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REVIEW

Transgenic maize event TC1507: Global status of food, feed, and environmental safety

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Maize (*Zea mays*) is a widely cultivated cereal that has been safely consumed by humans and animals for centuries. Transgenic or genetically engineered insect-resistant and herbicide-tolerant maize, are commercially grown on a broad scale. Event TC1507 (OECD unique identifier: DAS-Ø15Ø7–1) or the Herculex®# I trait, an insect-resistant and herbicide-tolerant maize expressing Cry1F and PAT proteins, has been registered for commercial cultivation in the US since 2001. A science-based safety assessment was conducted on TC1507 prior to commercialization. The safety assessment addressed allergenicity; acute oral toxicity; subchronic toxicity; substantial equivalence with conventional comparators, as well as environmental impact. Results from biochemical, physicochemical, and *in silico* investigations supported the conclusion that Cry1F and PAT proteins are unlikely to be either allergenic or toxic to humans. Also, findings from toxicological and animal feeding studies supported

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that maize with TC1507 is as safe and nutritious as conventional maize. Maize with TC1507 is not expected to behave differently than conventional maize in terms of its potential for invasiveness, gene flow to wild and weedy relatives, or impact on non-target organisms. These safety conclusions regarding TC1507 were acknowledged by over 20 regulatory agencies including United States Environment Protection Agency (US EPA), US Department of Agriculture (USDA), Canadian Food Inspection Agency (CFIA), and European Food Safety Authority (EFSA) before authorizing cultivation and/or food and feed uses. A comprehensive review of the safety studies on TC1507, as well as some benefits, are presented here to serve as a reference for regulatory agencies and decision makers in other countries where authorization of TC1507 is or will be pursued.

KEYWORDS. TC1507, Cry1F, GE maize, environmental safety, food and feed safety, global authorizations

ABBREVIATIONS. aa, amino acid; Bt, *Bacillus thuringiensis*; CFIA, Canadian Food Inspection Agency; Cry, crystalline; CTNBio, Comissão Técnica Nacional de Biossegurança; DA-BPI, Department of Agriculture-Bureau of Plant Industry; DNA, deoxyribonucleic acid; EFSA, European Food Safety Authority; ELISA, enzyme-linked immunosorbent assay; ERA, environmental risk assessment; EU, European Union; FAO, Food and Agriculture Organization of the United Nations; FDA, Food and Drug Administration; FFP, food, feed, and processing; FSANZ, Food Standards Australia New Zealand; GAIN, Global Agricultural Information Network; GE, genetically engineered; HGT, horizontal gene transfer; ISAAA, International Service for the Acquisition of Agri-biotech Applications; LD_{50}, median lethal dose; NCGA, National Corn Growers Association; NTOs, non-target organisms; *nptII*, neomycin phosphotransferase II; OECD, Organisation for Economic Co-operation and Development; PAT, phosphinothricin-N-acetyltransferase; PCR, polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SE, Substantial Equivalence; SGF, simulated gastric fluid; US EPA, United States Environment Protection Agency; USDA APHIS, US Department of Agriculture-Animal and Plant Health Inspection Service; WHO, World Health Organization

INTRODUCTION

Maize (*Zea mays*) is a widely grown cereal that has been safely consumed by humans and animals for millennia. Currently maize is predominantly used to feed livestock or as raw material for industrial products, while only 21% is consumed as human food (OECD, 2003). Between 1996 and 2013, transgenic or genetically engineered (GE) maize was grown on a cumulative 460 million hectares (based on data derived from ISAAA [2014]) and in 2013 alone GE maize occupied over 32% or 57 million hectares of maize area. GE maize was grown in 17 countries and the greatest hectarage (in millions of hectares) was in the US (35.6), Brazil (12.9), Argentina (3.2), South Africa (2.4), and Canada (1.7) at that time (James, 2013).

In the US, GE maize products were developed targeting lepidopteran insect pests due to the potential for substantial economic damage as a result of significant yield losses. These GE maize products were first commercialized in 1996. With the success of lepidopteran-resistant maize, GE maize products that protect against subterranean corn rootworm followed (Castle et al., 2006). McLaren and Copping (2011) have summarized the global registration status of different commercially available GE maize lines. Transgenic maize hybrids expressing 2 or more traits and combined through conventional breeding techniques, commonly referred to as breeding stacks, have been available since 2000. Insect-resistant and herbicide-tolerant traits enable farmers to use simplified crop management practices (Que et al., 2010); therefore, such breeding stacks occupied almost 73% of all GE maize hectares planted in 2012 (ISAAA, 2014).

The insect-resistant GE maize currently in the market expresses genes derived from

Bacillus thuringiensis (Bt), and these transgenic products are commonly referred to as Bt maize. Bt is a ubiquitous soil bacterium that has proven to be a rich source of insecticidal proteins, which are considered to be selective and generally active against insects within a specific taxonomic insect order (Van Frankenhuyzen, 2009). All of the commercially available Bt maize products express one or more crystalline (Cry) insecticidal or vegetative insecticidal proteins (Que et al., 2010). The mode of action of Cry proteins is well understood (Estruch et al., 1996; Whalon and Wingerd, 2003; OECD, 2007) though scientific studies continue to further elucidate these mechanisms (Vachon et al., 2012). In general, Cry proteins are ingested, processed by intestinal proteases, and converted to active toxin in the insect alkaline midgut. The active toxins bind to specific receptors present in the gut of susceptible insects. The bound toxin-receptor complex leads to gut membrane pore formation, subsequent septicemia, and ultimately death. Humans and other mammals lack the alkaline gut as well as Cry protein-binding receptors and are therefore not vulnerable to toxicity from corresponding Cry proteins. For over 50 years, several varieties of Bt containing Cry proteins have been safely used as insecticidal sprays in commercial agriculture (Siegel, 2001) and, for the past 14 years, products derived from GE crops expressing Bt proteins have been safely consumed as food and feed (Hammond and Koch, 2012). The Organisation for Economic Co-operation and Development (OECD) summarized the safety information regarding Bt proteins derived from GE crops, with a focus on human health assessment and impact on non-target species (OECD, 2007).

Safety of GE crops and the food/feed derived from them are assessed through an extensive and systematic process prior to authorization for cultivation or food and/or feed use. The safety assessment process followed by many countries, which includes protein safety and a demonstration of Substantial Equivalence (SE), is structured according to guidelines from international organizations, such as the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO), OECD, and the Codex Alimentarius Commission (Delaney, 2009). A fundamental step during the safety assessment of GE crops is to establish SE. The establishment of SE is not a safety assessment in itself, but rather a starting point from which to structure the safety assessment. A GE crop is considered substantially equivalent to its non-GE counterpart (also referred to as near-isoline, near-isogenic hybrid, unmodified conventional counterpart, etc.), when their agronomic characteristics and compositional profiles are shown to be comparable, with the exception of the introduced genes and proteins they express. This comparative approach assists in the identification of potential safety and nutritional issues and is currently regarded as the most appropriate strategy for the safety assessment of GE crops and foods derived from them (CAC, 2009). If a GE crop is substantially equivalent with a non-GE counterpart that has an established history of safe use, the focus of the safety assessment can shift to studying the potential impact of the introduced genes and proteins they express on human and animal health as well as the environment (Bajaj and Mohanty, 2005).

The safety of a transgenic event with respect to human health and the environment is evaluated through various laboratory and field experiments and toxicological studies with non-target organisms (e.g., arthropods, aquatic organisms, birds, rodents, large mammals) (Craig et al., 2008). Such regulatory studies, performed to investigate the safety of a GE product prior to commercialization, can cost up to US $13 – 18 million and take an average of 3 to 4 y to conduct (McDougall, 2011). The safety data required for product authorizations are generated from research in the discovery phase through product development and regulatory studies. The cost and time to conduct these studies can vary significantly depending on the crop (food or non-food crop), nature of the introduced trait(s), country requirements, etc. Moreover, the estimated total cost for biotechnology trait development from discovery to commercial entry is US $136 million with regulatory costs including authorizations projected at 26% of that total cost (McDougall, 2011).

The purpose of this paper is to review the robust food, feed, and environmental safety information that served as the basis for securing regulatory authorization of maize event TC1507 in more than 20 countries, as well as some beneficial aspects from TC1507 commercialization. Information sources reviewed herein include dossiers presented to regulatory agencies in several countries, regulator safety assessment reports, peer-reviewed literature, online databases, and technology developer product materials. This comprehensive review is assembled to aid authorities making regulatory decisions in countries where registrations are being pursued for maize event TC1507, as a single event product and in breeding stack products, and for others interested in the safety of TC1507.

Event TC1507 and Background

Insect-resistant transgenic maize event TC1507 (OECD identifier: DAS-Ø15Ø7–1), also referred to as 1507 in the regulatory context and the Herculex® I trait commercially, was jointly developed by Pioneer Hi-Bred International, Inc. (DuPont Pioneer) and Dow AgroSciences LLC. TC1507 maize was developed to provide farmers a simple and highly effective tool to control certain key lepidopteran larval pests while tolerating glufosinate herbicidal active ingredients.

The process used to assess the safety of TC1507 summarized here is consistent with the recommended international guidelines as reviewed by Delaney (2009). During the assessment process, a variety of data were considered including the history of safe use of maize; source of the introduced genes (donor organisms); molecular characterization of the event; genetic stability; inheritance pattern; protein expression; protein specificity and efficacy; protein biochemistry and bioinformatics; toxicology; substantial equivalence with conventional comparators; impact on non-target organisms; and fate in the environment. Safety data, as required, were submitted to multiple regulatory agencies including the US, Canada, EU, Japan, Australia, Brazil, Argentina, Philippines, and South Africa, before obtaining authorizations for cultivation, food, and/or feed use.

Genes, Donor Organisms and Their Safety

TC1507 maize was designed to express sufficient levels of Cry1F and phosphinothricin-N-acetyltransferase (PAT) proteins, encoded by *cry1F* and *pat* genes, respectively, to achieve efficacious insect resistance and herbicide tolerance. The *cry1F* gene was derived from *Bacillus thuringiensis* var. *aizawai*. A modified *cry1F* gene that was codon-optimized for more efficient *in planta* expression was used to produce TC1507 maize. The plant-expressed *cry1F* gene encodes a protein of 68 kDa that is a truncated version of the native protein with a single amino acid substitution (USDA APHIS, 2000). The Cry1F protein is active against certain lepidopterans including key pests such as European corn borer (*Ostrinia nubilalis*), fall armyworm (*Spodoptera frugiperda*), corn earworm (*Helicoverpa zea*), and black cutworm (*Agrotis ipsilon*). It is worth referring to Wolt (2011) for a detailed list of lepidopteran species and observed susceptibilities to Cry1F protein in laboratory studies.

The *pat* gene was derived from the aerobic, non-pathogenic, naturally occurring soil actinomycete *Streptomyces viridochromogenes*. The PAT protein acetylates phosphinothricin, the active isomer present in the non-selective glufosinate-ammonium herbicide, to a metabolite, N-acetyl phosphinothricin, that is non-phytotoxic (OECD, 2002). In this way, expression of the PAT protein in TC1507 maize confers tolerance to glufosinate-ammonium herbicidal active ingredient and serves as a marker to select transformed maize in the laboratory. Separate reviews demonstrate the safety of PAT to human health (Hérouet et al., 2005) and the environment (CERA, 2011).

The *pat* gene in event TC1507 is a modified version of the native bacterial gene that was codon-optimized for improved *in planta* expression. The amino acid sequence of the plant-derived PAT protein is identical to the native PAT protein (Meyer, 1999). Refer to OECD (1999), for a general review on the *pat* genes and their enzymatic proteins.

Transformation and Event Development

PHI8999A, a linear DNA fragment containing the *cry1F* gene and the *pat* selectable marker gene, was obtained from plasmid PHP8999. The *cry1F* and *pat* gene coding sequences were driven by regulatory sequences enabling constitutive expression of the Cry1F and PAT proteins throughout the plant. An inbred maize line was transformed with PHI8999A by a micro-projectile bombardment (biolistic) method. Positively transformed plants containing both the *cry1F* and *pat* genes were evaluated in greenhouse testing and in the field. Of these, line 1507, which would later be designated as event TC1507, was selected for its good agronomic characteristics and efficacy against target insects.

Results of the molecular characterization revealed event TC1507 consisted of an insert at a single genetic locus that included the nearly full-length intact copy of the DNA insert, which contained the *cry1F* and *pat* genes. In addition, there are a few non-functional rearranged *cry1F* and *pat* partial fragments that are interspersed among native maize genomic sequences on both flanking regions, which are commonly observed during genomic integration via micro-projectile bombardment transformation (Pawlowski and Somers, 1996; Makarevitch et al., 2003). The event TC1507 does not contain the antibiotic resistance gene (*nptII*) that was included in the plasmid backbone, but was not present in the PHI8999A fragment used for maize transformation (USDA APHIS, 2000; US EPA, 2005).

Stability of the inserted genes was studied over multiple generations. Event TC1507 was crossed and backcrossed with an elite inbred to produce hybrids that were tested for glufosinate tolerance and resistance to European corn borer. Southern blot analyses demonstrated the stability of the inserted genes in progenies across at least 6 generations, and inheritance followed a Mendelian segregation pattern for a single dominant gene (USDA APHIS, 2000).

Protein concentration

The concentration of the Cry1F and PAT proteins in TC1507 maize has been well characterized. To date, protein expression in TC1507 maize has been characterized in over 20 field studies, spanning multiple geographies (including Brazil, Canada, Chile, Spain, and the US) and years (2005–2013). Concentrations of Cry1F and PAT proteins in representative tissues and developmental growth stages (e.g., leaf, root, pollen, stalk, whole plant, grain) were analyzed by enzyme-linked immunosorbent assay (ELISA) methods (**Table 1**). The Cry1F protein was detected in leaf, root, pollen, stalk, whole plant, and grain tissues. The PAT protein was detected in leaf, root, stalk, whole

TABLE 1. Expression of Cry1F and PAT proteins in different tissues of TC1507 maize (based on Pioneer Hi-Bred International, Inc. internal studies conducted during 2005–2013, unpublished data)

	Mean Cry1F Protein (Range)*	Mean PAT Protein (Range)*	
Tissue (Growth stage)	(ng/mg tissue dry weight)		Number of Studies**
Leaf (R1)	20 (15 – 31)	9.0 (5.1 - 16)	21
Root (R1)	5.7 (1.5 – 7.9)	0.51 (0.090 - 1.4)	19
Pollen (R1)	27 (23 – 37)	<0.28	21
Stalk (R1)	8.8 (4.7 – 13)	0.16 (0.022 - 0.50)	19
Whole Plant (R1)	13 (8.3 – 21)	3.3 (1.6 - 6.9)	18
Grain (R6)	3.7 (2.1 – 5.7)	<0.069 (<0 .069 - 0.073)	21

*mean is reported as the overall mean of the reported mean protein concentrations across all studies; range spans the minimum and maximum reported mean protein concentrations across all studies.

R1 – stage of plant development when silks become visible.

R6 – stage of plant development regarded as physiological maturity.

**Field studies conducted in Brazil, Canada, Chile, Spain, and the US.

plant, and grain tissues. The PAT protein was close to the lower limit of the quantitative range of the ELISA in stalk, root, and grain, and was below the lower limit of the quantitative range of the ELISA in pollen (**Table 1**).

Diagnostic tools like gene- and event-specific polymerase chain reaction (PCR) methods, ELISA, and lateral flow strips are widely available to detect the introduced genes or the expressed Cry1F and PAT proteins in TC1507. These methods are employed for research, adventitious presence testing in seed or grain, inspection of food stuff, environmental monitoring and quarantine at ports (La Paz et al., 2006; Heide et al., 2008; Shrestha et al., 2008; Holck et al., 2009; Kim et al., 2010; Park et al., 2010; Rimachi et al., 2011; Takabatake et al., 2010).

Agronomic characteristics

Field trials were conducted in multiple key maize-growing regions in the US to evaluate the agronomic performance of TC1507 compared with an appropriate non-GE counterpart. Data on parameters such as yield, time to pollen shed, time to silking, grain density, plant height, ear height, early stand count, emergence, vigor, stalk lodging, root lodging, dropped ears, and integrity of the stalk were recorded from these field trials. Germination of TC1507 and control maize under cold and warm growing conditions was examined in laboratory studies. Results from these field trials show that the evaluated agronomic parameters were comparable between TC1507 and the non-GE comparator (USDA APHIS, 2000). Multiple additional field studies have been conducted in the US, Canada, Italy, France, Bulgaria, Spain, Chile, Philippines, and Indonesia (Pioneer Hi-Bred International, Inc. internal unpublished data), which all support the conclusion that TC1507 is substantially equivalent to non-GE maize.

Compositional characteristics

A field trial was conducted at sites in 4 major maize-growing regions in Chile to compare the nutritional composition of grain and whole plant tissue (referred to as forage) samples from TC1507 maize with an unmodified hybrid that had the same genetic background. Forage samples were analyzed to characterize levels of proximates (protein, fat, acid detergent fiber, neutral detergent fiber, carbohydrate, ash, and moisture level). Grain samples were analyzed to characterize proximates, minerals, fatty acid composition, amino acid levels, vitamins, secondary metabolites and anti-nutrients. Results from this study show that the nutrient composition of forage and grain were comparable between TC1507 and the non-GE control hybrid (Stauffer and Zeph, 2000). Multiple additional field studies have been conducted in the US, Canada, Italy, France, Bulgaria, Spain, and Argentina, which all support the results of this field trial (Pioneer Hi-Bred International, Inc. internal unpublished data).

Safety Assessment of Cry1F and PAT Proteins

Safety tests are conducted with the introduced protein(s) to evaluate potential risks of a transgenic event. These studies are used to assess the potential allergenicity and toxicity of the introduced proteins and to inform conclusions as to the safety of the proteins in the context of food and feed uses.

Cry1F protein equivalency

Safety assessments of GE crops include regulatory studies covering topics such as toxicology and fate of environmental exposure that require large quantities of protein. Low trait protein expression levels render it impractical to extract sufficient quantities of novel protein from GE plants. Hence, alternative systems such as microbes are engineered to express the novel proteins. The suitability of microbial-expressed protein to serve as a surrogate for use in safety studies is determined by demonstrating equivalence of the proteins derived from microbe and plant sources through biochemical and physicochemical evaluations (Evans, 2004; Raybould et al., 2013).

The Cry1F protein used for the safety assessment studies was produced in the bacteria

Pseudomonas fluorescens. Full-length Cry1F toxin was extracted, truncated with trypsin, purified by diafiltration, and concentrated by lyophilization. Comparisons were made between the microbial-expressed Cry1F protein and the Cry1F protein isolated from TC1507 maize to evaluate protein equivalency by mass spectrometry, N-terminal sequencing, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and western blot analyses. The amino acid (aa) sequence of the microbial-expressed Cry1F protein contained a single aa substitution and was 27 aa shorter (at the N-terminus) and 7 aa longer (at the C-terminus) in comparison with the plant-encoded Cry1F protein. Additionally, the Cry1F protein from both sources lacked detectable post-translational glycosylation (Evans, 1998). The bioactivity of the maize-expressed and microbial-expressed Cry1F proteins was tested in bioassays with susceptible insects such as European corn borer (*O. nubilalis*), tobacco budworm (*Heliothis virescens*), and fall armyworm (*S. frugiperda*). The results indicated that the bioactivity of Cry1F protein from plant and microbial sources was comparable (Evans, 1998).

A similar biochemical comparison was made with plant-derived and microbial-expressed PAT proteins. The results of mass spectrometry, SDS-PAGE, and western blot analyses demonstrated the biochemical equivalency of the PAT protein from the plant and microbial sources (CFIA, 2002). Cumulatively, these studies established that Cry1F and PAT proteins isolated from the microbial source are equivalent to the corresponding proteins isolated from TC1507 maize, and these findings support the use of microbial-expressed proteins for regulatory studies.

Allergenicity Assessment

No single factor has been recognized as the primary indicator for protein allergenicity and no validated animal model predictive of allergenic potential is available. Therefore, allergenic potential of proteins produced from the introduced genes in transgenic events is typically evaluated through a "weight-of-evidence" approach (CAC, 2009; Ladics, 2008; Ladics et al., 2011). The assessment of allergenic potential is based on the existing knowledge about allergens including the history of exposure and safety of the gene(s) source; the amino acid sequence similarity to known human allergens; and the thermolability, pepsin digestibility, and glycosylation status of the proteins (Ladics, 2008).

Bt (the source of the *cry1F* gene) has no history of causing allergy. In over 50 y of commercial use as a microbial pesticide on food crops, there have been no reports of allergenicity to proteins from Bt, including occupational allergy associated with the manufacture of products containing Bt (Hammond and Koch, 2012). These microbial formulations have been used on a wide variety of crops, including fresh vegetables, with no reported allergic concerns. *S. viridochromogenes* (the source of the *pat* gene) occurs widely in nature and is not known to cause allergy (Hérouet et al., 2005; OECD, 1999). This history establishes a sound basis for the lack of allergenic potential for the Cry1F and PAT proteins.

Bioinformatics in allergenicity assessment

Amino acid (aa) sequence similarity and structural comparisons of a novel protein to known allergenic proteins are important endpoints in the evaluation of allergenicity of GE foods. Bioinformatics analyses were conducted to compare whether the aa sequences of Cry1F and PAT proteins are similar to sequences in a database of food, dermal, and respiratory allergenic proteins. Such *in silico* analyses additionally examine the potential for cross-reactivity to known allergens (Ladics et al., 2011). Similarity (>35% shared identity over 80 aa or greater) was not detected and no contiguous sequence matches (8 aa or greater) were identified compared with sequences in the AllergenOnline database (University of Nebraska, Lincoln). However, a single contiguous match over 6 aa was identified between Cry1F and the Der p7 protein of the dust mite, *Dermatophagoides pteronyssinus,* but there was no evidence of cross-reactivity between the Cry1F and human sera reactive to Der p7 protein

(Ladics et al., 2006). The lack of any significant aa similarity indicates that the potential for cross-reactivity of either Cry1F or PAT proteins with known allergens is extremely low (Meyer, 1999; Ladics et al., 2006).

Thermolability of Cry1F and PAT proteins

Thermal stability of novel proteins is assessed based on the premise that proteins that are less stable and denatured by heat are less likely to be allergenic or cause adverse health effects (Craig et al., 2008; Delaney et al., 2008). Aliquots of microbial-expressed Cry1F protein were subjected to different temperature regimens and applied to the surface of an insect diet provided to neonates of tobacco budworm (*H. virescens*). Diminished larval growth inhibition, demonstrating loss of bioactivity, indicated Cry1F protein was labile to heat at and above 75 °C for 30 min (Herman, 2000). When heated at 55 °C for 10 min, the PAT protein was denatured as corroborated by a loss of enzymatic activity (Wehrmann et al., 1996). Notably, the denatured PAT protein did not show any similarities with IgE epitopes of known allergenic proteins (Hérouet et al., 2005).

Pepsin digestibility

Important protein allergens have been shown to be stable to peptic digestion; therefore, protein resistance to pepsin indicates that further testing is required to determine allergenic potential (CAC, 2009; Ladics, 2008). Cry1F and PAT proteins were incubated *in vitro* with simulated gastric fluid (SGF) containing pepsin for different time periods and the digested products were analyzed using SDS-PAGE and western blot analyses. The results demonstrated that Cry1F protein was degraded in less than 1 minute (Evans, 1998) and PAT protein was digested to non-detectable levels within 5 seconds after addition of SGF containing pepsin (FSANZ, 2003). As the Cry1F and PAT proteins are readily digested by pepsin, there is a lower probability they would cause adverse health effects due to limited persistence in the mammalian digestive environment. The Cry1F and PAT proteins are not from allergenic sources, are heat labile, are rapidly degraded in simulated gastric fluid, and are not glycosylated. This evidence supports the conclusion that the allergenic potential of Cry1F and PAT proteins is low (Hérouet et al., 2005; Ladics et al., 2006).

Mammalian Toxicology Assessment of Cry1F and PAT Proteins

Acute oral toxicity in mice

To date, no known mammalian health effects have been associated with Cry1 proteins from Bt microbial products (Siegel, 2001; Delaney et al., 2008). Safety of the Cry1F protein was demonstrated in a 14-day acute oral toxicity study in mice (Kuhn, 1998). No mortality or clinical/behavioral signs of pathology were observed during the study, and all mice achieved normal relative weight gain. No treatment-related adverse effects were observed at Cry1F protein levels of >576 mg/kg bodyweight. This is equivalent to a dose of pure Cry1F protein of 34.56 g per person, assuming a body weight of 60 kg. Assuming an expression level observed in TC1507 maize grain (3.7 ng/mg tissue dry weight, **Table 1**), a person would have to consume 9,341 kg of maize for an equivalent exposure to Cry1F protein as the mice received in the acute oral toxicity study.

Safety of PAT protein was also demonstrated in a 2-week acute oral toxicity study in mice (Brooks, 2000). All mice survived the 2-week study and there were no treatment-related clinical observations of toxicity. All mice, with the exception of one female, gained body weight over the duration of the study and there were no gross pathologic lesions observed upon necropsy for any animal. Under the study conditions, the acute oral LD_{50} (median lethal dose) of the PAT protein in mice was >6,000 mg/kg. These results are consistent with previous findings from acute oral toxicity studies indicating that the PAT protein presents no significant human health risk (US EPA, 1997; Health Canada, 1997). Cry1F and PAT proteins were not found to be acutely toxic to mice. These findings support the conclusion

that Cry1F and PAT proteins are unlikely to be toxic to humans and other mammals, and can be considered safe for mammalian health through dietary consumption.

Subchronic rodent feeding study

A subchronic (90-day) rodent feeding study conducted with whole grains or processed feed fractions from GE crops is routinely requested by regulatory agencies, such as in the EU (Kuiper et al., 2013). The objective of a subchronic (90-day) rodent feeding study is to detect potential toxicological effects of the test diet compared with a control diet. While this study may be required to assess safety of an introduced protein or to identify unintended changes in metabolic pathways attributable to the genetic modification (EFSA, 2008), recent publications have discussed the limited contribution of such studies in the safety assessment (Kuiper et al., 2013; Herman and Ekmay, 2014). A review of published 90-day subchronic feeding studies demonstrated that GE crops do not pose any health hazard (Snell et al., 2012).

The nutritional performance of rats fed diets containing TC1507 maize grain was evaluated in a 90-day subchronic feeding study, in accordance with OECD guidelines (MacKenzie et al., 2007). Standard toxicological response variables were compared between rats fed diet containing 11% or 33% TC1507 maize grain and those in rats fed diet containing either a near-isoline control or non-GE commercial maize grain. The maize grain from TC1507, control, and non-GE commercial hybrids were comparable with respect to content of proximates, amino acids, minerals, anti-nutrients, and secondary metabolites. Individual diets formulated from the 3 hybrids were found to be nutritionally equivalent. There were no toxicologically significant differences in body weight and feed intake between the treatment groups. Neither mortality nor clinical signs of toxicity were observed in any of the treatment groups. Additionally, there were no toxicologically significant differences identified in ophthalmological and neurobehavioral responses, organ weights, and pathology between the treatment groups. Minor differences in a subset of hematological parameters were observed in females, which were not treatment related or biologically significant (MacKenzie et al., 2007). These findings demonstrated that TC1507 maize grain does not have the potential to cause significant toxicological effects in rodents, and that it is as safe as non-GE maize grain for human and animal consumption.

Nutritional Feeding Studies

Demonstrating the nutritional quality and equivalence of food and feed derived from GE crops is critical to ensure the well-being of humans and animals consuming such products. Livestock feeding studies with food derived from GE crops aim to evaluate the nutritional quality and wholesomeness of the novel food (Delaney, 2009). Such studies are generally recommended when there are substantial compositional changes or improved nutritional characteristics as a result of the modification in a GE crop (EFSA, 2008). Due to the widespread cultivation of TC1507 maize and the resultant abundance of GE grain for use in animal feed, livestock studies with TC1507 were designed to demonstrate nutritional equivalency for the purpose of gaining market acceptance. Parameters such as feed consumption, growth performance, and product quality (e.g. milk, meat, eggs) are quantified in these livestock studies to determine the wholesomeness of grain derived from TC1507 maize relative to non-GE maize.

Broiler chickens

Due to their rapid growth, broiler chickens are good animal models for the detection of even small dietary nutritional imbalances (EFSA, 2008). The performance of commercial broiler chickens (Cobb x Cobb strain) was examined by feeding the animals with diet containing TC1507 and non-GE grains for 42 d (McNaughton and Zeph, 2004). The maize grain was incorporated at the rate of 54.2% in starter diets and 57% in grower diets, across all treatments. The TC1507 grain diet treatment group

was compared with treatment groups fed diets containing either grain from a non-GE control maize hybrid or from one of 4 non-GE commercial maize sources. These findings revealed performance, as indicated by mortality, mean body weight, and feed conversion, was statistically similar among treatment groups. Therefore, TC1507 maize grain was nutritionally equivalent to maize grain from commercial hybrids when fed to broiler chickens.

Laying hens

In a similar study, grain from TC1507 maize was compared with grain from a near-isoline maize line and 2 non-GE conventional maize lines, incorporated at approximately 60% of diet, in a 16-week feeding trial with laying hens (Bovans White) (Scheideler et al., 2008). Hen performance, as measured by parameters including egg production and production efficiency as well as egg qualities such as albumen and color, was evaluated in the different diet treatment groups. The results demonstrated that performance of hens fed TC1507 grain was comparable to those fed grain from near-isoline maize or non-GE conventional maize.

Beef heifers

Sindt *et al.* (2007) compared the growth performance (daily weight gain, dry matter intake, feed efficiency) and carcass traits (liver abscess score, yield and quality grade) of beef heifers fed diets containing grain from a TC1507 maize hybrid with those fed diets containing grain from a near-isoline or one of 2 non-GE commercial maize hybrids. Diets incorporating steam-flaked maize at approximately 75% were individually fed to 20 beef heifers in each of 4 treatment groups for 118 d. The results indicated that growth performance and carcass characteristics of beef heifers were not significantly altered when provided diet containing TC1507 maize grain.

Dairy cows

Faust *et al*. (2007) evaluated the health and performance of lactating dairy cows fed maize silage containing maize grain, incorporated at a concentration of approximately 30%, derived from either TC1507 or near-isoline maize. Parameters such as milk production, production efficiency, and milk composition were compared in a replicated experiment, where 20 Holstein cows in each of 2 treatment groups were fed the maize diets for 28 d. The results demonstrated that the source of the maize grain and silage did not influence dairy production or health of the cows as assessed by physical characteristics, blood chemistry, and hematological indicators.

Swine

Stein *et al*. (2009) evaluated the growth performance and carcass composition of 24 pigs (offspring of Duroc x Large White sires mated to Yorkshire x Duroc x Landrace dams) in each of 4 treatment groups provided diet containing grain from either TC1507, near-isoline, or one of 2 non-GE commercial maize sources. Ground maize grain was incorporated into diets fed at 3 different growth phases. The concentrations in these successive phases were 65.1, 73.5, and 80.6%, respectively. Diets were formulated by mixing maize grain, soybean meal, soybean oil, vitamins, and minerals. Average daily weight gain, average daily feed intake, and gain/feed ratio were calculated to measure growth performance. Live weights at slaughter and standard carcass measurements were used to calculate dressing and lean meat percentages. There were no significant differences observed in the growth performance and carcass measurements between the 4 dietary treatment groups. These findings indicated that the presence of TC1507 grain did not impact the growth performance or carcass composition of pigs, and that these indices were comparable with those evaluated in pigs fed dietary treatments containing non-GE grain. Taken together, the various animal feeding studies support the conclusion that the TC1507 maize is as safe as, and nutritionally equivalent to, non-GE maize.

Environmental Risk Assessment

The environmental risk assessment for TC1507 maize evaluated the potential for

invasiveness (weediness), gene flow to sexually compatible wild relatives, horizontal gene transfer, and ecological effects including the potential impact on non-target organisms. Modern-day maize (*Z. mays*) is highly domesticated and unable to establish self-sustaining populations without human intervention; therefore, cultivation of maize poses negligible risk to the environment as a weed (OECD, 2003; Raybould et al., 2012). It has been established that maize event TC1507 is substantially equivalent when compared with its non-GE counterpart. The Cry1F protein provides protection against insect damage from certain lepidopteran pests, which would not be expected to alter the persistence, invasiveness, or weediness of maize outside managed agriculture. The PAT protein confers tolerance to the glufosinate-ammonium herbicide active ingredient. Since glufosinate-ammonium is a broad-spectrum herbicide that is not routinely broadcast outside agricultural habitats, tolerance to this herbicide does not enhance the potential for persistence, invasiveness, or weediness of TC1507 maize in the environment. Thus, TC1507 is not expected to behave differently than conventional maize in terms of invasiveness potential. A 2-year field experiment in south Texas with 5 GE maize events, including TC1507, non-GE maize (4 near-isoline hybrids and a commercial hybrid), and 3 Mexican landraces demonstrated that the insect-resistant trait does not increase the invasiveness potential of TC1507 maize. Researchers concluded that cultivation of Bt maize, similar to non-GE maize, would pose negligible weediness risk (Raybould et al., 2012).

The potential for gene flow from TC1507 maize to its wild and weedy relatives was evaluated. Biology documents on the potential for gene flow in conventional maize have been published by the OECD (2003). Maize has a high outcrossing rate, and can pollinate sexually compatible varieties and hybrids (e.g., other cultivated maize hybrids, landraces, teosinte). However, gene flow in the environment is limited by environmental barriers (pollen viability, pollen dispersal, proximity, and synchrony of flowering) and genetic barriers (ability to outcross and produce fertile progeny). Outcrossing between domesticated maize and *Tripsacum* species is unlikely under natural field conditions (OECD, 2003; US EPA, 2001). None of the genetic modifications in TC1507 maize were intended to alter the agronomy or composition of the TC1507 maize, relative to non-GE maize. As the agronomic characteristics were comparable between TC1507 maize and non-GE maize, there is no evidence to suggest that TC1507 maize has different reproductive biology or would not be subject to the same environmental and genetic barriers to gene flow as conventional maize.

The potential for horizontal gene transfer (HGT) of GE crop transgenes of microbial origin to human gut has been reviewed by Kleter *et al.* (2005) to suggest that transfer of a gene from GE plants to intestinal microflora is improbable. Plant DNA traversing the gastrointestinal tract and tolerating digestive enzymes, while maintaining the original coding information, is unlikely. Using a weight-of-evidence approach, Kleter *et al*. (2005) concluded that even in a rare event, HGT of *cry* genes from GE crops to microbes is unlikely to cause pathogenicity in receiving microbes residing in humans and animals. Moreover, there is no evidence of HGT of *pat* genes from GE crops to microorganisms (Hérouet et al., 2005; Kleter et al., 2005). The US EPA surmised HGT of Bt crop transgenes to soil microflora would be extremely rare and unlikely to increase soil microbial fitness (Mendelsohn et al., 2003). To date, there are no reports in the literature demonstrating that HGT occurs from plants to microorganisms, plants, animals, and humans under typical environmental conditions. Therefore, HGT from GE plants poses negligible risks to animal and human health or the environment (Keese, 2008). The *cry1F* and *pat* genes in TC1507 were derived from naturally occurring soil bacteria and are not pathogenic; therefore, microorganisms, plants, animals, and humans are regularly exposed to these organisms and their components without adverse consequences. Even if HGT were to occur, due to the absence of any selective advantage of any of these transgenes, there would be no increased risk of adverse effects attributed to HGT of transgenes in TC1507 maize (Mendelsohn et al., 2003).

A thorough environmental risk assessment (ERA) for the cultivation of TC1507 maize was conducted for non-target organisms (NTOs) present in the maize agro-ecosystem. Groups of NTOs that could be exposed to the Cry1F protein from a cultivated maize field were identified, and factors that affect the magnitude and duration of exposure in the environment were considered. The potential hazard of the Cry1F protein to NTOs in the environment was assessed using a tiered testing approach (Romeis et al., 2008). If no adverse effects are detected in early tier testing using unrealistically high Cry1F protein concentrations (e.g., 10X higher concentrations than those that would be encountered in the field), it can be concluded that at realistic environmental concentrations the risk to NTOs would be low. Laboratory bioassays were conducted on NTOs at high concentrations of Cry1F protein or using TC1507 maize tissue and no adverse effects were detected. The risk to each group of organisms was assessed by considering both the likelihood of exposure in the environment and the potential hazard caused by the Cry1F protein (**Table 2**).

There are many factors that mitigate the magnitude and duration of exposure of pollinators and pollen feeders to the Cry1F protein in TC1507 maize pollen. For example, many non-target lepidopterans are known to feed on host plants and are exposed to maize pollen indirectly if pollen is present on the host plant. In this case, exposure to maize pollen is limited to host plants that grow in close proximity to maize fields. There is a relatively short period of time when maize pollen is shed, which limits the duration of exposure. The timing of when maize pollen is shed and when the most sensitive life stages are foraging (generally neonates and early instars) may not overlap, which limits exposure. Pollen deposition rates, Cry protein stability in pollen, host plant density, cropping area, temporal and spatial overlap, and larvae feeding behavior are all important considerations that mitigate the magnitude and duration of exposure of pollinators and pollen feeders to Cry proteins in maize pollen (Sears et al., 2001). Hazard studies on honeybee (*Apis mellifera*) (Maggi, 1999) and monarch butterfly (*Danaus plexippus*) (Bystrak, 2000) demonstrate low hazard of the Cry1F protein at concentrations that exceed realistic environmental concentrations. Therefore, the risk of cultivation of TC1507 maize on pollinators and pollen feeders is low. Predators and parasitoids could be exposed to the Cry1F protein through secondary (prey-mediated) transfer. In general, Cry proteins have not been found to bioaccumulate in prey (Romeis and Meissle, 2011); therefore, potential exposure to predators and prey to the Cry1F protein is low. Hazard studies on green lacewing (*Chrysoperla carnea*) (Hoxter, Porch et al., 1999b), parasitic hymenoptera (*Nasonia vitripennis*) (Hoxter, Krueger et al., 1999a), and ladybird beetle (*Hippodamia convergens*) (Hoxter, Krueger et al., 1999b) demonstrate low hazard of the Cry1F protein at concentrations that exceed realistic environmental concentrations. Therefore, the risk of cultivation of TC1507 maize on predators and parasitoids is low. In general, Cry proteins do not persist or accumulate in soil. The environmental fate of a variety of Cry proteins in various soil types has been well characterized (Clark et al., 2005; Icoz and Stotzky, 2008). Like other Cry proteins, the soil dissipation of Cry1F proteins can be characterized as rapid (Herman et al., 2002; Shan et al., 2008), and the magnitude and duration of exposure of aquatic organisms and soil-dwelling organisms to the Cry1F protein is low. Hazard studies on water flea (*Daphnia magna*) (Drottar and Krueger, 1999), earthworm (*Eisenia fetida*) (Hoxter, Porch, et al., 1999a), and springtail (*Folsomia candida*) (Halliday, 1998) demonstrate low hazard of the Cry1F protein at concentrations that exceed realistic environmental concentrations. Therefore, the risk of cultivation of TC1507 maize on aquatic organisms and soil dwellers is low.

Subsequent to the early-tier laboratory studies that were conducted as part of the original TC1507 safety assessment, several additional studies have been conducted on NTOs including honeybee (*A. mellifera*) (Hanley et al., 2003), green lacewing (*C. rufilabris*) (Tian et al., 2013), larval endoparasitoid (*Cotesia marginiventris*) (Tian et al.,

TABLE 2. Summary of non-target organism environmental risk assessment for Cry1F protein in TC1507 maize (details of the hazard studies on non-target organisms have been summarized previously; US EPA, 2005)

Surrogate Species for Hazard Testing (Common Name)	Exposure to Cry1F Protein from TC1507 Maize	Hazard of Cry1F Protein at Environmentally Relevant Concentrations	Environmental Risk Conclusion
Pollinators and pollen feeders			
Apis mellifera[a] (Honeybee)	Low; there are many mitigating factors that decrease the likelihood of exposure to Cry1F protein in TC1507 maize pollen.	Low; no hazard to *Apis mellifera* in laboratory testing using concentrations that exceed realistic environmental concentrations	Low risk to honeybee
Danaus plexippus[b] (Monarch butterfly)		Low; no hazard to *Danaus plexippus* in laboratory testing using concentrations that exceed realistic environmental concentrations	Low risk to non-target Lepidoptera
Predators and parasitoids			
Chrysoperla carnea[c] (Green lacewing)	Low; Cry proteins are not likely to bioaccumulate in prey items.[i]	Low; no hazard to *Chrysoperla carnea* in laboratory testing using concentrations that exceed realistic environmental concentrations	Low risk to predators and parasitoids
Nasonia vitripennis[d] (Parasitic hymenoptera)		Low; no hazard to *Nasonia vitripennis* in laboratory testing using concentrations that exceed realistic environmental concentrations	
Hippodamia convergens[e] (Ladybird beetle)		Low; no hazard to *Hippodamia convergens* in laboratory testing using concentrations that exceed realistic environmental concentrations	
Aquatic organisms			
Daphnia magna[f] (Water flea)	Low; the concentration of Cry1F protein in aquatic habitats is low.	Low; no hazard to *Daphnia magna* in laboratory testing using concentrations that exceed realistic environmental concentrations	Low risk to aquatic organisms
Soil-dwelling organisms			
Eisenia fetida[g] (Earthworm)	Low; the concentration of Cry1F protein in soil is low, indicating low magnitude of exposure. The dissipation of the Cry1F protein in soil is rapid, indicating low duration of exposure.[j]	Low; no hazard to *Eisenia fetida* in laboratory testing using concentrations that exceed realistic environmental concentrations	Low risk to soil-dwelling organisms
Folsomia candida[h] (Springtail)		Low; no hazard to *Folsomia candida* in laboratory testing using concentrations that exceed realistic environmental concentrations	

[a]Maggi (1999).
[b]Bystrak (2000).
[c]Hoxter, Porch et al. (1999b).
[d]Hoxter, Krueger et al. (1999a).
[e]Hoxter, Krueger et al. (1999b).
[f]Drottar and Krueger (1999).
[g]Hoxter, Porch et al. (1999a).
[h]Halliday (1998).
[i]Romeis and Meissle (2011).
[j]Herman et al. (2002); Shan et al. (2008).

2014), pale grass blue butterfly (*Pseudozizeeria maha*) (Wolt et al., 2005), and bobwhite quail (*Colinus virginianus*) (Gallagher et al., 1999) that support the lack of adverse effects of the Cry1F protein. Furthermore, multiple field studies have been conducted in different global geographies, including Vietnam, the US, Spain, France, Philippines, India, and Indonesia, to support regulatory submissions (Pioneer Hi-Bred International, Inc. internal unpublished data). These additional laboratory and field studies all support the conclusion of low environmental risk associated with the cultivation of TC1507 maize. The environmental fate, specificity to lepidopteran pest species, and lack of effects on NTOs of the Cry1F protein are well-characterized. Based on this characterization, the environmental risk associated with the cultivation of TC1507 maize is low.

Yield Increase/Economic Benefits

Invariably, increasing yield is the priority when any new crop technologies or hybrids are developed. In the US, data collected between 1964 and 2010 revealed GE traits, since 1996, have had a significant positive impact on maize yield trends (Xu et al., 2013). To this end, Bt maize offers a highly efficient pest control measure that allows growers to produce high-quality grain with reduced insecticide inputs and farm operations, which can contribute to the reduction of greenhouse gas emissions (Barfoot and Brookes, 2014).

Event TC1507 is a popular component among the many Bt maize breeding stacks planted. In maize yield evaluations held in the US during 2011 and 2012, there were 8,431 and 8,263 entries, respectively, under different categories. Among the entries with the highest yield, 59% of hybrids in 2011 and 56% of hybrids in 2012 contained event TC1507 stacked with other insect-resistant or herbicide-tolerant traits using conventional breeding techniques (NCGA, 2011; NCGA, 2012).

As event TC1507 is predominantly planted as part of a breeding stack (ISAAA, 2015), agronomic studies specifically evaluating the yield performance of TC1507 as a single event product compared with conventional hybrids

TABLE 3. Summary of global regulatory authorization status of maize event TC1507

	Country	Food direct use or additive	Feed direct use or additive	Cultivation domestic or non-domestic use
1	Argentina	2005	2005	2005
2	Australia	2003		
3	Brazil	2008	2008	2008
4	Canada	2002	2002	2002
5	China	2002[a]	2002[a]	
6	Colombia	2006	2006	2007
7	European Union	2006[b]	2006[b]	
8	Honduras			2009
9	Japan	2002	2002	2005
10	Malaysia	2013	2013	
11	Mexico	2003		
12	New Zealand	2003		
13	Panama	2012		2012
14	Paraguay	2012	2012	2012
15	Philippines	2003[c]	2003[c]	2013
16	Singapore	2014		
17	South Africa	2002	2002	2012
18	Korea	2002	2004	
19	Taiwan	2003		
20	Turkey		2011	
21	USA	2001	2001	2001
22	Uruguay	2011	2011	2011

[a]Renewal 2009, 2012; [b]Expires 2016; [c]Renewal 2008.

Based on ISAAA (2015).

are limited. However, published field studies have reported that TC1507 maize prevented significant yield loss due to *S. frugiperda* infestation compared with non-Bt maize as evidenced by reduced foliar injury, whorl damage, and larval survivorship (Buntin, 2008; Siebert et al., 2008; Hardke et al., 2011).

In the Philippines, two TC1507 maize hybrids, their near-isoline hybrids, and a conventional local hybrid were planted in 12 locations over 2 seasons during 2006–2007, to evaluate their performance against the Asian corn borer (*Ostrinia furnacalis*). Significantly lower insect damage and higher yields were observed with the TC1507 event compared with near-isoline hybrids across the multi-site trials (Thompson et al., 2010).

Global Regulatory Acceptance and Commercial Status of TC1507 Maize

Maize with the Herculex® I trait has been authorized in most of the major grain trading countries in the world. To date, more than 20 countries have authorized use of TC1507 for food and/or feed purposes, of which 10 grow it in commercial scale (**Table 3**) (ISAAA, 2015). In the US, field trials conducted from 1997 to 2000 in at least 20 States and in Puerto Rico demonstrated that event TC1507 exhibited the desired agronomic characteristics and did not pose a plant pest risk prior to authorization from the USDA and EPA, and prior to review by the US Food and Drug Administration (FDA) in 2001 (Mendelsohn et al., 2003; USDA APHIS, 2001; US FDA, 2001). Authorization from the Canadian Food Inspection Agency followed in 2002 (CFIA, 2002). Thereafter in 2003, TC1507 was launched for commercial cultivation and for food/feed uses in the US and Canada (Rowe et al., 2012). Subsequently, TC1507 has become a common component in breeding stack products in the US (**Table 4**). For example in 2010, more than 150 hybrids contained event TC1507 either as a single event product (26; number of US maize hybrids) or in breeding stack products: TC1507 × NK603 (10), TC1507 × 59122 (31), TC1507 × 59122 × NK603 (37), and MON88017 x MON89034 × TC1507 × 59122 (52) (McLaren and Copping, 2011).

TC1507 was first authorized for commercial planting in Argentina in 2005 and maize containing events stacked using conventional breeding techniques including TC1507 were authorized soon thereafter (Trigo, 2011). In Honduras, field trials with TC1507 were initiated in 2006, followed in 2009 by initial limited scale commercialization and subsequent full commercialization in 2010 (GAIN Honduras, 2012). TC1507 has been approved for import

TABLE 4. Breeding stack products authorized for cultivation or food/feed use that contain TC1507

	Breeding stack products with TC1507	Commercial name
1	TC1507 x 59122	Herculex® XTRA®
2	TC1507 x NK603	Herculex® I Roundup Ready®
3	TC1507 x 59122 x NK603	Herculex® XTRA® Roundup Ready®
4	TC1507 x MON810 x NK603	Optimum® Intrasect® Roundup Ready®
5	TC1507 x 59122 x MON810	Optimum® Intrasect® XTRA®
6	TC1507 x 59122 x MON810 x NK603	Optimum® Intrasect® XTRA® Roundup Ready®
7	MON89034 x TC1507 x NK603	Power Core™
8	MON89034 x TC1507 x NK603 x DAS40278	Power Core™ Enlist™
9	MON89034 x TC1507 x MON88017 x 59122 x DAS40278	SmartStax® Enlist™
10	TC1507 x 59122 x MON810 x MIR604 x NK603	Optimum® Intrasect® XTreme
11	TC1507 x MIR604 x NK603	Optimum® TRIsect®
12	Bt11 x MIR162 x TC1507 x GA21	Agrisure Viptera® 3220
13	Bt11 x 59122 x MIR604 x TC1507 x GA21	Agrisure® 3122
14	5307 x MIR604 x Bt11 x TC1507 x GA21 x MIR162	Agrisure Duracade™ 5222
15	5307 x MIR604 x Bt11 x TC1507 x GA21	Agrisure Duracade™ 5122
16	TC1507 x MON810	Optimum® Intrasect®

Based on ISAAA (2015).

in Colombia since 2006 and for plantings since 2007 (GAIN Colombia, 2013).

The Brazilian Regulatory Authority, Comissão Técnica Nacional de Biossegurança (CTNBio), assessed safety of TC1507 and authorized commercial cultivation in 2008 (CTNBIO, 2008). Subsequently, breeding stacks containing TC1507, such as TC1507 × NK603 and MON89034 × TC1507 × NK603, were also authorized in 2009 and 2010, respectively. As of early 2011, 448 total GE maize varieties, derived from 9 different transformation events, were registered in Brazil. Of these, 120 varieties carried TC1507 as a single event product and 44 were breeding stack products containing TC1507 × NK603 (Marinho et al., 2012).

Event TC1507 maize received full European Union (EU) authorization for import, food, and feed in March, 2006, following safety assessments from the European Food Safety Authority (EFSA) including molecular characterization, toxicology, and allergenicity as well as agronomic and compositional equivalence. These EFSA Opinions concluded that 1507 maize is unlikely to have an adverse effect on human health or the environment in the context of its use as or in food, feed, and processing (EFSA, 2004, 2005a, 2005b). The authorization was renewed in 2011 following a further positive EFSA Opinion in May, 2009 (EFSA, 2009a). Moreover, EFSA has published several other safety Opinions on breeding stack products containing the 1507 event, notably 59122 × 1507 × NK603 (EFSA, 2009b), MON89034 × 1507 × MON88017 × 59122, and all subcombinations of the individual events for food and feed uses, import, and processing (EFSA, 2010). Multiple positive EFSA Opinions regarding safety have been received on a currently pending 1507 maize EU cultivation submission made in 2001 (Rowe et al., 2012).

In Asia, GE maize is largely imported for food, feed, and processing (FFP) use in Japan, Korea, Taiwan, and China. As of 2013, Taiwan has authorized 18 single maize events and 32 breeding stack event combinations for FFP purposes only. The single event TC1507 was registered in 2003 followed by at least 12 breeding stack event combinations containing TC1507 (FDA, 2014). Korea imported 8.2 million metric tons of GE maize for food and feed use in 2012. The use of TC1507 maize for food and for feed in Korea was authorized in 2002 and 2004, respectively, which was followed by authorization of TC1507 × NK603 maize for food and for feed uses in 2004 and 2008, respectively. Later, 12 GE maize breeding stack products containing event TC1507 were authorized (GAIN Korea, 2013). To date, the Philippines is the only Asian country where GE maize is commercially cultivated and consumed, which has been on-going since 2003. In 2013, GE maize was planted in 750,000 hectares of which 90% was stacked maize expressing a Bt trait (James, 2013). Field trials to obtain cultivation authorization for TC1507 as a single event product and in the TC1507 × MON810 × NK603 breeding stack product were completed in 2012. In addition, TC1507 maize and TC1507 × MON810 × NK603 maize were determined to be as safe as conventional counterparts for FFP, and have been allowed for import since 2003 and 2012, respectively (DA-BPI, 2012, 2013a). TC1507 and the following breeding stack products: TC1507 × MON810 ×NK603, TC1507 × MON810, and TC1507 × NK603 were recently approved for cultivation in the Philippines (DA-BPI, 2013b).

Event TC1507 has also received full approval in South Africa including a general release permit for commercial cultivation in 2012 (DAFF, 2014). Efforts are underway to obtain authorizations to cultivate TC1507 in other countries where farmers will benefit from an insect control perspective.

Conclusions

Maize is an important food crop grown widely in many countries, while primarily cultivated in North and South America, EU, China, Indonesia, and India. Maize grain and its derivatives have been safely consumed by humans for centuries without health concern. GE maize was introduced in 1996 and has been commercially cultivated on millions of hectares in 17 countries without any reported safety incidents. In addition to countries with on-going

commercial cultivation, Japan, Mexico, Korea, Taiwan, and China use significant quantities of GE maize obtained through import for food and feed purposes (Rowe et al., 2012). Other than the presence of the introduced gene(s), GE maize varieties are comparable with their non-GE counterparts with respect to composition, nutrition, and safety.

The robust information generated from over a decade of food, feed, and environmental safety assessments has established that TC1507 is as safe as conventional maize. Laboratory and field experiments conducted with TC1507 demonstrated that the introduced genes are stably integrated and follow the expected Mendelian inheritance pattern for a dominant gene. Additionally, these studies indicated TC1507 maize is substantially equivalent to its non-GE counterpart. A thorough safety assessment has been conducted, and no adverse effects are anticipated on non-target organisms from cultivation of TC1507 maize. It is unlikely that TC1507 maize would become a weed or that the introduced genes would flow to related wild species or other microorganisms resulting in a deleterious environmental impact. Similar conclusions were drawn by OECD and Center for Environmental Risk Assessment-International Life Sciences Institute while reviewing the food, feed and environmental safety of Cry1F protein (OECD, 2007; CERA, 2013).

Results from safety studies on Cry1F and PAT proteins and grains derived from TC1507 suggest TC1507 is unlikely to impact mammalian health through dietary consumption. The safety information provided here supports the conclusion that TC1507 presents negligible risk to human health and low risk to the environment.

Safety data on TC1507 have been submitted to regulatory agencies in over 20 countries that have authorized its use for cultivation and/or food and feed uses; 10 of which have been consuming TC1507 grains for at least a decade. Testimony to TC1507 product safety includes the extensive cultivation of TC1507 maize hybrids in Argentina, Brazil, Canada, and the US as well as its consumption in over 20 countries without any safety issues. Increasing authorization of event TC1507 as a component of breeding stack products further demonstrates the safety and global acceptance of TC1507. As TC1507 maize has been widely cultivated and consumed by humans and animals without incident, and in combination with the extensive safety data available, it is therefore concluded that TC1507 maize has a history of safe use for cultivation and food/feed purposes.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

GBB, BD, TLF, GSL, RJL, MEHL, JS, and JAA are employed with DuPont Pioneer; RAH and SLE are employed with Dow AgroSciences LLC, both of which market transgenic seed, including TC1507 maize.

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