2010; Carstens *et al.*, 2012, 2014; CERA, 2012).

Assessments of the potential impacts to NTOs, and the regulatory decisions informed by these assessments, have been grounded in the long and well-documented history of the evaluation of chemical insecticides, including microbial formulations of *B. thuringiensis* (Rose, 2007; Romeis *et al.*, 2008, 2013; Carstens *et al.*, 2012, 2014; CERA, 2012; Sanvido *et al.*, 2012). 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 insect-resistant GE crops on NTOs (Dutton *et al.*, 2003; EFSA, 2006; Garcia-Alonso *et al.*, 2006; Raybould, 2006; Rose, 2007; Romeis *et al.*, 2008, 2013; Duan *et al.*, 2010; USEPA, 2011c).

So-called "Tier 1" studies are performed under controlled laboratory conditions and involve the exposure of NTOs, or surrogate species, to concentrations of the pesticide several times higher than are likely in the natural environment. These studies identify those species that may be significantly affected by the pesticide. When found, such effects may require further analysis at a higher tier level. Tier 1 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, 2006; Garcia-Alonso *et al.*, 2006; Rose, 2007; Romeis *et al.*, 2008, 2011; USEPA, 2011c)

Routes of Environmental Exposure

Fundamental to the assessment of impacts of Cry3Bb1 on NTOs is the determination of routes through which NTOs would be exposed to the toxin. Direct exposure typically occurs when NTOs feed on living crop tissues expressing Cry3Bb1, on seed, pollen, and other plant tissues that have fallen to the ground, or on post-harvest 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 Cry3Bb1 (Romeis *et al.*, 2009; Tian *et al.*, 2012). In addition, regulatory authorities may consider other routes of indirect exposure to the Cry3Bb1 toxin, e.g., exposure to toxin that is exuded into the soil from living maize roots or toxin released into the soil by decomposing plant material (USEPA, 2003, 2009; CFIA, 2006; EFSA, 2009a; Carstens *et al.*, 2012; CERA, 2012).

Regulators typically consider protein expression data to determine potential routes and levels of exposure. For example, plant tissues producing little or no Cry3Bb1 are unlikely to pose a hazard to NTOs (USDA, 2001, 2004; USFDA, 2001; CFIA, 2003, 2006; FSANZ, 2003; USEPA, 2003; EFSA, 2009a; b; Nguyen and Jehle, 2009). (See Annex I for Cry3Bb1 expression level data in the tissues of approved maize varieties.) Published data as well as data submitted to regulatory authorities indicate that Cry3Bb1 is quickly degraded once released from living maize roots as well as from decomposing plant tissue and is not likely to persist or accumulate in the soil nor in aquatic environments (USDA, 2001; Evans, 2002; CFIA, 2003; USEPA, 2003; Ahmad *et al.*, 2005; Fiorito *et al.*, 2008; Icoz and Stotzky, 2008; Prihoda and Coats, 2008a; b; Miethling-Graff *et al.*, 2010; Höss *et al.*, 2011).

Ecotoxicological Testing of Cry3Bb1 on Non-Target Organisms

As discussed above, ecotoxicological testing has been conducted for many years using a variety of well-characterized test organisms to determine the effects of chemical pesticides on NTOs. Data from these tests have been shown to effectively assess the environmental risks of chemical pesticides and to inform regulators' decisions regarding the safe development and use of pesticides. Analogous testing using many of the same organisms has been successfully used to assess impacts from the environmental release of transgenic crops expressing one or more Bt proteins (Dutton *et al.*, 2003; Garcia-Alonso *et al.*, 2006; Raybould, 2007; Rose, 2007; Romeis *et al.*, 2008; Gealy *et al.*, 2010; Yu *et al.*, 2011; Carstens *et al.*, 2012).

Regulators may require GE crop developers to provide data regarding adverse impacts on beneficial species, such as pollinators, predators, and decomposers; culturally important species, such as the Monarch butterfly; and representative soil dwelling species to demonstrate that there are no significant impacts to these species from exposure to Cry3Bb1. Test organisms have included *Apis mellifera* (honeybee); *Coleomegilla maculata* and *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 Cry3Bb1 several times higher than the highest exposure levels predicted from the observed tissue concentrations of Cry3Bb1 in GE maize plants (See Annex II). After evaluating these test results, regulators have concluded that no significant adverse effects were observed (USDA, 2001, 2004; USEPA, 2003, 2009).

Impacts on non-target coleopterans: Because Cry3Bb1 is used as a selective pesticide for the coleopteran target pests *Leptinotarsa decemlineata* and *Diabrotica* sp., several researchers have performed Tier 1 testing to determine whether Cry3Bb1 may have adverse impacts on non-target coleopterans. Lab studies have exposed non-target coleopteran insects[6] to Cry3Bb1 in several ways: the insects were fed a

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[6] Some of the coleopteran species that have been subjected to Tier 1 lab tests with Cry3Bb1 include *Adalia bipunctata*, *Atheta coriaria*, *Coleomegilla maculata*, *Epilachna vigintioctopunctata*, *Galerucella vittaticollis*, *Harpalus caliginosus*, *H. pensylvanicus*, *Oulema melanopus*, *Poecilus chalcites*, and *Stethorus punctillum*.

prepared diet containing Cry3Bb1 protein; insects were fed pollen collected from maize plants expressing the *cry3Bb1* gene; or herbivorous insects, such as aphids and mites, were allowed to feed on Cry3Bb1 maize plants and were then fed to coleopteran test subjects. Not all herbivorous species that feed on Bt crops actually ingest the Bt toxins.[7] The results of these laboratory studies indicate that there are no environmentally significant adverse effects from the consumption of Cry3Bb1 by non-target coleopterans (Duan *et al.*, 2002, 2006; Lundgren and Wiedenmann, 2002, 2005; Mullin *et al.*, 2005; Ahmad *et al.*, 2006b; Shirai, 2006; Li and Romeis, 2010; Alvarez-Alfageme *et al.*, 2011; García *et al.*, 2012; Meissle *et al.*, 2012).

Impacts on non-coleopteran species: Researchers have also performed many Tier 1 tests to determine if Cry3Bb1 has any adverse effects on non-coleopteran NTOs, including beneficial organisms that act as pollinators, predators, parasitoids, and decomposers, as well as culturally important species, such as the Monarch butterfly.[8] Test organisms were exposed to Cry3Bb1 in a variety of ways: they were fed Cry3Bb1 maize tissues, such as pollen, leaves, or roots; they were provided with synthetic diets containing Cry3Bb1 protein; or they were fed prey insects that had previously fed on Cry3Bb1 maize plants. These assays did not detect any environmentally significant adverse impacts from Cry3Bb1 exposure (Arpaia, 1996; Carter *et al.*, 2004; Mattila *et al.*, 2005; Li *et al.*, 2008, 2010; Lipiński *et al.*, 2008; Prihoda and Coats, 2008a; Duan *et al.*, 2008a; b; Meissle and Romeis, 2009b; Zurbrügg and Nentwig, 2009; Hönemann and Nentwig, 2009, 2010; Knecht and Nentwig, 2010; Höss *et al.*, 2010, 2013; Hendriksma *et al.*, 2011, 2012; Meissle, 2013).

Higher-tier assays of NTO impacts: 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.[9] However, numerous higher-tier studies of the effects of Cry3Bb1 maize on populations of NTOs have been performed, including both greenhouse and field studies. Some of these studies have looked at specific species,[10] while others have focused on impacts on communities of organisms, for example, impacts on all Collembola species. These studies sampled populations using a variety of trapping methods for aboveground species and various extraction methods for subterranean species. The tests found no significant differences between populations of the species associated with Cry3Bb1 maize and those associated with non-GE maize varieties. Therefore, the results of these studies corroborate the results of the Tier 1 studies: Cry3Bb1 does not adversely affect NTO populations (Al-deeb *et al.*, 2003; Carter *et al.*, 2004; Ahmad *et al.*, 2005, 2006a; McManus *et al.*, 2005; Ahmad *et al.*, 2006b; Bhatti *et al.*, 2005; Bitzer *et al.*, 2005; Wolfenbarger *et al.*, 2008; Rauschen *et al.*, 2009, 2011; Li *et al.*, 2010; Zeilinger *et al.*, 2010; Höss *et al.*, 2010, 2011; Schuppener *et al.*, 2012; Priesnitz *et al.*, 2013; Svobodová *et al.*, 2013; Hendriksma *et al.*, 2013).

Additionally, vertebrate toxicological testing of the Cry3Bb1 protein has been conducted on *Mus musculus* (mouse); *Ictalurus punctatu*s (catfish); *Gallus domesticus* (chicken); *Rattus norvegicus* (rat); *Bos taurus* (cattle); and *Colinus virginianus* (northern bobwhite quail) (See Annex II). From these test data, scientists and regulators have concluded that the Cry3Bb1 protein is not toxic to animals or to humans (USDA, 2001, 2004; ACRE, 2003; PDABPI, 2003, 2004a; b, 2005; USEPA, 2003; EFSA, 2004, 2005, 2007, 2009b; COGEM, 2005b; EU, 2005, 2006; Hammond *et al.*, 2006; FSANZ, 2006; Doull *et al.*, 2007; Healy *et al.*, 2008; Scheideler *et al.*, 2008; Sissener *et al.*, 2011).

The potential for harm to NTOs from exposure to Cry3Bb1 has been considered in risk assessments conducted by several regulatory authorities (USDA, 2001, 2004; USEPA, 2002, 2003, 2009, 2011a; b; ACRE, 2003; CFIA, 2003; FSANZ, 2003, 2006; CFIA, 2006; PDABPI, 2003, 2004a; b, 2005; UK, 2003; EFSA, 2009b; JBCH, 2004a; b; c; d, 2006a; b, 2008a; b, 2009a; b; EFSA, 2004, 2005, 2007, 2009a; EU, 2005, 2006; COGEM, 2005a; b; EC, 2011). Data collected from laboratory and field trials of GE maize producing Cry3Bb1 and submitted to regulators have established that the Cry3Bb1 protein is active specifically against the subset of coleopteran pests which feed on the below ground parts of maize plants and cause no significant harms to vertebrate species and other NTOs. Moreover, several studies have indicated that the use of traditional chemical pesticides to control Diabrotica sp. may result in more adverse impacts on NTOs and the environment than the use of Cry3Bb1, due to the specificity of Bt toxins and their rapid degradation in the environment (Evans, 2002; USEPA, 2003; Rice, 2004; McManus *et al.*, 2005; Hönemann *et al.*, 2008; Romeis *et al.*, 2009; Carpenter, 2011; Yu *et al.*, 2011).

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[7] Thrips and spider mites ingest Bt toxins, while aphids do not (Romeis and Meissle, 2011).

[8] Some of the non-coleopteran species that have been subjected to Tier 1 lab tests with Cry3Bb1 include *Apis mellifera*, *Arion lusitanicus*, *Arion vulgaris*, *Caecidotea communis*, *Caenorhabditis elegans*, *Chironomus dilutus*, *Chrysoperla carnea*, *Danaus plexippus*, *Deroceras reticulatum*, *Drosophila melanogaster*, *Enchytraeus albidus*, *Lepidostoma* sp., *Megaselia scalaris*, *Orius insidiosus*, *Pycnopsyche scabripennis*, *Rhizoglypus robini*, *Tetranychus urticae*, *Theridion impressum*, and *Tipula abdominalis*. These species include insects, spiders, aquatic arthropods, earthworms, nematodes, and mollusks.

[9] 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 (Rose, 2007).

[10] Some of the non-target species that have been studied in greenhouse or field tests with Cry3Bb1 maize include *Aglais urticae*, *Apis mellifera*, *Aporrectodea caliginosa*, *Aporrectodea trapezoides*, *Aporrectodea tuberculata*, *Chaetocnema pulicaria*, *Chrysoperla carnea*, *Coleomegilla maculata*, *Hippodamia convergens*, *Lumbricus terrestris*, *Macrocentrus cingulum*, *Orius insidiosus*, *Rhizoglypus robini*, *Rhopalosiphum maidis*, *Scymnus* sp., *Trigonotylus caelestialium*, and *Zyginidia scutellaris*.

Regulatory authorities have determined that adverse effects on NTOs are unlikely for several reasons. First, Cry3Bb1 has a narrow spectrum of pesticidal activity. Second, Tier I laboratory assays, employing a range of invertebrate species present in maize agricultural ecosystems, or surrogates for those species, have shown that Cry3Bb1 causes no significant observable effects in these species. Third, Tier I studies have demonstrated that Cry3Bb1 has no observable effect on representative vertebrate and aquatic species. Fourth, the levels of Cry3Bb1 used in these Tier I assays were much higher than those measured in GE maize tissues growing in the field. Fifth, field studies of maize varieties producing Cry3Bb1 showed no significant adverse effects on a wide range of arthropods, microbes, and other species. Sixth, when compared to insect control via Cry3Bb1, traditional insect control using chemical pesticides causes significantly more alterations to species diversity and poses greater harms non-target species. Together, these findings indicate that Cry3Bb1 is unlikely to have adverse effects on natural populations of organisms, except for the target coleopteran crop pests it is meant to control.

Impacts of Cry3Bb1 Maize on Soil Biology

It is common agronomic practice to leave maize crop residues on and in the soil to improve soil moisture and texture and to foster healthy microbial populations (Taylor *et al.*, 1964; Blevins *et al.*, 1971; Karlen *et al.*, 1994). Numerous field studies have been undertaken to assess any adverse effects from the practice of leaving Cry3Bb1 maize residues in the field to decompose after harvest. These studies have focused on two issues: whether Cry3Bb1 maize residues take longer to decompose than non-Bt varieties and whether crop residues from Cry3Bb1 maize varieties have higher levels of lignin, which would impede decomposition. None of these studies have found either a significantly different rate of decomposition for Cry3Bb1 maize residues or significantly different levels of lignin due solely to the presence of the *cry3Bb1* gene (Hönemann *et al.*, 2008; Lehman *et al.*, 2008a; b, 2010; Poerschmann *et al.*, 2008; Rauschen *et al.*, 2008; Lawhorn *et al.*, 2009; Swan *et al.*, 2009; Zurbrügg *et al.*, 2010; Xue *et al.*, 2011). Therefore the presence of Cry3Bb1 maize residues in the soil is highly unlikely to result in adverse impacts.

Although the studies discussed above indicate that Cry3Bb1 from GE maize plants does not accumulate or persist in the soil, additional studies have been performed to identify any potentially adverse effects of Cry3Bb1 maize cultivation on soil microbiology. These greenhouse and field studies have looked at the size, diversity, and biological activity of microbial populations in the soil surrounding the roots of Cry3Bb1 maize and conventional maize varieties. Some of the studies have looked specifically at effects of Cry3Bb1 on *Metarhizium anisopliae*, an entomopathic fungus that attacks western corn rootworm, and other studies have looked at possible effects on mycorrhizal fungi. None of these studies have found adverse impacts to soil microbes that would have significant environmental or agronomic consequences (Devare *et al.*, 2004, 2007; Icoz and Stotzky, 2008; Lawhorn *et al.*, 2009; Meissle and Romeis, 2009a; Meissle *et al.*, 2009; Xue *et al.*, 2011; Cheeke *et al.*, 2012; Dohrmann *et al.*, 2013).

ESTABLISHMENT AND PERSISTENCE IN THE ENVIRONMENT OF MAIZE PLANTS EXPRESSING Cry3Bb1

Biology of the Plant Species

As discussed earlier, a full understanding of the biology of maize and its interactions with its receiving environment are fundamental to the environmental risk assessment of a GE maize variety (CFIA, 1994; OECD, 2003; OGTR, 2008). Information about the biology of the non-GE version of a crop, including any known adverse environmental impacts associated with its commercial production, provides a basis of comparison with the GE version of the crop (Beadle, 1980; FSANZ, 2003; Raybould, 2007; FAO, 2008; Paoletti *et al.*, 2008; EFSA, 2010; Gealy *et al.*, 2010; Sanvido *et al.*, 2012; Devos *et al.*, 2013). The risk assessment process identifies any differences between the GE and non-GE versions of the crop that could result in significant adverse environmental impacts, such as the likelihood of a GE maize variety establishing and persisting outside of cultivation (OECD, 1992, 2007; EFSA, 2006).

Information about the phenotype of GE maize plants expressing Cry3Bb1 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 can help regulators identify characteristics of the plant that might enhance its survival or persistence (i.e., potential weediness), or characteristics 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 Cry3Bb1. 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). Data submitted to regulatory authorities by the developers of Cry3Bb1 maize varieties have indicated that the phenotypes of GE maize plants expressing Cry3Bb1 were within the reported ranges for non-GE maize varieties. Collectively, regulators have determined that the phenotypic data do not support the hypothesis that the expression of Cry3Bb1 had any unintended impact on the gross morphology or phenotypic characteristics of maize plants, besides conferring resistance to coleopteran insect pests (USDA, 2001, 2004, 2005; PDABPI, 2003, 2004a; b, 2005; USEPA, 2003; JBCH, 2004a; b; c; d, 2006a; b, 2008a; b, 2009a; b).

Weediness in Agricultural Environments

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 Cry3Bb1 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 Cry3Bb1 would not be expected to present any unusual weed management challenges and can be dealt with in the same manner as conventional maize volunteers (Carpenter *et al.*, 2002; CFIA, 2003, 2006; JBCH, 2006a; b, 2008a; b, 2009a; b; USDA, 2004, 2005; JBCH, 2004a; b; c; d; COGEM, 2005a; b; EFSA, 2009b; Raybould *et al.*, 2011).

Weediness in Non-Agricultural Environments

The primary mechanism by which the *cry3Bb1* gene might 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. 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 Cry3Bb1 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 (Mack, 1996; Keane and Crawley, 2002; Mason *et al.*, 2004; Blumenthal, 2005), regulatory decisions have determined that it is unlikely that resistance to coleopteran pests would allow maize producing Cry3Bb1 to become weedy or invasive in non-agricultural environments (Carpenter *et al.*, 2002; CFIA, 2003, 2006; JBCH, 2006a; b, 2008a; b, 2009a; b; USDA, 2004, 2005; JBCH, 2004a; b; c; d; COGEM, 2005a; b; EFSA, 2009b; Raybould *et al.*, 2011).

Movement of the Transgene to Sexually Compatible Relatives

The movement of transgenes from a GE crop plant to one of its wild relatives is both seed and pollen mediated, since the dispersal of seeds will create additional sources of pollen. However, the dispersal of pollen is only the first step in the pathway that could lead to the introgression of a transgene in a wild population of sexually compatible crop relatives (Carpenter *et al.*, 2002; Chandler and Dunwell, 2008). In addition to the dispersal of viable seed and pollen outside the field where the GE crop was grown, several other steps must occur:

- Wild relatives of the crop must be near enough for viable pollen from the crop to reach them.
- Pollen must reach the wild relatives at a time when they are producing flowers receptive to pollen.
- Cross pollination must not be barred by incompatibility mechanism and must result in the production of viable hybrid seed.
- Hybrid seed must bear a functional version of the gene and express the trait.
- The trait must be expressed so as to provide the hybrid progeny with a significant competitive advantage over its wild maternal parent.
- Significant selection pressure must exist in the wild population to favor the survival of progeny bearing the trait.

A thorough understanding of maize biology can determine the likelihood that one or more of these steps will occur. For example, cultivated maize varieties have been bred to retain their seeds on the plant for ease of harvest, and therefore they have lost the ability to disperse their seeds. Maize is predominantly wind pollinated, but maize pollen quickly loses viability once it is shed, and the likelihood of successful pollination falls off rapidly with increasing distance from the parent plant, therefore the potential for gene flow is highly unlikely (Carpenter *et al.*, 2002; OECD, 2003; USEPA, 2003; Devos *et al.*, 2005; Goggi *et al.*, 2006; EFSA, 2009b). In the four countries that have authorized maize expressing Cry3Bb1, regulators have imposed no confinement conditions on its cultivation, concluding that Cry3Bb1 maize is as safe as conventional corn varieties (CFIA, 2003, 2006; JBCH, 2004a; USDA, 2005; MinAgri, 2010).

Teosintes are a group of species in the *Zea* genus that are sexually compatible with cultivated maize. Wild teosinte populations are limited to Mexico, Guatemala, and a single population in Nicaragua. While teosinte is considered a 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). Although maize freely hybridizes with wild teosintes, the potential for gene introgression into teosinte is thought to be limited (Castillo-Gonzalez and Goodman, 1997; OECD, 2003; Baltazar *et al.*, 2005). Crosses between teosinte and GE maize expressing Cry3Bb1 are not expected to occur more frequently than those between teosinte and traditionally bred maize varieties (Carpenter *et al.*, 2002).

COMPOSITIONAL ANALYSIS OF MAIZE PLANTS EXPRESSING Cry3Bb1

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. 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 Cry3Bb1 maize has undergone proximate analysis to determine levels of crude protein, crude fat, fiber, moisture, and ash; and levels of select minerals, fatty acids, amino acids, and antioxidants have also been determined. Many common crop plants are known to produce toxins or anti-nutritive compounds, for example, maize is known to produce the anti-nutritive compounds phytic acid, raffinose, and trypsin inhibitor (OECD, 2003); and levels of these compounds have also been measured to determine whether the presence of the transgene has inadvertently resulted in significantly elevated levels. Composition data from maize varieties expressing Cry3Bb1 have been compared with data obtained from near isogenic comparators as well as data accumulated in various databases representing hundreds of commercial maize varieties. These studies have established that Cry3Bb1 maize is nutritionally equivalent to conventional maize varieties and that the presence of the *cry3Bb1* gene does not result in elevated levels of naturally occurring toxins and anti-nutritive compounds in maize (USDA, 2001, 2004; FSANZ, 2003, 2006; Taylor *et al.*, 2003, 2007; EFSA, 2004; George *et al.*, 2004; Poerschmann *et al.*, 2009; Lundry *et al.*, 2013). These data are presented in Annex III. Regulators in several jurisdictions have determined that these data revealed no differences relevant to environmental safety (USFDA, 2001, 2005; CFIA, 2003, 2006; PDABPI, 2003, 2004a; b, 2005; UK, 2003; FSANZ, 2003, 2006; Health Canada, 2003, 2006; EFSA, 2004, 2005, 2009a; b; Lundry *et al.*, 2013).

CONCLUSION

The Cry3Bb1 protein produced by insect-resistant GE maize plants is derived from the common soil bacterium *Bacillus thuringiensis* and is specifically toxic to coleopteran insects. Toxicity testing with a broad range of representative non-target organisms demonstrated that Cry3Bb1 produced no observable effects at concentrations significantly higher than the expected environmental concentrations of Cry3Bb1. Field data suggest that cultivation of GE maize plants expressing Cry3Bb1 does not adversely affect the abundance of non-target arthropods or impact soil microbial populations. Cry3Bb1 in plants can be toxic to non-target Coleoptera, 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 Cry3Bb1 expression in approved maize varieties does 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 maize varieties. To date, every regulatory body that has evaluated the safety of events MON863 and MON88017 has concluded that these varieties are as safe as conventionally bred maize varieties and pose no significant environmental or food safety concerns.

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ANNEX I: SUMMARY OF Cry3Bb1 PROTEIN EXPRESSION DATA

The tables that follow present summary data from applicant dossiers and regulatory decisions documents concerning maize events MON863 and MON88017 occurring alone and in combination with other transgenic traits. 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 Cry3Bb1 protein level measured in MON863 tissue samples collected from multiple field sites (CFIA, 2003; FSANZ, 2003; USDA, 2001; USFDA, 2001).

Tissue (days post-planting)	Parameter	Cry3Bb1[1] (µg/g fresh weight)
Young leaf[2] (21)	Mean ± SD Range n	81 ± 11 65 – 93 4
Forage[3] (90)	Mean ± SD Range n	39 ± 10 24 – 45 4
Mature Root[3] (90)	Mean ± SD Range n	41 ± 13 25 – 56 4
Grain[4] (125)	Mean ± SD Range n	70 ± 17 49 – 86 4
Silk[5] (58)	Mean ± SD n	10 1
Pollen[6] (60)	Mean ± SD Range n	62 ± 18 30 – 93 13

1 Limit of detection for Cry3Bb1 variant protein ranges from 0.08 µg/g in silk to 0.76 µg/g in root tissues.
2 Samples were a pool of tissues ranging from 37 to 50 plants collected from each site at approximately V-4 stage.
3 Forage (above ground portion only) and mature root were a composite of two plants collected from each site at early dent stage.
4 Process grain samples were combined from 28-41 corn ears collected from each site at plant maturity and dried to about 15% moisture content.
5 Silk was combined (n=1) from five plants at about 50% pollen shed from one field site.
6 In the US, one sample of pollen tissue was combined over a period of 7 days (about 60 days post planting or about 50% pollen shed). Samples of pollen from Argentina were combined as four replicates per site (three sites total) and collected about 65 days post-planting over about 5 days.

Table I.2. Production of Cry3Bb1 in MON863 maize (μg/gram fresh weight) (USEPA, 2003).

Leaf	Grain	Pollen	Root	Whole plant*
30-93	49-86	30-93	3.2-66	13-54

* Above ground portion

Table I.4. Cry3Bb1 expression levels in MON88017 X MON810 and MON88017 maize grain (μg/g dry weight) (EFSA, 2009a)

Cry3Bb1	MON88017 X MON810	MON88017
Mean (SD) Range	9.3 (3.4) 3.9 – 13	15 (3.6) 10 – 22

Table I.3. Cry3Bb1 protein levels in MON863 measured over the growth of the plant (FSANZ, 2003; USDA, 2001).

Collection Post-planting	Parameter	Cry3Bb1 in Leaf (μg/g fw)	Cry3Bb1 in Whole Plant* (μg/g fw)	Cry3Bb1 in Root (μg/g fw)
21 days	Mean ± SD Range n	81 ± 14 65 - 93 3	not collected	not collected
35 days	Mean ± SD Range n	79 ± 6.4 72 – 84 3	46 ± 7.8 38 – 54 3	58 ± 10 46 – 66 3
49 days	Mean ± SD Range n	43 ± 18 30 – 56 2	31 ± 3.3 28 – 33 2	57 ± 3.8 54 – 59 2
90 days	Mean ± SD Range n	not collected	37 ± 12 24 – 45 3	37 ± 11 25 - 47 3
126 days	Mean ± SD Range n	not collected	25 ± 11 13 – 35 3	24 ± 18 3.2 – 36 3

* Above ground portion

Table I.5. Cry3Bb1 levels in different tissues of MON88017 collected during four developmental stages in three growing seasons, 2005 – 2007 (Nguyen and Jehle, 2009).

Tissue	Parameter	Developmental Stage[1]			
		BBCH19	BBCH30	BBCH63	BBCH83
Root	Mean (SD)[2] Range n	129.7 (4.1) dA 76.8-175.3 48	99.0 (7.2) cA 36.6-289.3 48	65.8 (3.7) bB 33.1-149.8 48	40.3 (2.0) aC 15.8-74.5 48
Stalk	Mean (SD) Range n	184.0 (8.7) dB 70.1-320.8 48	113.9 (6.5) cAB 31.3-206.3 48	113.9 (6.5) cAB 31.3-206.3 48	37.4 (3.1) aBC 12.2-107.2 48
Lower leaf	Mean (SD) Range n		126.8 (8.0) bBC 57.0-298.4 48	117.0 (8.4) bC 31.7-242.3 48	33.51 (4.3) aAB 0.74-133.1 48
Upper leaf	Mean (SD) Range n	228.4 (11.0) dC 116.4-391.6 48	151.7 (8.6) cC 22.8-304.8 48	125.5 (4.1) bC 63.6-196.8 48	100.2 (3.5) aD 53.0-162.6 48
Anther	Mean (SD) Range n			65.8 (2.1) B 37.5-108.1 48	
Pollen (μg/g fresh wt)	Mean (SD) Range n			3.81 (0.2) 2.3-5.9 32	
Silk	Mean (SD) Range n			110.2 (6.5) C 25.9-205.0 32	
Grain	Mean (SD) Range n				27.1 (0.6) A 7.2-59.4 48

[1] BBCH19 = Nine or more leaves unfolded; BBCH30 = Beginning of stem elongation; BBCH63 = Flowering, anthesis; BBCH83 = Ripening, early dough: kernel soft, about 45% dry matter.

[2] SE = standard error; n = number of samples. The range gives the minimum and maximum value during the 3-year survey. Means within a row followed by the same lowercase letter are not significantly different. Means within a column followed by the same capital letter are not significantly different (P>0.05).

Table I.6. Levels of the Cry3Bb1 protein in tissues of MON88017 (CFIA, 2006; EFSA, 2009b; USDA, 2004).

Tissue Type	**Growth Stage**	**Cry3Bb1 Mean (SD)[1] [Range][2]**	
		(µg/g dwt)	**(µg/g fwt)**
Young leaf	V2-V3 (14-22 DAP[3])	570 (170) [230-820]	76 (23) [28-110]
Pollen	R1 (62-69 DAP)	25 (4.2) [17-32]	14 (2.5) [11-20]
Silk	R1 (62-69 DAP)	380 (65) [300-500]	37 (5.6) [30-45]
Forage	R4-R6 (early dent) (97-124 DAP)	95 (19) [75-130]	27 (5.5) [22-39]
Forage root	R4-R6 (early dent) (97-124 DAP)	130 (29) [98-170]	21 (3.1) [17-27]
Grain	R6 (133–146 DAP)	15 (3.6) [10-22]	13(3.1) [8.7-19]
Stover	R6 (after harvest) (133-147 DAP)	88 (13) [71-110]	30 (4.4) [25-39]

1 The mean and standard deviation were calculated across sites and replicates (n=9).
2 Minimum and maximum values were determined for each tissue type across sites.
3 DAP = days after planting.

ANNEX II: SUMMARY OF Cry3Bb1 ECOTOXICITY DATA

Table II.1. Summary of results from ecological toxicity tests with Cry3Bb1 proteins. MON863 plant tissue served as the test substance for some assays. All other assays employed an artificial diet containing the EG11231 variant of Cry3Bb1. Risk conclusions are based on protein concentrations in plant tissues from event MON863 (USDA, 2001).

Test Organism	**Test Substance**	**Results**	**Conclusions**
Cladoceran (*Daphnia magna*)	Pollen containing Cry3Bbl	NOEC ≥ 2.26 µg/l	NOEC > 141X predicted maximum concentration in surface water
Collembola (*Folsomia candida*)	Leaf containing Cry3Bbl	NOEC ≥ 872.5 µg/l protein/g diet	NOEC > 66X predicted maximum concentration in soil
Channel catfish (*Ictalurus punctatus*)	Grain containing Cry3Bbl	No effect on growth or survival at 35% of diet	No significant risk
Larval Ladybird Beetle (*Coleomegilla maculata*)	Pollen containing Cry3Bb1	No effect on development or survival at 50% of diet	No significant risk
Adult Ladybird Beetle (*Coleomegilla maculata*)	Pollen containing Cry3Bb1	No effect on survival at 50% of diet	No significant risk
Adult Ladybird Beetle (*Hippodamia convergens*)	Pollen containing Cry3Bb1	No effect on survival at 50% of diet	No significant risk
Adult Honey Bee (*Apis mellifera*)	EG11231 in an artificial diet	NOEC ≥ 360 µg/ml in diet	NOEC > 3.9X predicted maximum concentration in pollen
Larval Honey Bee (*Apis mellifera*)	EG11231 in water	NOEC ≥ 1790 µg/ml as a single dose	NOEC > 19X predicted maximum concentration in pollen
Green Lacewing Larvae (*Chrysoperla carnea*)	EG11231 in an artificial diet	NOEC ≥ 8000 µg/g in diet	NOEC > 86X maximum environmental concentration predicted in pollen
Parasitic Hymenoptera (*Nasonia vitripennis*)	EG11231 in an artificial diet	NOEC = 400 µg/ml in diet	NOEC > 4.3X maximum environmental concentration predicted in pollen
Earthworm (*Eisenia foetida*)	EG11231 in soil	NOEC = 57 mg/kg in soil	NOEC ≥ 4.3 X maximum estimated environmental exposure in soil

Table II.2. Results of non-target wildlife studies event MON863 maize (USEPA, 2003).

Guideline Number	Study	Results
USEPA OPPTS 885.405	Dietary Toxicity Study with the Northern Bobwhite	The dietary LC_{50} value for Cry3Bb1 corn grain to juvenile Northern Bobwhite was > 70,000 ppm (10% of the diet) in an 8-day study. No adverse effects on avian wildlife are expected from incidental field exposure to Cry3Bb1 corn.
885.42	Freshwater Fish Testing	No treatment mortality or behavior change was observed among channel catfish in an 8-week sub-chronic study when fed diets containing 35% Cry3Bb1 corn.
Series 72, Subdivision E	Acute Toxicity Test with *Daphnia magna*	The 48-hour LC_{50} value for Cry3Bb1 corn pollen when administered to neonate daphnids was >120 mg pollen/L, a maximum hazard dose. No adverse effects were noted.
885.438	Adult Honey Bee Testing	An adult honeybee maximum hazard dose feeding study showed the LC_{50} of the Cry3Bb1 protein to be >360 µg/mL (20X the concentration found in pollen).
885.434	Parasitic Hymenoptera Larva Testing	The LC_{50} for parasitic Hymenoptera was determined to be >400 ppm Cry3Bb1 protein. Although 400 ppm Cry3Bb1 protein is only 1X field concentration in plants rather than 10X, parasitic Hymenoptera are not expected to feed directly on corn plant tissue.
885.434	Dietary Toxicity Study with Green Lacewing Larvae	The LC_{50} for green lacewing larvae was determined to be >8,000 ppm Cry3Bb1 protein (20X field exposure). Based on these results it can be concluded that green lacewing will not be adversely affected when exposed to Cry3Bb1 in the field.[1]
885.434	Effects of Bt Protein on Adult Lady Beetles (*Hippodamia convergens*)	This maximum hazard dose study showed that the LC_{50} for Cry3Bb1when fed to adult *H. convergens* is >8,000 µg purified Bt protein/mL diet., equivalent to 20X the maximum Bt protein concentration in plant tissue.
885.434	Lady Beetle Larval Pollen Feeding Study (*Coleomegilla maculata*)	The LC_{50} for Cry3Bb1 expressed in pollen is >93 µg/g fresh pollen weight. The larvae were observed through pupation to adult emergence. It can be concluded from this study that *C. maculata* larvae will not be adversely affected by Cry3Bb1 field corn pollen.
885.434	Adult Lady Beetle Pollen Feeding Study (*C. maculata*)	No significant adverse effects were noted in a 30 day 50% pollen feeding study. Based on these results, no hazard to *C. maculata* is expected when feeding on Cry3Bb1 corn pollen in the field.
885.434	Adult Lady Beetle Pollen Feeding Study (*H. convergens*)	No significant adverse effects were noted in a 15 day 50% pollen in honey water feeding study. Based on these results, no hazard to *H. convergens* is expected if feeding on Cry3Bb1corn pollen in the field.
885.434	Collembola Chronic Dietary Toxicity Study	The LC_{50} of the Cry3Bb1 protein for Collembola was found to be >872.5 µg (50% corn leaf tissue in the diet). No adverse reproductive effects were noted. It can be concluded from this test that Cry3Bb1 protein does not pose a hazard to Collembola, a representative of a beneficial decomposer soil inhabiting species.
850.62	Earthworm Toxicity Study	A maximum hazard dose 14-day LC50 for earthworms exposed to Cry3Bb1 protein in an artificial soil substrate was determined to be > 570 mg Cry3Bb1 protein/kg dry soil, or greater than 10 times the maximum EEC of the protein. The data show that no adverse effects to earthworms are expected from exposure to Cry3Bb1 protein producing corn plants.
OECD Guideline 207	Earthworm Toxicity Study	There were no earthworm mortalities or other remarkable observations during the 14 day study. The LC50 value is greater than the highest maximum hazard concentration tested.
885.434	Monarch Butterfly Larval Pollen Feeding Study	This study has demonstrated that corn pollen expressing the Cry3Bb1 protein will not result in acute toxic or developmental effects to monarch larvae.

[1] USEPA requested that because of questionable ingestion of the test material, another species (e.g., minute pirate bug) that is more likely to be exposed to Cry3Bb1 should be tested.

Table II.3. Calculated margins of exposure to NTOs for the Cry3Bb1 protein produced in MON88017 (USDA, 2004).

Test Organism	Cry3Bb1 Variant	Origin (Tissue)	Results	Margins of Exposure[1]	
				MON 88017[2] (NOEC ≥)[3]	**MON 863 (NOEC ≥)**
Cladoceran (*Daphnia magna*)	11098 (Q349R)	MON 863 (pollen)	NOEC ≥ 2.26 μg/l	665x surface water MEEC	141x surface water MEEC
Collembola (*Folsomia candida*)	11098 (Q349R)	MON 863 (leaf)	NOEC ≥ 872.5 μg/g	88.6x soil MEEC	105x soil MEEC
Adult Honey Bee (*Apis mellifera*)	11231	*B.t.*	NOEC ≥ 360 μg/ml	18x max. pollen level	3.8x max. pollen level
Larval Honey Bee (*Apis mellifera*)	11231	*B.t.*	NOEC ≥ 1790 μg/ml as a single dose	89.5x max. pollen level	19x max. pollen level
Adult Ladybird Beetle (*Hippodamia convergens*)	11231	*B.t.*	NOEC ≥ 8000 μg/g	400x max. pollen level	86x max. pollen level
Green Lacewing Larvae (*Chrysoperla carnea*)	11231	*B.t.*	NOEC ≥ 8000 μg/g	400x max. pollen level	86x max. pollen level
Parasitic Hymenoptera (*Nasonia vitripennis*)	11231	*B.t.*	NOEC = 400 μg/ml	20x max. pollen level	4.3x max. pollen level
Earthworm (*Eisenia fetida*)	11231	*B.t.*	NOEC = 57 mg/kg	5.8x MEEC in soil	6.9x MEEC in soil

1 Margin of exposure = ratio of NOEC to MEEC.

2 Based on the following MEEC values for the Cry3Bb1 protein: 110 μg/g fresh weight in leaf, 20 μg/g fresh weight in pollen, 9.85 mg/kg in soil, and 0.0034 μg/l in aquatic environments.

3 NOEC = no observed effect concentration; MEEC – maximum expected environmental concentration.

Table II.4. Levels of the Cry3Bb1 protein in overseason tissues of MON88017 (USDA, 2004).

Tissues (9 samples)		**V2 – V3 (14 – 22 DAP1)**	**V5 (26 – 34 DAP)**	**V8 (40-45 DAP)**	**V11-V17 (55-62 DAP)**	**R4-R6 (97-124 DAP)**	**R6 (133-147 DAP)**
μg/g dry weight							
Leaf	Mean (SD)	570 (170)	430 (58)	310 (45)	260 (44)	NA	NA
	Range	230 – 820	310 – 510	240 – 380	190 – 340		
Whole plant	Mean (SD)	500 (64)	380 (170)	310 (48)	220 (23)	NA	NA
	Range	410 – 590	150 – 600	230 – 380	190 – 250		
Root	Mean (SD)	370 (80)	250 (71)	210 (78)	180 (37)	130 (29)	100 (19)
	Range	240 – 510	190 – 420	150 – 410	110 – 230	98 – 170	77 – 140
μg/g fresh weight							
Leaf	Mean (SD)	76 (23)	75 (10)	69 (12)	62 (9.2)	NA	NA
	Range	28 – 110	58 – 92	55 – 90	49 – 77		
Whole plant	Mean (SD)	50 (6.4)	37 (8.0)	34 (5.2)	32 (4.4)	NA	NA
	Range	41 – 59	26 – 48	25 – 42	26 – 38		
Root	Mean (SD)	39 (8.1)	34 (8.4)	29 (8.3)	26 (5.4)	21 (3.1)	18 (2.6)
	Range	24 – 51	25 – 55	21 – 50	16 – 34	17 – 27	14 - 22

1 DAP = days after planting, SD = standard deviation, NA = not applicable.

ANNEX III: SUMMARY OF COMPOSITIONAL ANALYSES OF GE PLANTS EXPRESSING Cry3Bb1, INCLUDING ANALYSES OF TOXINS, ANTI-NUTRIENTS, AND SECONDARY METABOLITES

Table III.1. Compositional analysis of the grain collected from corn event MON863, nontransgenic control corn, and commercial corn varieties (USDA, 2001).

Component	Unit	MON863 Mean	Control Mean	Commercial Range
Ash	% dry wt.	1.35	1.41	0.62 – 1.53
Carbohydrates	% dry wt.	83.3	82.8	82.5 – 87.8
Acid detergent fiber	% dry wt.	4.45	4.50	3.65 – 6.09
Neutral detergent fiber	% dry wt.	11.6	12.0	9.50 – 15.0
Moisture	% fresh wt.	10.0	10.2	8.75 – 15.7
Total fat	% dry wt.	3.77	3.64	2.18 – 3.86
Protein	% dry wt.	11.6	12.2	7.95 – 13.8
Calcium	% dry wt.	0.005	0.005	0.004 – 0.006
Copper	mg/kg dry wt.	2.26	2.19	1.03 – 2.15
Iron	mg/kg dry wt.	23.6	24.2	16.7 – 28.7
Magnesium	% dry wt.	0.13	0.14	0.091 – 0.14
Manganese	mg/kg dry wt.	5.81	6.15	3.51 – 9.80
Phosphorus	% dry wt.	0.40	0.42	0.33 – 0.43
Potassium	% dry wt.	0.43	0.44	0.33 – 0.43
Zinc	mg/kg dry wt.	22.2	23.7	12.8 – 31.2
Phytic Acid	% dry wt.	1.11	1.23	0.73 – 1.17
Trypsin inhibitor	TUI/mg dry wt.	2.30	2.48	0.58 – 3.05
Vitamin E	mg/kg dry wt.	0.011	0.013	0.004 – 0.014
16:0 Palmitic acid	% of total FA	12.0	11.9	9.07 – 12.1
18:0 Stearic acid	% of total FA	1.66	1.66	1.44 – 2.40
18:1 Oleic acid	% of total FA	22.0	21.9	21.3 – 32.1
18:2 Linoleic acid	% of total FA	62.2	62.5	54.2 – 63.6
18:3 Linolenic acid	% of total FA	1.20	1.24	0.97 – 1.36

Table III.2. Fiber, mineral, and proximate composition of grain from corn event MON863 (George *et al.*, 2004).

Component[3]	1999 U.S. Trials[1]			1999 Argentina Trials[2]			Literature Range	Historical Range[7]
	MON863 Mean (Range)[8]	Control[4] Mean (Range)[8]	Comm. Hybrids[5] Tolerance Interval[6]	MON863 Mean (Range)[8]	Control[4] Mean (Range)[8]	Comm. Hybrids[5] Tolerance Interval[6]		
Protein	11.60 (10.43 - 12.82)	12.19 (10.45 - 13.80)	5.47, 16.57	10.39 (9.54 - 11.36)	10.40 (9.30 - 10.92)	3.37, 16.57	6.0 - 12.0i 9.7 - 16.1j	9.0 - 13.6
Total Fat	3.77 (3.00 - 4.56)	3.64 (3.05 - 4.29)	1.68, 4.64	3.59 (3.00 - 4.42)	3.60 (2.83 - 3.94)	1.26, 6.25	3.1 - 5.7i 2.9 - 6.1j	2.4 - 4.2
Ash	1.35 (0.84 - 1.71)	1.41 (0.89 - 1.89)	0.26, 2.06	1.55 (1.34 - 1.81)	1.51 (1.32 - 1.80)	0.97, 1.76	1.1 - 3.9i	1.2 - 1.8
ADF[9]	4.45 (3.49 - 5.23)	4.50 (3.62 - 5.89)	1.98, 6.62	3.47 (2.65 - 4.84)	3.25 (2.58 - 4.44)	1.35, 5.75	3.3 - 4.3i	3.1 - 5.3
NDF[9]	11.64 (9.21 - 13.47)	12.02 (10.31 - 15.82)	6.51, 16.28	12.67 (9.70 - 19.86)	11.60 (8.49 - 18.12)	4.35, 17.20	8.3 - 11.9i	9.6 - 15.3
Carbohydrates	83.30 (81.83 - 85.00)	82.76 (80.70 - 84.80)	78.97, 90.36	84.58 (83.28 - 87.10)	84.49 (83.84 - 85.92)	77.60, 92.24	Not reported in this form	81.7 - 86.3
Moisture	10.03 (8.54 - 11.20)	10.23 (8.60 - 11.40)	5.09, 18.62	12.52 (11.10 - 15.10)	12.73 (11.60 - 15.30)	0, 20.94	7 - 23i	9.4 - 15.8
Calcium	0.0052 (0.0041 - 0.0064)	0.0053 (0.0043 - 0.0089)	0.0022, 0.0073	0.0041 (0.0028 - 0.0051)	0.0044 (0.0033 - 0.0055)	0.0016, 0.0090	0.01 - 0.1i	0.003 - 0.006
Copper	2.26 (1.72 - 3.18)	2.19 (1.60 - 2.88)	0.25, 2.70	2.29l (1.88 - 2.63)	2.82 (2.32 - 3.22)	0, 3.91	0.9 - 10i	Not available
Iron	23.55 (21.13 - 26.36)	24.18 (20.57 - 28.16)	12.52, 35.06	24.91 (21.97 - 31.67)	25.33 (22.84 - 27.19)	2.49, 37.25	1 - 100i	Not available
Magnesium	0.13 (0.12 - 0.14)	0.14 (0.12 - 0.16)	0.082, 0.17	0.13 (0.12 - 0.16)	0.13 (0.12 - 0.14)	0.074, 0.17	0.09 - 1.0i	Not available
Manganese	5.81 (3.75 - 7.40)	6.15 (4.01 - 8.28)	0, 12.84	7.74 (5.95 - 9.72)	7.58 (6.04 - 9.05)	0.90, 11.97	0.7 - 54i	Not available
Phosphorus	0.40 (0.37 - 0.45)	0.42 (0.39 - 0.46)	0.21, 0.47	0.35 (0.30 - 0.41)	0.36 (0.31 - 0.39)	0.25, 0.39	0.26 - 0.75i	0.288 - 0.363
Potassium	0.43 (0.40 - 0.48)	0.44 (0.39 - 0.48)	0.28, 0.48	0.43 (0.38 - 0.49)	0.43 (0.41 - 0.46)	0.23, 0.52	0.32 - 0.72i	Not available
Zinc	22.15 (17.95 - 25.25)	23.68 (18.77 - 28.14)	6.31, 37.95	27.15 (23.50 - 30.31)	28.13 (24.38 - 31.63)	6.10, 40.05	12 - 30i	na

1 Data from four replicated U.S. sites.
2 Data from four replicated sites in Argentina.
3 Percent dry weight of sample except for moisture as percent fresh weight and copper, iron, manganese, and zinc as mg/kg dry weight.
4 Nontransgenic control.
5 Commercial hybrids planted at each trial site. The commercial hybrids for Argentina also included six hybrids grown in the E.U. during 1999.
6 Tolerance interval is specified to contain 99% of the commercial line population; negative limits are set to zero.
7 Range for nontransgenic control hybrids planted in Monsanto Company field trials conducted between 1993 and 1995.
8 Range denotes the lowest and highest individual value across sites for each line.
9 ADF = acid detergent fiber; NDF = neutral detergent fiber.

Table III.3. Fiber and proximate composition of forage from corn event MON863 (George *et al.*, 2004).

Component[3]	1999 U.S. Trials[1]			1999 Argentina Trials[2]			Literature Range	Historical Range[7]
	MON863 Mean (Range)[8]	Control[4] Mean (Range)[8]	Comm. Hybrids[5] Tolerance Interval[6]	MON863 Mean (Range)[8]	Control[4] Mean (Range)[8]	Comm. Hybrids[5] Tolerance Interval[6]		
Protein	8.62 (6.91 - 10.40)	8.33 (5.99 - 10.55)	4.94, 11.97	8.92 (7.59 - 10.04)	9.52 (8.35 - 10.60)	0.22, 15.79	5.11 - 10.27	4.8 - 8.4
Total Fat	2.40 (0.92 - 3.16)	2.35 (1.30 - 3.33)	1.03, 3.24	1.59 (0.81 - 2.65)	1.56 (0.71 - 2.37)	0, 4.49	0.35 - 3.62	1.4 - 2.1
Ash	4.73 (3.62 - 5.65)	5.00 (3.81 - 6.27)	3.04, 5.58	6.51 (4.24 - 8.08)	6.32 (4.88 - 8.23)	2.33, 7.70	2.00 - 6.60	2.9 - 5.1
ADF[9]	28.67 (21.74 - 43.30)	28.41 (23.39 - 32.08)	9.33, 45.44	26.79 (22.55 - 31.27)	27.22 (22.83 - 30.32)	15.09, 34.96	18.32 - 40.99	21.4 - 29.2
NDF[9]	43.25 (37.97 - 49.67)	42.94 (37.32 - 51.85)	22.71, 56.02	42.87 (35.21 - 48.21)	43.20 (39.15 - 47.21)	24.59, 55.98	26.37 - 54.45	39.9 - 46.6
Carbohydrates	84.24 (82.29 - 86.32)	84.32 (80.78 - 87.21)	81.22, 88.97	82.98 (80.74 - 85.10)	82.61 (81.09 - 84.68)	78.37, 91.73	83.16 - 91.55	84.6 - 89.1
Moisture	71.09 (69.30 - 73.10)	71.68 (69.80 - 74.50)	62.70, 77.69	73.32 (70.10 - 75.10)	74.13 (70.20 - 77.70)	56.69, 87.10	55.30 - 75.30	68.7 - 73.5

1 Data from four replicated U.S. sites.
2 Data from four replicated sites in Argentina.
3 Percent dry weight of sample except for moisture.
4 Nontransgenic control.
5 Commercial hybrids planted at each trial site. The commercial hybrids for Argentina also included six hybrids grown in the E.U. during 1999.
6 Tolerance interval is specified to contain 99% of the commercial line population; negative limits are set to zero.
7 Range for nontransgenic control hybrids planted in Monsanto Company field trials conducted between 1993 and 1995.
8 Range denotes the lowest and highest individual value across sites for each line.
9 ADF = acid detergent fiber; NDF = neutral detergent fiber.

Table III.4. Fatty acid composition of grain from corn event MON863 (George *et al.*, 2004).

Fatty Acid[3]	1999 U.S. Trials[1]			1999 Argentina Trials[2]			Literature Range	Historical Range[7]
	MON863 Mean (Range)[8]	Control[4] Mean (Range)[8]	Comm. Hybrids[5] Tolerance Interval[6]	MON863 Mean (Range)[8]	Control[4] Mean (Range)[8]	Comm. Hybrids[5] Tolerance Interval[6]		
Arachidic (20:0)	0.41 (0.39 - 0.44)	0.40 (0.39 - 0.42)	0.30, 0.51	0.34 (0.32 - 0.37)	0.35 (0.32 - 0.39)	0.16, 0.60	0.1 - 2	0.3 - 0.5
Behenic (22:0)	0.18 (0.17 - 0.21)	0.18 (0.15 - 0.21)	0.055, 0.30	0.15 (0.073 - 0.18)	0.15 (0.086 - 0.17)	0.054, 0.28	Not reported	0.1 - 0.3
Eicosenoic (20.1)	0.30 (0.28 - 0.35)	0.30 (0.28 - 0.35)	0.18, 0.42	0.24[9] (0.22 - 0.27)	0.25 (0.24 - 0.27)	0.19, 0.39	Not reported	0.2 - 0.3
Linoleic (18:2)	62.23 (60.02 - 63.21)	62.47 (61.55 - 63.60)	50.21, 70.86	63.99[9] (62.14 - 65.09)	62.58 (61.41 - 63.63)	49.72, 69.67	35 - 70	55.9 - 66.1
Linolenic (18:3)	1.20 (1.13 - 1.29)	1.24 (1.09 - 1.45)	0.75, 1.51	1.17 (1.12 - 1.20)	1.19 (1.15 - 1.23)	0.76, 1.58	0.8 - 2	0.8 - 1.1
Oleic (18:1)	22.00 (20.97 - 23.55)	21.87 (21.00 - 22.53)	13.28, 36.31	21.53 (20.68 - 22.45)	22.03 (21.20 - 22.92)	18.41, 31.88	20 - 46	20.6 - 27.5
Palmitic (16:0)	12.01 (11.61 - 12.56)	11.88 (11.66 - 12.20)	7.74, 13.87	10.70[9] (9.86 - 11.47)	11.68 (11.35 - 12.06)	5.63, 17.42	7 - 19	9.9 - 12.0
Stearic (18:0)	1.66 (1.40 - 1.86)	1.66 (1.33 - 1.81)	1.04, 2.68	1.88[9] (1.67 - 2.34)	1.76 (1.64 - 1.91)	0.80, 2.44	1 - 3	1.4 - 2.2

1 Data from four replicated U.S. sites.
2 Data from four replicated sites in Argentina.
3 Value of fatty acids expressed as % of total fatty acid, except for palmitic acid (16:0), which is expressed as % of triglyceride fatty acids. The method included the analysis of the following fatty acids, which were not detected in the majority of samples analyzed: caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristic acid (14:0), myristoleic acid (14:1), pentadecanoic acid (15:0), pentadecenoic acid (15:1), palmitoleic acid (16:1), heptadecanoic acid (17:0), heptadecenoic acid (17:1), γ-linolenic acid (18:3), eicosadienoic acid (20:2), eicosatrienoic acid (20:3), and arachidonic acid (20:4).
4 Nontransgenic control.
5 Commercial hybrids planted at each trial site. The commercial hybrids for Argentina also included six hybrids grown in the E.U. during 1999.
6 Tolerance interval is specified to contain 99% of the commercial line population; negative limits are set to zero.
7 Range for control hybrids planted in Monsanto Company field trials conducted between 1993 and 1995; values are expressed as % of total fatty acids
8 Range denotes the lowest and highest individual value across sites.
9 Statistically and significantly different from the control at the 5% level (p<0.05).

Table III.5. Phytic acid, trypsin inhibitor, vitamin E, thiamin, riboflavin, folic acid, and secondary metabolite content of grain from corn event MON863 (George *et al.*, 2004).

Component	1999 U.S. Trials[1]			1999 Argentina Trials[2]			Literature Range	Historical Range[6]
	MON863 Mean (Range)[7]	Control[3] Mean (Range)[7]	Comm. Hybrids[4] Tolerance Interval[5]	MON863 Mean (Range)[7]	Control[3] Mean (Range)[7]	Comm. Hybrids[4] Tolerance Interval[5]		
Phytic acid (% dw)	1.11[8] (0.92 - 1.28)	1.23 (1.01 - 1.37)	0.39, 1.33	0.76[8] (0.61 - 1.05)	0.60 (0.42 - 0.76)	0.36, 0.97	to 0.9%	Not available
Trypsin inhibitor (TIU/mg dw)	2.30 (0.56 - 3.10)	2.48 (1.91 - 3.45)	0, 4.25	3.82 (2.89 - 4.76)	3.83 (2.19 - 5.05)	0, 6.98	Not available	Not available
Folic acid (µg/g dw)	Not available	Not available	Not available	0.71 (0.48 - 1.02)	0.68 (0.59 - 0.75)	Not available	Not available	Not available
Thiamin (mg/100 g dw)	Not available	Not available	Not available	0.28 (0.21 - 0.41)	0.27 (0.23 - 0.33)	Not available	0.3 - 0.86	Not available
Riboflavin (µg/g dw)	Not available	Not available	Not available	1.35 (0.93 - 1.76)	1.27 (0.91 - 1.74)	Not available	0.25 - 5.6	Not available
Vitamin E (mg/g dw)	0.011[8] (0.0062 - 0.014)	0.013 (0.0088 - 0.016)	0, 0.019	0.0089 (0.0070 - 0.014)	0.0080 (0.0060 - 0.011)	0, 0.028	0.017 - 0.047	0.008 - 0.015
Ferulic acid (% dw)	Not available	Not available	Not available	0.24 (0.20 - 0.40)	0.23 (0.19 - 0.27)	0.17, 0.28	Not available	0.17 - 0.27
Inositol (µg/g dw)	Not available	Not available	Not available	1564.01 (1355.93 - 1820.25)	1494.18 (1244.34 - 1704.55)	Not available	Not available	Not available
p-Coumaric acid (% dw)	Not available	Not available	Not available	0.023 (0.016 - 0.047)	0.020 (0.016 - 0.026)	0.0022, 0.037	Not available	0.011 - 0.030
Raffinose (% dw)	Not available	Not available	Not available	0.12 (0.10 - 0.15)	0.11 (0.091 - 0.13)	0, 0.35	0.028 - 0.074[8]	0.053 - 0.16

1 Data from four replicated U.S. sites.
2 Data from four replicated sites in Argentina.
3 Nontransgenic control.
4 Commercial hybrids planted at each trial site. The commercial hybrids for Argentina also included six hybrids grown in the E.U. during 1999.
5 Tolerance interval is specified to contain 99% of the commercial line population; negative limits are set to zero.
6 Range for control hybrids planted in Monsanto Company field trials conducted between 1993 and 1995.
7 Range denotes the lowest and highest individual value across sites for each hybrid.
8 Statistically and significantly different from the control at the 5% level ($p<0.05$).

Table III.6. Amino acid composition of grain from corn event MON863 (George *et al.*, 2004).

Amino Acid[3]	1999 U.S. Trials[1]			1999 Argentina Trials[2]			Literature Range	Historical Range[7]
	MON863 Mean (Range)[8]	**Control[4] Mean (Range)[8]**	**Comm. Hybrids[5] Tolerance Interval[6]**	**MON863 Mean (Range)[8]**	**Control[4] Mean (Range)[8]**	**Comm. Hybrids[5] Tolerance Interval[6]**		
Alanine	7.74 (7.65 - 7.85)	7.79 (7.46 - 7.98)	6.94, 8.46	7.74 (7.47 - 7.98)	7.84 (7.46 - 8.06)	7.09, 8.31	6.4 - 9.9	7.2 - 8.8
Arginine	4.43[9] (4.21 - 4.68)	4.33 (4.09 - 4.63)	3.38, 5.22	4.24 (3.14 - 4.87)	4.24 (3.49 - 5.33)	3.00, 5.75	2.9 - 5.9	3.5 - 5.0
Aspartic acid	6.51 (6.38 - 6.72)	6.45 (6.30 - 6.67)	5.54, 7.65	6.71 (6.25 - 7.22)	6.60 (6.30 - 6.99)	5.60, 7.68	5.8 - 7.2	6.3 - 7.5
Cysteine/Cystine	2.20[9] (1.98 - 2.40)	2.09 (1.99 - 2.29)	1.59, 2.65	2.22 (2.11 - 2.33)	2.20 (1.98 - 2.30)	1.31, 3.02	1.2 - 1.6	1.8 - 2.7
Glutamic acid	19.39 (18.99 - 19.91)	19.56 (18.97 - 20.26)	17.55, 21.25	18.97 (18.36 - 19.35)	19.21 (18.61 - 19.77)	15.91, 22.38	12.4 - 19.6	18.6 - 22.8
Glycine	3.60 (3.45 - 3.74)	3.53 (3.32 - 3.72)	2.81, 4.46	3.78 (3.59 - 4.01)	3.71 (3.58 - 3.89)	2.29, 5.26	2.6 - 4.7	3.2 - 4.2
Histidine	2.84 (2.70 - 2.95)	2.83 (2.72 - 2.94)	2.37, 3.35	3.02 (2.85 - 3.19)	2.99 (2.79 - 3.21)	2.22, 3.71	2.0 - 2.8	2.8 - 3.4
Isoleucine	3.67 (3.45 - 3.89)	3.74 (3.61 - 3.87)	3.20, 4.17	3.73 (3.54 - 3.91)	3.71 (3.55 - 3.88)	3.18, 4.13	2.6 - 4.0	3.2 - 4.3
Leucine	13.36[9] (12.88 - 13.65)	13.65 (13.27 - 14.17)	11.30, 15.63	12.90 (12.14 - 13.35)	12.99 (12.59 - 13.44)	9.76, 16.17	7.8 - 15.2	12.0 - 15.8
Lysine	2.92 (2.65 - 3.26)	2.88 (2.67 - 3.08)	1.87, 3.89	3.01 (2.69 - 3.40)	2.93 (2.68 - 3.21)	1.79, 4.28	2.0 - 3.8	2.6 - 3.5
Methionine	2.28 (1.89 - 2.49)	2.24 (1.96 - 2.58)	1.34, 2.74	2.01 (1.77 - 2.17)	2.08 (1.89 - 2.38)	1.03, 3.01	1.0 - 2.1	1.3 - 2.6
Phenylalanine	4.99 (4.93 - 5.06)	5.04 (4.95 - 5.23)	4.53, 5.66	5.03 (4.88 - 5.18)	5.02 (4.92 - 5.15)	4.25, 5.75	2.9 - 5.7	4.9 - 6.1
Proline	8.73 (8.30 - 9.21)	8.78 (8.60 - 9.05)	8.04, 10.35	9.35[9] (8.86 - 9.82)	9.68 (9.17 - 10.56)	8.47, 10.48	6.6 - 10.3	8.7 - 10.1
Serine	4.70 (3.93 - 5.09)	4.67 (4.20 - 4.94)	3.76, 5.69	4.93 (4.62 - 5.26)	4.92 (4.56 - 5.29)	4.11, 5.52	4.2 - 5.5	4.9 - 6.0
Threonine	3.41 (3.16 - 3.60)	3.36 (3.16 - 3.49)	2.93, 3.83	3.32 (2.76 - 3.60)	3.31 (2.87 - 3.61)	2.87, 3.99	2.9 - 3.9	3.3 - 4.2
Tryptophan	0.66 (0.60 - 0.83)	0.65 (0.60 - 0.68)	0.37, 0.90	0.56 (0.51 - 0.61)	0.58 (0.51 - 0.66)	0.23, 0.94	0.5 - 1.2	0.4 - 1.0
Tyrosine	3.63 (3.33 - 3.77)	3.48 (2.71 - 3.82)	2.15, 4.65	3.45 (2.81 - 3.66)	3.00 (1.93 - 3.66)	2.38, 4.19	2.9 - 4.7	3.7 - 4.3
Valine	4.94 (4.71 - 5.13)	4.94 (4.64 - 5.12)	4.15, 5.63	5.03 (4.82 - 5.19)	4.98 (4.77 - 5.16)	4.49, 5.47	2.1 - 5.2	4.2 - 5.3

1 Data from four replicated U.S. sites.
2 Data from four replicated sites in Argentina.
3 Values are percent of total protein.
4 Nontransgenic control.
5 Commercial hybrids planted at each trial site. The commercial hybrids for Argentina also included six hybrids grown in the E.U. during 1999.
6 Tolerance interval is specified to contain 99% of the commercial line population; negative limits are set to zero.
7 Range for nontransgenic control hybrids planted in Monsanto Company field trials conducted between 1993 and 1995; values are percent of total protein.
8 Range denotes the lowest and highest individual value across sites for each line.
9 Statistically and significantly different from the control at the 5% level ($p<0.05$).

Table III.7. Combined site statistical comparison of fiber and proximate content in MON863 corn and control grain (FSANZ, 2003).

Constituent	MON863[1] Mean ± S.E.[2] (Range)	Control[1] Mean ± S.E. (Range)	Difference (MON863 minus Control)			Comm. Range[4] (95% T.I.[5] Lower, Upper)	Literature Range	Historical Range[6]
			Mean ± S.E. (Range)	p-Value	95% C.I.[3] (Lower, Upper)			
Ash (% DW)	1.35 ± 0.12 (0.84 – 1.71)	1.41 ± 0.12 (0.89 – 1.89)	-0.064 ± 0.047 (-0.45 – 0.31)	0.196	-0.17, 0.037	0.62 – 1.53 (0.26, 2.06)	1.1 – 3.9	1.2 – 1.8
Carbohydrates (% DW)	83.30 ± 0.56 (81.83 – 85.00)	82.76 ± 0.56 (80.70 – 84.80)	0.54 ± 0.27 (-0.78 – 2.43)	0.138	-0.32, 1.40	82.51 – 87.84 (78.97, 90.36)	NA	81.7 – 86.3
ADF (% DW)	4.45 ± 0.15 (3.49 – 5.23)	4.50 ± 0.15 (3.62 – 5.89)	-0.050 ± 0.18 (-1.77 – 1.16)	0.778	-0.43, 0.33	3.65 – 6.09 (1.98, 6.62)	3.3 – 4.3	3.1 – 5.3
NDF (% DW)	11.64 ± 0.54 (9.21 – 13.47)	12.02 ± 0.54 (10.31 – 15.82)	-0.37 ± 0.61 (-4.32 – 2.30)	0.585	-2.33, 1.58	9.50 – 14.95 (6.51, 16.28)	8.3 – 11.9	9.6 – 15.3
Moisture (% FW)	10.03 ± 0.50 (8.54 – 11.20)	10.23 ± 0.50 (8.60 – 11.40)	-0.20 ± 0.13 (-0.90 – 0.26)	0.216	-0.61, 0.21	8.75 – 15.70 (5.09, 18.62)	7 – 23	9.4 – 15.8
Total fat (% DW)	3.77 ± 0.20 (3.00 – 4.56)	3.64 ± 0.20 (3.02 – 4.29)	0.13 ± 0.18 (-0.77 – 1.02)	0.520	-0.44, 0.70	2.18 – 3.86 (1.68, 4.64)	3.1 – 5.7, 2.9 – 6.1	2.4 – 4.2
Protein (% DW)	11.60 ± 0.48 (10.43 – 12.82)	12.19 ± 0.48 (10.45 – 13.80)	-0.59 ± 0.22 (-1.52 – 0.12)	0.071	-1.28, 0.097	7.95 – 13.83 (5.47, 16.57)	6.0 – 12.0, 9.7 – 16.1	9.0 – 13.6

1 MON863 and Control mean values are for 16 replicates collected from 4 sites.
2 S.E. = standard error of the mean
3 C.I. = confidence interval
4 Comm. Range = the range of sample values for commercial hybrids grown at the same field sites
5 T.I. = tolerance interval, specified to contain 95% of the commercial line population
6 Historical range for control lines refers to data collected on Monsanto field trials conducted between 1993 and 1995.

Table III.8. Combined site statistical comparison of amino acid levels in MON863 and control grain (FSANZ, 2003).

Constituent	MON863[1] Mean ± S.E.[2] (Range)	Control[1] Mean ± S.E. (Range)	Difference (MON863 minus Control)			Comm. Range[4] (95% T.I.[5] Lower, Upper)	Literature Range	Historical Range[6]
			Mean ± S.E. (Range)	p-Value	95% C.I.[3] (Lower, Upper)			
Alanine	7.74 ± 0.032 (7.65 – 7.85)	7.79 ± 0.032 (7.46 – 7.98)	-0.045 ± 0.031 (-0.23 – 0.24)	0.247	-0.14, 0.055	7.30 – 8.06 (6.94, 8.46)	6.4 – 9.9	7.2 – 8.8
Arginine	4.43 ± 0.062 (4.21 – 4.68)	4.33 ± 0.062 (4.09 – 4.63)	0.10 ± 0.044 (-0.16 – 0.51)	0.030	-0.0099, 0.19	3.86 – 4.83 (3.38, 5.22)	2.9 – 5.9	3.5 – 5.0
Aspartic acid	6.51 ± 0.053 (6.38 – 6.72)	6.45 ± 0.053 (6.30 – 6.67)	0.061 ± 0.021 (-0.11 – 0.23)	0.064	-0.0070, 0.13	6.05 – 7.14 (5.54, 7.65)	5.8 – 7.2	6.3 – 7.5
Cystine	2.20 ± 0.027 (1.98 – 2.40)	2.09 ± 0.027 (1.99 – 2.29)	0.11 ± 0.029 (-0.15 – 0.39)	<0.001	0.054, 0.17	1.84 – 2.35 (1.59, 2.65)	1.2 – 1.6	1.8 – 2.7
Glutamic acid	19.39 ± 0.16 (18.99 – 19.91)	19.56 ± 0.16 (18.97 – 20.26)	-0.17 ± 0.090 (-0.76 – 0.24)	0.157	-0.46, 0.12	18.31 ± 20.25 (17.55, 21.25)	12.4 – 19.6	18.6 – 22.8
Glycine	3.60 ± 0.048 (3.45 – 3.74)	3.53 ± 0.048 (3.32 – 3.72)	0.072 ± 0.030 (-0.075 – 0.31)	0.100	-0.025, 0.17	3.20 ± 4.13 (2.81, 4.46)	2.6 – 4.7	3.2 – 4.2
Histidine	2.84 ± 0.032 (2.70 – 2.95)	2.83 ± 0.032 (2.72 – 2.94)	0.011 ± 0.023 (-0.082 – 0.24)	0.665	-0.063, 0.085	2.60 – 3.20 (2.37, 3.35)	2.0 – 2.8	2.8 – 3.4
Isoleucine	3.67 ± 0.033 (3.45 – 3.89)	3.74 ± 0.033 (3.61 – 3.87)	-0.064 ± 0.033 (-0.33 – 0.15)	0.072	-0.13, 0.0065	3.47 – 3.94 (3.20, 4.17)	2.6 – 4.0	3.2 – 4.3
Leucine	13.36 ± 0.081 (12.88 – 13.65)	13.65 ± 0.081 (13.27 – 14.17)	-0.29 ± 0.084 (-0.75 – 0.13)	0.039	-0.56, -0.026	11.94 – 14.47 (11.30, 15.63)	7.8 – 15.2	12.0 – 15.8
Lysine	2.92 ± 0.061 (2.65 – 3.26)	2.88 ± 0.061 (2.67 – 3.08)	0.042 ± 0.036 (-0.19 – 0.32)	0.328	-0.073, 0.16	2.40 – 3.52 (1.87, 3.89)	2.0 – 3.8	2.6 – 3.5
Methionine	2.28 ± 0.060 (1.89 – 2.49)	2.24 ± 0.060 (1.96 – 2.58)	0.034 ± 0.035 (-0.20 – 0.25)	0.348	-0.040, 0.11	1.61 – 2.29 (1.34, 2.74)	1.0 – 2.1	1.3 – 2.6
Phenylalanine	4.99 ± 0.015 (4.93 – 5.06)	5.04 ± 0.015 (4.95 – 5.23)	-0.048 ± 0.017 (-0.17 – 0.041)	0.052	-0.096, 0.0010	4.80 – 5.35 (4.53, 5.66)	2.9 – 5.7	4.9 – 6.1
Proline	8.73 ± 0.054 (8.30 – 9.21)	8.78 ± 0.054 (8.60 – 9.05)	-0.052 ± 0.046 (-0.32 – 0.38)	0.267	-0.15, 0.045	8.57 – 9.61 (8.04, 10.35)	6.6 – 10.3	8.7 – 10.1
Serine	4.70 ± 0.11 (3.93 – 5.09)	4.67 ± 0.11 (4.20 – 4.94)	0.031 ± 0.094 (-0.77 – 0.89)	0.743	-0.17, 0.23	4.24 – 4.99 (3.76, 5.69)	4.2 – 5.5	4.9 – 6.0
Threonine	3.41 ± 0.035 (3.16 – 3.60)	3.36 ± 0.035 (3.16 – 3.49)	0.049 ± 0.024 (-0.15 – 0.23)	0.056	-0.0016, 0.099	3.19 – 3.59 (2.93, 3.83)	2.9 – 3.9	3.3 – 4.2
Tryptophan	0.66 ± 0.015 (0.60 – 0.83)	0.65 ± 0.015 (0.60 – 0.68)	0.013 ± 0.012 (-0.043 – 0.17)	0.295	-0.013, 0.039	0.54 – 0.82 (0.37, 0.90)	0.5 – 1.2	0.4 – 1.0
Tyrosine	3.63 ± 0.057 (3.33 – 3.77)	3.48 ± 0.057 (2.71 – 3.82)	0.15 ± 0.078 (-0.14 – 0.92)	0.073	-0.016, 0.32	2.60 – 3.73 (2.15, 4.65)	2.9 – 4.7	3.7 – 4.3
Valine	4.94 ± 0.043 (4.71 – 5.13)	4.94 ± 0.043 (4.64 – 5.12)	-0.0091 ± 0.043 (-0.36 – 0.50)	0.833	-0.097, 0.079	4.49 – 5.30 (4.15, 5.63)	2.1 – 5.2	4.2 – 5.3

[1] MON863 and Control mean values are for 16 replicates collected from 4 sites.

[2] S.E. = standard error of the mean

[3] C.I. = confidence interval

[4] Comm. Range = the range of sample values for commercial hybrids grown at the same field sites

[5] T.I. = tolerance interval, specified to contain 95% of the commercial line population

[6] Historical range for control lines refers to data collected on Monsanto field trials conducted between 1993 and 1995.

Table III.9. Combined site statistical comparison of fatty acid levels in MON863 and control grain (FSANZ, 2003).

Constituent	**MON863[1] Mean ± S.E.[2] (Range)**	**Control[1] Mean ± S.E. (Range)**	**Difference (MON863 minus Control)**			**Comm. Range[4] (95% T.I.[5] Lower, Upper)**	**Literature Range**	**Historical Range[6]**
			Mean ± S.E. (Range)	**p-Value**	**95% C.I.[3] (Lower, Upper)**			
16:0 palmitic	12.01 ± 0.11 (11.61 – 12.56)	11.88 ± 0.11 (11.66 – 12.20)	0.12 ± 0.11 (-0.21 – 0.79)	0.337	-0.22, 0.47	9.07 – 12.14 (7.74, 13.87)	7 – 19	9.9 – 12.0
18:0 stearic	1.66 ± 0.083 (1.40 – 1.86)	1.66 ± 0.083 (1.33 – 1.81)	0.0044 ± 0.013 (-0.087 – 0.078)	0.738	-0.023, 0.032	1.44 – 2.40 (1.04, 2.68)	1 – 3	1.4 – 2.2
18:1 oleic	22.00 ± 0.36 (20.97 – 23.55)	21.87 ± 0.36 (21.00 – 22.53)	0.13 ± 0.12 (-0.16 – 1.05)	0.365	-0.26, 0.52	21.26 – 32.06 (13.28, 36.31)	20 – 46	20.6 – 27.5
18:2 linoleic	62.23 ± 0.38 (60.02 – 63.21)	62.47 ± 0.38 (61.55 – 63.60)	-0.23 ± 0.18 (-1.83 – 0.32)	0.293	-0.81, 0.35	54.15 – 63.64 (50.21, 70.86)	35 – 70	55.9 – 66.1
18:3 linolenic	1.20 ± 0.020 (1.13 – 1.29)	1.24 ± 0.020 (1.09 – 1.45)	-0.037 ± 0.021 (-0.30 – 0.071)	0.079	-0.080, 0.0047	0.97 – 1.36 (0.75, 1.51)	0.8 – 2	0.8 – 1.1
20:0 arachidic	0.41 ± 0.0068 (0.39 – 0.44)	0.40 ± 0.0068 (0.39 – 0.42)	0.0052 ± 0.0062 (-0.017 – 0.027)	0.460	-0.014, 0.025	0.35 – 0.45 (0.30, 0.51)	0.1 – 2	0.3 – 0.5
20:1 eicosenoic	0.30 ± 0.011 (0.28 – 0.35)	0.30 ± 0.011 (0.28 – 0.35)	0.0011 ± 0.0037 (-0.039 – 0.040)	0.783	-0.011, 0.013	0.25 – 0.39 (0.18, 0.42)	NA	0.2 – 0.3
22:0 behenic	0.18 ± 0.0068 (0.17 – 0.21)	0.18 ± 0.0068 (0.15 – 0.21)	0.0043 ± 0.0056 (-0.023 – 0.029)	0.498	-0.013, 0.222	0.089 – 0.21 (0.055, 0.30)	NA	0.1 – 0.3

[1] MON863 and Control mean values are for 16 replicates collected from 4 sites.
[2] S.E. = standard error of the mean
[3] C.I. = confidence interval
[4] Comm. Range = the range of sample values for commercial hybrids grown at the same field sites
[5] T.I. = tolerance interval, specified to contain 95% of the commercial line population
[6] Historical range for control lines refers to data collected on Monsanto field trials conducted between 1993 and 1995.

Table III.10. Combined site statistical comparison of mineral, vitamin, and anti-nutritive levels in MON863 and control grain (FSANZ, 2003).

Constituent	MON863[1] Mean ± S.E.[2] (Range)	Control[1] Mean ± S.E. (Range)	Difference (MON863 minus Control)			Comm. Range[4] (95% T.I.[5] Lower, Upper)	Literature Range	Historical Range[6]
			Mean ± S.E. (Range)	p-Value	95% C.I.[3] (Lower, Upper)			
Calcium (% DW)	0.0052 ± 0.00041 (0.0041 – 0.0064)	0.0053 ± 0.00041 (0.0043 – 0.0089)	-0.00013 ± 0.00020 (-0.0027 – 0.00081)	0.538	-0.00056, 0.00031	0.0039 – 0.0060 (0.0022, 0.0073)	0.01 – 0.1	0.003 – 0.006
Copper (mg/kg DW)	2.26 ± 0.17 (1.72 – 3.18)	2.19 ± 0.17 (1.60 – 2.88)	0.078 ± 0.076 (-0.58 – 1.10)	0.315	-0.078, 0.23	1.03 – 2.15 (0.25, 2.70)	0.9 – 10	NA
Iron (mg/kg DW)	23.55 ± 1.16 (21.13 – 26.36)	24.18 ± 1.16 (20.57 – 28.16)	-0.63 ± 0.80 (-3.92 – 1.83)	0.490	-3.18, 1.92	16.74 – 28.69 (12.52, 35.06)	1 – 100	NA
Magnesium (% DW)	0.13 ± 0.0034 (0.12 – 0.14)	0.14 ± 0.0034 (0.12 – 0.16)	-0.0049 ± 0.0024 (-0.018 – 0.0049)	0.135	-0.013, 0.0028	0.091 – 0.14 (0.082, 0.17)	0.09 – 1.0	NA
Manganese (mg/kg DW)	0.13 ± 0.0034 (0.12 – 0.14)	0.14 ± 0.0034 (0.12 – 0.16)	-0.0049 ± 0.0024 (-0.018 – 0.0049)	0.122	-0.84, 0.17	0.091 – 0.14 (0.082, 0.17)	0.7 – 54	NA
Phosphorus (% DW)	0.4 ± 0.0068 (0.37 – 0.45)	0.42 ± 0.0068 (0.39 – 0.46)	-0.022 ± 0.0094 (-0.070 – 0.019)	0.065	-0.045, 0.0020	0.27 – 0.41 (0.21, 0.47)	0.26 – 0.75	0.288 – 0.363
Potassium (% DW)	0.43 ± 0.0088 (0.40 – 0.48)	0.44 ± 0.0088 (0.39 – 0.48)	-0.0074 ± 0.0087 (-0.056 – 0.037)	0.457	-0.035, 0.020	0.33 – 0.43 (0.28, 0.48)	0.32 – 0.72	NA
Zinc (mg/kg DW)	22.15 ± 1.44 (17.95 – 25.25)	23.68 ± 1.44 (18.77 – 28.14)	-1.53 ± 0.69 (-4.60 – 0.90)	0.112	-3.73, 0.66	12.84 – 31.22 (6.31, 37.95)	12 – 30	NA
Vitamin E (mg/g DW)	0.011 ± 0.0012 (0.0062 – 0.014)	0.013 ± 0.0012 (0.0088 – 0.016)	-0.0015 ± 0.00047 (-0.0077 – 0.00090)	0.002	-0.0025, -0.00058	0.0041 – 0.014 (0, 0.019)	0.017 – 0.047	0.008 – 0.015
Phytic Acid (% DW)	1.11 ± 0.033 (0.92 – 1.28)	1.23 ± 0.033 (1.01 – 1.37)	-0.12 ± 0.034 (-0.31 – 0.19)	0.001	-0.91, -0.050	0.73 – 1.17 (0.39, 1.33)	To 0.9%	NA
Trypsin Inhibitor (TIU/mg DW)	2.30 ± 0.16 (0.56 – 3.10)	2.48 ± 0.16 (1.91 – 3.45)	-0.18 ± 0.16 (-1.70 – 0.63)	0.288	-0.53, 0.17	0.58 – 3.05 (0, 4.25)	NA	NA

[1] MON863 and Control mean values are for 16 replicates collected from 4 sites.
[2] S.E. = standard error of the mean
[3] C.I. = confidence interval
[4] Comm. Range = the range of sample values for commercial hybrids grown at the same field sites
[5] T.I. = tolerance interval, specified to contain 95% of the commercial line population
[6] Historical range for control lines refers to data collected on Monsanto field trials conducted between 1993 and 1995.

Table III.11. Combined site statistical comparison of fiber and proximate content in MON863 corn and control forage (FSANZ, 2003).

Constituent	MON863[1] Mean ± S.E.[2] (Range)	Control[1] Mean ± S.E. (Range)	Difference (MON863 minus Control)			Comm. Range[4] (95% T.I.[5] Lower, Upper)	Literature Range
			Mean ± S.E. (Range)	p-Value	95% C.I.[3] (Lower, Upper)		
Ash (% DW)	4.73 ± 0.22 (3.62 – 5.65)	5.00 ± 0.22 (3.81 – 6.27)	-0.27 ± 0.16 (-1.29 – 1.09)	0.106	-0.61, 0.066	3.74 – 5.02 (3.04, 5.58)	2.9 – 5.1
Carbohydrates (% DW)	84.24 ± 0.53 (82.29 – 86.32)	84.32 ± 0.53 (80.78 – 87.21)	-0.084 ± 0.43 (-2.70 – 2.52)	0.859	-1.47, 1.30	82.59 – 87.10 (81.22, 88.97)	84.6 – 89.1
ADF (% DW)	28.67 ± 1.66 (21.74 – 43.30)	28.41 ± 1.66 (23.39 – 32.08)	0.26 ± 2.06 (-7.90 – 14.03)	0.907	-6.29, 6.81	19.78 – 39.00 (9.33, 45.44	21.4 – 29.2
NDF (% DW)	43.25 ± 1.26 (37.97 – 49.67)	42.94 ± 1.26 (37.32 – 51.85)	0.31 ± 1.25 (-10.81 – 12.34)	0.807	-2.25, 2.87	30.30 – 47.75 (22.71, 56.02)	39.9 – 46.6
Moisture (% FW)	71.09 ± 0.46 (69.30 – 73.10)	71.68 ± 0.46 (69.80 – 74.50)	-0.58 ± 0.43 (-3.70 – 2.90)	0.269	-1.95, 0.79	67.00 – 74.10 (62.70, 77.69)	68.7 – 73.5
Total fat (% DW)	2.40 ± 0.23 (0.92 – 3.16)	2.35 ± 0.23 (1.30 – 3.33)	0.053 ± 0.15 (-0.91 – 1.14)	0.721	-0.26, 0.36	1.39 – 2.62 (1.03, 3.24)	1.4 – 2.1
Protein (% DW)	8.62 ± 0.53 (6.91 – 10.40)	8.33 ± 0.53 5.99 – 10.55)	0.30 ± 0.37 (-2.54 – 2.42)	0.478	-0.87, 1.47	6.45 – 10.14 (4.94, 11.97)	4.8 – 8.4

1 MON863 and Control mean values are for 16 replicates collected from 4 sites.
2 S.E. = standard error of the mean
3 C.I. = confidence interval
4 Comm. Range = the range of sample values for commercial hybrids grown at the same field sites
5 T.I. = tolerance interval, specified to contain 95% of the commercial line population
6 Historical range for control lines refers to data collected on Monsanto field trials conducted between 1993 and 1995.

Table III.12. Summary of the statistical differences for the comparison of MON88017 grain to control corn, grown at three different trial sites (FSANZ, 2006; USDA, 2004).

Tissue/Site/Component (Units)	Mean MON8807	Mean Control	Mean Difference (% of Control Value)	Significance (p-Value)	MON88017 (Range)	99% Tolerance Interval
Iowa						
16:0 palmitic (% total fatty acids)	10.16	12.94	-21.50	0.029	(10.11 – 10.23)	[6.51, 16.50]
18:2 linoleic (% total fatty acids)	63.25	60.41	4.70	0.017	(62.73 – 63.72)	[41.22, 74.09]
18:3 linolenic (% total fatty acids)	1.25	1.57	-20.26	0.036	(1.24 – 1.26)	[0.42, 1.95]
Methionine (% total amino acids)	2.20	2.16	-6.39	<0.001	(1.96 – 2.05)	[1.37, 2.60]
Moisture (% fresh weight)	9.38	9.93	-5.54	0.034	(9.03 – 9.70)	[4.67, 17.56]
Vitamin B1 (mg/kg dry weight)	2.54	3.07	-17.37	<0.001	(2.42 – 2.65)	[1.96, 4.38]
Illinois						
18:1 oleic (% total fatty acids)	22.53	23.29	-3.26	<0.001	(22.50 – 22.56)	[9.25, 44.14]
18:2 linoleic (% total fatty acids)	63.11	62.15	1.55	0.003	(62.84 – 63.29)	[41.22, 74.09]
Niacin (mg/kg dry weight)	21.10	22.52	-6.30	0.014	(20.39 – 21.52)	[3.19, 34.49]
Vitamin B1 (mg/kg dry weight)	2.30	3.10	-25.63	<0.001	(2.30 – 2.30)	[1.96, 4.38]
Nebraska						
Copper (mg/kg dry weight)	1.57	2.21	-28.80	0.023	(1.48 – 1.68)	[0.17, 3.00]
Serine (% total amino acids)	4.80	4.97	-3.37	0.042	(4.80 – 4.81)	[4.60, 5.43]
Vitamin B1	2.58	3.56	-27.53	<0.001	(2.47 – 2.69)	[1.96, 4.38]
All Sites Combined						
18:2 linoleic (% total fatty acids)	62.85	61.52	2.17	0.038	(61.86 – 63.72)	[41.22, 74.09]
20:0 arachidic (% total fatty acids)	0.37	0.38	-2.24	0.012	(0.35 – 0.39)	[0.31, 0.49]
Vitamin B1 (mg/kg dry weight)	2.47	3.24	-23.72	<0.001	(2.30 – 2.69)	[1.96, 4.38]

Table III.13. Comparison of proximates, fiber, and mineral content in forage from MON88017 and conventional corn for combined field sites (USDA, 2004).

Component (Units)[1]	**MON88017 Mean ± S.E. (Range)**	**Control Mean ± S.E. (Range)**	**Difference (MON 88017 Minus Control)**			**Commercial (Range) [99% T.I.]**[2]
			Mean ± S.E. (Range)	**95% C.I. (Lower, Upper)**	**p-Value**	
Ash (% dwt)	3.99 ± 0.24 (3.30 - 5.53)	4.04 ± 0.24 (3.59 - 4.67)	-0.051 ± 0.28 (-1.37 – 1.55)	-0.74,0.64	0.861	(2.62 - 6.78) [0.72,7.42]
Carbohydrates (% dwt)	86.19 ± 0.62 (83.54 - 87.88)	86.48 ± 0.62 (84.43 - 87.71)	-0.29 ± 0.40 (-2.58 – 1.73)	-1.11,0.54	0.478	(81.86 - 89.90) [78.70,93.43]
Fat, total (% dwt)	1.61 ± 0.29 (0.80 - 3.13)	1.65 ± 0.29 (0.83 - 2.97)	-0.039 ± 0.25 (-1.47 – 1.99)	-0.56,0.48	0.878	(0.69 - 2.92) [0.80,2.95]
Moisture (% fwt)	70.86 ± 0.66 (68.50 - 72.70)	70.66 ± 0.66 (69.10 - 72.70)	0.20 ± 0.39 (-1.40 – 1.90)	-0.61,1.01	0.615	(65.20 - 78.60) [59.37,80.83]
Protein (% dwt)	8.20 ± 0.31 (7.44 - 8.97)	7.82 ± 0.31 (6.79 - 8.54)	0.38 ± 0.25 (-0.99 – 1.65)	-0.13,0.88	0.137	(6.31 - 9.96) [4.17,11.81]
ADF (% dwt)	26.54 ± 1.25 (24.29 - 29.97)	25.45 ± 1.25 (23.34 - 28.13)	1.10 ± 1.76 (-2.58 – 4.08)	-2.97,5.16	0.549	(19.16 - 35.55) [13.95,38.96]
NDF (% dwt)	37.34 ± 1.22 (33.44 - 45.05)	38.33 ± 1.22 (35.86 - 41.18)	-0.99 ± 1.42 (-4.63 – 6.97)	-3.90,1.91	0.490	(30.27 - 57.93) [23.80,54.73]
Calcium (% dwt)	0.22 ± 0.014 (0.19 - 0.26)	0.23 ± 0.014 (0.18 - 0.31)	-0.0092 ± 0.014 (-0.054 – 0.024)	-0.044,0.026	0.542	(0.13 - 0.32) [0.11,0.32]
Phosphorus (% dwt)	0.25 ± 0.011 (0.21 - 0.30)	0.25 ± 0.011 (0.20 - 0.30)	0.0017 ± 0.013 (-0.060 - 0.079)	-0.029,0.032	0.899	(0.16 - 0.31) [0.095,0.38]

[1] dwt = dry weight; ADF = acid detergent fiber; NDF = neutral detergent fiber; S.E. = standard error of the mean; C.I. = confidence interval; T.I. = tolerance interval

[2] With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table III.14. Comparison of the amino acid content[1] in grain from MON88017 and conventional corn for combined field sites (FSANZ, 2006; USDA, 2004).

Component	MON88017 Mean ± S.E.[2] (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.][3]
			Mean ± S.E. (Range)	95% C.I. (Lower, Upper)	p-Value	
Alanine	7.55 ± 0.084 (7.29 - 7.70)	7.55 ± 0.084 (7.34 - 7.79)	-0.0026 ± 0.039 (-0.19 - 0.18)	-0.097, 0.092	0.949	(7.24 - 8.16) [6.66,8.49]
Arginine	4.42 ± 0.11 (4.10 - 4.74)	4.29 ± 0.11 (4.01 - 4.63)	0.13 ± 0.060 (-0.12 - 0.36)	-0.013, 0.28	0.066	(3.72 - 5.08) [3.34,5.67]
Aspartic acid	6.22 ± 0.050 (6.09 - 6.34)	6.25 ± 0.050 (6.04 - 6.45)	-0.032 ± 0.067 (-0.34 - 0.18)	-0.20, 0.13	0.648	(6.18 - 6.81) [5.77,7.16]
Cystine	2.14 ± 0.054 (1.93 - 2.26)	2.15 ± 0.054 (1.93 - 2.30)	-0.013 ± 0.042 (-0.20 - 0.17)	-0.098,0.073	0.766	(1.82 - 2.58) [1.46,2.89]
Glutamic acid	20.40 ± 0.18 (19.80 - 20.87)	20.44 ± 0.18 (19.91 - 20.84)	-0.036 ± 0.086 (-0.52 - 0.48)	-0.25, 0.17	0.686	(19.46 - 21.57) [18.01,22.15]
Glycine	3.45 ± 0.063 (3.32 - 3.62)	3.45 ± 0.063 (3.18 - 3.61)	0.0061 ± 0.031 (-0.081 - 0.19)	-0.058, 0.070	0.844	(3.29 - 4.03) [2.81,4.54]
Histidine	2.99 ± 0.049 (2.90 - 3.10)	2.95 ± 0.049 (2.83 - 3.14)	0.032 ± 0.022 (-0.056 - 0.10)	-0.023, 0.087	0.200	(2.50 - 3.12) [2.16,3.60]
Isoleucine	3.59 ± 0.037 (3.43 - 3.71)	3.57 ± 0.037 (3.45 - 3.76)	0.025 ± 0.044 (-0.15 - 0.25)	-0.065, 0.11	0.577	(3.39 - 3.79) [3.30,3.84]
Leucine	13.28 ± 0.20 (12.69 - 13.62)	13.31 ± 0.20 (12.76 - 14.11)	-0.037 ± 0.098 (-0.69 - 0.56)	-0.28, 0.20	0.717	(12.11 - 14.35) [10.72,15.18]
Lysine	2.69 ± 0.058 (2.42 - 2.87)	2.66 ± 0.058 (2.49 - 2.82)	0.024 ± 0.047 (-0.072 - 0.11)	-0.074, 0.12	0.614	(2.44 - 3.27) [2.06,3.73]
Methionine	1.98 ± 0.059 (1.85 - 2.05)	2.01 ± 0.059 (1.83 - 2.20)	-0.030 ± 0.043 (-0.15 - 0.12)	-0.14, 0.076	0.515	(1.70 - 2.47) [1.37,2.60]
Phenylalanine	5.18 ± 0.059 (4.97 - 5.31)	5.14 ± 0.059 (5.01 - 5.32)	0.035 ± 0.055 (-0.13 - 0.25)	-0.10, 0.17	0.545	(4.82 - 5.39) [4.57,5.71]
Proline	9.39 ± 0.094 (9.02 - 9.69)	9.34 ± 0.094 (8.85 - 9.80)	0.046 ± 0.11 (-0.61 - 0.71)	-0.18, 0.27	0.676	(8.35 - 9.72) [7.60,10.37]
Threonine	3.22 ± 0.040 (3.10 - 3.38)	3.25 ± 0.040 (3.06 - 3.37)	-0.026 ± 0.045 (-0.25 - 0.24)	-0.12, 0.067	0.572	(2.96 - 3.55) [2.89,3.84]
Tryptophan	0.54 ± 0.027 (0.48 - 0.60)	0.55 ± 0.027 (0.41 - 0.68)	-0.0090 ± 0.018 (-0.17 - 0.096)	-0.046, 0.028	0.627	(0.44 - 0.83) [0.36,0.77]
Tyrosine	3.35 ± 0.16 (2.35 - 3.66)	3.43 ± 0.16 (2.58 - 3.66)	-0.079 ± 0.23 (-1.18 - 0.98)	-0.61, 0.46	0.743	(2.26 - 3.80) [2.62,4.26]
Valine	4.79 ± 0.039 (4.60 - 4.92)	4.74 ± 0.039 (4.60 - 4.94)	0.043 ± 0.052 (-0.25 - 0.26)	-0.064, 0.15	0.414	(4.44 - 5.04) [4.22,5.27]

[1] % total amino acids

[2] S.E. = standard error of the mean; C.I. = confidence interval; T.I. = tolerance interval

[3] With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table III.15. Comparison of the fatty acid content[1] in grain from MON88017 and conventional corn for combined field sites (FSANZ, 2006; USDA, 2004).

Component	MON88017 Mean ± S.E.[2] (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.][3]
			Mean ± S.E. (Range)	95% C.I. (Lower, Upper)	p-Value	
16:0 palmitic (% total FA)	10.24 ± 0.43 (10.07 - 10.52)	11.27 ± 0.43 (10.14 - 14.57)	-1.03 ± 0.60 (-4.35 - 0.36)	-2.42, 0.37	0.128	(9.29 - 17.81) [6.51,16.50]
16:1 pamitoleic (% total FA)	0.18 ± 0.010 (0.16 - 0.21)	0.18 ± 0.010 (0.16 - 0.22)	-0.0030 ± 0.0064 (-0.029 - 0.025)	-0.019, 0.013	0.655	(0.054 - 0.21) [0.0017,0.28]
18:0 stearic (% total FA)	2.01 ± 0.073 (1.80 - 2.19)	2.07 ± 0.073 (1.76 - 2.23)	-0.052 ± 0.046 (-0.28 - 0.25)	-0.15, 0.042	0.266	(1.68 - 2.30) [1.41,2.53]
18:1 oleic (% total FA)	22.74 ± 0.23 (22.20 - 23.53)	22.87 ± 0.23 (21.43 - 23.51)	-0.13 ± 0.24 (-0.94 - 1.13)	-0.71, 0.46	0.613	(19.79 - 34.46) [9.25,44.14]
18:2 linoleic (% total FA)	62.85 ± 0.39 (61.86 - 63.72)	61.52 ± 0.39 (59.10 - 63.18)	1.34 ± 0.53 (-0.64 - 4.19)	0.093, 2.58	0.038	(51.64 - 64.12) [41.22,74.09]
18:3 linolenic (% total FA)	1.21 ± 0.062 (1.15 - 1.26)	1.32 ± 0.062 (1.19 - 1.77)	-0.11 ± 0.077 (-0.53 - 0.043)	-0.30, 0.079	0.205	(0.84 - 1.91) [0.42,1.95]
20:0 arachidic (% total FA)	0.37 ± 0.010 (0.35 - 0.39)	0.38 ± 0.010 (0.35 - 0.41)	-0.0085 ± 0.0032 (-0.028 - 0.0088)	-0.015, -0.0019	0.012	(0.36 - 0.45) [0.31,0.49]
20:1 eicosenoic (% total FA)	0.24 ± 0.0056 (0.23 - 0.26)	0.25 ± 0.0056 (0.24 - 0.26)	-0.0034 ± 0.0034 (-0.019 - 0.019)	-0.010, 0.0036	0.323	(0.24 - 0.36) [0.18,0.40]
22:0 behenic (% total FA)	0.15 ± 0.0027 (0.14 - 0.16)	0.15 ± 0.0027 (0.14 - 0.17)	-0.0062 ± 0.0038 (-0.018 - 0.014)	-0.014, 0.0016	0.116	(0.074 - 0.24) [0.071,0.25]

[1] 5% of total fatty acids

[2] S.E. = standard error of the mean; C.I. = confidence interval; T.I. = tolerance interval

[3] With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table III.16. Comparison of the mineral content in grain from MON88017 and conventional corn for combined field sites (FSANZ, 2006; USDA, 2004).

Component (Units)[1]	MON88017 Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.][2]
			Mean ± S.E. (Range)	95% C.I. (Lower, Upper)	p-Value	
Calcium (% dwt)	0.0054 ± 0.00035 (0.0047 - 0.0060)	0.0058 ± 0.00035 (0.0049 - 0.0069)	-0.00040 ± 0.00025 (-0.0013 - 0.00006)	-0.0010, 0.00021	0.159	(0.0032 - 0.0060) [0.0017,0.0062]
Copper (mg/kg dwt)	1.73 ± 0.086 (1.48 - 2.05)	1.99 ± 0.086 (1.64 - 2.63)	-0.26 ± 0.12 (-0.95 - 0.41)	-0.54, 0.016	0.061	(1.01 - 2.34) [0.17,3.00]
Iron (mg/kg dwt)	21.51 ± 0.59 (20.07 - 22.92)	21.84 ± 0.59 (20.31 - 23.93)	-0.33 ± 0.62 (-2.16 - 2.12)	-1.60, 0.93	0.595	(16.42 - 26.03) [12.60,31.26]
Magnesium (% dwt)	0.14 ± 0.0034 (0.13 - 0.15)	0.14 ± 0.0034 (0.13 - 0.16)	-0.0022 ± 0.0044 (-0.024 - 0.018)	-0.011, 0.0069	0.618	(0.10 - 0.14) [0.088,0.16]
Manganese (mg/kg dwt)	9.72 ± 0.38 (9.01 - 10.76)	9.37 ± 0.38 (7.55 - 10.44)	0.35 ± 0.38 (-0.39 - 1.56)	-0.57, 1.27	0.384	(4.96 - 9.81) [2.45,10.60]
Phosphorus (% dwt)	0.39 ± 0.010 (0.37 - 0.41)	0.39 ± 0.010 (0.36 - 0.43)	-0.0042 ± 0.013 (-0.052 - 0.042)	-0.032, 0.023	0.754	(0.28 - 0.41) [0.24,0.44]
Potassium (% dwt)	0.41 ± 0.012 (0.39 - 0.44)	0.42 ± 0.012 (0.38 - 0.47)	-0.0063 ± 0.012 (-0.052 - 0.037)	-0.030, 0.018	0.592	(0.29 - 0.43) [0.27,0.48]
Zinc (mg/kg dwt)	24.53 ± 0.98 (22.31 - 27.27)	24.92 ± 0.98 (22.02 - 27.18)	-0.39 ± 0.62 (-3.87 - 1.90)	-1.67, 0.89	0.534	(17.15 - 26.18) [13.42,31.37]

[1] dwt – dry weight; S.E. = standard error of the mean; C.I. = confidence interval; T.I. = tolerance interval

[2] With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table III.17. Comparison of the proximates and fiber content in grain from MON88017 and conventional corn for combined field sites (FSANZ, 2006; USDA, 2004).

Component (Units)[1]	MON88017 Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.][2]
			Mean ± S.E. (Range)	95% C.I. (Lower, Upper)	p-Value	
Ash (% dwt)	1.54 ± 0.077 (1.31 - 1.68)	1.59 ± 0.077 (1.23 - 1.97)	-0.049 ± 0.087 (-0.45 - 0.43)	-0.23, 0.13	0.573	(1.04 - 1.86) [0.94,1.73]
Carbohydrates (% dwt)	82.32 ± 0.40 (81.61 - 83.39)	82.33 ± 0.40 (80.67 - 83.62)	-0.019 ± 0.25 (-1.39 - 0.94)	-0.62, 0.58	0.940	(81.46 - 86.68) [79.39,89.67]
Fat, total (% dwt)	3.64 ± 0.13 (3.44 - 3.96)	3.79 ± 0.13 (3.53 - 4.36)	-0.16 ± 0.080 (-0.63 - 0.15)	-0.35, 0.041	0.100	(2.38 - 4.43) [0.74,6.01]
Moisture (% fwt)	11.10 ± 0.99 (9.03 - 13.20)	11.60 ± 0.99 (9.73 - 14.20)	-0.49 ± 0.35 (-1.10 - -0.10)	-1.36, 0.37	0.212	(9.15 - 14.90) [4.67,17.56]
Protein (% dwt)	12.51 ± 0.35 (11.63 - 13.00)	12.28 ± 0.35 (11.22 - 13.82)	0.23 ± 0.24 (-0.82 - 1.37)	-0.36, 0.82	0.379	(9.26 - 13.37) [6.20,15.35]
ADF (% dwt)	3.77 ± 0.16 (3.31 - 4.40)	3.54 ± 0.16 (2.97 - 4.69)	0.23 ± 0.18 (-0.62 - 1.16)	-0.13, 0.59	0.203	(2.39 - 4.89) [1.89,5.23]
NDF (% dwt)	12.44 ± 0.62 (10.99 - 13.58)	11.87 ± 0.62 (10.38 - 14.29)	0.57 ± 0.50 (-1.21 - 2.64)	-0.66, 1.79	0.299	(8.41 - 16.54) [3.51,21.65]
TDF (% dwt)	16.24 ± 0.71 (13.57 - 18.64)	15.40 ± 0.71 (13.18 - 17.84)	0.84 ± 0.96 (-2.39 - 4.19)	-1.51, 3.20	0.414	(11.80 - 23.04) [5.72,27.10]

[1] ADF = acid detergent fiber; NDF = neutral detergent fiber; TDF = total dietary fiber; S.E. = standard error of the mean; C.I. = confidence interval; T.I. = tolerance interval

[2] With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table III.18. Comparison of the vitamin content[1] in grain from MON88017 and conventional corn for combined field sites (FSANZ, 2006; USDA, 2004).

Component[2]	MON88017 Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.][3]
			Mean ± S.E. (Range)	95% C.I. (Lower, Upper)	p-Value	
Folic acid	0.48 ± 0.021 (0.38 - 0.60)	0.48 ± 0.021 (0.42 - 0.59)	0.0012 ± 0.030 (-0.074 - 0.11)	-0.072, 0.075	0.969	(0.28 - 0.61) [0.12,0.77]
Niacin	20.94 ± 1.20 (17.04 - 24.14)	21.75 ± 1.20 (19.08 - 23.92)	-0.81 ± 0.42 (-2.04 - 0.23)	-1.67, 0.050	0.063	(14.11 - 27.77) [3.19,34.49]
Vitamin B1	2.47 ± 0.14 (2.30 - 2.69)	3.24 ± 0.14 (2.99 - 3.60)	-0.77 ± 0.12 (-1.02 - -0.35)	-1.06, -0.48	<0.001	(2.69 - 3.73) [1.96,4.38]
Vitamin B2	1.10 ± 0.041 (0.98 - 1.22)	1.13 ± 0.041 (0.99 - 1.33)	-0.025 ± 0.037 (-0.17 - 0.14)	-0.12, 0.066	0.524	(0.88 - 1.32) [0.67,1.51]
Vitamin B6	7.16 ± 0.22 (6.57 - 8.06)	7.10 ± 0.22 (5.65 - 8.54)	0.063 ± 0.28 (-1.27 - 2.40)	-0.59, 0.72	0.828	(4.93 - 7.24) [4.29,7.84]
Vitamin E	14.15 ± 1.70 (6.08 - 16.93)	14.15 ± 1.70 (6.08 - 16.93)	0.070 ± 1.46 (-11.15 - 14.39)	-2.93, 3.07	0.962	(8.09 - 21.97) [0,29.69]

[1] mg/kg dry weight

[2] Vitamin B1 =Thiamine; Vitamin B2 =Riboflavin; Vitamin B6 =Pyridoxine; S.E. = standard error of the mean; C.I. = confidence interval; T.I.= tolerance interval

[3] With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table III.19. Comparison of the secondary metabolites and anti-nutrients content in grain from MON88017 and conventional corn for combined field sites (FSANZ, 2006; USDA, 2004).

Component	**MON88017 Mean ± S.E. (Range)**	**Control Mean ± S.E. (Range)**	**Difference (MON 88017 Minus Control)**			**Commercial (Range) [99% T.I.][1]**
			Mean ± S.E. (Range)	**95% C.I. (Lower, Upper)**	**p-Value**	
Ferulic acid (µg/g dwt)	2175.34 ± 46.31 (1986.75 - 2275.48)	2121.05 ± 46.31 (1927.55 - 2339.71)	54.29 ± 49.66 (-200.92 - 347.92)	-47.14, 155.72	0.283	(1717.17 - 2687.57) [1415.19,3173.90]
p-Coumaric acid (µg/g dwt)	169.26 ± 7.26 (148.45 - 215.25)	154.83 ± 7.26 (141.41 - 173.24)	14.43 ± 9.88 (-14.72 - 72.55)	-9.75, 38.61	0.194	(152.30 - 319.15) [43.13,384.34]
Phytic acid (% dwt)	0.95 ± 0.043 (0.83 - 1.05)	0.89 ± 0.043 (0.72 - 1.03)	0.058 ± 0.056 (-0.15 - 0.24)	-0.058, 0.17	0.309	(0.45 - 1.00) [0.28,1.12]
Raffinose (% dwt)	0.17 ± 0.013 (0.14 - 0.20)	0.17 ± 0.013 (0.14 - 0.23)	0.00080 ± 0.0081 (-0.035 - 0.036)	-0.019, 0.021	0.924	(0.073 - 0.22) [0,0.32]

1 With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table III.20. Fatty acid profiles from the of MON88017 and an isogenic comparator (Poerschmann, Rauschen, Langer, Augustin and Górecki, 2009).

Fatty Acid (as the methyl ester)	**MON88017 µg/g dry weight**	**Near Isogenic Comparator µg/g dry weight**
Σ FAME (C_{12-20})	2860	2765
Σ saturated FAME (C_{12-20})	2250	2140
DBI[1]	0.52	0.48
Lauric (12:0)	59	38
Myristic (14:0)	116	126
C15-branched	~25	~20
Pentadecanoic (15:0)	57	45
Palmitic (16:0)	1200	1060
Heptadecanoic (17:0)	40	33
Stearic (18:0)	545	720
Σ 18:1	410	350
Linoleic (18:2)	305	285
Linolenic (18:3)	51	38
Arachic (20:0)	39	37
3-OH-butyric	33	21
Benzoic	39	24
Malonic	67	54
Levulinic	7430	7080
Methyl maleate + Methylene succinate	205	215
Malic[2]	325	300
Aconitic	285	275

1 DBI = double bond index = [(1 * % monoen) + (2 * % dien) + (3 * % trien)]/Σ(% saturated fatty acids).

2 Sum of the enantiomers (L-isomer prevailing).

Table III.21. Nutrient composition of forage from SmartStax (MON89034 × TC1507 × MON88017 × DAS-59122-7) maize (Lundry, Burns, Nemeth and Riordan, 2013).

Component	SmartStax mean (Range)[1]	Control Mean (Range)	Commercial Hybrids (Range)[2] [99% TI][3]	Literature Range[4]
Ash (% dry weight)	3.85 (3.08 – 4.68)	4.12 (2.98 – 6.01)	(2.80 – 6.54) [0.16, 8.68]	1.527 – 9.638
Carbohydrate (% dry weight)	86.52 (84.00 – 88.57)	86.82 (84.12 – 89.13)	(83.38 – 88.33) [80.33, 92.03]	76.4 – 92.1
Protein (% dry weight)	7.69 (6.80 – 8.60)	7.20 (5.39 – 8.32)	(6.27 – 8.80) [5.01, 10.55]	3.14 – 11.57
Total fat (% dry weight)	1.94 (0.16 – 3.19)	1.86 (0.46 – 2.97)	(0.91 – 2.72) [0, 3.67]	0.296 – 4.570
ADF (% dry weight)	30.26 (24.19 – 39.07)	29.90 (24.33 – 36.05)	(25.84 – 39.37) [16.73, 47.63]	16.13 – 47.39
NDF (% dry weight)	41.68 (31.57 – 51.88)	43.56 (36.34 – 47.76)	(36.09 – 65.15) [13.81, 78.53]	20.29 – 63.71
Calcium (mg/kg dry weight)	0.19 (0.11 – 0.34)	0.20 (0.13 – 0.31)	(0.15 – 0.31) [0.0028, 0.41]	0.0714 – 0.5768
Phosphorus (mg/kg dry weight)	0.20 (0.13 – 0.24)	0.18 (0.11 – 0.23)	(0.13 – 0.24) [0.067, 0.33]	0.0936 – 0.3704

1 The mean and range of 12 values (three replicates from each of four field sites).
2 The range of 14 values for commercial hybrids grown concurrently (three hybrids from each of four field sites and two hybrids from one field site).
3 TI = tolerance interval, specified to contain 99% of the commercial hybrid population with 95% confidence; negative limits set to zero.
4 from: Crop Composition Database (CCDB). Crop Composition Database, Version 4.2; CCDB: Washington D.C., 2011.

Table III.22. Fatty acid composition of grain from SmartStax (MON89034 × TC1507 × MON88017 × DAS-59122-7) maize (Lundry *et al.*, 2013).

Component	SmartStax Mean (Range)[1]	Control Mean (Range)	Commercial Hybrids (Range)[2] [99% TI][3]	Literature Range[4]
Palmitic acid	10.64 (10.18–11.05)	10.56 (10.09–11.14)	(8.96–12.73) [6.60, 15.00]	7.94–20.71
Stearic acid	2.05 (1.96–2.28)	1.93[5] (1.90–1.98)	(1.39–2.38) [0.58, 2.89]	1.02–3.40
Oleic acid	30.40 (29.60–31.71)	31.24[5] (29.85–32.92)	(21.00–34.20) [10.72, 42.79]	17.4–40.2
Linoleic acid	55.09 (53.39–56.03)	54.53 (52.23–56.02)	(51.11–63.09) [44.51, 73.33]	36.2–66.5
Linolenic acid	1.00 (0.95–1.05)	0.96[5] (0.90–1.00)	(0.86–1.31) [0.53, 1.54]	0.57–2.25
Arachidic acid	0.42 (0.40–0.46)	0.40[5] (0.37–0.42)	(0.30–0.43) [0.23, 0.53]	0.279–0.965
Eicosenoic acid	0.26 (0.24–0.28)	0.27[5] (0.25–0.28)	(0.20–0.30) [0.13, 0.34]	0.170–1.917
Behenic acid	0.14 (0.067–0.22)	0.12 (0.064–0.22)	(0.060–0.24) [0, 0.39]	0.110–0.349

1 The mean and range of 12 values (three replicates from each of four field sites).
2 The range of 14 values for commercial hybrids grown concurrently (three replicates from each of four field sites and two replicates from one field site.)
3 TI = tolerance interval, specified to contain 99% of the commercial conventional hybrid population with 95% confidence; negative limits set to zero.
4 from: Crop Composition Database (CCDB). International Life Science Institute Crop Composition Database, Version 4.2; CCDB: Washington D.C., 2011.
5 Statistically and significantly different from the control at the 5% level (p<0.05).

Table III.23. Vitamin composition of grain from SmartStax (MON89034 × TC1507 × MON88017 × DAS-59122-7) maize (Lundry *et al.*, 2013).

Component (mg/kg dry weight)	SmartStax Mean (Range)[1]	Control Mean (Range)	Commercial Hybrids (Range)[2] [99% TI][3]	Literature Range[4]
Folic acid	0.39 (0.33–0.46)	0.36 (0.29–0.43)	(0.28–0.45) [0.15, 0.57]	0.147–01.464
Niacin	24.02 (20.11–29.35)	23.77 (19.14–27.84)	(13.88–27.09) [6.69, 34.92]	9.36–4.290
β-Carotene	1.05 (0.89–1.19)	1.02 (0.79–1.20)	(0.54–1.48) [0, 1.98]	0.19–46.81
Vitamin B1	2.33 (2.05–2.70)	2.63[5] (2.36–3.20)	(2.13–3.73) [1.24, 4.86]	1.26–40.00
Vitamin B2	1.91 (1.23–2.76)	2.30 (1.30–2.94)	(1.28–3.68) [0, 5.68]	0.50–2.36
Vitamin B6	5.80 (5.39–6.14)	5.79 (5.30–6.49)	(4.51–7.24) [2.23, 8.85]	3.68–11.32
Vitamin E	8.42 (6.57–9.97)	7.72 (6.27–8.63)	(5.95–15.52) [0, 22.92]	1.537–68.672

1 The mean and range of 12 values (three replicates from each of four field sites).

2 The range of 14 values for commercial hybrids grown concurrently (three replicates from each of four field sites and two replicates from one field site.)

3 TI = tolerance interval, specified to contain 99% of the commercial conventional hybrid population with 95% confidence; negative limits set to zero.

4 from: Crop Composition Database (CCDB). Crop Composition Database, Version 4.2; CCDB: Washington D.C., 2011.

5 Statistically and significantly different from the control at the 5% level ($p<0.05$).

Table III.24. Proximate, fiber, and mineral composition of grain from SmartStax (MON89034 × TC1507 × MON88017 × DAS-59122-7) maize (Lundry *et al.*, 2013).

Component (mg/kg dry weight)	**SmartStax Mean (Range)**[1]	**Control Mean (Range)**	**Commercial Hybrids (Range)**[2] **[99% TI]**[3]	**Literature Range**[4]
Ash	1.24 (1.08–1.36)	1.22 (1.02–1.51)	(1.07–1.53) [0.77, 1.81]	0.616–6.282
Carbohydrate	85.39 (84.45–85.96)	85.41 (84.60–86.53)	(82.35–86.70) [79.24, 90.01]	77.4–89.5
Protein	9.85 (9.22–10.62)	9.78 (9.01–10.39)	(9.21–12.80) [6.20, 15.18]	6.15–17.26
Total fat	3.52 (3.18–3.98)	3.60 (3.13–4.04)	(2.77–4.60) [1.35, 5.45]	1.742–5.900
ADF	2.93 (2.32–4.56)	2.97 (2.02–4.22)	(2.55–3.92) [1.60, 4.68]	1.82–11.34
NDF	11.68 (10.29–14.85)	11.62 (9.77–14.43)	(8.62–12.88) [6.22, 15.51]	5.59–22.64
Total dietary fiber	16.91 (13.74–21.83)	16.48 (12.33–21.89)	(12.78–20.65) [8.28, 24.21]	9.01–35.31
Calcium	37.67 (30.70–45.23)	39.67 (31.54–50.92)	(27.46–60.23) [5.86, 83.14]	12.7–208.4
Copper	2.33 (1.63–4.21)	1.93 (1.34–3.95)	(1.51–3.42) [0, 4.96]	0.73–18.50
Iron	21.11 (18.79–23.37)	21.86 (18.63–24.16)	(15.63–24.35) [11.51, 29.14]	10.42–49.07
Magnesium	1159.84 (988.75–1300.90)	1170.40 (1023.97–1282.63)	(936.10–1346.80) [659.92, 1708.83]	594.0–1940.0
Manganese	5.98 (5.14–6.46)	6.22 (5.22–7.41)	(5.50–7.15) [4.24, 8.21]	1.69–14.30
Phosphorus	2990.96 (2440.94–3438.91)	2923.70 (2596.81–3234.96)	(2522.62–3697.86) [1776.54, 4654.30]	1470.0–5330.0
Potassium	3185.16 (2800.90–3472.85)	3135.51 (2984.05–3442.07)	(2802.26–3887.01) [2003.91, 4604.37]	1810.0–6030.0
Zinc	20.81 (17.44–24.44)	22.66 (18.86–27.03)	(18.64–34.20) [10.42, 37.84]	6.5–37.2

[1] The mean and range of 12 values (three replicates from each of four field sites).

[2] The range of 14 values for commercial hybrids grown concurrently (three replicates from each of four field sites and two replicates from one field site.)

[3] TI = tolerance interval, specified to contain 99% of the commercial conventional hybrid population with 95% confidence; negative limits set to zero.

[4] from: Crop Composition Database (CCDB). Crop Composition Database, Version 4.2; CCDB: Washington D.C., 2011.

Table III.25. Amino acid composition of grain from SmartStax (MON89034 × TC1507 × MON88017 × DAS-59122-7) maize (Lundry *et al.*, 2013).

Component (mg/kg dry weight)	SmartStax Mean (Range)[1]	Control Mean (Range)	Commercial Hybrids (Range)[2] [99% TI][3]	Literature Range[4]
Alanine	0.71 (0.65–0.78)	0.72 (0.62–0.80)	(0.67–0.96) [0.44, 1.18]	0.44–1.39
Arginine	0.42 (0.38–0.46)	0.40 (0.30–0.45)	(0.38–0.54) [0.25, 0.61]	0.12–0.64
Aspartic acid	0.64 (0.58–0.69)	0.63 (0.51–0.70)	(0.60–0.82) [0.39, 0.97]	0.33–1.21
Cystine/cysteine	0.20 (0.18–0.21)	0.19 (0.15–0.21)	(0.16–0.25) [0.11, 0.30]	0.13–0.51
Glutamic acid	1.83 (1.67–1.99)	1.85 (1.60–2.03)	(1.68–2.54) [1.08, 3.07]	0.97–3.54
Glycine	0.36 (0.33–0.38)	0.35 (0.29–0.37)	(0.34–0.45) [0.24, 0.50]	0.18–0.54
Histidine	0.26 (0.24–0.28)	0.26 (0.22–0.28)	(0.25–0.35) [0.19, 0.38]	0.14–0.43
Isoleucine	0.33 (0.30–0.36)	0.33 (0.28–0.38)	(0.31–0.45) [0.20, 0.53]	0.18–0.69
Leucine	1.20 (1.09–1.31)	1.22 (1.06–1.37)	(1.11–1.73) [0.67, 2.12]	0.64–2.49
Lysine	0.29 (0.26–0.32)	0.28 (0.21–0.30)	(0.26–0.36) [0.17, 0.40]	0.17–0.67
Methionine	0.18 (0.17–0.19)	0.19 (0.17–0.20)	(0.17–0.26) [0.10, 0.30]	0.12–0.47
Phenylalanine	0.49 (0.45–0.53)	0.49 (0.41–0.55)	(0.46–0.67) [0.28, 0.83]	0.24–0.93
Proline	0.84 (0.77–0.93)	0.85 (0.72–0.96)	(0.75–1.17) [0.47, 1.41]	0.46–1.63
Serine	0.48 (0.43–0.51)	0.48 (0.43–0.52)	(0.43–0.66) [0.26, 0.80]	0.24–0.77
Threonine	0.33 (0.30–0.35)	0.33 (0.26–0.36)	(0.31–0.44) [0.20, 0.51]	0.22–0.67
Tryptophan	0.065 (0.050–0.077)	0.063 (0.054–0.075)	(0.051–0.084) [0.032, 0.10]	0.027–0.22
Tyrosine	0.31 (0.24–0.34)	0.30 (0.18–0.35)	(0.19–0.42) [0.11, 0.56]	0.10–0.64
Valine	0.45 (041–0.48)	0.45 0.37–0.49)	(0.43–0.59) [0.30, 0.68]	0.27–0.86

1 The mean and range of 12 values (three replicates from each of four field sites).

2 The range of 14 values for commercial hybrids grown concurrently (three replicates from each of four field sites and two replicates from one field site.)

3 TI = tolerance interval, specified to contain 99% of the commercial conventional hybrid population with 95% confidence; negative limits set to zero.

4 from: Crop Composition Database (CCDB). Crop Composition Database, Version 4.2; CCDB: Washington D.C., 2011.

Table III.26. Anti-nutrient and secondary metabolite composition of grain from SmartStax (MON89034 × TC1507 × MON88017 × DAS-59122-7) maize (Lundry *et al.*, 2013).

Component	SmartStax Mean (Range)[1]	Control Mean (Range)	Commercial Hybrids (Range)[2] [99% TI][3]	Literature Range[4]
Antinutrient				
Phytic acid (% dry weight)	0.73 (0.53–0.87)	0.71 (0.57–0.80)	(0.53–0.90) [0.25, 1.25]	0.111–1.570
Raffinose (% dry weight)	0.095 (0.074–0.12)	0.088 (0.028–0.12)	(0.089–0.18) [0.026, 0.23]	0.020–0.320
Secondary Metabolite				
Ferulic acid (mg/kg dry weight)	1614.21 (956.82–1974.89)	1477.59 (930.26–1874.30)	(1422.12–2085.20) [858.39, 2495.12]	291.9–3885.8
p-Coumaric acid (mg/kg dry weight)	54.49 (28.12–82.91)	45.94 (28.12–86.95)	(91.06–219.73) [0, 281.45]	53.4–576.2

1 The mean and range of 12 values (three replicates from each of four field sites).
2 The range of 14 values for commercial hybrids grown concurrently (three replicates from each of four field sites and two replicates from one field site.)
3 TI = tolerance interval, specified to contain 99% of the commercial conventional hybrid population with 95% confidence; negative limits set to zero.
4 from: Crop Composition Database (CCDB). Crop Composition Database, Version 4.2; CCDB: Washington D.C., 2011.