A Review of the Environmental Safety of Vip3Aa

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INTRODUCTION

This document provides a comprehensive review of information and data relevant to the environmental risk assessment of Vip3Aa proteins[1] and presents a summary statement about the environmental safety of Vip3Aa. All sources of information reviewed herein are publically available and include: dossiers presented to regulatory authorities; decision summaries prepared by regulatory authorities; peer reviewed literature; and product summaries prepared by product developers.

Environmental risk assessments related to the introduction of genetically engineered (GE) plants are conducted on a case-by-case basis taking into account the biology of the plant; the nature of the transgene and the protein it produces; the phenotype conferred by the transgene; the intended use of the plant; and the environment where it will be introduced (*i.e.*, the receiving environment). These assessments typically involve comparisons to an untransformed parent line or closely related isoline (NRC, 1989; OECD, 1992; CBD, 2000a, 2000b; Codex, 2003a, 2003b; EFSA, 2006). The point of these comparisons is to identify risks the GE plant might present beyond what is already accepted for similar plants in the environment, so that the consequences of such risks, if any, can be assessed.

Regulatory approvals for environmental release of GE plants producing Vip3Aa have been issued in five countries: Argentina, Brazil, Canada, Japan, and the United States. This includes two transformation events, one in maize and one in cotton.

ORIGIN AND FUNCTION OF Vip3Aa

Bacillus thuringiensis and the Vip3A Insecticidal Proteins

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. The species has been studied extensively and used commercially for many years due to its ability to synthesize proteins with pesticidal properties (Hofte and Whiteley, 1989; Schnepf *et al.*, 1998; OECD, 2007). Preparations of natural isolates of *B. thuringiensis* were first used as a commercial insecticide in France in 1938, and *B. thuringiensis* subspecies *kurstaki* has been registered with USEPA since 1961 (Kumar, Sharma, and Malik, 1996; Schnepf *et al.*, 1998; USEPA, 2001). Microbial preparations of *B. thuringiensis* are currently approved for use around the world including in Australia, Canada, the European Union, and the United States (USEPA, 2001; PMRA,

Table 1. Regulatory approvals[a] for the environmental release of GE plants containing Vip3Aa (as of 6/30/2012).

Event	OECD Unique Identifier	Argentina	Brazil	Canada	European Union	Japan	United States
COT102 (cotton)	SYN-IR102-7						2005 (USDA) 2008 (USEPA) [b]
MIR162 (maize)	SYN-IR162-4	2011	2009	2010	2012	2010	2010 (USDA) 2009 (USEPA)[c]

a. Regulations may require periodic renewal of pesticide registrations. Current status of USEPA registrations can be found at http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm.
b. In 2008, USEPA approved a tolerance exemption for a cotton line producing both a Vip3Aa protein and the Cry1Ab protein, combined via conventional breeding.
c. In 2009, USEPA approved a tolerance exemption for a corn line producing Vip3Aa20, Cry1Ab, and Cry3A, combined via conventional breeding.

1 The term "Vip3Aa" describes a family of closely related pesticidal proteins and "Vip3Aa" and "Vip3Aa proteins" are used interchangeably in this paper.

Key words

Vip3Aa, vegetative insecticidal protein, *Bacillus thuringiensis*, insect resistance, genetically engineered, environmental risk assessment

2008; AVPMA, 2012; SANCO, 2012). These preparations contain a mixture of microbial pesticides that interact extensively with each other to influence toxicity and insect specificity[2] (Schnepf *et al.*, 1998; OECD, 2007).

The pesticidal proteins produced by *B. thuringiensis* display a tremendous variety with regard to the mode of action, target specificity, and mechanism of expression (Hofte and Whiteley, 1989; Schnepf *et al.*, 1998; OECD, 2007). Pesticidal proteins expressed by *B. thuringiensis* strains include antifungal compounds, δ-exotoxins,[3] and the δ-endotoxins, which include the insecticidal Cry (crystalline) proteins and the structurally unrelated Cyt (cytolytic) proteins (Hofte and Whiteley, 1989; Schnepf *et al.*, 1998; OECD, 2007). Several hundred insecticidal proteins have been identified (Crickmore *et al.*, 2012), and some (notably δ-exotoxins and Cyt proteins) have a wide spectrum of activity (Hofte and Whiteley, 1989; Schnepf *et al.*, 1998; OECD, 2007).

In 1996, a new 88 kDa protein with insecticidal properties was isolated from *B. thuringiensis* that shared no sequence homology to the Cry proteins (Estruch *et al.*, 1996; C.-G. Yu, Mullins, Warren, Koziel, and Estruch, 1997). The Cry proteins are so named because they are stored as parasporal crystals during spore formation (OECD, 2007), but the new protein was produced by *B. thuringiensis* during its vegetative stage of growth, in addition to during sporulation, so the protein was termed "Vip," for vegetative insecticidal protein. In addition, while the Cry proteins are isolated as crystals, Vip proteins are secreted by the bacteria and can be isolated directly from the culture medium (Estruch *et al.*, 1996; Lee *et al.*, 2003; OECD, 2007). *B. thuringiensis* strains from diverse environments have been screened for Vip-mediated insecticidal activity (Fang *et al.*, 2007; Franco-Rivera *et al.*, 2004; Guttmann and Ellar, 2000; Mesrati, Tounsi, and Jaoua, 2005; X. Yu *et al.*, 2003). It has been determined that there are actually many variants of Vip, falling into three classes based on amino acid sequence similarity, and a nomenclature system has been established (Selvapandiyan *et al.*, 2001; Bhalla *et al.*, 2005; Crickmore *et al.*, 2012). The taxonomic group of Vip proteins currently used in the production of insect-resistant GE crop plants is Vip3Aa. The Vip3Aa proteins are the only members of the Vip group to date that have been developed to confer an insect control trait in transgenic plants. There is conflicting information in the literature concerning the location of *vip3Aa* genes in the *B. thuringiensis* genome; evidence exists supporting both a chromosomal location and a position on the same plasmid bearing the *cry1A* gene (Franco-Rivera *et al.*, 2004; Mesrati *et al.*, 2005; Cai *et al.*, 2006).

The Vip3Aa proteins, like the Cry1A family of proteins, are active against a broad range of lepidopteran pests. However, the range of organisms susceptible to the Vip3Aa toxin is not the same as those affected by Cry1A, and the two toxins have an additive effect. A strain bearing genes for both the Cry1 protein and Vip3Aa was found to be measurably less toxic to insects after the *Vip3Aa* gene was knocked out, and particularly less toxic to *Agrotis ipsilon* (black cutworm) and *Spodoptera exigua* (beet armyworm) (Donovan, Donovan, and Engleman, 2001). Vip3Aa insecticidal activity has been demonstrated against *Agrotis ipsilon*, *Helicoverpa zea* (corn earworm), *Heliothis virescens* (tobacco budworm), and *Spodoptera frugiperda* (fall armyworm), and several other pest species, but most notably, it is not effective against *Ostrinia nubilalis* (European corn borer), a pest species susceptible to the Cry1A family of proteins, nor is it effective against *Pieris brassica* (large white) (Estruch *et al.*, 1996; C.-G. Yu *et al.*, 1997; Donovan *et al.*, 2001; Selvapandiyan *et al.*, 2001; Loguercio *et al.*, 2002; M. K. Lee *et al.*, 2003; Cai *et al.*, 2006; Jackson, Marcus, Gould, Bradley, and Van Duyn, 2007; Sena, Hernández-Rodríguez, and Ferré, 2009; Burkness, Dively, Patton, Morey, and Hutchison, 2010; Ali and Luttrell, 2011). Considerable attention has been given to potential impacts of *B. thuringiensis* proteins on the Monarch butterfly (*Danaus plexippus*), a well-known and charismatic non-pest lepidopteran species in North America, and studies have indicated that Vip3Aa proteins are not toxic to this butterfly species (M. K. Lee *et al.*, 2003; USEPA, 2008, 2009a).

Mechanism of Vip3Aa Insecticidal Activity

A prerequisite for Vip3Aa insecticidal activity is an enzymatic proteolysis occurring in the insect gut, which processes the 88 kDa protein into the active 62 kDa form. This processing can be duplicated via trypsin digestion (M. K. Lee *et al.*, 2003). Interestingly, Vip3Aa proteins are processed by insect gut fluids to the active form regardless of whether the insect is susceptible to the toxin (M. K. Lee *et al.*, 2003). Once the Vip3Aa protein is in the active form, it binds to the midgut brush border membrane vesicles of susceptible species. The Vip3Aa binding site is not the same site bound by Cry1A, and binding to the gut is correlated with toxicity (Lee, Walters, Hart, Palekar, and Chen, 2003a; Sena, Hernández-Rodríguez, and Ferré, 2009; Abdelkefi-Mesrati *et al.*, 2009). Subsequent to binding, Vip3Aa causes gut paralysis, followed by lysis of gut epithelium cells, presumably by disruption of the transmembrane potential, resulting in cell death. The activated protein is able to form transmembrane pores, and it is thought that these pores contribute to lysis and death of midgut epithelial cells (C.-G. Yu *et al.*, 1997; M. K. Lee *et al.*, 2003). Vip3Aa toxicity symptoms develop more slowly than with Cry1A (48 to 72 hours, as compared to 16 to 24 hours), but the toxicity of Vip3Aa is comparable to that of Cry1 per unit weight (Yu *et al.* 1997; Ali and Luttrell, 2011).

Expression of Vip3Aa in Insect Resistant GE Cotton and Maize

The level of expression of a transgene in a GE plant can be influenced by several factors, including the promoter and terminating sequences used and the gene insert site. Data from enzyme-linked immunosorbent assays (ELISA), showing levels of Vip3Aa protein expression in GE cotton plants have been made available in publicly accessible regulatory submissions and decision documents associated with regulatory approval processes (USDA/APHIS, 2003; FSANZ, 2004; USDA/APHIS, 2005; USEPA, 2008). Similar

2 The activity of bacterial foliar sprays is due to a combination of multiple toxins and qualities of the spore itself that can have an impact on selectivity and host range (Tabashnik, 1992; Schnepf *et al.*, 1998). Therefore, the exposure profile for foliar sprays of bacterial preparations may differ from expression of *B. thuringiensis* proteins in a GE plant (OECD, 2007).

3 also called thuringiensin

data showing Vip3Aa protein expression in GE maize tissues has also been made publicly available (USDA/APHIS, 2007; FSANZ, 2008; CTNBio, 2009; USEPA, 2009a, 2009b; CFIA, 2010; USDA/APHIS, 2010; MAFF, 2011; MAGP, 2011). Samples were collected from several tissue types and at multiple growth stages providing data from plants over time and from multiple locations. Typically, samples of plant tissue were taken at a field trial site and pooled for analysis. The amount of Vip3Aa was generally determined on a dry weight basis (*e.g.*, micrograms of Vip3Aa protein per gram of dry weight) then calculated to provide environmentally relevant values relative to the total fresh weight of the sample. These data may be used to predict exposure of various organisms to Vip3A via cultivation of the event. In most cases the data were presented as a mean value; additional statistics, such as the range and standard deviation were sometimes provided. For some analyses, the data were also expressed as total Vip3Aa produced per hectare (USDA/APHIS, 2003, 2005, 2007, 2010). Table 2 includes the highest reported values of expression of Vip3Aa in cotton and maize plants. Additional protein expression data is contained in Annex I. The tables in Annex I indicate when protein expression values were adjusted to reflect extraction efficiencies.

Table 2. Highest reported protein concentrations of Vip3Aa in GE plant tissues.

Event	Tissue	µg Vip3Aa/g dry weight (growth stage)
COT102	Leaf	24 (squaring)
	Bolls	2 (peak bloom)
	Squares	5 (1st white bloom)
	Roots	3 (pre-harvest)
	Whole plant	15 (4-leaf stage)
MIR162	Leaf	50 (seed maturity)
	Kernels	30 (seed maturity)
	Roots	5 (senescence)
	Pollen	43 (anthesis)
	Whole plant	17 (seed maturity)

Modifications to the *vip3Aa* gene and Vip3Aa protein in GE plants

To produce GE insect-resistant cotton, a *vip* gene designated *vip3Aa19*, derived from *B. thuringiensis* strain AB88, was selected (Estruch *et al.*, 1996; USDA/APHIS, 2003; FSANZ, 2004; USDA/APHIS, 2005; USEPA, 2008). A synthetic version of the gene, encoding 789 amino acids, was produced with two modifications. First, the sequence was altered to reflect preferred codon usage in plants (Murray, Lotzer, and Eberle, 1989). This modification does not change the final amino acid sequence and is done to improve translation efficiency (Perlak, Fuchs, Dean, McPherson, and Fischhoff, 1991). The second change was a substitution of glutamine for lysine at position 284. This alteration has no effect on the protein structure or function and was done to align the amino acid sequence with new sequence data, published after the original isolation of the vip3Aa gene (Estruch *et al.*, 1996; USDA/APHIS, 2003; FSANZ, 2004; USDA/APHIS, 2005; USEPA, 2008). Transcription of the *vip3Aa* gene was directed by a modified version of the *actin-2* promoter from *Arabidopsis thaliana*. This modified sequence was then used to create cotton transformation event COT102.

To produce GE insect-resistant maize, a *vip* gene designated *vip3Aa20*, also derived from *B. thuringiensis* strain AB88, was used. The *vip3Aa20* sequence contains the glutamine substitution at position 284 and an isoleucine substitution for the native methionine at position 129.[4] Transcription of the *vip3Aa* gene was directed by the polyubiquitin gene (*ZmUbilnt*) from maize (Estruch e*t al.*, 1996; USDA/APHIS, 2007; FSANZ, 2008; USEPA, 2009a, 2009b; CFIA, 2010; USDA/APHIS, 2010; MAFF, 2011; MAGP, 2011). This modified sequence was then used to create maize transformation event MIR162.

NON-TARGET ORGANISM (NTO) TESTING AND IMPACTS OF EXPOSURE TO Vip3Aa PROTEIN

Vip3Aa proteins have insecticidal properties against lepidopteran insects, and when used in genetically engineered crops, the proteins target lepidopteran insect pests to reduce feeding damage (Estruch *et al.*, 1996; C.-G. Yu *et al.*, 1997; M. K. Lee *et al.*, 2003; Bhalla *et al.*, 2005; Jackson *et al.*, 2007; Burkness *et al.*, 2010; Ali and Luttrell, 2011). Organisms in the environment, directly or indirectly exposed to Vip3Aa proteins, that are not pests in the agricultural system are called non-target organisms (NTOs). Direct exposure occurs when NTOs feed on live crop tissues or on crop detritus above ground or in the soil. Indirect exposure occurs when one organism feeds on another organism which has consumed plant tissues containing Vip3Aa proteins. The potential for harm to NTOs by Vip3Aa has been considered as a part of regulatory risk assessments for GE crops expressing the gene for *vip3Aa*, and data has been submitted to demonstrate that NTOs exposed to Vip3Aa proteins in the environment, either directly or indirectly, are not harmed by it. Regulatory decisions have been guided by the long history of use of microbial insecticidal formulations of *B. thuringiensis* as well as data collected from field trials of GE crops producing one of the Vip3Aa proteins. These data have established that Vip3Aa proteins are active specifically against the subset of lepidopteran pests which consume the crop and are harmless to vertebrate species and other NTOs (FSANZ, 2004; USDA/APHIS, 2005; FSANZ, 2008; USEPA, 2008, 2009a, 2009b; USDA/APHIS, 2010).

4 The codon change at position 129 was noted when the T-DNA containing the *Vip3Aa20* gene was sequenced by the developer. The change in amino acid sequence did not affect the pesticidal activity of the Vip3Aa20 protein (USDA/APHIS, 2007, 2010).

The assessment begins with a determination of which organisms are likely to be directly or indirectly exposed to Vip3Aa in the environment. Special consideration is given to NTOs with beneficial environmental functions, such as natural enemies, pollinators, designated threatened or endangered species, and species with unique cultural value. These species or surrogate species are then tested for adverse effects from that exposure. Government agencies assess NTO impacts from chemical pesticides using tiered testing, and the tiered approach has been determined by these agencies to be appropriate for assessing NTO impacts from GM crops. While the nature of each tier depends on the pesticide, the crop, and the routes of exposure, the early tier studies involve controlled laboratory environments where NTO or surrogate species are exposed to high concentrations of the pesticide being studied to identify those species which are adversely affected by the pesticide and require further analysis at a higher tier (EFSA, 2006; Garcia-Alonso *et al*., 2006; USEPA, 2007; Romeis *et al*., 2008; USEPA, 2011). Early tier testing also identifies those NTOs which are unaffected by the pesticide and for which higher tier testing is unnecessary. Testing at higher tiers involves increasing levels of complexity and increasingly realistic assay conditions. Higher level tier testing may also be appropriate when the results of early tier testing are uncertain (EFSA, 2006; Garcia-Alonso *et al*., 2006; USEPA, 2007; Romeis *et al*., 2008; USEPA, 2011).

Routes of Environmental Exposure

Regulatory decisions have generally considered three primary routes of exposure in addition to direct contact with the GE plant expressing one of the Vip3Aa proteins: exposure to pollen containing Vip3Aa, exposure to Vip3Aa deposited in the soil by decomposing plant material, and tritrophic exposure via feeding on herbivores which have been feeding on the GE plant (USDA/APHIS, 2003, 2005, 2007; USEPA, 2008, 2009a, 2009b; CFIA, 2010; USDA/APHIS, 2010; MAFF, 2011). Exposure through pollen can occur but is limited by the generally low expression levels of Vip3Aa in the pollen of varieties that have received regulatory approvals as well as the rapidly decreasing density of pollen deposition with increasing distance from the source plant (See Annex I for expression level data in pollen of approved varieties). Although some biologically significant exposure may occur within a short distance of crop fields, regulatory agencies have generally only requested data for the impacts of Vip3Aa on representative pollinator species (*i.e.*, honey bee).

Similarly, the specificity of Vip3Aa toxicity to Lepidoptera and evidence suggesting the potential for exposure in the soil has led regulators to require testing for representative soil dwelling arthropod species, and some regulatory authorities also require data to be collected on non-arthropod species, such as earthworms (USDA/APHIS, 2003, 2005; EFSA, 2006; USDA/APHIS, 2007; USEPA, 2008, 2009a, 2009b; USDA/APHIS, 2010).The United States requires data regarding the longevity of Bt proteins in the soil, and the data suggest that Vip3Aa is quickly degraded once released from decomposing plant tissue and is not likely to persist or accumulate in the soil environment (USEPA, 2009a).

Ecotoxicological Testing of Vip3Aa on Non-Lepidopteran NTOs

Ecotoxicological testing of Vip3Aa on non-lepidopteran NTOs has been conducted on a variety of well-characterized test organisms that are typically used for ecotoxicological testing of chemical pesticides. Government regulatory agencies may also recommend the use of specific species for testing (USDA/APHIS, 2003; FSANZ, 2004; USDA/APHIS, 2005, 2007; FSANZ, 2008; USEPA, 2008, 2009a, 2009b; CFIA, 2010; USDA/APHIS, 2010; Raybould and Vlachos, 2011). Test organisms have included adult and larval *Apis mellifera* (honey bee); Hemiptera: *Orius indiosus* (minute pirate bug); Coleoptera: *Coccinella septempunctata* or *Coleomegilla maculata* (ladybird beetle) and *Aleochara bilineata* (rove beetle); Neuoptera: *Chrysoperla carnea* (green lacewing); soil dwelling Collembola: *Folsomia candida* (springtail); aquatic *Daphnia magna*; and *Eisenia foetida* (earthworms), (USDA/APHIS, 2003; FSANZ, 2004; USDA/APHIS, 2005, 2007; FSANZ, 2008; USEPA, 2008, 2009a, 2009b; CFIA, 2010; USDA/APHIS, 2010; Raybould and Vlachos, 2011). None of these organisms showed a significant response to Vip3Aa, resulting in determinations of a No Observed Effects Level (NOEL) or No Observed Effects Concentration (NOEC) at concentrations ranging from less than 1 ppm to 7250 ppm (USDA/APHIS, 2003; FSANZ, 2004; USDA/APHIS, 2005, 2007; FSANZ, 2008; USEPA, 2008, 2009a, 2009b; CFIA, 2010; USDA/APHIS, 2010; Raybould and Vlachos, 2011). This can be compared with worst case scenario exposure estimates based on the highest observed tissue concentrations of Vip3Aa in GE plants ranging from 2-50 ppm (see Table 2). Additionally, acute mammalian toxicological testing has been conducted on *Mus musculus* (mouse) (USDA/APHIS, 2003; FSANZ, 2004; USDA/APHIS, 2005, 2007; FSANZ, 2008; Li, Meissle, and Romeis, 2008; USEPA, 2008, 2009a, 2009b; CFIA, 2010; USDA/APHIS, 2010; Raybould and Vlachos, 2011); *Colinus virginianus* (bobwhite quail) (USDA/APHIS, 2005; USEPA, 2008, 2009a; USDA/APHIS, 2010; Raybould and Vlachos, 2011); *Ictalurus punctatus* (channel catfish) (USDA/APHIS, 2003, 2005, 2007; USEPA, 2008, 2009a, 2009b; CFIA, 2010; USDA/APHIS, 2010; Raybould and Vlachos, 2011); and *Gallus domesticus* (chicken) (USDA/APHIS, 2003, 2005, 2007; Miller, Morandini, and Ammann, 2008; USEPA, 2008, 2009a, 2009b; CFIA, 2010; USDA/APHIS, 2010; Raybould and Vlachos, 2011). The results of these studies are summarized in Table 4. These results from Tier 1 tests indicate that no higher tier testing is necessary from a regulatory standpoint, because no adverse effects were noted;[5] however, as discussed below, studies of the effects of Vip3Aa on natural populations of NTOs have been performed (Dively, 2005; Whitehouse, Wilson, and Constable, 2007).

Field Studies of Vip3Aa on NTOs

Regulatory authorities have considered the potential impact of the Vip3Aa protein on natural populations of NTOs and determined that adverse effects on NTOs are unlikely for several reasons. First, Vip3Aa proteins have a narrow spectrum of pesticidal activity.

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

Table 3. Summary of ecotoxicological tests of Vip3Aa on non-lepidopteran non-target organisms reviewed in regulatory decisions.

Species	Method of Exposure	Duration of Exposure	Results
Aleochara bilineata (rove beetle)	Exposure at 500 µg/g diet	35 days	NOEC > 500 µg/g
Apis mellifera (honeybee) larvae	Exposure to protein at 500µg/g diet	24 days	NOEC> 500µg/g
Chrysoperla carnea (green lacewing) larvae	Exposure at 21.7 ppm	13 days	NOEC > 21.7 ppm
	Exposure at 7.25 mg/g diet	30 days	NOEC > 7.25 mg/g
Coccinella septempunctata (ladybird beetles)	Exposure at 7.25 mg/g diet	15 days	NOEC >7.25 mg/g
Coleomegilla maculata (ladybird beetles)	Exposure at 7.24 µg/g diet	20 days	NOEL > 7.24 µg/g
Colinus virginianus (bobwhite quail)	Exposure at 400 mg/kg body weight	single dose	NOEL > 400 mg/kg
Daphnia magna	Exposure to 10 µg/L water	48 hours	NOEC > 10 µg/L
Eisenia foetida (earthworm)	Exposure to Vip3Aa protein at 3.6 µg/g artificial soil	14 days	NOEL > 3.6 µg/g
Folsomia candida (Collembola)	Exposure at 43.2 µg/g diet	28 days	NOEC > 43.2 µg/g
*Gallus domesticus (*chicken)	Exposure at 0.588 µg/g diet	49 days	NOEC > 0.588 µg/g
Ictalurus punctatus (channel catfish)	Exposure at 7.1 µg/g diet	30 days	NOEC > 7.1 µg/g
Mus musculus (mouse)	Acute oral gavage at 1250–3675 mg/kg	single dose	NOEL > 3675 mg/kg
Orius insidiosus.(minute pirate bug)	Exposure at 7.25 mg/g diet	21 days	NOEC > 7.25 mg/g

(Sources: (USDA/APHIS, 2003, 2005, 2007; USEPA, 2008, 2009a, 2009b; CFIA, 2010; USDA/APHIS, 2010; Raybould and Vlachos, 2011)

Second, Tier I laboratory assays, employing a range of invertebrate species present in maize and cotton agricultural ecosystems, or surrogates for those species, have shown that the Vip3Aa proteins cause no observable effects in these species. Third, Tier I studies have also demonstrated that the Vip3Aa proteins have no observable effect on representative vertebrate and aquatic species. Fourth, the levels of Vip3Aa used in these Tier I assays were much higher than those measured in GE maize and cotton tissues growing in the field. Fifth, field studies of corn and cotton varieties producing Vip3Aa show no significant adverse effects on biodiversity of non-target arthropods or beneficial species, including egg parasitoids, although populations of lepidopteran-specific predators and parasites were slightly reduced. Sixth, when compared to insect control via Vip3Aa, traditional insect control using chemical pesticides significantly alters species diversity and harms non-target species. Together, these findings indicate that the Vip3Aa proteins are unlikely to have adverse effects on natural populations of organisms, except for lepidopteran crop pests (USDA/APHIS, 2003; Dively, 2005; USDA/APHIS, 2005, 2007; Whitehouse *et al.*, 2007; USEPA, 2008, 2009a, 2009b; CFIA, 2010; USDA/APHIS, 2010; Raybould and Vlachos, 2011).

ESTABLISHMENT AND PERSISTENCE OF Vip3Aa-EXPRESSING PLANTS IN THE ENVIRONMENT

Biology of the Plant Species

The biology of the non-GE plant species in the receiving environment is typically the starting point for environmental risk assessments of GE plants (OECD, 2003, 2007, 2008). Information about the biology of the non-GE plant can be used to assess whether a genetically engineered variety of the plant may become weedy, invasive, or otherwise harmful to the environment. It can also provide details on significant interactions between the plant and other organisms that may be important when considering potential harms. Whether a crop produces seed via self-pollination or outcrossing can provide insights into the potential for gene flow between the GE crop and other sexually compatible plants. By considering the biology of the host plant, a risk assessor can identify potential hazards that may be associated with the expression of the novel protein (*e.g.*, Vip3Aa) and then be able to assess the likelihood of these hazards. For example, whether the plant is an annual or perennial species or whether the plant is self-pollinated or wind pollinated can bear on the assessment of the likelihood of the GE plant establishing and persisting outside of cultivation (OECD, 1992, 2003; EFSA, 2006; OECD, 2007, 2008).

Phenotypic Data

Information about the phenotype of GE plants expressing Vip3Aa is collected from laboratory, greenhouse, and field trial studies and is presented in regulatory submissions to (1) identify any intentional changes to the phenotype that might impact the environmental safety of the plant and (2) to identify any unintended changes to the biology of the plant that might impact environmental safety. Phenotypic data in regulatory submissions and peer reviewed publications have focused on characteristics of the plant that might contribute to its survival or persistence (*i.e.*, potential weediness), or that negatively affect agricultural performance (*e.g.*, disease susceptibility and yield data) (CFIA, 2010; USDA/APHIS, 2003, 2005, 2007, 2010; USEPA, 2008, 2009a, 2009b). The phenotypic observations take into account the desired phenotype resulting from the transgenic trait, in this case insect predation resistance mediated by Vip3Aa. Some of the collected data are quantitative (*e.g.*, plant height or percent seed germination) while other data are qualitative and observational (*e.g.*, no differences in disease susceptibility). Statistically significant differences between GE plants expressing Vip3Aa and controls were observed, but these differences were not consistent among the field trial locations and fell within the reported range for non-GE maize and cotton varieties (USDA/APHIS, 2003, 2005, 2007; USEPA, 2008, 2009a, 2009b; CFIA, 2010; USDA/APHIS, 2010). Collectively, regulators have deter-

mined that the phenotypic data do not support the hypothesis that the introduction of the Vip3Aa protein had any unintended impact on the gross morphology or phenotypic characteristics of plants, besides conferring insect resistance to lepidopteran pests (USDA/APHIS, 2003, 2005, 2007; Romeis *et al.*, 2008; USEPA, 2008, 2009a, 2009b; CFIA, 2010; USDA/APHIS, 2010; Raybould and Vlachos, 2011).

Weediness in Agricultural Environments

Cotton Cultivated cotton lacks weedy or aggressive characteristics, and it is not generally considered to be an economically important agricultural weed, although it can grow as a perennial in areas lacking a cold season (USDA/APHIS, 2003, 2005; OECD, 2008; USEPA, 2008). Researchers and regulators have evaluated the potential for insect-resistant GE cotton varieties to become weeds, including cotton producing the Vip3Aa protein, and they have found that there are no characteristics of insect-resistant cotton that would increase its potential to become an agricultural weed, because volunteer cotton plants would be readily controlled using conventional weed management techniques (CSIRO, 2002; Carpenter *et al.*, 2002; USDA/APHIS, 2003, 2005; Eastick and Hearnden, 2006; USEPA, 2008).

Maize Maize is not generally regarded as a weed, although it has some potential to volunteer in subsequent growing seasons (Carpenter *et al.*, 2002; OECD, 2003; USEPA, 2009a, 2009b; USDA/APHIS, 2010). Maize possesses very few of the characteristics that increase the likelihood of a plant to volunteer or to become a weed, such as seed dormancy, shattering or competitiveness (Baker, 1974; OECD, 2003; USDA/APHIS, 2010). There are no data indicating that Vip3Aa protein expression results in altered seed dormancy, over wintering capacity, or other characteristics that would alter the prevalence of volunteer maize in subsequent growing seasons (Carpenter *et al.*, 2002; OECD, 2003; USDA/APHIS, 2007; USEPA, 2009a, 2009b; CFIA, 2010; USDA/APHIS, 2010). Following-season volunteers producing Vip3Aa would not be expected to present any management difficulty and can be dealt with in the same manner as conventional volunteers of maize.

Weediness in Non-Agricultural Environments

The primary mechanisms by which Vip3Aa may be introduced into a non-agricultural environment are through the movement of propagules outside of cultivated areas and through gene flow from the GE plant to a naturalized population of sexually compatible relatives (D. Lee and Natesan, 2006). Risk assessments for GE cotton and maize expressing Vip3Aa have considered the potential impacts associated with both types of movement (USDA/APHIS, 2005; USEPA, 2008, 2009a, 2009b; USDA/APHIS, 2010).

Cotton While all plants may exhibit weedy characteristics under certain conditions, commercial varieties of cotton are not considered to pose a significant weed risk in non-agricultural environments. Selective breeding has resulted in modern cotton varieties' dependence on human intervention, and factors such as water stress and cold severely limit the ability of commercial varieties to survive in non-agricultural environments (CSIRO, 2002; Carpenter *et al.*, 2002; USDA/APHIS, 2005; Eastick and Hearnden, 2006; OECD, 2008; USEPA, 2008). Although insect resistance mediated through the Vip3Aa protein may provide some fitness advantage to an escaped GE cotton plant, researchers and regulators have determined that such an advantage would be insufficient to allow GE cotton expressing Vip3Aa to persist in a non-agricultural environment (Carpenter *et al.*, 2002; USDA/APHIS, 2005; USEPA, 2008).

Maize As a result of extensive selective breeding, commercial maize varieties are severely restricted in their ability to persist in non-agricultural environments without human intervention, and maize is not considered to be an invasive or aggressive weed outside of agricultural systems (Carpenter *et al.*, 2002; OECD, 2003; Raybould *et al.*, 2011). Agronomic data show that Vip3Aa does not have a significant impact on traits associated with weediness (OECD, 2003; USDA/APHIS, 2007; USEPA, 2009a, 2009b; CFIA, 2010; USDA/APHIS, 2010). 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, Braun, Warwick, Zhu, and Stewart Jr., 2004; Blumenthal, 2005), regulatory decisions have determined that it is unlikely that the addition of resistance to lepidopteran pests would allow maize producing Vip3Aa to become invasive in non-agricultural environments (Carpenter *et al.*, 2002; OECD, 2003; USDA/APHIS, 2007; USEPA, 2009a, 2009b; CFIA, 2010; USDA/APHIS, 2010).

Movement of the Transgene to Sexually Compatible Relatives

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

Cotton The *Gossypium* genome is very complex and is organized into eight diploid species groups and one tetraploid species group, which includes *G. hirsutum*. Crosses within groups can occur, but crosses between groups are rare, and offspring display meiotic abnormalities and infertility, including crosses between *G. hirsutum* and members of the diploid species. Hybridization between *G. hirsutum* and the three wild tetraploid species (*G. mustelinum*, *G. darwinii*, and *G. tomentosum*) as well as crosses with feral populations of *G. barbadense* and *G. hirsutum* can be readily made experimentally and result in fertile offspring. Under the favorable conditions discussed above, spontaneous hybridizations can occur when commercial varieties of *G. hirsutum* are grown near natural populations of tetraploid species (OECD, 2008). However, the frequency of such crosses between transgenic *G. hirsutum* and sexually compatible wild relatives is considered to be no greater than crosses between traditionally bred varieties of *G. hirsutum* and wild species (Carpenter *et al.*, 2002; USDA/APHIS, 2003, 2005, 2007; OECD, 2008; USEPA, 2008).

Maize Maize does not have sexually compatible relatives that are considered invasive or weedy (Carpenter *et al.*, 2002; OECD, 2003). Maize freely hybridizes with wild teosintes, but gene introgression is thought to be limited (Castillo-Gonzalez and Goodman, 1997; OECD, 2003; Baltazar, de Jesús Sánchez-Gonzalez, de la Cruz-Larios, and Schoper, 2005). Wild teosinte populations are limited to Mexico, Guatemala and a single population in Nicaragua, and while teosinte is considered a serious weed by some farmers in Mexico, it is treated as a beneficial by others (González and Corral, 1997). Crosses between teosinte and Vip3Aa maize are not expected to occur more frequently than those between teosinte and traditionally bred maize varieties (Carpenter *et al.*, 2002; USDA/APHIS, 2007; USEPA, 2009a, 2009b; USDA/APHIS, 2010).

COMPOSITIONAL ANALYSIS OF Vip3Aa PLANTS

A compositional analysis is required by 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 over the course of more than one year, 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 from Vip3Aa cotton event COT102 and Vip3Aa maize event MIR162 has undergone proximate analysis to determine levels of crude protein, crude fat, fiber, moisture, and ash. In addition, levels of select minerals, fatty acids, and amino acids have been determined. Some crop plants produce toxins or anti-nutritive compounds, and levels of these compounds are also measured to determine whether the presence of the transgene has inadvertently resulted in elevated production of these substances. Cotton is known to produce the toxins gossypol and cyclopropenoid fatty acids, and the levels of these substances were measured in COT102 and a non-transformed comparator. Similarly, maize is known to produce the anti-nutritive compounds phytic acid, raffinose, and trypsin inhibitor, and levels of these substances in MIR162 were determined. Data from publicly available sources are summarized in Annex II. All differences noted between the Vip3Aa cotton and maize events analyzed and the comparator varieties were within the normal range of variation, and these differences were deemed irrelevant to environmental safety (USDA/APHIS, 2003; FSANZ, 2004; USDA/APHIS, 2005, 2007; FSANZ, 2008; USDA/APHIS, 2010).

CONCLUSION

The Vip3Aa proteins produced by insect-resistant GE plants are derived from the common soil bacterium *Bacillus thuringiensis* and are specifically toxic to Lepidoptera. Toxicity testing with a range of representative non-target organisms produced NOEL values at concentrations significantly higher than the expected environmental concentrations of Vip3Aa. Field studies suggest that cultivation of GE maize plants expressing Vip3Aa does not affect the abundance of non-target arthropods, with the possible exception of specialist predators of the target pests controlled by Vip3Aa. Vip3Aa in plants can be toxic to non-target Lepidoptera, but regulatory risk assessments for approved products have concluded that the risk is likely reduced when compared to other insect-control practices. The weight of evidence from analyses of phenotypic and compositional data demonstrates that Vip3Aa expression in approved maize and cotton events do not alter the gross physiology of the crop plants and indicates that these plants are not more likely to become weedy or invasive than their conventional counterparts.

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ANNEX I: SUMMARY OF Vip3Aa PROTEIN EXPRESSION DATA

The tables that follow present summary data from peer-reviewed publications and regulatory submissions. The data is presented in the format in which it is available in the cited document in order to facilitate cross-referencing. Additional information on collection and sampling methodologies can be found in the referenced sources.

Summary Data for GE Cotton

Table I.1. Vip3Aa protein levels[1] in cotton seeds and cotton fiber from pre-harvest stage (22 weeks post emergence) during the development of COT102 plants (USDA/APHIS, 2003; FSANZ, 2004; USDA/APHIS, 2005)

Tissue	Location	Mean[2] µg Vip3Aa/g fresh weight ± SD[3] (Range)	Mean µg Vip3Aa/ g dry weight ± SD (Range)
Seeds	**Georgia**	2.88 ± 0.28 (2.52 – 3.28)	3.23± 0.31 (2.86 – 3.65)
	Texas	2.70 ± 0.27 (2.41 – 3.05)	2.99 ± 0.29 (2.65 – 3.65)
	Arizona	2.51 ± 0.25 (2.14 – 2.82)	2.72 ± 0.28 (2.33 – 3.08)
Fiber	**Georgia**	NM[4]	ND[5]
	Texas	NM	ND
	Arizona	NM	ND

1 As determined by ELISA. Values not corrected for extraction efficiency. Values for all control plants corresponded to 0 ng Vip3Aa/g fresh or dry weight.
2 n = 5
3 SD = standard deviation
4 NM = not measured
5 ND = not detected

Table I.2. Vip3A Protein levels[1] in nectar and pollen, collected from greenhouse-grown cotton plants derived from event COT102 (USDA/APHIS, 2003, 2005)

Tissue	µg Vip3a/g sample
Pollen[2]	1.09
Nectar[3]	Not Detected

1 Values were determined by ELISA and were not corrected for extraction efficiency.
Values for all control plants corresponded to 0 ng Vip3Aa/g sample.
Value represents a composite of pollen or nectar collected from 15-25 plants.
2 Pollen values are reported on a g air-dried pollen basis.
3 Nectar values are on a g nectar (as collected) basis.

Table I.3. Vip3A levels1 in processed cottonseed products (FSANZ, 2004)

Vip3A µg/g	Non-Toasted meal	Non-Toasted meal	Toasted meal	Refined Oil
COT102	2.75 ± 0.12	2.57 ± 0.03	0.23 ± 0.02	ND[2]
Coker 312	ND	ND	ND	ND

1 Values were determined by ELISA and were not corrected for extraction efficiency.
2 ND = not detected (the mean absorbance generated during ELISA did not exceed that of the controls.)
The limit of detection was from 40 ng/g to 270 ng/g fresh weight, depending on the tissue.

Table I.4. Mean µg Vip3A[1] per gram fresh weight during development of COT102 plants (USDA/APHIS, 2003, 2005)

Tissue	Location	4-Leaf	Squaring	1st White Bloom	Peak Bloom	1st Open Boll	Pre-Harvest
Leaves	**Georgia**	15.08 ± 3.22[2] (9.39 – 15.08)	18.26 ± 4.92 (11.12 – 22.40, N=4)	9.86 ± 2.28 (7.66 – 13.31)	5.92 ± 2.84 (1.87 – 9.05)	NGE[2]	3.29 ± 3.31 (0.95 – 5.63, N=2)
	Texas	18.51 ± 2.45 (15.47 – 20.78)	21.51 ± 2.06 (19.01 – 23.75)	10.78 ± 1.22 (9.99 – 12.92)	4.66 ± 0.84 (3.73 – 5.46)	8.82 ± 1.49 (6.79 – 10.68)	7.24 ± 1.18 (5.41 – 8.50)
	Arizona	12.35 ± 6.26 (1.20 – 15.92)	12.87 ± 3.39 (7.04 – 15.70)	8.56 ± 3.48 (3.94 – 13.31)	NGE	NGE	3.65 ± 1.94 (1.08 – 5.17, N=4)
Roots	**Georgia**	1.27 ± 0.36 (0.82 – 1.78)	NA[3]	NA	1.18 ± 0.13 (1.01 – 1.35)	NA	1.21 ± 0.46 (0.53 – 1.78)
	Texas	1.57 ± 0.16 (1.31 – 1.73)	NA	NA	1.82 ± 0.69 (1.05 – 2.53)	NA	2.15 ± 0.32 (1.88 – 2.69)
	Arizona	<1.33 (DNQ[4] – 1.86)	NA	NA	NGE	NA	<0.17 (DNQ – 0.35)
Bolls	**Georgia**	NA	NA	NA	1.09 ± 0.10 (0.94 – 1.19)	NGE	ND[5]
	Texas	NA	NA	NA	1.38 ± 0.82 (0.44 – 2.18)	0.45 ± 0.17 (0.33 – 0.74)	<0.20 (DNQ)
	Arizona	NA	NA	NA	NGE	NGE	<0.36 (DNQ – 0.47)
Squares	**Georgia**	NA	NA	3.72 ± 1.25 (2.01 – 5.45)	2.77 ± 0.12 2.58 – 2.85)	NGE	<0.22 (DNQ)
	Texas	NA	NA	1.64 ± 0.43 (0.88 – 1.92)	2.64 ± 0.92 (1.42 – 3.54)	2.10 ± 0.34 (1.69 – 2.41)	4.00 ± 1.48 (1.62 – 5.64)
	Arizona	NA	NA	3.11 ± 0.41 (2.68 – 3.70)	NGE	NGE	1.51 ± 0.31 (1.17 – 2.01)
Whole Plant	**Georgia**	13.22 ± 1.68 (11.70 – 15.13)	NA	4.53 ± 0.84 (3.63 – 5.91)	6.35 ± 0.35 (5.90 – 6.79)	NGE	0.72 ± 0.16 (0.47 – 0.86)
	Texas	12.37 ± 1.40 (10.53 – 14.30)	NA	5.46 ± 1.10 (4.24 – 6.86)	4.64 ± 0.75 (3.92 – 5.70)	5.28 ± 2.07 (1.87 – 7.36)	2.96 ± 1.04 (1.82 – 4.19)
	Arizona	10.75 ± 2.05 (7.72 – 12.65)	NA	5.16 ± 1.83 (2.39 – 7.49)	NGE	NGE	1.59 ± 0.35 (1.30 – 2.12)

1 As determined by ELISA. Values are not corrected for extraction efficiency.
2 Mean ± standard deviation; Range: n = 5 unless noted.
3 NGE = sampled plants were determined to be non-genetically engineered.
4 NA = not analyzed
5 DNQ = detected but not quantifiable. Means were calculated by assuming Vip3A was present at the lower limit of quantification, and means are preceded by "<" to indicate that the mean is less than the quantity indicated.
6 ND = tested, but no Vip3A detected.

Table I.5. Mean µg Vip3A[1] per gram dry weight during development of COT102 plants (USDA/APHIS, 2003, 2005)

Tissue	Location	4-Leaf	Squaring	1st White Bloom	Peak Bloom	1st Open Boll	Pre-Harvest
Leaves	**Georgia**	77.29 ± 19.452 (44.67– 95.97)	118.22 ± 28.03 (68.67 – 135.60)	40.52 ± 9.53 (32.34 – 56.15)	30.05 ± 14.93 (10.40 – 46.88)	NGE[2]	4.58 ± 4.76 (1.21 – 7.95, N=2)
	Texas	96.26 ± 11.15 (81.42 – 108.81)	87.41 ± 9.40 (76.85 – 99.66)	50.42 ± 5.67 (46.47 – 60.10)	17.89 ± 2.60 (14.79 – 20.44)	35.31 ± 4.76 (28.19 – 40.14)	9.01 ± 1.56 (6.28 – 10.06)
	Arizona	64.61 ± 32.82 (6.17 – 83.67)	58.50 ± 15.22 (32.59 – 71.70)	40.70 ± 15.00 (18.26 – 59.69)	NGE	NGE	6.17 ± 2.08 (3.38 – 8.41, N=4)
Roots	**Georgia**	6.30 ± 0.81 (5.08 – 7.28)	NA[3]	NA	4.37 ± 0.58 (3.76 – 5.19)	NA	3.39 ± 1.40 (1.32 – 5.12)
	Texas	7.03 ± 0.76 (6.16 – 7.84)	NA	NA	5.13 ± 1.95 (3.14 – 7.32)	NA	4.83 ± 0.58 (4.14 – 5.68)
	Arizona	<5.09 (DNQ[4] – 7.45)	NA	NA	NGE	NA	<0.43 (DNQ – 0.94)
Bolls	**Georgia**	NA	NA	NA	8.66 ± 1.09 (7.34 – 10.05)	NGE	ND[5]
	Texas	NA	NA	NA	6.99 ± 4.04 (2.18 – 11.28)	2.12 ± 0.82 (1.66 – 3.56)	<0.30 (DNQ)
	Arizona	NA	NA	NA	NGE	NGE	<0.91 (DNQ – 1.36)
Squares	**Georgia**	NA	NA	16.57 ± 5.44 (8.81 – 23.69)	16.86 ± 1.36 15.43 – 18.75)	NGE	<0.30 (DNQ)
	Texas	NA	NA	7.99 ± 2.02 (4.51 – 9.50)	11.42 ± 3.77 (6.84 – 15.55)	8.63 ± 1.42 (7.02 – 10.23)	4.75 ± 1.81 (1.77 – 6.43)
	Arizona	NA	NA	16.69 ± 1.61 (14.93 – 19.06)	NGE	NGE	3.64 ± 1.91 (2.04 – 5.92)
Whole Plant	**Georgia**	72.82 ± 8.43 (62.76 – 81.81)	NA	17.74 ± 3.25 (13.49 – 22.63)	35.53 ± 2.81 (31.04 – 38.17)	NGE	1.29 ± 0.30 (0.82 – 1.55)
	Texas	65.36 ± 6.70 (57.84 – 76.07)	NA	26.88 ± 5.02 (21.51 – 32.99)	18.20 ± 2.53 (15.56 – 21.67)	19.96 ± 8.33 (6.54 – 28.54)	5.27 ± 1.98 (2.94 – 7.68)
	Arizona	54.97 ± 12.17 (35.42 – 65.90)	NA	26.76 ± 7.65 (14.96 – 36.34)	NGE	NGE	4.27 ± 0.94 (3.30 – 5.60)

1 As determined by ELISA. Values are not corrected for extraction efficiency.
2 Mean ± standard deviation; Range: n = 5 unless noted.
3 NGE = sampled plants were determined to be non-genetically engineered.
4 NA = not analyzed
5 DNQ = detected but not quantifiable. Means were calculated by assuming Vip3A was present at the lower limit of quantification, and means are preceded by "<" to indicate that the mean is less than the quantity indicated.
6 ND = tested, but no Vip3A detected.

Table I.6. Mean Vip3Aa levels[1] across all cotton developmental stages and locations (FSANZ, 2004)

Mean Vip3A concentration	Fresh weight (µg/g)	Dry weight (µg/g)
Whole plant	1-13	1-73
Leaves	3-22	5-118
Squares	<4	<17
Roots	<2	<7
Bolls	<1	<9
Pollen		1
Seeds	3	3

1 Values were determined by ELISA and were not corrected for extraction efficiency.

Table I.7. Vip3A Protein levels[1] in young leaves of event COT102-derived cotton plants grown at two locations and sampled at four developmental stages (USDA/APHIS, 2003, 2005)

Location	Stage	µg Vip3a/g fresh weight	µg Vip3a/g dry weight
NorthCarolina	**Squaring**	13.88	44.85
	1st White Bloom	18.87	65.61
	Peak Bloom	12.33	45.20
	1st Open Boll	3.90	15.37
Texas	**Squaring**	6.86	27.19
	1st White Bloom	5.54	22.52
	Peak Bloom	1.55	7.11
	1st Open Boll	1.33	5.04

1 Values were determined by ELISA and were not corrected for extraction efficiency. Each sample represented a composite of ten leaves from different plants.

Summary Data for GE Maize

Table I.8. Mean µg Vip3Aa20 per gram fresh weight in various MIR162 plant tissues at different developmental stages (USDA/APHIS, 2007, 2010)

Tissue	V9-V12[2]	Anthesis	Seed Maturity	Senescence
Leaves	17.63 (13.11 – 22.35)3	24.44 (21.08 – 29.07)	50.41 (35.85 – 60.92)	13.40 (8.87 – 18.20)
Roots	5.23 (3.99 – 7.07)	4.32 (4.18 – 4.69)	4.81 (2.27 – 6.60)	5.29 (4.61 – 5.82)
Pith	NA[4]	3.54 (3.17 – 4.16)	11.47 (10.21 – 12.75)	NA
Kernels	NA	NA	29.81 (27.78 – 34.13)	28.65 (25.06 – 32.42)
Silk	NA	12.55 (8.05 – 18.91)	NA	NA
Pollen	NA	43.21 (37.42 – 49.70)	NA	NA
Whole Plants	11.98 (8.96 – 15.39)	12.16 (11.51 – 12.97)	20.84 (15.54 – 25.98)	17.35 (13.24 – 24.07)

1 As determined by ELISA. Values are corrected for extraction efficiency.
2 Approximately 8 weeks after planting
3 Range
4 NA = not analyzed at this stage

Table I.9. Mean µg Vip3Aa20 per gram dry weight in MIR162 maize plant tissues at different developmental stages (FSANZ, 2004; USDA/APHIS, 2007, 2010)

Tissue	V9-V12[2]	Anthesis	Seed Maturity	Senescence
Leaves	97.26 (76.12 – 119.12)3	107.74 (97.10 – 118.80)	121.79 (77.25 – 159.66)	21.31 (12.93 – 30.28)
Roots	31.80 (28.10 – 35.65)	28.34 (26.30 – 30.20)	20.29 (9.87 – 27.48)	21.66 (11.58 – 32.13)
Pith	N/A[4]	31.71 (29.43 – 36.18)	58.21 (52.74 – 63.68)	N/A
Kernels	N/A	N/A	43.56 (40.47 – 50.50)	34.24 (30.90 – 37.67)
Silk	N/A	97.40 (60.54 – 149.00)	N/A	N/A
Pollen	N/A	47.13 (41.45 – 53.52)	N/A	N/A
Whole Plants	91.53 (88.68 – 96.51)	67.61 (61.68 – 72.63)	49.04 (34.84 – 63.14)	34.30 (21.12 – 55.17)

1 As determined by ELISA. Values were corrected for extraction efficiency.
2 Approximately 8 weeks after planting
3 Range
4 NA = not analyzed at this stage

ANNEX II: SUMMARY OF COMPOSITIONAL ANALYSES OF GE PLANTS EXPRESSING Vip3Aa

The tables that follow present summary data from peer-reviewed publications and regulatory submissions. The data is presented in the format in which it is available in the cited document in order to facilitate cross-referencing. Additional information on collection and sampling methodologies can be found in the referenced sources.

Summary Data for Cotton

Table II.1. Proximate analysis of COT101 and Coker 312 cottonseed (FSANZ, 2004; USDA/APHIS, 2007, 2010)

Component[1]	COT102	Coker 312	Reference Range 1[2]	Reference Range 2[3]
Moisture	8.844 (8.06-9.27)5	9.27 (8.01-11.47)	3.97-7.49	3.97-8.47
Fat	21.90 (20.89-23.47)	22.12 (21.78-22.35)	15.44-23.64	15.44-23.83
Protein	29.87 (28.92-31.72)	29.34 (27.73-31.02)	21.76-27.79	21.76-28.15
Fiber	15.25 (14.79-15.98)	15.81 (14.13-17.05)	15.38-19.31	15.38-20.89
Ash	4.06 (3.37-4.69)	4.21 (3.85-4.63)	3.76-4.85	3.76-4.85

1 All values expressed as % dry weight, except for moisture, which is % fresh weight.
2 Reference Range 1 = Range included data from four commercial, non-transgenic varieties.
3 Reference Range 2 = Range included data from ten commercial, non-transgenic and transgenic varieties.
4 Mean
5 Range

Table II.2. Mineral analysis of COT102 and Coker 312 cottonseed (FSANZ, 2004; USDA/APHIS, 2007, 2010)

Component	**COT102**	**Coker 312**	**Reference Range 1[1]**
Phosphorus (%)	0.64[2] (0.54 – 0.72)[3]	0.68 (0.64 – 0.75)	0.61-0.88
Calcium (%)	0.11 (0.10 – 0.12)	0.12 (0.09 – 0.15)	0.12-0.33
Sodium (ppm)	969 (562 – 1300)	929 (529 – 1300)	54-3000
Iron (ppm)	82.1 (79.3 – 84.3)	81.7 (67.4 – 93.7)	41.84-72.15
Magnesium (%)	0.33 (0.33 – 0.34)	0.34 (0.33 – 0.37)	0.37-0.49
Manganese (ppm)	13.6 (13.2 – 14.1)	13.6 (13.3 – 14.1)	11.17-18.31
Potassium (%)	0.81 (0.72 – 0.88)	0.82 (0.76 – 0.89)	1.08-1.25
Zinc (ppm)	30.3 (29.3 – 32.0)	31.6 (31.5 – 31.6)	27.39-51.20
Copper (ppm)	9.14 (8.7 – 9.43)	9.4 (9.1 – 9.8)	4.39-10.35
Chromium (ppm)	<1	<1	

1 Range includes data from ten commercially available transgenic and non-transgenic varieties.
2 Mean, n = 3
3 Range

Table II.3. Fatty acid analysis of COT102 and Coker 312 cottonseed (FSANZ, 2004; USDA/APHIS, 2007, 2010)

Component (g/100 g)	**COT102**	**Coker 312**
14:0 myristic	0.837[1] (0.59-0.99)[2]	0.813 (0.54-0.96)
16:0 palmitic	24.84 (22.81-25.87)	24.27 (22.59-25.64)
16:1 palmitoleic	0.587 (0.57-0.62)	0.570 (0.55-0.59)
18:0 stearic	2.51 (2.39-2.58)	2.51 (2.41-2.58)
18:1 oleic	15.25 (13.53-16.14)	15.51 (13.94-16.73)
18:2 linoleic	55.04 (52.97-59.14)	55.94 (52.59-58.14)
18:3 linolenic	0.393 (0.27-0.53)	0.513 (0.48-0.58)
20:0 arachidic	0.240 (0.21-0.26)	0.237 (0.20-0.27)
22:0 behenic	0.120 (0.1-0.13)	0.123 (0.11 – 0.14)

1 Mean, n = 3
2 Range

Table II.4. Amino acid analysis of COT102 and Coker 312 cottonseed (FSANZ, 2004)

Component (g/100 g)	COT102	Coker 312	Component (g/100 g)	COT102	Coker 312
Asp	423[1] (360 – 460)[2]	400 (360 – 460)	**Met**	1013 (920 – 1090)	987 (930 – 1090)
Thr	400 (370 – 420)	403 (380 – 430)	**Ile**	733 (670 – 790)	710 (670 – 780)
Ser	2340 (2160 – 2490)	2270 (2180 – 2420)	**Leu**	1330 (1200 – 1430)	1297 (1220 – 1410)
Glu	787 (720 – 840)	770 (740 – 820)	**Tyr**	540 (480 – 590)	520 (490 – 560)
Pro	1057 (950 – 1130)	1023 (950 – 1110)	**Phe**	1197 (1060 – 1300)	1153 (1060 – 1280)
Gly	4597 (4060 – 5000)	4450 (4090 – 4930)	**His**	657 (590 – 710)	643 (600 – 700)
Ala	880 (800 – 960)	850 (800 – 950)	**Lys**	1003 (930 – 1070)	990 (960 – 1040)
Cys	963 (880 – 1030)	940 (890 – 1010)	**Arg**	2630 (2280 – 2890)	2523 (2290 – 2800)
Val	953 (900 – 1010)	950 (900 – 1020)	**Trp**	310 (280 – 330)	313 (310 – 320)

1 Mean, n = 3
2 Range

Summary Data for GE Maize

Table II.5. Amino acid analysis of cottonseed from COT102 and Coker 312 (USDA/APHIS, 2003, 2005)

Year	Line	Ala	Arg	Asp	Cys	Glu	Gly	His	Ile	Leu
2001	**COT102**	880[1]	2630	423	963	787	4597	657	733	1330
	Coker 312	850	2523	407	940	770	4450	643	710	1297
2002	**COT102**	1065	3123	2503	455	5760	1141	770	913	1604
	Coker 312	1090	3251	2546	466	5904	1161	788	925	1636
Year	**Line**	**Lys**	**Met**	**Phe**	**Pro**	**Ser**	**Thr**	**Trp**	**Tyr**	**Val**
2001	**COT102**	1003	1013	1197	1057	2340	400	310	540	953
	Coker 312	990	987	1153	1023	2270	403	313	520	950
2002	**COT102**	**1214**[2]	400	1458	1055	**1105**	779	265	710	1300
	Coker 312	1260	398	1500	1080	1180	775	273	735	1324

1 mg/100 g
2 Values in bold differ significantly from the control (p≤0.05)

Table II.6. Gossypol and cyclopropenoid fatty acid analysis of COT102 and Coker 312 cottonseed across locations (FSANZ, 2004; USDA/APHIS, 2007, 2010)

Component (g/100 g)	COT102	Coker 312
Total Gossypol	0.906	0.940
Free Gossypol	0.700	0.728
Sterculic	0.263	0.258
Malvalic	0.349	0.364
Dihydrosterculic	0.104	0.109

Table II.7. Proximate composition of forage from MIR162 and a non-transgenic hybrid (FSANZ, 2004; USDA/APHIS, 2007, 2010)

Line	Moisture % FW	Protein % DW	Fat % DW	Ash % DW	Carbohydrate % DW	Acid Detergent Fiber % DW	Neutral Detergent Fiber % DW
MIR162	71.2[1] (66.2 – 77.2)[2]	7.2 (3.1 – 10.1)	1.5 (0.8 – 1.9)	4.1 (3.1 – 5.8)	87.3 (82.9 – 90.7)	28.2 (23.6 – 34.2)	43.2 (35.1 – 56.1)
Non-GE	70.5 (64.1 – 75.8)	7.3 (3.4 – 8.9)	1.6 (0.4 – 2.3)	4.0 (2.9 – 5.7)	87.1 (83.6 – 91.7)	28.8 (23.3 – 34.8)	38.8 (32.1 – 46.9)
CCDB[1]	70.2 (49.1 – 81.3) N = 945	7.78 (3.14 – 11.57) N = 945	2.039 (0.296 – 4.570) N = 921	4.628 (1.527 – 9.638) N = 945	85.6 (76.4 – 92.1) N = 945	27.0 (16.13 – 47.39) N = 945	41.51 (20.29 – 63.71) N = 945
OECD[2]	(62 – 78)	(4.7 – 9.2)	(1.5 – 3.2)	(2.9 – 5.7)		(25.6 – 34)	(40 – 48.2)

1 Mean
2 Range
3 CCDB (2006) Crop Composition Database Version 3.0. http://www.cropcomposition.org.
4 OECD (2002) Consensus document on compositional considerations for new varieties of maize (*Zea mays*): Key food and feed nutrients, antinutrients and secondary plant metabolites. Series on the safety of novel foods and feeds, No. 6. Organisation for Economic Co-operation and Development, Paris.

Table II.8. Calcium and phosphorus composition of forage from MIR162 and a non-transgenic hybrid (FSANZ, 2004; USDA/APHIS, 2007, 2010)

Line	Calcium (mg/kg DW)	Phosphorus (mg/kg DW)
MIR162	21061 (1720 – 2930)2	1997 (1270 – 2240)
Non-GE	2039 (1440 – 2620)	2079 (1760 – 2560)
CCDB[3]	2028.6 (713.9 – 5767.9) N = 481	2066.1 (936.2 – 3704.1) N = 481
OECD[4]	0.15 – 0.31% dry weight	0.20 – 0.27% dry weight

1 Mean
2 Range
3 CCDB (2006) Crop Composition Database Version 3.0. http://www.cropcomposition.org.
4 OECD (2002) Consensus document on compositional considerations for new varieties of maize (*Zea mays*): Key food and feed nutrients, antinutrients and secondary plant metabolites. Series on the safety of novel foods and feeds, No. 6. Organisation for Economic Co-operation and Development, Paris.

Table II.9. Proximate analysis of grain from MIR162 and a non-transgenic hybrid (FSANZ, 2004; USDA/APHIS, 2007, 2010)

Line	Moisture % FW	Protein % DW	Fat % DW	Ash % DW	Carbohydrate % DW	Acid Detergent Fiber % DW	Neutral Detergent Fiber % DW	Total Dietary Fiber % DW	Starch % DW
MIR162	10.3[1] (9.5 – 11.5)[2]	9.8 (7.5 – 11.2)	3.8 (3.3 – 4.6)	1.4 (1.1 – 1.6)	85.0 (83.2 – 87.1)	5.0 (3.3 – 7.0)	11.7 (10.1 – 13.0)	16.8 (14.1 – 19.4)	63.1 (54.8 – 68.1)
Non-GE	10.5 (9.4 – 12.0)	9.6 (7.1 – 11.0)	3.8 (3.0 – 4.4)	1.3 (1.1 – 1.5)	85.3 (83.3 – 88.1)	4.6 (3.3 – 6.2)	11.1 (9.5 – 12.8)	16.3 (14.3 – 17.8)	64.9 (60.6 – 69.2)
CCDB[3]	11.3 (6.1 – 40.5) N = 1434	10.30 (6.15 – 17.26) N = 1434	3.555 (1.742 – 5.823) N = 1174	1.439 (0.616 – 6.282) N = 1410	84.6 (77.4 – 89.5) N = 1410	4.05 (1.82 – 11.34) N = 1350	11.23 (5.59 – 22.64) N = 1349	16.43 (8.85 – 35.31) N = 397	57.7 (26.5 – 73.8) N = 68
OECD[4]	7.0 – 23	6 – 12.7	3.1 – 5.8	1.1 – 3.9	82.2 – 82.9	3.0 – 4.3	8.3 – 11.9	11.1	

1 Mean
2 Range
3 CCDB (2006) Crop Composition Database Version 3.0. http://www.cropcomposition.org.
4 OECD (2002) Consensus document on compositional considerations for new varieties of maize (*Zea mays*): Key food and feed nutrients, antinutrients and secondary plant metabolites. Series on the safety of novel foods and feeds, No. 6. Organisation for Economic Co-operation and Development, Paris.

Table II.10. Mineral composition of grain from MIR162 and a non-transgenic hybrid (FSANZ, 2004; USDA/APHIS, 2007, 2010)

Line	Calcium (mg/kg DW)	Copper (mg/kg DW)	Iron (mg/kg DW)	Magnesium (mg/kg DW)	Manganese (mg/kg DW)	Phosphorus (mg/kg DW)	Potassium (mg/kg DW)	Selenium (mg/kg DW)	Sodium (mg/kg DW)	Zinc (mg/kg DW)
MIR162	38.1[1] (29.4 – 47.2)[2]	1.3 (0.96 – 1.95)	20.2 (17.3 – 22.9)	125 (1090 – 1480)	6.3 (4.14 – 7.97)	3173 (2810 – 3550)	3352 (3160 – 3710)	<LOQ[3] – 0.414 NA	<LOQ – <LOQ NA	21.7 (18.8 – 24.3)
Non-GE	35.3 25.7 – 44.0	1.2 1.00 – 1.58	19.2 15.7 – 22.5	1218 960 – 1470	6.1 4.59 – 8.01	3073 2710 – 3400	3357 2950 – 3660	<LOQ – 0.531 NA	<LOQ – <LOQ NA	21.5 19.2 – 23.8
CCDB[4]	46.4 (12.7 – 208.40) N = 1344	1.75 (0.73 – 18.5) N = 1249	21.81 (10.42 – 49.07) N = 1255	1193.8 (594.0 – 1940.0) N = 1257	6.18 (1.69 – 14.30) N = 1256	3273.5 (1470.0 – 5330.0) N = 1349	3842 (1810.0 – 6030.0) N =1257	0.2 (0.05 – 0.75) N = 89	31.75 (0.17 – 731.54) (N = 223)	21.6 (6.5 – 37.2) (N = 1257)
OECD[5]	3 – 100	0.09 – 1.0	0.1 – 10	82 – 1000		234 – 750	320 – 720	0.001 – 0.1	0 – 150	1.2 – 3.0

1 Mean
2 Range
3 "LOQ" = Limit of quantitation
4 CCDB (2006) Crop Composition Database Version 3.0. http://www.cropcomposition.org.
5 OECD (2002) Consensus document on compositional considerations for new varieties of maize (*Zea mays*): Key food and feed nutrients, antinutrients and secondary plant metabolites. Series on the safety of novel foods and feeds, No. 6. Organisation for Economic Co-operation and Development, Paris.

Table II.11. Vitamin analysis of grain from MIR162 and a non-transgenic hybrid (FSANZ, 2004; USDA/APHIS, 2007, 2010)

Line	ß-Carotene (mg/100g DW)	Thiamine (mg/100g DW)	Riboflavin (mg/100g DW)	Niacin (mg/100g DW)	Pyridoxine (mg/100g DW)	Folic Acid (mg/100g DW)	α-Tocopherol (mg/100g DW)
MIR162	0.277[1] (0.241 – 0.316)[2]	0.393 (0.358 – 0.433)	0.190 (0.112 – 0.238)	2.37 (2.11 – 2.8)	0.565 (0.434 – 0.694)	0.028 (0.021 – 0.034)	0.01 (0.97 – 1.54)
Non-GE	0.294 (0.244 – 0.358)	0.392 (0.339 – 0.443)	0.180 (0.144 – 0.226)	2.47 (2.03 – 3.15)	0.605 (0.486 – 0.738)	0.028 (0.024 – 0.033)	0.01 (1.10 – 1.54)
CCDB[3]	0.684 (0.019 – 4.681) N = 276	0.530 (0.126 – 4.000) N = 894	0.125 (0.050 – 0.236) N = 704	2.376 (1.037 – 4.694) N = 415	0.644 (0.368 – 1.132) N = 415	0.0651 (0.0147 – 0.1464) N = 895	0.0103 (0.0015 – 0.0687) N = 863
OECD[4]		0.23 – 0.86	0.025 – 0.53	0.93 – 0.70	0.46 – 0.96		

1 Mean
2 Range
3 CCDB (2006) Crop Composition Database Version 3.0. http://www.cropcomposition.org.
4 OECD (2002) Consensus document on compositional considerations for new varieties of maize (*Zea mays*): Key food and feed nutrients, antinutrients and secondary plant metabolites. Series on the safety of novel foods and feeds, No. 6. Organisation for Economic Co-operation and Development, Paris.

Table II.12. Amino Acid composition of grain from MIR162 and a non-transgenic hybrid (FSANZ, 2004; USDA/APHIS, 2007, 2010)

Line	Ala (mg/g DW)	Arg (mg/g DW)	Asp (mg/g DW)	Cys (mg/g DW)	Glu (mg/g DW)	Gly (mg/g DW)	His (mg/g DW)	Ile (mg/g DW)	Leu (mg/g DW)
MIR162	7.701 (5.59 – 9.17)[2]	4.77 (3.89 – 5.30)	6.66 (5.29 – 7.72)	2.31 (1.96 – 2.62)	19.54 (14.0 – 23.3)	3.84 (3.26 – 4.27)	2.87 (2.28 – 3.26)	3.38 (2.55 – 4.00)	12.85 (8.86 – 15.6)
Non-GE	7.55 (5.24 – 8.89)	4.68 (3.64 – 5.27)	6.54 (4.85 – 7.45)	2.29 (1.96 – 2.65)	19.16 (13.2 – 22.5)	3.79 (3.13 – 4.10)	2.85 (2.20 – 3.14)	3.31 (2.35 – 3.85)	12.57 (8.28 – 15.1)
CCDB[3]	7.9 (4.39 – 13.93) N = 1350	4.33 (1.19 – 6.39) N = 1350	6.88 (3.35 – 12.08) N = 1350	2.21 (1.25 – 5.14) N = 1350	20.02 (9.65 – 35.36) N = 1350	3.85 (1.84 – 5.39) N = 1350	2.96 (1.37 – 4.34) N = 1350	3.68 (1.79 – 6.92) N = 1350	13.41 (6.42 – 24.92) N = 1350
OECD[4] (% dw)	0.56 – 1.04	0.22 – 0.64	0.48 – 0.85	0.08 – 0.32	1.25 – 2.58	0.26 – 0.49	0.15 – 0.38	0.22 – 0.71	0.79 – 2.41
Line	**Lys (mg/g DW)**	**Met (mg/g DW)**	**Phe (mg/g DW)**	**Pro (mg/g DW)**	**Ser (mg/g DW)**	**Thr (mg/g DW)**	**Trp (mg/g DW)**	**Tyr (mg/g DW)**	**Val (mg/g DW)**
MIR162	3.05 2.52 – 3.44	2.15 1.76 – 2.54	5.09 3.70 – 6.04	9.12 6.79 – 10.8	5.21 3.95 – 6.06	3.55 2.83 – 4.06	0.570 0.453 – 0.645	3.42 2.58 – 4.09	4.81 3.78 – 5.61
Non-GE	2.96 2.47 – 3.29	2.10 1.71 – 2.42	4.99 3.43 – 5.84	8.96 6.51 – 10.3	5.11 3.68 – 5.84	3.47 2.64 – 3.96	0.562 0.479 – 0.636	3.35 2.35 – 3.86	4.74 3.52 – 5.37
CCDB[3]	3.15 1.72 – 6.68 N = 1350	2.09 1.24 – 4.68 N = 1350	5.25 2.44 – 9.30 N = 1350	9.51 4.62 – 16.32 N = 1350	5.12 2.35 – 7.69 N = 1350	3.75 2.24 – 6.66 N = 1350	0.627 0.271 – 2.150 N = 1350	3.36 1.03 – 6.42 N = 1350	4.90 2.66 – 8.55 N = 1350
OECD[4] (% dw)	0.05 – 0.55	0.1 – 0.46	0.29 = 0.64	0.63 – 1.36	0.35 – 0.91	0.27 – 0.58	0.04 – 0.13	0.12 – 0.79	0.21 – 0.85

1 Mean
2 Range
3 CCDB (2006) Crop Composition Database Version 3.0. http://www.cropcomposition.org.
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Table II.13. Fatty Acid composition (% of total fatty acids) of MIR162 and a non-transgenic hybrid (FSANZ, 2004; USDA/APHIS, 2007, 2010)

Line	16:0 Palmitic	18:0 Stearic	18:1 Oleic	18:2 Linoleic	18:3 Linolenic
MIR162	12.78[1] (12.25 – 13.09)[2]	1.84 (1.56 – 1.99)	25.49 (22.67 – 26.57)	56.99 (55.86 – 59.74)	1.81 (1.72 – 1.89)
Non-GE	12.69 (12.29 – 13.12)	1.88 (1.625 – 2.07)	25.22 (23.38 – 26.77)	57.36 (56.26 – 59.47)	1.75 (1.64 – 1.86)
CCDB[3]	11.50 (7.94 – 20.71) N = 1344	1.82 (1.02 – 3.40) N = 1344	25.8 (17.4 – 40.2) N = 1344	57.60 (36.2 – 66.5) N = 1344	1.20 (0.57 – 2.25) N = 1344
OECD[4] %dw	0.29 – 0.79	0.04 – 0.17	0.70 – 1.39	0.67 – 2.81	0.03 – 0.10

1 Mean
2 Range
3 CCDB (2006) Crop Composition Database Version 3.0. http://www.cropcomposition.org.
4 OECD (2002) Consensus document on compositional considerations for new varieties of maize (*Zea mays*): Key food and feed nutrients, antinutrients and secondary plant metabolites. Series on the safety of novel foods and feeds, No. 6. Organisation for Economic Co-operation and Development, Paris.

Table II.14. Secondary metabolite and anti-nutrient analysis of grain from MIR162 and a non-transgenic comparator (FSANZ, 2004; USDA/APHIS, 2007, 2010)

Line	Ferulic Acid (mg/kg DW)	ρ-Coumaric Acid (mg/kg DW)	Inositol (ppm)	Phytic Acid (% DW)	Trypsin Inhibitor (TIU/mg DW)	Furfural (mg/kg DW)	Raffinose (% DW)
MIR162	2682[1] (2490 – 2980)[2]	179 (148 – 202)	2957 (2410 – 3530)	0.745 (0.621 – 0.871)	2.82 (2.27 – 3.72)	<LOQ - <LOQ NA	<LOQ – 0.116 NA
Non-GE	2453 (2010 – 2760)	157 (137 – 179)	2792 (2180 – 3610)	0.727 (0.593 – 0.919)	2.92 (2.38 – 3.48)	<LOQ - <LOQ NA	<LOQ – 0.137 NA
CCDB[3]	2201.1 (291.9 – 3885.8) N = 817	218.4 (53.4 – 576.2) N = 817	1331.5 (89.0 – 3765.4) N = 504	0.745 (0.111 – 1.570) N = 1196	2.73 (1.09 – 7.18) N = 696	3.697 (3.000 – 6.340) N = 14	0.132 (0.020 – 0.320) N = 701
OECD[4] %dw	0.02 – 0.3	0.003 – 0.03		0.45 – 1.0		<0.01 ppm	0.21 – 0.31

1 Mean
2 Range
3 CCDB (2006) Crop Composition Database Version 3.0. http://www.cropcomposition.org.
4 OECD (2002) Consensus document on compositional considerations for new varieties of maize (*Zea mays*): Key food and feed nutrients, antinutrients and secondary plant metabolites. Series on the safety of novel foods and feeds, No. 6. Organisation for Economic Co-operation and Development, Paris.

Table II.15. Statistically significant differences between MIR162 and control comparators (USDA/APHIS, 2007, 2010)

Analyte	MIR162	Control	% Difference	CCDB Database	Literature Range
Neutral Detergent Fiber[1] (%)	43.2 (35.1 – 56.1)[2]	38.8 (32.13 – 46.9)	11.34	41.51 (20.29 – 63.71)	40 – 48.2
Ash (%)	1.4 (1.1 – 1.6)	1.3 (1.1 – 1.5)	7.69	1.439 (0.616 – 6.282)	1.1 – 3.9
Neutral Detergent Fiber (%)	11.7 (10.1 – 13.0)	11.1 (9.5 – 12.8)	5.41	11.23 (5.59 – 22.64)	8.3 – 11.9
Starch (%)	63.1 (54.8 – 68.1)	64.9 (60.6 – 69.2)	-2.77	57.7 (26.5 – 73.8)	
Calcium (mg/kg)	38.1 (29.4 – 47.2)	35.3 (25.7 – 44.0)	7.93	46.4 (12.7 – 208.4)	3 – 100 g /100 g
Iron (mg/kg)	20.2 (17.3 – 22.9)	19.2 (15.7 – 22.5)	5.21	21.81 (10.42 – 49.07)	0.1 – 10 g /100 g
Phosphorus (mg/kg)	3173 (2810 – 3550)	3073 (2710 – 3400)	3.25	3273.5 (1470.0 – 5330.0)	234 – 750 mg /100 g
Vitamin A (mg/100 g)	0.277 (0.241 – 0.316)	0.294 (0.244 – 0.358)	-5.78	0.684 (0.019 – 4.681)	
Vitamin B_6 (mg/100 g)	0.565 (0.434 – 0.694)	0.605 (0.486 – 0.738)	-6.61	0.644 (0.368 – 1.132)	4.6 – 9.6
18:2 Linoleic Acid (% total FA[3])	56.99 (55.86 – 59.74)	57.36 (56.26 – 59.47)	-0.65	57.60 (36.2 – 66.5)	0.67 – 2.81% DW
18:3 Linolenic Acid (% total FA)	1.81 (1.72 – 1.89)	1.75 (1.64 – 1.86)	3.43	1.20 (0.57 – 2.25)	0.03 – 0.10% DW
Ferulic Acid (mg/kg)	2682 (2490 – 2980)	2453 (2010 – 2760)	9.33	2201.1 (291.9 – 3885.8)	200 – 3000
ρ-Coumaric Acid (mg/kg)	179 (148 – 202)	157 (137 – 179)	14.01	218.4 (53.4 – 576.2)	3 – 300

1 Measured in forage. All other components were measured in grain.
2 Range
3 FA = fatty acid