SAFETY OF *Bacillus thuringiensis* Proteins Used to Control Insect Pests in Agricultural Crops

I. INTRODUCTION

This document reviews the safety assessment for *Bacillus thuringiensis* (*Bt*) delta-endotoxin proteins that have been introduced into agricultural crops to provide protection against insect pests. Information is provided on the scientific basis for U.S. EPA approvals for the introduction of *Bt* proteins into agricultural crops. Relevant to these approvals is the extensive data base on *Bt* protein safety. These proteins are the insect control components of *Bt* microbial formulations that have a history of safety when used on agricultural crops for over 30 years. A summary of the extensive data base on *Bt* protein safety is provided in tables at the end of this document.

II. BACKGROUND

The Environmental Protection Agency (EPA) has approved the commercial use of the *Bacillus thuringiensis* (*Bt*) delta-endotoxin proteins Cry1Ab, Cry1Ac, and Cry3A as expressed in genetically engineered corn, cotton, and potato, respectively. A Cry2Aa plant pesticide is currently under review at EPA. Cry5 and Cry9 proteins have been reported to exhibit useful insecticidal properties and may also be developed for expression in plants (Tailor et al. 1992, and Lambert et al. 1996, respectively).

To assess the implications of human dietary exposure to these plant pesticides, EPA has asked registrants to submit results of acute oral mammalian toxicology studies (oral LD_{50}) and in vitro digestibility studies. These tests have been conducted using microbially produced *Bt* proteins that have been shown to be equivalent to the plant-expressed protein. In all cases, the test results have shown that Cry proteins are not toxic to mammals, even at the highest dose tested, and that they are rapidly degraded in simulated gastric fluid. Thus, the Agency has been able to conclude that Cry protein plant pesticides pose no foreseeable risks to human health and has granted exemptions from the requirement of a tolerance for all of the plant pesticides registered thus far (60 FR 21725, May 3, 1995; 60 FR 42443, August 16, 1995; 60 FR 47871, September 15, 1995; 61 FR 40340, August 2, 1996; 62 FR 17720, April 11, 1997).

The first exemptions from tolerance were limited to a specific Cry protein as expressed in a single crop, such as Cry3A in potato and Cry1Ac in cotton. More recently, in approving Monsanto's Cry1Ab expressed in corn (61 FR 40340, August 2, 1996) and Dekalb's Cry1Ac expressed in corn (62 FR 17720, April 11, 1997), EPA established a broad tolerance exemption for Cry1Ab and Cry1Ac proteins in all plant raw agricultural commodities. No additional exemptions from tolerance for the Cry1Ab or Cry1Ac proteins will be necessary to support other crops engineered to express either of these proteins. However, under current Agency policy, the acute oral toxicity and in vitro digestibility studies must be conducted on

each new Cry protein to be registered as a plant pesticide (e.g., Cry1Aa, Cry1Ba). An exemption from the requirement of a tolerance must also be established for each new Cry protein.

Cry proteins are named according to their amino acid similarity to established holotype proteins (Crickmore et al. 1995). Cry proteins with similar amino acid sequences are grouped together. This nomenclature scheme replaces that of Hofte and Whitely (1989), in which *Bt* insecticidal crystal proteins are classified as CryI, CryII, or CryIII proteins, based on their insecticidal activities.

As defined by the current nomenclature (Crickmore et al. 1995),[1] Cry proteins with the same Arabic numeral (e.g., Cry1) share at least a 45 percent amino acid sequence identity. Those with the same Arabic numeral and upper case letter (e.g., Cry1A) share at least a 75 percent sequence identity. The same Arabic numeral and upper and lower case letter (e.g., Cry1Ab) designates a greater than 95 percent sequence identity. Therefore, one can apply safety conclusions from testing one or a few representative Cry proteins to a broader, but closely related, group of proteins — proteins that by definition share significant amino acid sequence identity.

There currently exists an extensive body of scientific data demonstrating the safety of Cry proteins. A review of the literature establishes that many different Cry proteins have been evaluated in a variety of mammalian toxicology tests over the past 35 years. No adverse effects have been observed in mammals upon oral exposure to any of these Cry proteins. Briefly, the following sets of data and lines of scientific reasoning support EPA making generic human health safety determinations for these classes of *Bt* Cry proteins:

- Studies on representative proteins from three classes of Cry proteins (Cry1, Cry2, and Cry3) indicate that these materials are not toxic to mammals when administered orally at high doses, and that they degrade rapidly in simulated gastric fluid.

- Modified Cry proteins, a priori, pose no unique human health concerns. Natural and modified Cry proteins warrant the same regulatory considerations.

- Cry proteins have a complex, highly specific mode of action and have specific, effective binding sites in invertebrates. Immunocytochemical analyses of Cry1A have revealed no comparable binding sites in mammals.

- Results of extensive acute oral or dietary studies representing numerous commercial *Bt* microbial pesticide products containing different combinations of Cry proteins establish no mammalian toxicity.

[1] Current information concerning the *Bt* holotype protein nomenclature and a continually updated database of *Bt* holotype proteins can be found on the world wide web at: http://epunix.biols.susx.ac.uk/Home/Neil_Crickmore/*Bt*/holo.html.

- *Bt* microbial products have a long history (> 30 years) of safe use. Reports of adverse effects in humans from the use of microbial *Bt* products are rare. None of the incidents are attributable to exposure to Cry proteins (EPA, 1988).

A. Cry Proteins Are Nontoxic to Mammals and Are Rapidly Digested

Dietary exposure is the major route by which humans can be exposed to Cry proteins expressed in plants. Dermal and inhalation exposures are anticipated to be negligible because Cry proteins are expressed and contained within the cells of the plants and are not volatile. This is consistent with EPA's testing scheme for Cry proteins (McClintock et al. 1992), which reflects a focus on dietary exposure. Acute oral mammalian toxicity and protein digestibility are the end points for EPA's human health risk assessment.

All of the mammalian toxicity testing of *Bt* proteins that are expressed in plants has, to the best of our knowledge, yielded negative results. Thus, no further mammalian toxicology testing (beyond acute and digestibility studies) has been required to support registration and exemptions from tolerance. No treatment-related adverse effects have been observed in any of the acute oral mammalian toxicity studies conducted with microbially produced Cry1Ab, Cry1Ac, Cry2A, and Cry3A proteins (Table 1). Six oral gavage studies in mice established the LD_{50} to be >3,280 mg/kg to >5,200 mg/kg for these proteins. Based on these results there is a safety factor of greater than 50,000 for human dietary exposure to Cry1Ab and Cry1Ac proteins in corn or cottonseed, greater than one million for Cry3A protein in potato, and greater than two million for Cry1Ac protein in tomato.

The no-observed-effect-level (NOEL) for Cry1Ab was > 0.45 mg/kg/day in a 28-day repeated dose oral toxicity study in mice and > 0.06 mg/kg/day in a 31-day repeated dose study in rabbits. Treatment doses in the 28-day and 31-day studies were estimated to be 1,000 to 4,000 times the maximum anticipated human exposure from consuming tomatoes genetically engineered to produce Cry1Ab (Noteborn et al. 1993). Based on the lack of toxic effects and the large margins of safety for both acute and 30-day exposures, these Cry proteins pose no foreseeable risks to human health. Moreover, these proteins are unlikely to cause endocrine effects because they exhibit no structural or functional similarity to estrogen or estrogen-mimicking compounds.

EPA has stated that when proteins are toxic, they are known to act via acute mechanisms and at very low dose levels (Sjoblad et al. 1992). The Cry proteins tested so far are judged to be nontoxic to mammals. In addition, we believe that the acute toxicity data on these representative Cry proteins and the extensive data base on microbial *Bt* products support a broader conclusion: All Cry proteins classified by their amino acid sequence to be Cry1, Cry2, or Cry3 are highly unlikely to be toxic to humans.

Further scientific evidence of the safety of Cry proteins is that they have been shown to be rapidly degraded *in vitro* using simulated gastric fluids (Table 2). Results of seven in vitro

assays conducted with representative Cry1, Cry2, and Cry3 proteins establish that the proteins are rapidly degraded, usually within 30 seconds. These results support the broader conclusion that members of these groups of Cry proteins (that share significant amino acid sequence identity) are likely to be rapidly degraded following ingestion by humans.

Another area of consideration is whether Cry proteins may induce an allergenic reaction. The demonstrated rapid *in vitro* degradation of Cry proteins should minimize the potential for such an occurrence. By comparison, food allergens generally persisted in the *in vitro* gastrointestinal model, whereas common food proteins with no allergenic history degraded rapidly in simulated gastric fluid (Metcalfe et al. 1996). To further investigate the potential for allergenicity, searches of allergen sequence databases have been conducted. These searches have shown no significant matches with the Cry proteins (Metcalfe et al. 1996). Moreover, Cry proteins do not share characteristics often exhibited by known food allergens. Unlike many known food allergens, the Cry proteins as expressed in plants are present in relatively low concentrations, and are heat labile. In addition, in the more than 30-year history of their commercial use, there have been no reported cases of allergenic reactions to the microbial Bt products (61 FR 40430, August 2, 1996).

B. Modified Cry Proteins Pose No Unique Human Health Concerns

There is no reason to expect that genetically modified proteins designated as Cry1, Cry2, or Cry3, according to the Crickmore nomenclature, pose any unique human health concerns compared to their naturally occurring counterparts. There is evidence that modified Cry proteins have already been generated in nature. The Cry1Ab delta endotoxin appears to have arisen from a recombination event between ancestral *cry1Aa* and *cry1Ac*-like toxin genes (Geiser et al. 1986).

Similarly, amino acid sequence alignments of Cry1Ca, Cry1Cb, Cry1Ea, and Cry1Eb provide evidence that *cry1Ea* and *cry1Cb* could have arisen from a recombination event between ancestral *cry1Ca* and *cry1Eb* toxin genes (Thompson et al. 1995). Multiple alignments of the Cry1Ca, Cry1Cb, Cry1Ea, and Cry1Eb amino acid sequences highlight the probable recombination site near amino acid 450. Analyses such as this suggest that recombination between related *cry* genes is a normal process in *cry* gene evolution. Regardless of its origin (i.e., naturally occurring or genetically engineered), a protein with sufficient amino acid identity with a holotype *Bt* protein to be classified as a Cry1, Cry2, or Cry3 protein warrants the same level of regulatory treatment.

EPA has registered microbial *Bt* products containing Cry proteins modified through genetic engineering. Two examples are Ecogen's Lepinox and Mycogen's Mattch bioinsecticides. The Agency's regulatory decisions related to these products are consistent with, and in support of, the rationale presented herein for applying the same standards to products containing either natural or modified Cry proteins. Lepinox is exempt from the requirement of a tolerance under the existing broad *Bt* tolerance exemption (40 CFR 180.1011). The Agency concluded that although Lepinox was developed using recombinant DNA

technology, its properties are no different from the range of properties of strains of *Bt* that might be found in nature. EPA required an acute oral toxicity/pathogenicity study and an in vitro digestibility study to support the tolerance exemption for the Cry1Ac and Cry1C derived delta endotoxins in Mattch (40 FR 47487, September 13, 1995).

C. No Cry Protein Binding Sites Have Been Identified in Mammals

Cry proteins are surface-acting insecticidal proteins that exhibit a complex, multicomponent mode of action (English and Slatin 1992). Ultimately, the proteins bind to specific sites in the midgut epithelium cells of susceptible insects, opening cation-selective channels in the cell membrane. The cells swell due to an influx of ions and water, leading to cell lysis and ultimately the death of the insect (Hofte and Whitely 1989).

Mammalian species are not susceptible to Cry proteins. This may be explained, in part, by the fact that conditions required for the complex steps in the mode of action described by English and Slatin (1992) do not exist in mammals or most invertebrates. Delta-endotoxins must first be solubilized. Some of the Cry proteins must then be proteolytically digested to the insecticidally active form. They must remain active rather than being further degraded. Receptor-mediated binding to the brush border membrane in midgut epithelium cells leads to membrane-bound forms of the endotoxin. This is believed to take place in three steps: binding to midgut receptor proteins, partitioning into the brush-border membrane, and finally, formation of channels and pores. The number of Cry proteins required to form a channel is uncertain. Only a relatively small subset of insects has been identified that supports this complex series of events that leads to cell death.

Noteborn et al. (1993) detected no specific binding of Cry1Ab protein to mouse and rat gastrointestinal tract tissue in vivo. These researchers also adapted an in vitro immunocytochemical assay (for detecting Cry protein binding in insect cells) to evaluate binding of Cry1Ab protein to mammalian gut tissue sections. Their analysis of mouse, rat, monkey, and human tissue sections did not reveal any Cry1Ab binding sites in these tissues. These findings further support the safety of Cry proteins exposed to mammals through the diet.

D. *Bacillus thuringiensis* Microbial Pesticides Are Nontoxic to Mammals

Results of testing microbial *Bt* preparations for oral mammalian toxicity over the past 35 years provide an extensive body of scientific data that supports their safe use. Collectively, these studies demonstrate the total lack of acute, subchronic, and chronic oral toxicity associated with *Bt* microbial pesticides (Table 3). These findings are directly relevant to this petition because these microbial preparations contain genes encoded for the production of at least four different classes of Cry proteins, including seven Cry1 proteins and two each of the Cry2, Cry3, and Cry9 proteins (Table 4). Therefore, these studies reflect mammalian oral exposures to some or all of the 13 Cry proteins expressed by these genes.

Acute oral studies conducted in rats and rabbits revealed no mortalities at the highest doses tested, which ranged from 10^8 to 10^{11} colony forming units per animal or >2,670 to 5,050 mg *Bt* product/kg. There were no deleterious effects based on observations including body weight, food consumption, and gross necropsy. Four subchronic oral studies in rats demonstrated NOELs of > 4,000 to >8,400 mg *Bt* product/kg/day and > 10^9 *Bt* spores/kg/day. The rat two-year chronic NOEL was also >8,400 mg *Bt* product/kg/day, the highest dose tested. Humans fed one gram of a *Bt* preparation per day for three days exhibited no symptoms of toxicity or other ill effects. The *Bt* preparation used in the latter study was a *Bt* subspecies *kurstaki* strain HD-1 containing genes encoded for the production of four Cry proteins: Cry1Aa, Cry1Ac, Cry1Ab, and Cry2A.

The literature contains numerous additional references to mammalian toxicology studies in which animals have been administered *Bt* microbial preparations via one of several other routes of exposure, such as pulmonary, dermal, ocular, intraperitoneal, subcutaneous, intravenous, or intracerebral injection. Some of these studies report adverse effects in test animals, including mortalities, often related to the microbes themselves and not the Cry proteins in the microbial formulations. These studies are not summarized in this report because we do not believe they are relevant to assessing risks from oral exposure to Bt proteins in plants. EPA's plant pesticide testing scheme focuses on oral ingestion via the diet for plant pesticide risk assessments.

Finally, EPA's interpretation of the microbial *Bt* mammalian toxicology database has led them to conclude that laboratory findings and favorable use history of *Bt* microbial pesticides "clearly argue for the human safety of these active microbial pesticide ingredients" (McClintock et al. 1995).

E. *Bacillus thuringiensis* Microbial Products Have a Long History of Safe Use

Bt microbial products were first registered in 1961 and have been applied continuously since then for an expanding number of uses in agriculture, disease vector control, and forestry. McClintock et al. (1995) reviewed the adverse effects that have been suggested might occur in humans from *Bt* microbial products. None of these reported incidents involved or implicated Cry proteins as the causative agent, nor did the authors (McClintock et al. [1995]) find them to be significant concerns in view of the currently registered products and the quality assurance safeguards that are in place for these products. They cited favorable use history in support of the overall human safety of the *Bt* microbial pesticides. In establishing the existing tolerance exemptions for Cry protein plant pesticides, EPA has stated that FIFRA section 6(a)2 reports claiming allergic reactions "were not due to *Bacillus thuringiensis* itself or any of the Cry toxins."

III. Conclusion

The Environmental Protection Agency has exempted from tolerance several *Bt* proteins for introduction into agricultural crops to control insect pests. These tolerance exemptions were based on the extensive safety data base and history of safe use that exist for microbial pesticide formulations that contain *Bt* proteins. Each of these proteins were also subjected to safety testing to confirm that they posed no meaningful risks to human health. Development of agricultural crop varieties that contain *Bt* proteins provides a safe alternative to the use of chemical insecticides to control insect pests.

Table 1. Mammalian Toxicity of *Bacillus thuringiensis* Cry Proteins (Oral Exposure in Mice)

Cry Protein	Doses Tested	Results (Acute LD_{50})	Dietary Exposure Safety Factor[1]	Findings	Reference
Cry1Ab (tryptic fragment)	400, 1000, 4000 mg/kg	>4000 mg/kg	>50,000 (corn)	No deleterious effects based on body weight gain, food consumption, and gross necropsy.	Monsanto and EPA Fact Sheet 1996
Cry1Ab	3280 mg/kg	>3280 mg/kg	_____	No mortalities and no treatment-related clinical signs of toxicity. One female failed to gain weight between day 7 and day 14. All animals gained weight by the end of the study (males gained more weight over the study than females).	EPA Memo 1995 [Fact Sheet] (Ciba Seeds)
Cry1Ab	0.045, 0.45 mg/kg/day via drinking water	NOEL > 0.45 mg/kg/day	>1000 to >4000 (tomato)	In the 28-day oral mouse study, no toxicity was observed. There was no production of immunological responses.	Noteborn et al. 1994
Cry1Ab	0.06 mg/kg/day via drinking water	NOEL > 0.06 mg/kg/day	>1000 to >4000 (tomato)	In the 31-day oral rabbit study, no toxicity was observed	Noteborn et al. 1994
Cry1Ac (full length)	500, 1000, 4200 mg/kg	>4200 mg/kg	>50,000 (cottonseed; cottonseed oil) >2,000,000 (tomato)	No deleterious effects based on body weight gain, food consumption, and gross necropsy.	Monsanto and EPA Fact Sheet,1995 (Monsanto)
Cry1Ac	5000 mg/kg (3325 mg of Cry1Ac protein/kg)	>5000 mg/kg	_____	No treatment-related toxicity or clinical abnormalities were observed.	Spenser et al. 1996 (Dekalb)
Cry2A	399, 1001, 4011 mg/kg	>4011 mg/kg	>50,000 (cottonseed; cottonseed oil)	No deleterious effects based on body weight gain, food consumption, and gross necropsy.	Monsanto
Cry3A	100; 500, 1000, 5220 mg/kg	>5220 mg/kg	>1,000,000 (potato)	No deleterious effects based on body weight gain, food consumption, and gross necropsy.	Monsanto 1995

[1] Safety Factor Calculation:

$$\text{Safety Factor} = \frac{\text{Mammalian } LD_{50} \text{ or NOEL } (\mu g/kg \text{ BW/day})}{\text{Human Cry Protein Consumption } (\mu g/kg \text{ BW/day})}$$

$$\text{Human Cry Protein consumption } (\mu g/kg \text{ body weight/day}) = \frac{\text{Average Human Consumption of Food Item (grams/day) x Maximum Cry Protein Concentration } (\mu g/g)}{\text{Average Human Body Weight (60kg)}}$$

Table 2. *In vitro* Digestibility of *Bacillus thuringiensis* Cry Proteins in Simulated Gastric Fluid

Cry Protein	Results	Findings	Reference
Cry1Ab (tryptic fragment)	Degraded within 30 seconds	No longer detectable after digestion for 30 seconds in gastric fluid; in intestinal fluid, converted to trypsin-resistant core, as expected.	Monsanto,1996
Cry1Ab	Degraded within 1 minute	Rapidly degraded in the presence of pepsin.	EPA Memo,1995 [Fact Sheet] (Ciba Seeds)
Cry1Ab	Substantially degraded	Study of the activity of Cry1Ab under simulated G.I. tract conditions and applying multienzymatic methods the Cry1Ab protein was substantially degraded via digestion.	Noteborn et al. 1994
Cry1Ac (full length)	Degraded within 30 seconds	No longer detectable after digestion for 30 seconds in gastric fluid; in intestinal fluid, converted to trypsin-resistant core, as expected.	Monsanto and EPA Fact Sheet (Monsanto) 1995
Cry1Ac	Degraded within 30 seconds	The protein was found to rapidly degrade in full strength and diluted simulated gastric fluid; degraded to below detection limits after a few seconds in full strength simulated gastric fluid; in simulated gastric fluid in which the pepsin concentration had been reduced 110-fold, Cry1Ac degraded to below detection in 5 minutes.	Spenser et al. 1996 (Dekalb)
Cry2A	Degraded within 30 seconds	No longer detectable after digestion for less than 30 seconds in gastric fluid; in intestinal fluid, converted to trypsin-resistant core, as expected.	Monsanto
Cry3A	Degraded within 30 seconds	No longer detectable after digestion for less than 30 seconds in gastric fluid; in intestinal fluid, converted to trypsin-resistant core, as expected.	Monsanto,1995

Table 3. Mammalian Toxicity of *Bacillus thuringiensis*—Microbial Pesticides (Oral Exposure)

Bt Microbial	Cry Gene Content	Test Substance or Formulation	Type of Study	Results (LD_{50})	Findings	Reference
kurstaki (Crymax)	Cry1Ac Cry2A Cry1C	technical (Ecogen)	Acute Oral Toxicity/ Pathogenicity (Rat)	2.5-2.8 x 10^8 CFU	Not pathogenic, toxic, nor infectious; EG7841 showed no evidence of toxicity or infectivity/pathogenicity to rats and rapid clearance was demonstrated.	Carter and Liggett 1994 and EPA Fact Sheet 1996 (Ecogen)
kurstaki (Crymax)	Cry1Ac Cry2A Cry1C	WDG formulation (Ecogen)	Acute Oral Toxicity	>5050 mg/kg	_____	EPA Fact Sheet 1996 (Ecogen)
kurstaki (Lepinox)	Cry1Aa Cry1Ac Cry3A Cry3Ba	technical (Ecogen)	Acute Oral Toxicity/ Pathogenicity (Rat)	> 1.19 x 10^8 CFU	There was no evidence of toxicity or pathogenicity at any of the doses tested.	Barbera 1995
kurstaki (Raven)	Cry1Ac Cry3A Cry3Ba	technical (Ecogen)	Acute Oral Toxicity/ Pathogenicity (Rat)	>4 x 10^8 spores/animal	There was no evidence of toxicity or infectivity/pathogenicity.	Carter et al. 1993
kurstaki (Cutlass OF)	Cry1Aa Cry1Ab Cry1Ac Cry2A Cry2B	formulation-Cutlass OF (Ecogen)	Acute Oral Toxicity/ Pathogenicity (Rat)	>10^8 CFU/ml	There was no evidence of toxicity or infectivity/pathogenicity.	David 1989a
kurstaki (Foil OF)	Cry1Ac Cry3A	formulation-Foil OF (Ecogen)	Acute Oral Toxicity/ Pathogenicity (Rat)	>10^8 CFU/ml	There was no evidence of toxicity or infectivity/pathogenicity.	David 1989b
kurstaki (Dipel)	Cry1Aa Cry1Ab Cry1Ac Cry2A		Acute Oral-Rat	≥2.67 g/kg	No acute oral infectivity.	EPA Fact Sheet 1986 (Abbott)
kurstaki (Dipel)			Acute Oral-Rat	≥4.7 x 10^{11} spores/kg	No acute oral infectivity, pathogenicity, or toxicity.	EPA Fact Sheet 1986 (Abbott)

(Continued) Table 3. Mammalian Toxicity of *Bacillus thuringiensis*—Microbial Pesticides (Oral Exposure)

Bt Microbial	Cry Gene Content	Test Substance or Formulation	Type of Study	Results (LD_{50})	Findings	Reference
kurstaki (Dipel)	Cry1Aa Cry1Ab Cry1Ac Cry2A	_____	Acute Oral-Rat	>3.4 x 10^{11} spores/kg	No acute oral infectivity.	EPA Fact Sheet 1986 (Abbott)
kurstaki (Dipel)	Cry1Aa Cry1Ab Cry1Ac Cry2A	_____	Acute Oral-Rat	>4.6 x 10^{11} spores/kg	No acute oral infectivity.	EPA Fact Sheet 1986 (Abbott)
kurstaki (Dipel)	Cry1Aa Cry1Ab Cry1Ac Cry2A	_____	Acute Oral-Rat	>5 g/kg	No toxic or virulent effects.	EPA Fact Sheet, 1986 (Abbott)
kurstaki (Dipel)	Cry1Aa Cry1Ab Cry1Ac Cry2A	_____	13-Week Oral-Rat	NOEL=1.3 x 10^{9} spores/kg/day	No toxicity or infectivity.	McClintock et al. 1995
kurstaki (Dipel)	Cry1Aa Cry1Ab Cry1Ac Cry2A	technical	90-Day Oral-Rat	NOEL>8400 mg/kg/day	_____	McClintock et al. 1995
kurstaki (Dipel)	Cry1Aa Cry1Ab Cry1Ac Cry2A	technical	2-Year Chronic- Rat	NOEL=8400 mg/kg/day	Statistically significantly decreased body weight gain in females from week 10 to week 104; no infectivity/pathogenicity was found.	McClintock et al. 1995

(Continued) Table 3. Mammalian Toxicity of *Bacillus thuringiensis*—Microbial Pesticides (Oral Exposure)

Bt Microbial	Cry Gene Content	Test Substance or Formulation	Type of Study	Results (LD_{50})	Findings	Reference
kurstaki	Crystal delta-endotoxin	_____	Oral Administration to Mice	15-30 µg of protein/g [0.1, 0.5, 1.0 mg]	*Bt kurstaki* crystal delta-endotoxin produced no pathological effects by oral administration. When administered per os, the alkali-solubilized crystal delta-endotoxin was not toxic to adult (0.5 mg protein/animal) or suckling (200 µg protein/animal) mice. In addition, native subspecies *kurstaki* crystal delta-endotoxin and soluble protein of *kurstaki* crystal delta-endotoxin produced no effects in adult (1.0 mg/animal) or suckling (200 µg/animal) mice by oral administration.	Thomas and Ellar 1983
kurstaki (Dipel)	Cry1Aa Cry1Ab Cry 1Ac Cry2A	_____	Acute Oral-Rabbit	$\geq 2.0 \times 10^9$ spores/animal	No acute oral infectivity.	EPA Fact Sheet 1986 (Abbott)
kurstaki (Dipel)	Cry1Aa Cry1Ab Cry 1Ac Cry2A	_____	Acute Oral-Rabbit	$\geq 6.9 \times 10^7$ spores/kg	No acute oral infectivity.	EPA Fact Sheet 1986 (Abbott)

(Continued) Table 3. Mammalian Toxicity of *Bacillus thuringiensis*—Microbial Pesticides (Oral Exposure)

Bt Microbial	Cry Gene Content	Test Substance or Formulation	Type of Study	Results (LD_{50})	Findings	Reference
kurstaki (Dipel and Thuricide-HP)	Cry1Aa Cry1Ab Cry 1Ac Cry2A	(Abbott and Sandoz)	5-Month Dietary Toxicity/ Infectivity Study in Sheep	NOEL = 500 mg/kg (~10^{12} spores/day)	The results of this study conclude that *Bt* was not a pathogen in sheep following oral administration. All sheep exposed to the test substances were blood and tissue culture positive for *Bt* in the absence of any pathological change, which suggests that *Bt* is an essentially avirulent bacterium in sheep. Considering the eating habits of sheep, the absence of lung lesions suggests that *Bt* is incapable of producing disease when introduced by inhalation. Treatment-related occasional loose stools were observed and may have been caused by either the carrier or the observed change in the bacterial content of the rumen. Mild to marked lymphoid hyperplasia in Peyer's patches of the colon and cecum was noted but could not be definitely linked to the test substances. The hyperplasia was not considered to be clinically significant.	Hadley et al. 1987
kurstaki	Cry1Aa Cry1Ab Cry 1Ac Cry2A	_____	Human-Oral	NOEL = 1 g (1 x 10^{10} viable spores)/day for 3 days	No toxicity/infectivity; all blood cultures were negative; 5 out of 10 patients showed viable *Bt* 30 days post-feeding.	EPA Fact Sheet 1986 (Abbott) and McClintock et al. 1995

(Continued) Table 3. Mammalian Toxicity of *Bacillus thuringiensis*—Microbial Pesticides (Oral Exposure)

Bt Microbial	Cry Gene Content	Test Substance or Formulation	Type of Study	Results (LD_{50})	Findings	Reference
aizawai (Strain GC-91)	_____	technical (CGA 237218)	Acute Oral Toxicity	20 ml/kg or ~10^8 CFU/animal	Not toxic, pathogenic, or infective.	EPA Fact Sheet 1993 (Ciba)
aizawai (Agree, Design)	Cry1Aa Cry 1Ac Cry1B-like Cry1C Cry1D Cry2B Cry9	Formulation	Acute Oral Toxicity	5050 mg/kg	_____	EPA Fact Sheet 1993 (Ciba)
aizawai (Xentari)	Cry9 Cry1D Cry1Aa Cry1Ab Cry1C Cry2B	technical	Acute Oral Toxicity	Not toxic	_____	Unknown
aizawai (Xentari)	Cry9 Cry1D Cry1Aa Cry1Ab Cry1C Cry2B	formulation	Acute Oral Toxicity	1000 mg/kg (1.3-1.4 x 10^{11} CFU/rat)	_____	Unknown
israelensis	Cry4A Cry4B Cry10A Cry11A Cyt1Aa	_____	Acute Oral-Rat	≥6.9 x 10^7 spores/kg	No acute oral infectivity.	EPA Fact Sheet 1986 (Abbott)

(Continued) Table 3. Mammalian Toxicity of *Bacillus thuringiensis*—Microbial Pesticides (Oral Exposure)

Bt Microbial	Cry Gene Content	Test Substance or Formulation	Type of Study	Results (LD_{50})	Findings	Reference
israelensis	Cry4A Cry4B Cry10A Cry11A Cyt1Aa	_____	Acute Oral-Rat	≥2.67 g/kg	_____	McClintock et al. 1995
israelensis	Cry4A Cry4B Cry10A Cry11A Cyt1Aa	_____	Single Oral Administration to Weanling Rats	> 410^{8} CFM	No rats became ill or died; the authors concluded that *Bt israelensis* is not a virulent or invasive mammalian pathogen.	Siegel et al. 1987
israelensis	Cry4A Cry4B Cry10A Cry11A Cyt1Aa	_____	3-Month Feeding-Rat	NOEL=4 g/kg/day	No toxicity.	McClintock et al. 1995
israelensis	Crystal delta-endotoxin	_____	Oral Administration to Mice	> 0.5 mg/animal	In a series of preliminary toxicity tests with adult and suckling mice, the native subspecies, *israelensis* crystal delta-endotoxin, produced no pathological effects by oral administration. When administered per os, the alkali-solubilized crystal delta-endotoxin was not toxic to adult (0.5 mg protein/animal) or suckling (200 µg protein/animal) mice	Thomas and Ellar 1983
israelensis	Cry4A Cry4B Cry10A Cry11A Cyt1Aa	_____	Acute Oral-Rabbit	≥2 x 10^{9} spores/animal	No acute oral infectivity.	McClintock et al. 1995

(Continued) Table 3. Mammalian Toxicity of *Bacillus thuringiensis*—Microbial Pesticides (Oral Exposure)

Bt Microbial	Cry Gene Content	Test Substance or Formulation	Type of Study	Results (LD_{50})	Findings	Reference
israelensis	Cry4A Cry4B Cry10A Cry11A Cyt1Aa	_____	Acute Oral-Rabbit	≥6.28 g/kg	No acute oral infectivity.	EPA Fact Sheet 1986 (Abbott)
Berliner	Cry1Ab Cry1B	technical	5-Day Human Oral Exposure	NOEL = 1 g of *Bt* (3×10^9 viable spore/gram) in capsules daily for 5 days	All subjects remained well during the course of the experiment (˜5 weeks) and all laboratory findings were negative (subjects were evaluated before treatment, after the 5-day treatment period, and 4 to 5 weeks post treatment).	Fisher and Rosner 1959

Table 4. Gene Content of *Bacillus thuringiensis* Commercial Products

***Bt* Product**	**Organism**	**Cry1Aa**	**Cry1Ab**	**Cry1Ac**	**Cry1Ba-like**	**Cry1Ca**	**Cry1Da**	**Cry1F**	**Cry2Aa**	**Cry2A6**	**Cry3A**	**Cry3Ba**	**Cry9B**	**Cry9-like**
DiPel 2X	*kurstaki*	X	X	X					X					
DiPel 6AF	*kurstaki*	X	X	X					X					
DiPel ES	*kurstaki*	X	X	X					X					
DiPel ES-NT	*kurstaki*	X	X	X					X					
Raptor	*kurstaki*	X			X		X		X	X				
Condor G	*kurstaki*	X		X					X	X				
Condor OF	*kurstaki*	X		X					X	X				
Foil BFC	*kurstaki*			X							X			
MVP	*kurstaki*			X										
Biobit FC	*kurstaki*	X	X	X					X					
Biocot	*kurstaki*	X	X	X					X					
Foray 48B	*kurstaki*	X	X	X					X					
Delfin WG	*kurstaki*	X	X	X					X					
Javelin WG	*kurstaki*	X	X	X					X					
B1781	*kurstaki*	X	X	X					X					
CryMax	*kurstaki*			X		X			X					
Raven	*kurstaki*			X							X	X		
Lepinox	*kurstaki*	X		X				X						
Agree	*kurstaki/ aizawai*			X		X	X			X			X	
Design	*kurstaki/ aizawai*	X		X	X	X	X			X				
Cutlass WP	*kurstaki/ aizawai*	X	X	X					X	X				
XenTari	*aizawai*	X	X			X	X			X			X	
Florbac AF	*aizawai*		X	X		X	X			X				X
Novodor FC	*tenebrionis*	X	X	X					X		X			
M-1	*tenebrionis*										X			
Novodor	*tenebrionis*										X			

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