Chapter 16
A Review of the Food Safety of *Bt* Crops

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Abstract There is a 50-year history of safe use and consumption of agricultural food crops sprayed with commercial *Bt* (*Bacillus thuringiensis*) microbial pesticides and a 14 year history of safe consumption of food and feed derived from *Bt* crops. This review summarizes the published literature addressing the safety of Cry insect control proteins found in both *Bt* microbial pesticides and those introduced into *Bt* agricultural crops. A discussion on the species-specific mode of action of Cry proteins to control target insect pests is presented. This information provides the scientific basis for the absence of toxicity of Cry proteins towards non-target organisms that has been confirmed in numerous mammalian toxicology studies. A human dietary exposure assessment for Cry proteins has also been provided which includes information that food processing of *Bt* crops such as maize leads to loss of functionally active Cry proteins in processed food products. Lastly the food and feed safety benefits of *Bt* crops are briefly summarized including lower insecticide use and reduction in fumonisin mycotoxin contamination of grain.

Keywords Food safety · Cry proteins · Toxicity · Bt crops · Consumption of Cry proteins

16.1 Background

Bt is a common Gram positive, spore-forming aerobic bacterium that is found in a variety of environmental sources such as soil, water, plant surfaces, grain dust, dead insects etc. (Federici and Siegel 2008). As part of its normal life cycle, the bacteria produce one or more insecticidal proteins in parasporal bodies when nutrients become insufficient to support bacterial growth. *Bt* microorganisms have been used for many years as a tool to control larval insect pests that feed on agricultural crops. They are also widely used in certified organic agricultural food production in the United States, Europe, and other countries. When the *Bt* microbial formulations are applied to the leaves of agricultural crops, the vegetative cells, spores,

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and insecticidal proteins in the formulation are consumed by the larval insect. The insecticidal proteins exist as protoxins and are converted to active insect toxins by proteases in the alkaline environment of the lepidopteran insect gastrointestinal tract. The activated toxins bind to specific receptors on the membranes of target insect mid-gastrointestinal tract epithelial cells and form pores in the membranes allowing water and electrolytes from the gastrointestinal tract juices to enter the cell. The epithelial cells swell and lyse, leading to electrolyte imbalance in the insect hemolymph causing paralysis so that the insect stops eating and dies. The *Bt* spores can also germinate and colonize the insect body, allowing the bacteria to reproduce (WHO/IPCS 1999; Betz et al. 2000; OECD 2007; Federici and Siegel 2008).

Bt insecticidal crystal proteins, Cry (for crystal) and Cyt (for cytolytic) proteins, as well as VIPs (vegetative insecticidal proteins produced during the vegetative phase) are the major insecticidal proteins. There can be considerable variations in the amino acid content and structure of each of these proteins as they exist in different strains of *Bt*.

As discussed previously, *Bt* Cry proteins are one of the insecticidal components in *Bt* microbial commercial products widely used as biological insecticides for over 50 years. *Bt* was first discovered in Japan in 1901 and later rediscovered in Germany (Sanchis 2010). Field trials were subsequently carried out in Europe and the United States with *Bt* microbes to investigate their insecticidal properties; the first commercial *Bt* microbial formulation was launched in France in 1938 (Sanchis 2010). *Bt* microbial pesticides are highly regarded as environmentally-friendly due to their species-specificity (controlling only target insect pest species) and their lack of environmental persistence (WHO/IPCS 1999; Betz et al. 2000; OECD 2007; Federici and Siegel 2008). China has been probably the biggest user of *Bt* microbial pesticides where, over the last few decades, tens of thousands of tons of various *Bt* microbial formulations have been topically applied on agricultural food crops (rice, vegetables, maize), in forests and to potable water to control mosquitoes and other larval insects that are vectors of human disease (WHO/ICPS 1999; Ziwen 2010). According to recent data, there were at least 180 registered *Bt* microbial products in the United States (EPA 1998) and over 120 microbial products in the European Union. There are reported to be approximately 276 *Bt* microbials registered in China (Huang et al. 2007). *Bt* microbial pesticides were first registered in the US in 1961 (Betz et al. 2000).

The efficacy of *Bt* microbials applied to the surface of leaves is limited by the fact that the formulation can be washed off by rain and the Cry proteins are inactivated by sunlight within a few days of application (Federici and Siegel 2008). With the development of biotechnology, it has been possible to introduce the genes coding for Cry proteins into plants so that Cry proteins are expressed in the plant and are produced throughout the growing season to provide protection against insect pests. At present, most commercial *Bt* crops are based on Cry proteins, although VIPs are now being introduced into agricultural crops (EPA 2004). To date, Cyt proteins have not been introduced into commercial *Bt* crops.

16.2 Regulatory Guidance for the Safety Assessment of Cry Proteins

According to guidance from the US Environmental Protection Agency (Mendelsohn et al. 2003), the European Food Safety Agency (2011), and Codex (2009), the food safety assessment of any insecticidal protein introduced into food/feed crops through genetic engineering should include:

1. information on the biochemical characterization of the introduced protein including the amino acid sequence, molecular weight, post-translation modifications (if any), and a description of the function.
2. Assessment of amino acid sequence similarity between the protein and any known protein mammalian toxins using bioinformatics tools to search curated data bases of amino acid sequences of proteins (eg. NCBI Entrez Protein, PIR, UniProt-Swiss-Prot etc.).
3. Assessment of stability of the protein to heat or food processing conditions.
4. Assessment of potential degradation in appropriate and validated *in vitro* gastric and intestinal model systems.
5. High dose acute toxicology testing to confirm the absence of toxicity to mammals (EPA requirement only). The US EPA requires high dose acute toxicology testing in rodents with either *Bt* microbial pesticides or Cry proteins that end up in food and feed crops (McClintock et al. 1995; Betz et al. 2000). The rationale for requiring high dose acute testing is that Cry proteins act through an acute mode of action to kill insect pests.

EFSA (2011) considers that acute toxicology testing provides little value, but may require a repeat dose 28 day toxicology study where there is considered to be insufficient safety information on the introduced protein.

16.3 The Species-specific Acute Mode of Action of Cry Proteins

The general mode of action of Cry proteins has been studied extensively and reviewed in a number of publications (WHO/IPCS 1999; Betz et al. 2000; Siegel 2001; OECD 2007; Bravo et al. 2007; Federici and Siegel 2008; Soberón et al. 2010). Cry proteins are not contact insecticides like chemical pesticides, but must be ingested and activated by proteases in the gastrointestinal tract of target insect pests. The activated Cry toxins bind to specific receptors on mid-gastrointestinal tract epithelial cells of target larval insects and this, results in oligomerization of the Cry toxin monomers. The toxin complex translocates into the cellular membrane of the gastrointestinal tract epithelial cells and forms pores that cause osmotic shock and cell lysis leading to death of the insect (Federici and Siegel 2008; Soberón et al. 2010). Some Cry protein binding receptors have been identified such as cadherin-

like glycoproteins, and glycosylphosphatidyl-inositol (GPI) membrane anchored receptors such as aminopeptidase N or alkaline phosphatase (Soberón et al. 2010). Binding of Cry proteins to the aforementioned receptors is not sufficient of itself to cause toxicity to the insect as oligomerization must also occur to form the pores in the membrane. This may explain the observation that some Cry proteins can bind to insect mid-gastrointestinal tract epithelial cells, but since oligomerization does not follow, no toxicity to the insect occurs (Federici and Siegel 2008; Soberón et al. 2010).

Non-target organisms such as humans, rhesus monkeys, cattle, mice, rats, rabbits, other non-target insects etc., lack high affinity Cry protein binding-receptors (Sacchi et al. 1986; Hofmann et al. 1988a, b; Wolfersberger et al. 1986; Van Rie et al. 1989, 1990; Lambert et al. 1996; Mendelsohn et al. 2003; Griffiths et al. 2005; Shimada et al. 2006; OECD 2007). In contrast to these studies in mammals demonstrating an absence of specific high affinity receptors to bind Cry proteins, one study reported binding of Cry1Ac protein to the mouse jejunum (Vazquez-Padron et al. 2000). However, this binding appeared to be non-specific because extremely high (non-physiological) concentrations of Cry1Ac protein were incubated *in vitro* with mouse intestinal brush border membrane vesicles (BBMV) at 1 µg Cry protein/1 µg BBMV. In target insects, Cry proteins bind avidly to receptors on insect BBMV at much lower concentrations (0.00001–0.001 µg Cry protein/µg BBMV) and surface plasmon resonance experiments indicate that binding to the cadherin receptor occurs at nM concentrations (Hofmann et al. 1988a; Sacchi et al. 1986; Soberón et al. 2010). This is supported by the observation that the LD50 dose of Cry proteins is in the low ng/larval insect range (Federici and Siegel 2008). Low affinity binding of Cry proteins to rat BBMV has been reported but was considered to be non-specific as it was not displaceable by non-iodinated Cry protein, whereas Cry protein binding to target insect BBMV was readily displaced (Hofmann et al. 1988b). In bovine epithelial cells (Shimada et al. 2006), low level binding of Cry1Ab protein to the cytoskeletal protein actin was detected. However, no binding to extracellular proteins such as aminopeptidase N, cadherins and alkaline phosphatase was detected on bovine epithelial cells; these aforementioned proteins have been identified as receptors for Cry protein binding on target insect mid-gastrointestinal tract epithelia. The absence of high affinity binding of Cry proteins on mammalian gastrointestinal tract epithelial cells may be due in part to the absence of a glycosylating enzyme, BL2, in mammalian gastrointestinal tract cells. This enzyme, which is present in target insect gastrointestinal tract cells, produces the specific sugar residues that facilitates recognition and binding by Cry proteins to the aforementioned aminopeptidase N and alkaline phosphatase receptors (Federici and Siegel 2008; Soberón et al. 2010). The absence of specific Cry binding receptors on the mammalian gastrointestinal tract epithelial cells can explain, in part, the absence of cellular toxicity when high, non-physiological concentrations of Cry1Ab protein were incubated with sheep rumen epithelial cells, whereas incubation with a positive control toxin, valinomycin, caused apoptosis and reduced cell viability (Bondzio et al. 2008).

Furthermore, the *in vitro* concentration of Cry protein used by Vazquez-Padron (2000) was many orders of magnitude higher than the potential dietary exposures the mammalian digestive tract might encounter from consumption of food derived from *Bt* maize (Hammond and Cockburn 2008). As will be discussed later in section 16.6, processing (*e.g.* cooking, etc.) of *Bt* maize into human food has been reported to denature and inactivate Cry proteins further reducing residual functionally active Cry protein residues in food (Hammond and Jez 2011). Any residual (~ppb) levels of functionally active Cry protein that survived food processing would also be expected to be digested in the gastrointestinal tract (see Sect. 16.4).

16.4 Potential Digestibility of Cry Proteins

The normal fate of most ingested dietary proteins is hydrolytic digestion and/or degradation to either individual amino acids or small peptides that are subsequently absorbed to provide amino acids for protein synthesis in the body (Delaney et al. 2008). A validated assay to assess the potential digestibility of proteins has been developed *in vitro* using a fixed ratio of pepsin to protein and at pH 1.2 and 2.0 that is designed to simulate conditions in the stomach (Thomas et al. 2004). A similar *in vitro* test to simulate intestinal digestion using pancreatin has also been developed. Cry proteins are readily degraded by pepsin when tested *in vitro* using the aforementioned pepsin assay (Okunuki et al. 2001; Herman et al. 2003; EPA 2001; Thomas et al. 2004; Cao et al. 2010; Guimaraes, et al. 2010). An alternative *in vitro* digestive model has been recently proposed using higher pH and a lower pepsin/Cry protein ratio where Cry1Ab protein is more slowly degraded (Guimaraes et al. 2010). Others have suggested that differences in pH and pepsin concentration using *in vitro* digestibility assays had only small effects on digestion of proteins of intermediate stability to pepsin and no effects on proteins that were either stable or resistant to pepsin digestion (Ofori-Anti et al. 2008)

In pigs and calves, Cry protein fragments were observed in the GI tract, but none were detected in the liver, spleen and lymph nodes (Chowdhury et al. 2003a, b) indicating they were too large to be systemically absorbed intact from the gastrointestinal tract. Farm animals are generally fed much higher levels of maize in the diet than humans, and the maize is generally not processed resulting in higher dietary exposure to Cry proteins. Human dietary exposure, in contrast, would be much lower due to the lower consumption of maize and the fact that human food derived from maize is processed. Maize is subjected to a variety of processing conditions such as cooking that denatures Cry proteins causing them to lose insecticidal activity (Sect. 16.6). Denaturation also makes proteins more susceptible to degradation by proteases (Herman et al. 2006) including Cry1Ab protein (Okunuki et al. 2001) so that potential dietary exposure to functionally active Cry proteins in food derived from maize is very low.

16.5 Toxicology Testing of Cry Proteins

Genes coding for Cry proteins that were similar to those derived from *Bt* microorganisms have been introduced into a variety of different agricultural crops. These Cry proteins are expressed at low (ppm) levels in plants since those levels are sufficient to control targeted insect pests (Hammond and Cockburn 2008). As described earlier, only Cry and VIP proteins are currently used in registered *Bt* crops, making the insecticidal complexity of the crop much simpler than that of the components in *Bt* microbial pesticide formulations. As a group, the Cry protein family contains considerable diversity, enabling *Bt* strains to kill different kinds of larval insect pests. The currently commercialized *Bt* crops used for food and feed are mainly *Bt* maize and cotton. Cry proteins produced by *Bt* plants registered in the US and other countries include Cry1Ab, Cry1Ac, Cry1F, Cry1A.105, Cry1Ac, Cry2Ab2, Cry3Bb and Cry34Ab1, and Cry35Ab1. VIP proteins have also been registered in the US for use in agricultural crops (EPA 2004). New *Bt* soybean varieties (US) that have the *cry1Ac* gene and *Bt* rice varieties (China) with the *cry1C* gene are currently going through regulatory review. The food safety of introduced Cry1Ac and Cry1C proteins has already been demonstrated (Betz et al. 2000; Cao et al. 2010).

16.5.1 Acute Toxicity Testing

As shown in Table 16.1, mice are not adversely affected even when fed acute, high dosages of Cry proteins that are thousands to millions of times higher than doses acutely toxic to target insect pests (Hammond and Cockburn 2008). The mouse is a relevant model for such testing because it is known to be susceptible to the toxicity of known mammalian protein toxins (Delaney et al. 2008). In such testing the Cry1Ab protein was administered to mice at a dose level of 4000 mg/kg/day and produced no adverse effects. An adult human would have to consume approximately 900,000 kg of uncooked *Bt* maize grain in 1 day to attain a similar acute dose of Cry1Ab protein administered to mice (Hammond and Cockburn 2008).

Numerous reviews summarizing results of animal toxicology studies with *Bt* microbial pesticides and individual Cry proteins and the long history of safe use of *Bt* microbial products and *Bt* crops support the safety of Cry proteins (Fisher and Rosner 1959; Siegel and Shadduck 1989; McClintock et al. 1995; WHO/ICPS 1999; Betz et al. 2000; Siegel 2001; OECD 2007; Federici and Siegel 2008). In contrast to certain chemical insecticides, no significant human illnesses have been attributed to the use of *Bt* microbial pesticides in agriculture (WHO/ICPS 1999; Federici and Siegel 2008). This correlates well with the results in human safety testing conducted on volunteers in the early days of safety assessment of *Bt* microbials. These individuals were fed 10^{10} *Bt* spores for 5 days or inhaled 10^{9} *Bt* spores with no reported adverse effects (Siegel and Shadduck 1989).

Table 16.1 Acute toxicity studies in mice with Cry proteins

Cry protein	NOAEL (mg/kg)	Reference
Cry1Ab	4000	Betz et al. (2000)
Cry1Ab/Cry1Ac fusion protein	5000	Xu et al. (2010)
Cry1A.105	2072	EPA (2008a)
Cry 1Ac	4200	Betz et al. (2000)
Cry1C	5000	Cao et al. (2010)
Cry2Aa	4011	Betz et al. (2000)
Cry2Ab	1450	Betz et al. (2000)
Cry2Ab2	2198	EPA (2008b)
Cry3A	5220	Betz et al. (2000)
Cry3Bb	3780	Betz et al. (2000)
Cry1F	576	EPA (2001)
Cry34Ab1	2700	Juberg et al. (2009)
Cry35Ab1	1850	Juberg et al. (2009)
VIP3A	3675	EPA (2004)

16.5.2 Allergenicity and Immunogenicity Assessment

According to the aforementioned Codex guidelines (2009), the assessment of potential allergenicity of introduced proteins is carried out by comparing the biochemical characteristics of the introduced protein to characteristics of known allergens. A protein is not likely to be allergenic if: (1) the protein is from a non-allergenic source; (2) the protein represents only a very small portion of the total plant protein; (3) the protein does not share structural similarities to known allergens based on amino acid sequence homology comparisons to known allergens using bioinformatics search tools, and (4) the protein has the potential to be digested as confirmed when incubated *in vitro* with simulated digestion fluids. All Cry proteins expressed in *Bt* crops have been assessed for potential allergenicity according to the recommendations of the aforementioned Codex guidelines. Those that are used in commercial *Bt* crops do not fit the profile of known protein allergens, *ie.* they are digested in simulated gastric fluid, are generally present at low (ppm) levels in grain and much lower levels in food, and are not structurally related to known allergens based on bioinformatics searches. Following commercial production and use of thousands of tons of *Bt* microbial formulations over the last few decades, there is no evidence of allergic reactions in workers who manufacture or apply *Bt* microbials to agricultural crops and forests (Siegel 2001; Federici and Siegel 2008). Similarly, for the millions of tons of *Bt* crops produced since the 1990s, there have been no reports of allergenic reactions in those that handle or consume the grain/seed. There has been a report of immunologic responses in workers who apply *Bt* microbial formulations to agricultural crops, but this reaction is classically observed when humans are exposed to "foreign" or non-human proteins. However, the immunologic responses were attributed to other bacterial proteins present in the *Bt* microbial formulation, and not the Cry proteins (Siegel 2001; Federici and Siegel 2008).

Cry1Ac protein that is present in *Bt* microbial formulations has been shown to be immunogenic in mice following intraperitoneal (IP), intragastric (IG), intranasal (IN) or intra-rectal (IR) administration (Moreno-Fierros et al. 2000; Vazquez-Padron et al. 2000). Systemic and mucosal immune responses with production of specific IgG, IgM and IgA antibodies were reported. In a separate study, administration of GM rice containing Cry1Ab protein (88% amino acid sequence homology to Cry1Ac protein), or spiked with Cry1Ab protein, was also reported to have induced an immune response in rats (Kroghsbo et al. 2008). Another study reported that feeding mice either 1 month or 18 months of age with MON810 maize resulted in some alterations in the intestinal and peripheral immune cell populations (Finamore et al. 2008).

The biological relevance of these studies to assessing potential health risks from human consumption of foods derived from *Bt* crops can be questioned on several fronts. Administration of Cry proteins by IN, IP and IR routes of exposure do not necessarily predict risks from IG or dietary intake because in some cases, the researchers bypassed the protective barrier of the gastrointestinal tract by injecting or administering proteins by other routes. When they did employ the IG route in mice, they gave doses of Cry proteins far in excess of potential human intakes and included Maalox® to neutralize the pH of the gastrointestinal tract, thereby compromising normal physiological conditions for digestion of Cry1Ac protein by pepsin. Mice were often dosed with 100 µg Cry1Ac protein which exceeds potential human intake by approximately 5000-fold.[1] This discrepancy may be greater still, since maize is normally processed into human foods and not consumed raw. Processing (e.g., cooking) denatures Cry1Ab protein (see Sect. 16.6), so the actual dietary intakes of intact Cry1Ab protein from consumption of processed foods is anticipated to be far less. It has been estimated that grain processing could reduce levels of functionally active protein by approximately two orders of magnitude (Hammond and Jez 2011). Based on the calculations in footnote 1, this would result in an actual human intact Cry1Ab intake of approximately 0.008 µg/kg body weight; a level 500,000-fold lower than the levels used on the study demonstrating immunogenic effects. In addition to human dietary irrelevance of the doses tested in mice, an attempt to reproduce this work in mice given Cry1Ab protein, failed to detect anti-Cry IgG antibodies at the 100 µg/mouse dose (these authors did not include Maalox along with the oral dosed Cry1Ab protein) (Adel-Patient et al. 2010). These authors attributed the discrepancy between their study and earlier studies (Moreno-Fierros et al. 2000; Vazquez-Padron et al. 2000) to the possible presence of *E. coli* endotoxin in the Cry1Ac preparations used on the earlier studies. The previous authors used engineered *E. coli* to produce Cry1Ac but did not apparently check the preparations for endotoxin contamination. Adel-Patient et al. (2010) reported that

[1] 100 µg Cry1Ac/25 gm mouse ~ 4000 µg/kg body weight; human intake of Cry1Ab protein from consumption of MON 810 maize (YIELDGARD Corn Borer®) was estimated to be 0.008 µg/kg body weight (Hammond and Jez 2011).

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IG administration of purified Cry1Ab protein had no impact on immune response in mice and confirmed the earlier reports of the immunogenicity of Cry1Ac administered by IP injection to mice, without any evidence of allergenicity. The other study (Finamore et al. 2008) reporting possible alterations of intestinal and peripheral immune cell populations in young and old mice fed large amounts of MON 810 maize in the diet can be questioned since the alterations were often of small magnitude, and sometimes in opposite direction when measured at different study intervals. It is difficult to interpret the biological relevance of the changes that were observed since no historical information was provided on normal variation of the measured parameters in control mice. A much bigger question is the relevance of findings in mice to predicting possible immune effects in humans.

While the mouse model has some similarities to human immunological mechanisms (Adel-Patient et al. 2010), in general, animal models have not been considered to have been sufficiently validated to be able to accurately predict potential allergenic or immunologic effects in humans from dietary exposure to proteins (Goodman et al. 2008; Thomas et al. 2009; Codex 2009). As a practical matter, there has been widespread dietary exposure to Cry proteins from application of *Bt* microbial formulations applied to vegetables and other crops for many decades (see Sect. 16.7) and there have been no reports that the immune system of humans is at risk from dietary exposures. Given the comparatively low dietary exposures to Cry proteins from consumption of foods derived from *Bt* crops (or for that matter, application of *Bt* microbials to food crops) the potential to induce an immune response in humans was considered to be unlikely (Guimaraes et al. 2010).

16.6 Assessment of Food Processing on Cry Protein Biological Activity

The functional activity of proteins including Cry proteins is dependent on their three-dimensional structure and the combination of various environmental forces (electrostatic forces, van der Waals interactions, hydrogen bonds, and hydrophobic interactions) that help to maintain that structure in the cell (Branden and Tooze 1991; Creighton 1993). In general, protein structures are only marginally stable under a limited range of physiological conditions and are easily disrupted by changes such as increases in temperature, variation of pH, or physical disruption that overcome the forces keeping them folded properly (Creighton 1993). These changes typically result in denaturation of proteins which leads to a drastic change in protein structure. Typically, denaturation does not involve changes in the primary structure (amino acid sequence) of a protein (i.e., degradation of the polypeptide chain), but disrupts the secondary, tertiary, and quaternary (if applicable) structure of denatured proteins. Consequently, there is a complete loss of biological function, as the denatured polypeptide is more like a random coil than a folded protein.

Changes such as increased temperature, altered pH, physical disruption occur routinely during processing of the seed/grain into human food. For example, during

the processing of maize and soybeans into food fractions, heating, high pressure extrusion, mechanical shearing, changes in pH, and the use of reducing agents are all employed and will unfold a native protein structure and/or alter the primary structure of a protein by hydrolysis of peptide bonds (Kilara and Sharkasi 1986; Meade et al. 2005). In typical processing of maize and soybeans into food fractions, temperatures of 95–100°C are commonly encountered (Berk 1992; Duensing et al. 2003; Rooney and Serna-Saldivar 2003). These elevated temperatures can lead to irreversible denaturation and loss of protein function (de Luis et al. 2009; Thomas et al. 2007), although this does not alter the nutritional value of the denatured protein as a source of dietary amino acids. Cooking proteins aids their digestion in the gastrointestinal tract as proteases (e.g., pepsin and trypsin), are able to cleave the random coil of a denatured protein more quickly and efficiently compared with the same protein in its native three dimensional conformation (Herman et al. 2006).

Precipitation is often used to remove proteins from other food fractions like lipids, as changes in the physical properties of a protein can reduce its solubility leading to aggregation or precipitation. Multimeric proteins can also dissociate into monomers resulting in loss of function (Schultz and Liebman 2002; Meade et al. 2005). It may still be possible to detect epitopes on denatured, aggregated or precipitated proteins using immunologic detection methods. This has been observed for some introduced proteins but not others (Terry et al. 2002; Grothaus et al. 2006; Margarit et al. 2006; Thomas et al. 2007; de Luis et al. 2009; Codex 2010). In general, immunologic methods for detecting introduced proteins is not often done because of the effects of denaturation of proteins in processed food; immunologic detection methods are mostly reserved for testing raw agricultural commodities (Margarit et al. 2006; Bogani et al. 2008; de Luis et al. 2009). Even if you can still detect proteins through antibody recognition of sequence-specific epitopes, the introduced proteins most likely have lost their functional activity as discussed below.

Cry proteins have been subjected to *in vitro* heat stability studies to determine if they maintain their insecticidal activity after cooking. Cry1Ab, Cry1F, Cry34Ab1 and Cry35Ab1 proteins were cooked to temperatures ranging from 60–90°C for periods ranging from 10–30 min (EFSA 2005a, 2007; de Luis et al. 2009). *Bt* soybean seeds containing Cry1Ac protein were also subjected to cooking that approximated temperatures used during processing of soybeans into meal (EPA 2010). All Cry proteins lost insecticidal activity after cooking when tested in insect bioassays. These Cry proteins were subjected to similar cooking conditions used when maize grain and soybeans are processed into human foods. The impact of food processing on the functional activity of introduced proteins may be relevant for other processed crops such as rice that are cooked before consumption. It is likely that there is minimal, if any dietary exposure to functionally active Cry proteins when food products derived from processed food crops are consumed.

Overall, the impact of harsh processing conditions on protein structure and function support the EFSA conclusion that risk assessors should consider the impact of food processing on the levels of the introduced protein, otherwise they may overestimate potential dietary exposure "…food products are often processed into ingredients and/or incorporated in formulated processed food products, where the

new protein and/or the novel secondary gene product attrition will occur. This may result in significant reduction in the theoretical maximum daily intake (TMDI) of the novel gene product, resulting in over-estimated exposure levels and even larger margins of safety for man" (EFSA 2008).

16.7 Human Dietary Exposure Assessment for Cry Proteins in *Bt* Crops

There is a history of safe consumption of Cry proteins. As mentioned earlier, *Bt* microbial pesticides have also been added to drinking water in outdoor storage facilities in various locations around the world to control insect (mosquito) vectors of disease (WHO/ICPS 1999). In locations where water has to be stored in containers for use in drinking *Bt* microbials have been added to the drinking water to control mosquito larvae etc (WHO/IPCS 1999; Bravo et al. 2007). Because of their safety profile and long history of safe use in agriculture, *Bt* microbials have been exempted from the requirement of a setting a tolerance in countries which they have been registered (OECD 2007).

They have also been applied topically to vegetables in organic agriculture to control insect pests (WHO/IPCS 1999). Residual levels of up to 10^4 CFUs (colony forming units, i.e., viable *Bt* microbes)/gram of plant tissue have been found on fresh vegetables marketed in Europe following application of commercial *Bt* microbial formulations (Frederiksen et al. 2006). In a recent review on the safety of *Bt*, it stated: "In many regions of the world where fresh vegetable crops are marketed within a few days of harvest, these have been recently sprayed with *Bt*. This is especially true of vegetables grown using organic methods. It is quite common for vegetables treated with *Bt*, such as broccoli, tomatoes, cucumbers, cauliflower and lettuce to be eaten raw with only minimal washing. In these cases, humans are directly consuming thousands of *Bt* spores and insecticidal crystals" (Federici and Siegel 2008).

A "back-of-the-envelope" estimate of potential human dietary exposure to Cry proteins from application of commercial *Bt* microbial pesticide formulations was undertaken. It was assumed that the *Bt* microbial formulation was applied shortly before harvest and that the broccoli was consumed raw (e.g. salads) and was not cooked. If the *Bt* microbial formulation was applied weeks prior to harvest, or the broccoli was cooked, the potential dietary intake of functionally active Cry proteins would be much lower. According to WHO/GEMs, the highest acute dietary intake of broccoli in the countries that responded to the WHO survey was in the United States. The WHO/GEMs US adult acute dietary intake (97th percentile) of broccoli is 5.8 gm/kg body weight. In a recent review, it was reported that chronic intake of broccoli in the US was 8.2 g/capita/day (Latte et al. 2011). For purposes of this assessment, it was assumed that the broccoli was consumed raw and not cooked (eg. in salads). The commercial application rate for the *Bt* microbial formulation to control insect pests was based on the label directions from the supplier which could

range up to 32 oz/acre. The *Bt* microbial formulation contained ~10% Cry protein (w/w) and was applied once. For purposes of the exposure calculation, it was assumed that 10% of the applied *Bt* microbial formulation was deposited on broccoli heads. Based on these assumptions, the acute adult intake of Cry protein from consumption of uncooked broccoli (heads) was estimated to range between 50–1 μg/kg body weight for acute and chronic consumption respectively. This estimated exposure is considerably higher than the previous estimate (~0.008 μg/kg body weight) of dietary intake of functionally active Cry protein from food derived from *Bt* maize (page 9). For other kinds of Bt maize, the levels of Cry proteins in grain were higher than 0.3 ppm ranging from approximately 15–115 ppm (Hammond and Cockburn 2008). Using the same assumptions (Hammond and Jez 2011), the potential human dietary intake of functionally active Cry protein for these Bt maize varieties could range up to approximately 2 ug/kg body weight/day. It seems likely that dietary exposure to functionally active Cry proteins from application of Bt microbial formulations to vegetables (shortly before harvest) could be similar to or even higher than dietary exposure from consumption of foods derived from Bt crops.

Regulatory agencies have confirmed the history of safe consumption of Cry protein residues on crops and in drinking water "The use patterns for *B. thuringiensis* may result in dietary exposure with possible residues of the bacterial spores on raw agricultural commodities. However, in the absence of any toxicological concerns, risk from the consumption of treated commodities is not expected for both the general population and infants and children" (EPA 1998) and "*Bt* has not been reported to cause adverse effects on human health when present in drinking-water or food" (WHO/IPCS 1999).

Although Cry proteins have been considered to be safe as mentioned above, even from organic agricultural use, questions still arise challenging their safety when incorporated in biotechnology-derived crops. For example, a recent paper gained attention by reporting the detection of Cry1Ab protein in the serum of non-pregnant women, pregnant women, and the cord blood of their fetuses at mean levels of detection of 0.19, 0.13 and 0.04 ng/ml respectively (Aris and Leblanc 2011). The authors used a commercially available ELISA immunoassay kit which has been validated for use in detecting Cry1Ab protein in grain/seed samples but not human serum. Considering the vastly different composition of these matrices, it cannot be overlooked that the authors did not report that they had validated the assay for use in human serum. Furthermore, the majority of the serum "detects" in the Aris and Leblanc paper were at, or below, the limit of detection (LOD) for this commercial kit for use in grain and seed. This raises serious questions about the accuracy of their data especially in light of previous reports on the topic including, (1) a commercial immunoassay kit that was not validated to detect Cry1Ab protein in porcine blood produced invalid results (Chowdhury et al. 2003a), and (2) a validated immunoassay to quantify Cry1Ab protein in plasma (LOD 1 ng/ml) was unable to detect Cry1Ab protein in any of the plasma samples collected from cows fed MON 810 maize at 70% w/w (dry matter) in the diet for 1 or 2 months (Paul et al. 2008). The amount of MON 810 (YIELDGARD Corn Borer®) maize consumed by dairy cows greatly exceeds potential human intake. In consideration of the much lower

Table 16.2 Examples of subchronic toxicity studies with *Bt* crops

Bt crop	% in diet[a]	References
Bt tomato	10	Noteborn et al. (1995)
Bt/HT[b] maize (ECB[c]/RR[d])	11/33	EFSA (2005a)
Bt/HT maize (CRW[e]/RR)	11/33	EFSA (2005b)
Bt/HT maize (ECB/CRW/RR)	11/33	EFSA (2005c)
Bt maize(ECB/CRW)	11/33	EFSA (2005d)
Bt maize (ECB)	11/33	Hammond et al. (2006a)
Bt maize (CRW)	11/33	Hammond et al. (2006b)
BT maize (ECB)	11/13	MacKenzie et al. (2007)
Bt cotton	10	Dryzga et al. (2007)
Bt rice	60	Schroder et al. (2007)
Bt/HT maize (CRW/Gluf[f])	35	Malley et al. (2007)
Bt/HT maize (CRW/RR)	11/33	Healy et al. (2008)
Bt maize (CRW)	50/70	He et al. (2008)
Bt/HT maize (ECB/CRW)	34	Appenzeller et al. (2009)

[a] percent (w/w) maize, rice or cottonseed meal added to the diet
[b] HT—herbicide tolerant
[c] ECB—European maize borer
[d] RR—Roundup Ready® (tolerant to ROUNDUP herbicide)
[e] CRW—maize rootworm
[f] Gluf—glufosinate (tolerant to glufosinate herbicide)
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potential human dietary intake of MON 810 maize compared to farm animals fed high levels in the diet, and in consideration of the digestibility of Cry1Ab protein when exposed to pepsin, the reported levels of intact Cry1Ab detected in human serum must be questioned.

16.8 Toxicology Feeding Studies in Rodents Fed *Bt* Crops

In the United States and Canada, there has been no requirement to routinely feed *Bt* crops to animals to confirm their safety for human and farm animal consumption. The composition and agronomic performance of *Bt* crops has been shown in many field trials to be similar to conventional non-biotech comparators. As a consequence, following review of submitted dossiers summarizing all relevant data by registrants, the USFDA and EPA have, to date, not considered additional animal toxicology studies as necessary to confirm safety. However, 90 day rodent subchronic feeding studies were often required by the EU to confirm the safety of the first generation of *Bt* crops that were registered for import or production in Europe. Some of these studies are shown in Table 16.2.

In 2008, EFSA published a review of its safety assessment of biotech crops over the last several years, and concluded that, where the safety of the introduced pro-

tein had been confirmed and no other unintended changes had been detected, the conduct of animal feeding studies added little to the overall safety assessment. They concluded that the majority of studies they had reviewed confirmed the food safety of biotech crops. Ninety-day rat toxicology studies with Bt biotech crops are listed in Table 16.2. EFSA also acknowledged that some published toxicology studies reported adverse effects when biotech crops were fed to animals, but EFSA concluded that because of deficiencies in these studies, the results were not interpretable (EFSA 2008).

No evidence of any treatment related adverse effects were observed in 90 day rat toxicology studies carried out with *Bt* crops whether the crops contained one or more Cry proteins. The maize grain that was added to rat diets was simply ground into meal and not further processed.

In a subchronic gastrointestinal impairment model in rats, chemically-induced gastrointestinal impairment (treated with famotidine to reduce gastric acid secretion and indomethacin to cause damage to the intestinal epithelium) was induced in rats fed Cry1Ab protein in the diet (10 ppm) for 2 weeks (Onose et al. 2008). Controls were treated in the same manner except they were not fed Cry1Ab protein. It was expected that Cry1Ab protein would not have been digested due to reduction of gastric acid production allowing intact Cry1Ab protein to enter systemic circulation via the intestinal tract damaged by indomethacin pretreatment. As expected, there was no evidence of meaningful toxicological effects (changes in clinical blood parameters and histologic appearance of organs) reported in Cry1Ab dosed animals.

Additionally, there have also been a number of feeding studies in laboratory and farm animals such as poultry, swine and ruminants fed both either *Bt* microbial pesticides and/or *Bt* crops (Betz et al. 2000; Flachowsky et al. 2005a, b, 2007; Federici and Siegel 2008; WHO/IPCS 1999; McClintock et al. 1995; OECD 2007; Siegel 2001; Brake et al. 2003; EFSA 2008; Scheideler et al. 2008; McNaughton et al. 2007; Taylor et al. 2005, 2007). No evidence of adverse effects were reported in these studies as the animals responded similarly in growth and feed consumption to controls fed non-biotech derived crops.

16.9 Food and Feed Safety Benefits of *Bt* Crops

16.9.1 Reduced Insecticide Application

The adoption of *Bt* crops in various world areas has contributed to a significant reduction in chemical insecticide applications. In Burkino Faso in West Africa, the planting of *Bt* cotton has enabled farmers to reduce the number of chemical insecticide sprays during a growing season from 6–2 applications (James 2010). India, which is now the world's biggest producer of *Bt* cotton with an estimated 23.2 million acres planted in 2010, reported pesticide use has been cut at least in half. In the most comprehensive survey conducted to date (2002–2008), Indian farmers report-

ed *Bt* cotton use prevented at least 2.4 million cases of pesticide poisoning saving $ 14 million (US dollar equivalent) in annual health costs (Kouser and Qaim 2011).

16.9.2 Reduced Mycotoxin Contamination of Grain

In addition to protecting crops from insect feeding damage, grain from *Bt* maize was found to have lower levels of the mycotoxin fumonisin based on field trials in various countries (Ostry et al. 2010; Folcher et al. 2010). Various mycotoxin producing fungi such as fusarium species exist in the environment wherever maize is grown. They can enter the maize plant through insect damaged tissue allowing the fungi to enter plant tissue (e.g., stalks, ears), producing fumonisins which are common mycotoxin contaminants of maize wherever it is grown (Miller 2001). Since Cry proteins can reduce feeding damage by controlling insect pests, they can lower the potential for fungal colonization and therefore mycotoxin contamination. Dietary exposure to fumonisin can cause a variety of adverse health effects in farm animals and possibly humans (Li et al. 2001; CAST 2003; Wang et al. 2003; Marasas et al. 2004; Wu et al. 2004), and lower levels of contamination associated with *Bt* maize should be considered a beneficial aspect of this technology.

16.10 Conclusions

There is a 50-year history of safe use and consumption of foods sprayed with commercial *Bt* microbial pesticide products and 14 year history of safe consumption of food and feed derived from *Bt* crops. Many toxicology studies conducted with *Bt* microbial pesticides and *Bt* crops have confirmed their safety for consumption. There is a history of safe consumption of Cry proteins from use of *Bt* microbial pesticides on vegetable food crops, and dietary exposures may be comparable or higher than that from consumption of foods derived from *Bt* crops. In either case, dietary intake of intact Cry proteins is very low. Use of *Bt* crops has secondary benefits by significantly reducing insecticide use, lowering insecticide exposure to applicators and improving the food security of maize grain by reducing contamination by fumonisin mycotoxins.

References

Adel-Patient K, Guimaraes VD, Paris A, Drumare MF, Ah-Leung S, Lamourette P, Nevers MC, Canlet C, Molina J, Bernard H, Créminon C, Wal JM (2010) Immunological and metabolomic impacts of administration of Cry1Ab protein and MON 810 maize in mouse. PLoS One 6(1):e16346. doi:10.1371/journal.pone.0016346

Appenzeller LM, Malley LA, MacKenzie SA, Everds N, Hoban D, Delaney B (2009) Subchronic feeding study with genetically modified stacked trait lepidopteran and coleopteran resistant (DAS Ø15Ø7 1 x DAS-59122-7) maize grain in Sprague-Dawley rats. Food Chem Toxicol 47:1512–1520

Aris A, Leblanc S (2011) Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. Reprod Toxicol 31:528–533

Berk Z (1992) Technology of production of edible flours and protein products from soybeans. FAO Agricultural Services Bulletin No. 97

Betz FS, Hammond BG, Fuchs RL (2000) Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. Regul Toxicol Pharmacol 32:156–173

Bogani P, Minunni M, Spiriti M, Zavaglia M, Tombelli S (2008) Transgenes monitoring in an industrial soybean processing chain by DNA-based conventional approaches and biosensors. Food Chem 113:658–664

Bondzio A, Stumpff F, Schön J, Martens H, Einspanier R (2008) Impact of *Bacillus thuringiensis* toxin Cry1Ab on rumen epithelial cells (REC)—A new in vitro model for safety assessment of recombinant food compounds. Food Chem Toxicol 46:1976–1984

Branden C, Tooze J (2001) Introduction to protein structure. Garland Publishing, New York

Brake J, Faust MA, Stein J (2003) Evaluation of transgenic event Bt 11 hybrid maize in broiler chickens. Poult Sci 82:551–559

Bravo A, Gill SS, Soberón M (2007) Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. Toxicon 49(4):423–435

Cao S, He X, Xu W, Ran W, Liang L, Luo Y, Yuan Y, Zhang N, Zhou X, Huang K (2010) Safety assessment of Cry1C protein from genetically modified rice according to the national standards of PR China for a new food source. Regul Toxicol Pharmacol 58(3):474–481

CAST (2003) Mycotoxins. Risks in plant, animal, and human systems. Council for Agricultural Science and Technology. Task Force Report No. 139

Chowdhury EH, Kuribara H, Hino A, Sultana P, Mikami O, Shimada N, Guruge NS, Saito M, Nakajima Y (2003a) Detection of maize intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified maize Bt11. J Anim Sci 81:2546–2551

Chowdhury EH, Shimada N, Murata H, Mikami O, Sultana P, Miyazaki S, Yoshioka M, Yamanaka N, Hirai N, Nakajima Y (2003b) Detection of Cry1Ab protein in the gastrointestinal contents but not in the visceral organs of genetically modified Bt11-fed calves. Vet Hum Toxicol 45:72–75

Codex (2009) WHO/FAO. Foods derived from modern biotechnology, 2nd edn. Rome

Codex (2010) Committee on Sampling and Detection Methods (CCMAS). Proposed draft guidelines on performance criteria and validation of methods for detection, identification, and quantification of specific DNA sequences and specific proteins in food (At step 5/8 of the procedure). ALINORM 10/33/23. Appendix III, pp 47–68

Creighton TE (1993) Proteins: structures and molecular properties. W.H. Freeman and Company, New York

Delaney B, Astwood JD, Cunny H, Eichen Coon R, Herouet-Guicheney C, MacIntosh S, Meyer LS, Privalle L, Gao Y, Mattsson J, Levine M (2008) Evaluation of protein safety in the context of agricultural biotechnology. Food Chem Toxicol 46:S71–S97

de Luis R, Lavilla M, Sánchez L, Calvo MD, Pérez M (2009) Immunochemical detection of Cry1A(b) protein in model processed foods made with transgenic maize. Eur Food Res Technol 229:15–19

Dryzga MD, Yano BL, Andrus AK, Mattsson JL (2007) Evaluation of the safety and nutritional equivalence of a genetically modified cottonseed meal in a 90 day dietary toxicity study in rats. Food Chem Toxicol 45:1994–2004

Duensing WJ, Roskens AB, Alexander RJ (2003) Chapter 11. Maize dry milling: processes, products, and applications. In: White P, Johnson L (eds) Maize chemistry and technology, 2nd edn. AAOCS, St Paul

EFSA (2005a) Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA GMO UK 2004 01) for the placing on the market of glyphosate tolerant and insect-resistant genetically modified maize NK603 × MON810, for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 from Monsanto (Question No EFSA Q-2004-086). EFSA J 309:1–22

EFSA (2005b) Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA GMO UK 2004 06) for the placing on the market of insect-protected glyphosate tolerant genetically modified maize MON863 × NK603, for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 from Monsanto (Question No EFSA Q 2004 154). EFSA J 255:1–21

EFSA (2005c) Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA GMO BE 2004 07) for the placing on the market of insect protected glyphosate tolerant genetically modified maize MON863 × MON810 × NK603, for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 from Monsanto (Question No EFSA Q 2004 159). EFSA J 256:1–25

EFSA (2005d) Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Notification (Reference C/DE/02/9) for the placing on the market of insect-protected genetically modified maize MON 863 x MON 810, for import and processing, under Part C of Directive 2001/18/EC from Monsanto. EFSA J 251:1–22

EFSA (2007) Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-NL-2005-12) for the placing on the market of insect-resistant genetically modified maize 59122, for food and feed uses, import and processing under Regulation (EC) No 1829/2003, from Pioneer Hi-Bred International, Inc. and Mycogen Seeds, c/o Dow Agrosciences LLC. EFSA J 470:1–25

EFSA (2008) Safety and nutritional assessment of GM plants and derived food and feed: the role of animal feeding trials. Food Chem Toxicol 46:S2–S70

EFSA (2011) Guidance for risk assessment of food and feed from genetically modified plants. EFSA J 9(5):2150

EPA (1998) EPA Registration Eligibility Decision (RED) *Bacillus thuringiensis* EPA 738-R-98-004

EPA (2001) *Bacillus thuringiensis* Cry1F protein and the genetic material necessary for its production in maize; exemption from the requirement for a tolerance. Fed Regist 66(109):30321

EPA (2004) *Bacillus thuringiensis* VIP3A insect control protein and the genetic material necessary for its production; notice of a filing to a pesticide petition to amend the exemption from the requirement for a tolerance for a certain pesticide chemical in the food. Fed Regist 69(178):55605

EPA (2008a) Exemption from the requirement of a tolerance for the Bacillus Thuringiensis Cry1A.105 protein in maize. 40 CFR 174.502

EPA (2008b) Exemption from the requirement of a tolerance for the Bacillus Thuringiensis Cry-2Ab2 protein in maize. 40 CFR 174.503

EPA (2010) Biopesticide Registration Action Document. *Bacillus thuringiensis* Cry1Ac Protein and the Genetic Material (Vector PV-GMIR9) Necessary for Its Production in MON 87701 (OECD Unique Identifier: MON 877Ø1-2) Soybean (PC Code 006532). U.S. Environmental Protection Agency Office of Pesticide Programs Biopesticides and Pollution Prevention Division. http://www.epa.gov/oppbppd1/biopesticides/pips/bt-cry1ac-protien.pdf. Accessed 8 Oct 2010

Federici B, Siegel J (2008) Safety assessment of *Bacillus thuringiensis* and Bt crops used in insect control. In: Hammond BG (ed) Food safety of proteins in agricultural biotechnology. CRC Press, Boca Raton

Finamore A, Roselli M, Britti S, Monastra G, Ambra R, Turrini A, Mengheri E (2008) Intestinal and peripheral immune response to MON810 maize ingestion in weaning and old mice. J Agric Food Chem 56:11533–11539

Fisher R, Rosner L (1959) Toxicology of the microbial insecticide, Thuricide. J Agric Food Chem 7:686–688

Flachowsky G, Chesson A, Aulrich K (2005a) Animal nutrition with feeds from genetically modified plants. Arch Anim Nutr 59:1–40

Flachowsky G, Halle I, Aulrich K (2005b) Long term feeding of Bt-maize—a ten generation study with quails. Arch Anim Nutr 59:449–451

Flachowsky G, Aulrich K, Bohme H, Halle I (2007) Studies on feeds from genetically modified plants (GMP)—Contributions to nutritional and safety assessment. Anim Feed Sci Technol 133:2–30

Folcher L, Delos M, Marengue E, Jarry M, Weissenberger A, Eychenne N, Regnault-Roger C (2010) Lower mycotoxin levels in Bt maize. Agron Sustain Dev 30:711–719

Frederiksen K, Rosenquist H, Jørgensen K, Wilcks A (2006) Occurrence of natural *Bacillus thuringiensis* contaminants and residues of *Bacillus thuringiensis*-based insecticides on fresh fruits and vegetables. Appl Environ Microbiol 72(5):3435–3440

Goodman R, Vieths S, Sampson HA, Hill D, Ebisawa M, Taylor SL, Ree R van (2008) Allergenicity assessment of genetically modified crops—what makes sense? Nat Biotechnol 26:73–81

Griffiths JS, Haslam SM, Yang T, Garczynski SF, Mulloy B, Morris H, Cremer PS, Dell A, Adang MJ, Aroian RV (2005) Glycolipids as receptors for *Bacillus thuringiensis* crystal toxin. Science 307:922–925

Grothaus GD, Bandla M, Currier T, Giroux R, Jenkins GR, Lipp M, Shan G, Stave JW, Pantella V (2006) Immunoassay as an analytical tool in agricultural biotechnology. AOAC Int 89(4):913–928

Guimaraes V, Drumare MF, Lereclus D, Gohar M, Lamourette P, Nevers MC, Vaisanen-Tunkelrott ML, Bernard H, Guillon B, Creminon C, Wal JM, Adel-Patient K (2010) In vitro digestion of Cry1Ab proteins and analysis of the impact on their immunoreactivity. J Agric Food Chem 58:3222–3231

Hammond B, Cockburn A (2008) The safety assessment of proteins introduced into crops developed through agricultural biotechnology: a consolidated approach to meet current and future needs. In: Hammond BG (ed) Food safety of proteins in agricultural biotechnology. CRC Press, New York

Hammond BG, Jez JM (2011) Impact of food processing on the safety assessment for proteins introduced into biotechnology-derived soybean and maize crops. Food Chem Toxicol 49(4):711–721

Hammond BG, Dudek R, Lemen JK, Nemeth MA (2006a) Results of a 90-day safety assurance study with rats fed grain from maize borer-protected maize. Food Chem Toxicol 44(7):1092–1099

Hammond B, Lemen J, Dudek R, Ward D, Jiang C, Nemeth M, Burns J (2006b) Results of a 90-day safety assurance study with rats fed grain from maize rootworm-protected maize. Food Chem Toxicol 44(2):147–160

He XY, Huang KL, Li X, Qin W, Delaney B, Luo YB (2008) Comparison of grain from maize rootworm resistant transgenic DAS 59122-7 maize with non transgenic maize grain in a 90 day feeding study in Sprague Dawley rats. Food Chem Toxicol 46:1994–2002

Healy C, Hammond B, Kirkpatrick J (2008) Results of a 90 day safety assurance study with rats fed grain from maize rootworm protected, glyphosate tolerant MON 88017 maize. Food Chem Toxicol 46:2517–2524

Herman RA, Schafer BW, Korjagin VA, Ernest AD (2003) Rapid digestion of Cry34Ab1 and Cry35Ab1 in simulated gastric fluid. J Agric Food Chem 51:6823–6827

Herman RA, Storer NP, Gao Y (2006) Acid-induced unfolding kinetics in simulated gastric digestion of proteins. Regul Toxicol Pharmacol 46:93–99

Hofmann C, Luthy P, Hutter R, Piska V (1988a) Binding of the delta endotoxin from *Bacillus thuringiensis* to brush-border membrane vesicles of the Cabbage Butterfly (Pieris brassicae). Eur J Biochem 173:85–91

Hofmann C, Vanderbruggen H, Hofte H, Van Rie J, Jansens S, Van Mellaert H (1988b) Specificity of B. thuringiensis delta-endotoxins is correlated with the presence of high affinity binding sites in the brush-border membrane of target insect midgastrointestinal tracts. Proc Natl Acad Sci USA 85:7844–7848

Huang DF, Zhang J, Song FP, Lang ZH (2007) Microbial control and biotechnology research on *Bacillus thuringiensis* in China. J Invertebr Pathol 95:175–180

James C (2010) Global Status of Commercialized Biotech GM Crops: 2010 International Service for the Acquisition of Agri-biotech Applications (ISAAA). ISAAA Briefs, brief 42

Juberg DR, Herman RA, Thomas J, Delaney B (2009) Acute and Repeated Dose (28 Day) Mouse Oral Toxicology Studies with Cry34Ab1 and Cry35Ab1 *Bt* Proteins Used in Coleopteran Resistant DAS-59122-1 Maize. Regul Toxicol Pharmacol 54:154–163

Kilara A, Sharkasi T (1986) Effects of temperature on food proteins and its implications on functional properties. Crit Rev Food Sci Nutr 23(4):323–395

Kroghsbo S, Madsen C, Poulsen M, Schroder M, Kvist PH, Taylor M, Gatehouse A, Shu Q, Knudsen L (2008) Immunotoxicological studies of genetically modified rice expressing PHA-E lectin or *Bt* toxin in Wistar rats. Toxicology 245:24–34

Kouser S, Qaim M (2011) Impact of Bt cotton on pesticide poisoning in smallholder agriculture: a panel data analysis. Ecol Econ 70(11):2105–2113

Lambert B, Buysse L, Decock C, Jansens S, Piens C, Saey B, Seurinck J, Van Audenhove K, Van Rie J, Van Vliet A, Peferoen M (1996) A *Bacillus thuringiensis* insecticidal crystal protein with a high activity against members of the family Noctuidae. Appl Environ Microbiol 62:80–86

Latte KP, Appel KE, Lampen A (2011) Health benefits and possible risks of broccoli. Food Chem Toxicol 49:3287–3309

Li FQ, Yoshizawa T, Kawamura O, Luo XY, Li YW (2001) Aflatoxins and fumonisins in maize from the high-incidence area for human hepatocellular carcinoma in Guangxi, China. J Agric Food Chem 49:4122–4126

MacKenzie SA, Lamb I, Schmidt J, Deege L, Morrisey MJ, Harper M, Layton RJ, Prochaska LM, Sanders C, Locke M, Mattsson JL, Fuentes A, Delaney B (2007) Thirteen week feeding study with transgenic maize grain containing event DASO15O7 1 in Sprague–Dawley rats. Food Chem Toxicol 45:551–562

Marasas WFO, Riley RL, Hendricks KA, Stevens VL, Sadler TW, Waes JG van, Missmer SA, Cabrera J, Torres O, Gelderblom WCA, Allegood J, Martínez C, Maddox J, Miller JD, Starr L, Sullards MC, Roman AV, Voss KA, Wang E, Merrill AH Jr (2004) Fumonisins disrupt sphingolipid metabolism, folate transport, and neural tube development in embryo culture and in vivo: a potential risk factor for human neural tube defects among populations consuming fumonisin-contaminated maize. J Nutr 134:711–716

Malley LA, Everds NE, Reynolds J, Mann PC, Lamb I, Rood T, Schmidt J, Layton RL, Prochaska LM, Hinds M, Locke M, Chui CF, Claussen F, Mattsson JL, Delaney B (2007) Subchronic feeding study of DAS 59122 7 maize grain in Sprague Dawley rats. Food Chem Toxicol 45:1277–1292

Margarit E, Reggiardo M, Vallejos R, Permingeat H (2006) Detection of *Bt* transgenic maize in foodstuffs. Food Res Int 39:250–255

McClintock J, Schaffer C, Sjoblad R (1995) A comparative review of the mammalian toxicity of *Bacillus thuringiensis*-based pesticides. Pestic Sci 45:95–105

McNaughton JL, Roberts M, Rice D, Smith B, Hinds M, Schmidt J, Locke M, Bryant A, Rood T, Layton R, Lamb I, Delaney B (2007) Feeding performance in broiler chickens fed diets containing DAS-59122-7 maize grain compared to diets containing non-transgenic maize grain. Anim Feed Sci Technol 132:227–239

Meade S, Reid E, Gerrard J (2005) The impact of processing on the nutritional quality of food proteins. J AOAC Int 88(3):904–922

Mendelsohn M, Kough J, Vaituzis Z, Matthews K (2003) Are *Bt* crops safe? Nat Biotechnol 21:1003–1009

Miller JD (2001) Factors That affect the occurrence of fumonisin. Environ Health Perspect 109(Suppl 2):321–324

Moreno-Fierros L, Garcia N, Gastrointestinal tractierrez R, Lopez-Revilla R, Vazquez-Padron RI (2000) Intranasal, rectal and intraperitoneal immunization with protoxin Cry1Ac from *Bacillus thuringiensis* induces compartmentalized serum, intestinal, vaginal and pulmonary immune responses in Balb/c mice. Microbes Infect 2:885–890

Noteborn HPJ, Bienenmann-Ploum ME, Berg JHJ van den, Alink GM, Zolla L, Reynaerts A, Pensa M, Kuiper HA (1995) Safety Assessment of *Bacillus thuringiensis* insecticidal protein

CRYIA(b) expressed in transgenic tomato. In: Engel K-H, Takeoka GR, Teranishi R (eds) Genetically modified foods: safety issues. American Chemical Society, Washington,DC

OECD (2007) Consensus document on safety information on transgenic plants expressing *Bacillus thuringiensis*—derived insect control proteins. In Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology, Paris, France

Ofori-Anti AO, Ariyarathna H, Chen L, Lee HL, Pramod SN, Goodman RE (2008) Establishing objective detection limits for pepsin digestion assay used in the assessment of genetically modified foods. Regul Toxicol Pharmacol 52:94–103

Okunuki H, Teshima R, Shigeta T, Sakushima J, Akiyama H, Goda Y, Toyoda M, Sawada J (2001) Increased digestibility of two products in genetically modified food (CP-4 EPSPS and Cry-1Ab) after preheating. J Food Hyg Soc Jpn 43(2):68–73

Onose J, Imai T, Hasumura M, Ueda M, Ozeki Y, Hirose M (2008) Evaluation of subchronic toxicity of dietary administered Cry1Ab protein from *Bacillus thuringiensis var. Kurustaki* HD-1 in F344 male rats with chemically induced gastrointestinal impairment. Food Chem Toxicol 46:2184–2189

Ostry V, Ovesna J, Skarkova J, Pouchova V, Ruprich J (2010) A review on comparative data concerning Fusarium mycotoxins in *Bt* maize and non-Bt isogenic maize. Mycotox Res 26:141–145

Paul V, Steinke K, Meyer HH (2008) Development and validation of a sensitive enzyme immunoassay for surveillance of Cry1Ab toxin in bovine blood plasma of cows fed *Bt*-maize (MON 810). Anal Chim Acta 607:106–113

Rooney LW, Serna-Saldivar SO (2003) Chapter 13. Food use of whole maize and dry-milled fractions. In: While PJ, Johnson LA (eds) Maize chemistry and technology, 2nd edn. American Association of Cereal Chemists, Inc., St Paul

Sacchi VF, Parenti P, Hanozet GM, Giordana B, Luthy P, Wolfersberger MG (1986) *Bacillus thuringiensis* toxin inhibits K+-gradient-dependent amino acid transport across the brush border membrane of Pieris brassicae midgastrointestinal tract cells. FEBS Lett 204:213–218

Sanchis V (2010) From microbial sprays to insect-resistant transgenic plants: a history of the biopesticide *Bacillus thuringiensis*. A review. Agron Sustain Dev 1–15. doi:10.1051/agro/2010027

Scheideler SE, Rice D, Smith B, Dana G, Sauber T (2008) Evaluation of nutritional equivalency of maize grain from DAS-Ø15Ø7-1 (Herculex I) in the diets of laying hens. J Appl Poult Res 17:383–389

Schrøder M, Poulsen M, Wilcks A, Kroghsbo S, Miller A, Frenzel T, Danier J, Rychlik M, Emami K, Gatehouse A, Shu Q, Engel KH, Altosaar I, Knudsen I (2007) A 90-day safety study of genetically modified rice expressing Cry1Ab protein (*Bacillus thuringiensis* toxin) in Wistar rats. Food Chem Toxicol 45(3):339–349

Schultz RM, Liebman MN (2002) Chapter 3. Proteins I: composition and structure. In: Devlin TM (ed) Textbook of biochemistry with clinical correlations, 5th edn. Wiley-Liss, NY

Shimada N, Miyamoto K, Kanda K, Murata H (2006) *Bacillus thuringiensis* insecticidal Cry1Ab toxin does not affect the membrane integrity of the mammalian intestinal epithelial cells: an in vitro study. Vitro Cell Dev Biol Anim 42:45–49

Siegel JP (2001) The mammalian safety of *Bacillus thuringiensis*-based insecticides. J Invertebr Pathol 77:13

Siegel JP, Shadduck JA (1989) Safety of microbial insecticides to vertebrates, Chapter 8. In: Laird M, Lacey L, Davidson E (eds) Safety of microbial insecticides. CRC Press, Boca Raton

Soberón M, Pardo L, Muñóz-Garay C, Sánchez J, Gómez I, Porta H, Bravo A (2010) Chapter 11. Pore formation by Cry toxins. In: Anderluh G, Lakey J (eds) Proteins: membrane binding and pore formation. Landes Bioscience and Springer Science+Business Media, NY

Taylor ML, Hartnell G, Nemeth M, Karunanandaa K, George B (2005) Comparison of broiler performance when fed diets containing maize grain with insect-protected (maize rootworm and European maize borer) and herbicide-tolerant (glyphosate) traits, control maize, or commercial reference maize—revisited. Poult Sci 84:1893–1899

Taylor M, Hartnell G, Nemeth M, Lucas D, Davis S (2007) Comparison of broiler performance when fed diets containing grain from second-generation insect-protected and glyphosate-tolerant, conventional control or commercial reference. Maize. Poult Sci 86:1972–1979

Terry C, Harris N, Parkes HC (2002) Detection of genetically modified crops and their derivatives: critical steps in sample preparation and extraction. J AOAC Int 85:768–774

Thomas K, Aalbers M, Bannon GA, Bartels M, Dearman RJ, Esdaile DJ, Fu TJ, Glatt CM, Hadfield N, Hatzos C, Hefle SL, Heylings JR, Goodman RE, Henry B, Herouet C, Holsapple M, Ladics GS, Landry TD, MacIntosh SC, Rice EA, Privalle LS, Steiner HY, Teshima R, Ree R van, Woolhiser M, Zawodnyk J (2004) A multi-laboratory evaluation of a common in vitro pepsin digestion assay protocol used in assessing the safety of novel proteins. Regul Toxicol Pharmacol 39:87–98

Thomas K, Herouet-Guicheney C, Ladics G, Bannon G, Cockburn A, Crevel R, Fitzpatrick J, Mills C, Privalle L, Veiths S (2007) Evaluating the effect of food processing on the potential human allergenicity of novel proteins: international workshop report. Food Chem Toxicol 45:1116–1122

Thomas K, MacIntosh S, Bannon G, Herouet-Guicheney C, Holsapple M, Ladics G, McClain S, Vieths S, Woolhiser M, Privalle L (2009) Scientific advancement of novel protein allergenicity evaluation: an overview of the HESI Protein Allergenicity Technical Committee (2000–2008). Food Chem Toxicol 47:1041–1050

Van Rie J, Jansens S, Hofte H, Degheele D, Van Mellaert H (1989) Specificity of *Bacillus thuringiensis* delta-endotoxins, importance of specific receptors on the brush border membrane of the mid-gastrointestinal tract of target insects. Eur J Biochem 186:239–247

Van Rie J, Jansens S, Hofte H, Degheele D, Van Mellaert H (1990) Receptors on the brush border membrane of the insect midgastrointestinal tract as determininants of the specificity of *Bacillus thuringiensis* delta-endotoxins. Appl Environ Microbiol 56:1378–1385

Vazquez-Padron RI, Gonzales-Cabrera J, Garcia-Toyar C, Neri-Bazan L, Lopez-Revilla R, Hernandez M, Moreno-Fierro L, De La Riva GA (2000) Cry1Ac protoxin from *Bacillus thuringiensis* sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. Biochem Biophys Res Commun 271:54–58

Wang J, Wang S, Su J, Huang T, Hu X, Yu J, Wei Z, Liang Y, Liu Y, Luo H, Sun G (2003) Food contamination of fumonisin B1 in high-risk area of esophageal and liver cancer. Toxicol Sci 72:188

WHO/GEMs Food Programme. http://www.who.int/foodsafety/chem/gems/en/. Accessed 20 Aug 2011

WHO/IPCS (International Programme on Chemical Safety) (1999) Environmental health criteria 217: microbial pest control agent *Bacillus thuringiensis*. Geneva, Switzerland

Wolfersberger MG, Hofmann C, Luthy P (1986) In: Falmagne P, Alouf JEF, Fehrenbach J, Jeljaszewics J, Thelestam M (eds) Bacterial protein toxins. Fischer, NY

Wu F, Miller JD, Casman EA (2004) The economic impact of *Bt* maize resulting from mycotoxin reduction. J Toxicol 23:393–419

Xu W, Cao S, Hea X, Luoa Y, Guoa X, Yuan Y, Huanga K (2010) Safety Assessment of Cry1Ab/Ac fusion protein. Food Chem Toxicol 47(7):1716–1721

Ziwen Y (2010) Hubei Bt Research and Development. Commercialization of *Bacillus thuringiensis* Insecticides in China. http://www.authorstream.com. Accessed 24 Sept 2010