

MINIREVIEW

The Mammalian Safety of *Bacillus thuringiensis*-Based Insecticides¹

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Received October 6, 2000; accepted December 1, 2000

The United States Environmental Protection Agency between the years 1961 and 1995 registered 177 products containing viable *Bacillus thuringiensis* (Bt). Numerous laboratory studies have demonstrated that Bt and Bt products are noninfectious and are toxic to mammals only at a dose $\geq 10^8$ colony forming units (cfu) per mouse (a human equivalent based on the weight of $>10^{11}$ cfu). In contrast, as few as three vegetative cells of *Bacillus anthracis* can kill mice (a human equivalent of $>10^3$ cfu). There are only two literature reports of Bt infection in man between the year 1997 and the present, and all infected individuals had experienced either extensive burns or a blast injury, which predisposed them to infection. Two epidemiology studies conducted during large-scale aerial Bt serovar *kurstaki* spray campaigns reported no increased incidence of illness. Some recent papers have expressed concern about the production of *Bacillus cereus* enterotoxins by Bt isolates. Laboratory studies found no evidence of illness in rats and sheep fed Bt products, nor have epidemiology studies found increased incidence of diarrhea during Bt aerial spray campaigns. Increases in human antibody levels following exposure to Bt products have been reported but there was no increased incidence in asthma or other illness. Based on laboratory studies and field experience, Bt insecticides have an excellent safety record.

Key Words: *Bacillus thuringiensis*; mammalian safety; infection of animals and man; *Bacillus cereus* enterotoxins; persistence of spores; clearance of spores.

INTRODUCTION

Commercial insecticides based on the bacterium *Bacillus thuringiensis* (Bt) have been registered in the United States since 1961. Many Bt products have been used widely in the United States and other countries for the control of caterpillars that attack vegetable crops and forests, and more recently, for the control of mosquito and black fly larvae. Bt insecticides have the advantage of being much more specific than broad-spectrum synthetic chemical insecticides and are thus considered environmentally friendly insect control agents. In addition, extensive laboratory studies required by the U.S. Environmental Protection Agency and regulatory authorities in other countries have shown that Bt insecticides are safe for vertebrates at doses several thousand-fold higher than would be encountered when these insecticides are used in accordance with recommendations provided by their manufacturers. Forty years of use in operational control programs has resulted in a remarkable safety record unparalleled by synthetic chemical insecticides. Despite this excellent record, concerns about the safety of Bt-transgenic crops as well as the taxonomic relationship of Bt to other bacilli that can cause illness in humans have raised questions about the safety of Bt insecticides. The purpose of this brief overview, therefore, is to summarize the literature on the safety of Bt insecticides with specific reference to mammals.

LABORATORY SAFETY TESTS

The United States Environmental Protection Agency between the years 1961 and 1995 registered 177 products containing viable *Bacillus thuringiensis* (Bt). All products were tested for infectivity and pathogenicity to mammals (McClintock *et al.*, 1995) and the earliest Bt products registered also underwent tests designed to assess carcinogenicity, such as long-term dietary

¹ Mention of a commercial product does not constitute an endorsement by the author.

studies. Ignoffo (1973) reported that rats were fed 10^9 Bt spores daily for 730 days without adverse reaction. Some of these early studies also utilized human volunteers. Fisher and Rosner (1959) reported that 18 humans ingested 1000 mg of Thuricide (Bt serovar *thuringiensis*) daily for 5 days and that 5 humans inhaled 100 mg of the technical powder daily for 5 days without incident. All safety studies of Bt products included injection (intraperitoneal or intravenous) of Bt into laboratory animals and the subsequent quantitative recovery of Bt from their tissues.

It is important to note that safety tests by design introduce large quantities of Bt spores and/or vegetative cells into test animals and their clearance is not instantaneous. Typically, there is a decline in the number of colony forming units (cfu) recovered over time, but in short-term studies (4 weeks or less), depending on the route of administration, at almost any point in time cfu may be recovered from tissue and the inoculum may be transported from the injection site by tissue fluids and circulate in the reticuloendothelial system (Adlersberg *et al.*, 1969). Recovery of cfu does not automatically constitute infection. Infection can be defined as disruption to the host caused by multiplication of the inoculum in tissue, toxin production, or both. When there is no evidence of infection but cfu are recovered, then a different condition, persistence, has occurred (Siegel, 1997). Examples of persistence include the recovery of Bt serovars *kurstaki* and *israelensis* from the spleens of mice 37 and 49 days, respectively, after intraperitoneal injection, recovery of Bt serovar *israelensis* from the lungs of mice 2.5 days after inhalation exposure (Siegel *et al.*, 1987), and recovery of Bt from the lungs of rats 21 days after intratracheal instillation (Tsai *et al.*, 1997). When evaluating the results of laboratory studies or assessing the significance of a report that Bt was recovered from mammals, it is essential to differentiate between infection and persistence.

Mortality may occur during safety studies and if it does occur, it is essential to assess its significance through the use of the proper benchmark. In the genus *Bacillus*, the most virulent mammalian pathogen is *B. anthracis*. Lamanna and Jones (1963), using albino Namru mice, reported LD₅₀ values following intraperitoneal and subcutaneous injection as low as 2.64 spores and 3.0 vegetative cells, respectively. In contrast, the dosages used to assess the safety of Bt products and other entomopathogens are one million- to one trillion-fold higher (10^{12}) than the *B. anthracis* LD₅₀ for mice, depending on the route of administration (ocular, dermal, dietary, feeding tube, inhalation, intranasal, intraperitoneal, intratracheal, intravenous, or subcutaneous). A product can be registered if it causes mortality, providing that the mortality occurs above the threshold dose specified by the regulatory agency (typically 10^6 cfu administered intravenously or intraperi-

toneally to mice). It is essential to convert these doses to a human equivalent on a cfu/kg basis to evaluate possible hazard. For example, a dose of 1×10^6 cfu in a 20-g mouse is equivalent to a dose of 5×10^9 cfu in a 100-kg man. Burges (1981) stated an important caveat for evaluating the results of regulatory testing of microbial insecticides: "a 'no risk' situation does not exist, certainly not with chemical pesticides and even with biological agents one cannot absolutely prove a negative. Registration of a chemical is essentially a statement of usage in which the risks are acceptable. The same must apply to biological agents."

REPORTS OF HUMAN AND ANIMAL INFECTION

From the 1960's through the early 1990's, literature searches for mammalian infections associated with Bt would have uncovered three cases; only one case was associated with a commercial product. In the first case, Gordon (1977) reported that Bt was recovered from a fatal case of bovine mastitis and no further details were provided. In the second case, which involved Dipel, a Bt serovar *kurstaki* product manufactured by Abbott Laboratories, Samples and Buettner (1983) reported that a farmer splashed in the face developed a corneal ulcer. The ulcer healed following injection of gentamicin and cephalosporin. It is difficult to evaluate the significance of this report because the farmer applied a corticosteroid ointment to relieve irritation in his eye for 7 days before the ulcer developed. One of the side effects of corticosteroids is delayed flattening and movement of the corneal epithelium, which is essential to wound healing. This may have contributed to the formation of the ulcer. Additionally, in the case report the physicians neglected the possibility that Bt may have simply persisted in the farmer's eye following exposure and that a different microorganism was responsible for the ulcer. The ability of commercial microbial products to persist in the conjunctival cul-de-sac of rabbits despite flushing was reported by Siegel and Shaddock (1990a). In their study, commercially produced Bt serovar *israelensis* (10^6 cfu) and *B. sphaericus* strain 2362 (10^8 cfu) were applied to rabbit eyes that were either unflushed or flushed with water for 30 s after exposure and the eyes were swabbed weekly. Bt serovar *israelensis* was recovered from both flushed and unflushed eyes for 1 week and *B. sphaericus* strain 2362 was recovered from flushed and unflushed eyes for 8 weeks. There was no evidence of infection. In the third case, Warren *et al.* (1984) reported that a British researcher who accidentally injected the webspace between his third/fourth finger with a mixed culture of Bt serovar *israelensis* and *Acinetobacter calcoaceticus* var. *anitratus* developed lymphangitis. The patient received intravenous injection of erythromycin, gentamicin, and benzylpenicillin, accompanied by decompression of the flexor tendon

sheath, and recovered after 5 days. In the report the authors speculated that proteases from *A. calcoaceticus* var. *anitrat* activated Bt serovar *israelensis* protoxin, but in a vascular permeability reaction test that evaluated each isolate separately, *A. calcoaceticus* var. *anitrat* produced a strong reaction with necrosis. Culture filtrates of neither strain were lethal when injected intravenously in mice but a filtrate from a mixed culture was consistently lethal when injected intravenously.

During the latter half of the 1990's, three reports of Bt infection were published. Two of these reports involved patients who had experienced serious burns or blast injuries and the patients may have been immunocompromised. It is important to emphasize that these case reports do not involve serovars used in commercial products and, furthermore, one report is the first note of Bt infecting burns. The susceptibility of immunocompromised mice to infection by Bt was investigated in two earlier studies that utilized serovar *israelensis*. In these studies 10^7 cfu were injected intraperitoneally in athymic and corticosteroid-treated mice and Bt was not infectious (Siegel *et al.*, 1987; Siegel and Shadduck, 1990a). It is possible that this animal model was inappropriate for assessing the likelihood of Bt infecting burn victims or that the isolates differed in pathogenicity.

In the first report, Damgaard *et al.* (1997) reported that a nonmotile Bt (serovar undeterminable, nontoxic to *Pieris brassicae* and *Aedes aegypti*) was recovered from two Italians with deep burns. Investigation by hospital staff revealed that the water used to treat the burns was contaminated with this isolate. The authors speculated that the immunosuppressed condition of the patients made them susceptible to infection when the contaminated water was used to wash their burns. In the second report, a French soldier wounded by a land mine blast developed abscesses on his leg and Bt serovar *konkukian* was isolated from the abscesses (Hernandez *et al.*, 1998). To test the hypothesis that this isolate was infectious in the absence of an effective immune response, 10^5 – 10^7 cfu were injected subcutaneously into immune intact and immunosuppressed (cyclophosphamide, 200 mg/kg) mice. Cutaneous inflammatory lesions appeared in all mice at the dose of 10^7 cfu, but the lesions healed in the immune intact mice. All mice were sacrificed after 48 h and Bt serovar *konkukian* was recovered only from the tissue samples from immunosuppressed mice. In a follow-up laboratory study, Hernandez *et al.* (1999) reported that a single intranasal instillation in mice of 10^8 spores of Bt serovars *konkukian*, *kurstaki*, *israelensis*, and *thompsoni* caused 100, 80, 40, and 0% mortality, respectively. No mortality occurred when 10^7 spores were administered. The authors stated that this was the first report that Bt applied at high concentration by the pulmonary route could kill mice. Salamitou *et al.* (2000) conducted

an intranasal instillation study which utilized a Bt serovar *thuringiensis* mutant (Cry) and the type strain of *B. cereus* (ATCC 14579). In this study, mortality occurred within 4–6 h following a single intranasal instillation of 10^8 or 5×10^7 spores or vegetative cells, but no mortality occurred when 10^7 spores were instilled. When a Bt serovar *thuringiensis* mutant (Δ plcR) containing a disrupted plcR gene was instilled there was no mortality, but a *B. cereus* Δ plcR strain still killed mice. Salamitou *et al.* (2000) stated that the cause of death was not due to bacterial multiplication but that factors dependent on the plcR regulon were responsible for the mortality. The applicability of these studies to human health is low because of the extremely high doses used. First, a dose of 10^8 spores in mice is equivalent to $\geq 10^{11}$ spores in man. Human inhalation of a dose of this magnitude during a single exposure is highly unlikely. Elliott *et al.* (1988) conducted a study of occupational and general public exposure to Bt serovar *kurstaki* used in a spray campaign. In their study, which utilized portable sampling pumps with membrane filters and subsequent analysis of the membrane filters, occupational exposure results ranged from 0 to 5600 Bt cfu/m³ of sampled air and general public exposure was 0 to 1600 Bt cfu/m³ of sampled air. These exposure levels are 100 million-fold lower for humans on a cfu/kg basis than the maximum dose used in the study by Hernandez *et al.* (1999) and Salamitou *et al.* (2000). Noble *et al.* (1992), in an epidemiology study of a Bt serovar *kurstaki* spray campaign in British Columbia, reported that spray workers experienced mean exposures (two cassette readings were averaged to represent the level in the breathing zone area) from 3000 to 5.9×10^6 cfu/m³ of sampled air. The maximum cumulative worker exposure for the entire spray season was 7.2×10^8 cfu. Even this cumulative value is at least 1000-fold lower than the maximum dose (human equivalent) used in the single exposure intranasal instillation laboratory studies cited above. Moreover, Hernandez *et al.* (1999) were not the first to report that intranasal exposure of 10^8 cfu of Bt is toxic to mice. McClintock *et al.* (1995) in their review, which included studies submitted to the United States Environmental Protection Agency for registration of Bt products, reported that a preparation of Bt serovar *kurstaki* containing 50% spores and 50% vegetative cells administered to mice intranasally (10^8 cfu) caused mortality. Toxicity and lethality after pulmonary exposure was strain dependent and attributed to presporulation proteins. Neither Hernandez *et al.* (1999) nor Salamitou *et al.* (2000) cited this report in their discussion of their findings. These two studies underscore the importance of placing mortality in perspective by utilizing a proper benchmark such as *B. anthracis*, as well as the need to convert the dose used in a laboratory study to the human equivalent in order to assess hazard. Unfortunately, since this was not done, these pa-

pers may cause unintended controversy about the safety of Bt insecticides.

When evaluating studies such as those by Hernandez *et al.* (1999), it is important to remember that growth conditions can influence the toxicity of a Bt inoculum in a laboratory study. Siegel and Shaddock (1990b) reported that rats injected intracerebrally with Bt serovars *kurstaki* and *israelensis* (10^7 cfu) were 2.12 times more likely to die (32/41 vs 21/57 dead) when the cultures were grown at 30°C than when grown at 38°C ($P < 0.001$). Cultures grown on brain heart infusion (BHI) broth were 1.69 times more likely to kill (33/38 vs 20/39 dead) than cultures grown on BHI agar ($0.005 > P > 0.001$). Mortality dramatically decreased in this study when 10^6 cfu were injected (3/30 dead). Although laboratory studies may reveal a potential for a Bt strain to cause mortality when administered at high doses, commercial fermentation conditions are quite different from those used in laboratory studies and may not allow production or degrade toxic factors.

The final report of human infection by Bt (Helgason *et al.*, 2000) is problematic because the authors considered *Bacillus cereus* and Bt to be the same species. The study analyzed the genetic diversity of 35 strains isolated from humans, none of which produced a parasporal protein crystal (the crystal contains the Cry proteins), which is the character that distinguishes Bt from *B. cereus*. According to the current systematic classification of the genus *Bacillus*, these isolates are *B. cereus*; therefore use of Bt in the title of this article is inaccurate (Sneath, 1986). Bergey's Manual is currently being revised and it is possible that Bt and *B. cereus* may be combined into a single species. Baumann *et al.* (1984) in their numerical analysis of Bt and *B. cereus* utilized 99 traits and the only trait that could distinguish between the strains of these two species was production of parasporal crystals. Carlson *et al.* (1994) used pulsed-field gel electrophoresis and concluded that Bt and *B. cereus* should be regarded as one species. Ripabelli *et al.* (2000) used amplified fragment length polymorphism (AFLP) analysis to characterize *B. cereus* strains associated with food poisoning and reported that, within serotype H1, responsible for the majority of emetic illness, three different AFLP profiles were discernible. Depending on the criteria used, Bt, *B. cereus*, and even *B. anthracis* can be divided into five species based on AFLP analysis (website of Dr. Paul Jackson and collaborators, <http://www.cbnpl.lanl.gov/>). In the dendrogram posted on this website, strains from all three of these current species cluster in five groupings. Bt serovar *kurstaki* (the most widely used Bt serovar) is distantly related to the *B. anthracis* isolates in this analysis. Future publications from these researchers will elucidate these relationships. Ultimately, distinctions between Bt, *B. cereus*, and *B. anthracis* will still be made based on their ecological

niches and mammalian infectivity, but it will make it more difficult to interpret reports of mammalian infection if the names *B. cereus* and Bt are used interchangeably. If commercial strains of Bt can be characterized by DNA analysis, AFLP analysis, or other means and these fingerprints can be used to distinguish among commercial and wild-type strains, then it will be easier to determine the significance of any future reports of Bt recovery from humans.

ENTEROTOXIN PRODUCTION

From the viewpoint of food safety, the close relationship between Bt and *B. cereus* has taken on new significance because of the production by *B. cereus* of emetic and diarrhea-causing enterotoxins (Kramer and Gilbert, 1988). There has also been an increased incidence in non-food-poisoning infections attributed to *B. cereus* (Drobniewski, 1993). In the United States between 1973 and 1987, according to the Center for Disease Control, *B. cereus* was responsible for 2% of all food poisoning outbreaks with confirmed etiology. Kramer and Gilbert (1988) reported that in the Netherlands from 1977 to 1982, as many as 22.4% of the outbreaks were attributed to *B. cereus*. Ripabelli *et al.* (2000) reported that there were 2715 cases of *B. cereus* food poisoning in England and Wales between 1980 and 1997. The Public Health Laboratory Service Food Safety Microbiology Laboratory of the United Kingdom recognizes 42 serotypes of *B. cereus* based on flagellar antigens (Ripabelli *et al.*, 2000). Typically, $>10^5$ *B. cereus* per gram are needed to cause food poisoning.

Spira and Goepfert (1972) were the first to report that Bt strains had the potential to produce enterotoxins based on laboratory studies. They used a young rabbit ileal loop assay for detecting enterotoxins. In their study 19 of 22 *B. cereus* cultures and 4 of 6 Bt cultures (only 1 culture is readily identified, the type strain for Bt serovar *thuringiensis*) gave a positive response (fluid accumulation in the loop). Cultures introduced directly into unligated ilea or by stomach catheter failed to elicit diarrhea. Abdel-Hameed and Landen (1994) reported that Bt strains isolated from Swedish soil produced *B. cereus* enterotoxins, based on a BCET-RPLA test kit. Damgaard (1995), using the Tecra VIA kit, tested isolates of Bt originating from eight commercial products and reported that all produced diarrheal enterotoxins. Rivera *et al.* (2000) reported that hemolytic toxin, nonhemolytic toxin, and a single component toxin cytotoxic to Vero cells were present in 24 Bt serovars. Bishop *et al.* (1999) reported that six strains of Bt (including serovars *kurstaki*, *israelensis*, and *morrisoni*) that were tested with two commercially available kits (BCET-RPLA [Unipath] and VIA ELISA [Tecra]) produced the diarrheal enterotoxin. However, when the isolate that produced the highest level of enterotoxin was fed to rats at 10^{12}

spores per rat, or when 10^6 spores were injected subcutaneously in rats, there was no evidence of illness and no weight loss occurred. This dietary study is consistent with the data reported by Ignoffo (1973) as well as the study by Hadley *et al.* (1987). In the latter study, mixed rambouillet/merino sheep received 10^{12} spores of Bt serovar *kurstaki* daily for 5 months without experiencing illness.

In contrast, Fares and El-Sayed (1998) reported the results of a feeding trial in which mice were fed spores and crystals of Bt HD14 (concentration was unspecified), as well as transgenic potatoes containing a "Cry1 gene." Semithin sections of the ileum were analyzed morphometrically and significant differences were seen in the size of enterocytes, as well as changes in the luminal microvilli. The greatest changes were associated with mice fed HD14. It is difficult to interpret the findings of this study. First, the authors used what they described as a *kurstaki* serovar; yet, HD14 belongs to serovar *thuringiensis* (*B. thuringiensis* cultures available from the U.S. Department of Agriculture, Technical Bulletin Number 1738). It is possible that their isolate produced beta exotoxin, which would confound their study of delta endotoxin effects (beta exotoxin is prohibited in commercially produced strains). Second, the authors never specified either the number of spores or the quantity of insecticidal crystal proteins (ICPs) that the mice received. Finally, numerous studies that were used for the registration of Bt products included microscopic pathologic observations of preserved tissue and no significant changes occurred. The study of Fares and El-Sayed (1998) must be repeated with a defined dose and a correctly identified isolate of Bt before its findings can be accepted without challenge.

In the medical literature, there is one case report associating Bt with food poisoning. Jackson *et al.* (1995) reported that Bt (serotype undetermined) was recovered from the stools of four patients following a gastroenteritis outbreak in a chronic care institution. The stool specimen from one of these patients was also positive for Norwalk virus, a known enteric pathogen, and another patient was positive for Norwalk virus but negative for Bt. *B. cereus* and Bt were both isolated from food and spice samples in the institution, but these isolates belonged to different phage types than the stool isolates. Since a known human pathogen, Norwalk virus, was recovered from two of these patients, and neither Bt nor *B. cereus* matching the stool isolates were recovered from samples of food, it is more parsimonious (Ockham's razor) to attribute the gastroenteritis to the virus than to Bt.

Given the ability of Bt isolates to produce enterotoxins in laboratory studies, why has it not been associated with food-borne illness in man? The simplest explanation is either that the isolates used in commercially produced Bt do not produce enterotoxins under

commercial fermentation conditions or that enterotoxins are absent from the final product because they are degraded by the end of the fermentation run. This is supported by the numerous laboratory safety studies that were used to register Bt insecticides. Even if these commercial isolates have the potential to produce enterotoxins in the field, Bt spores do not readily germinate in soil (germination requires specific alkaline conditions; H. D. Burges, personal communication) and vegetative Bt cells disappear rapidly from soil (Petras and Casida, 1985). A second possibility is that food safety laboratories and hospital clinics do not distinguish between *B. cereus* and Bt; consequently, Bt isolations are misidentified. Although this is possible, food safety manuals such as the "Bad Bug Book" of the United States Food and Drug Administration (<http://vm.cfsan.fda.gov/~mow/intro.html>) clearly state that differentiation between *B. cereus* and Bt is based on the presence of toxin crystals. Consequently, testing laboratories are or should be aware that light microscopy is necessary to differentiate between the two species. The misidentification hypothesis can be readily tested by obtaining isolates previously identified as *B. cereus* associated with food poisoning and then examining these cultures for the presence of parasporal crystals. Misidentification could also occur if Bt strains lose their plasmids encoding the insecticidal crystal proteins or do not form parasporal crystals at mammalian body temperatures. In this scenario, Bt enters but *B. cereus* exits. However, temperatures $\geq 42^\circ\text{C}$ are necessary to cure plasmids (Mikesell and Vodkin, 1985) and the normal human body temperature of 37°C is well below this threshold. Furthermore, crystal-producing Bt was isolated from both the burn patients and the land mine victim. This hypothesis can also be easily addressed in a laboratory study.

EPIDEMIOLOGY STUDIES OF HUMANS EXPOSED TO AERIAL APPLICATION OF Bt

When the focus is shifted from the laboratory to the field, there have been two epidemiology studies that specifically addressed the possibility of increased incidence of infection and food poisoning associated with large-scale spraying of Bt serovar *kurstaki*. In a 2-year study (1985–1986) in the United States, Green *et al.* (1990) conducted a passive surveillance program in which the four largest medical laboratories in a spray area (population of approximately 80,000 people in 1985 and 40,000 people in 1986) provided cultures obtained from humans during spraying periods. A non-sprayed community located approximately 100 km away served as a control. Telephone complaints received by the health department were also tabulated and evaluated. Ninety-five cultures were received from the spray area of which 55 were identified as Bt. However, 52 of these Bt specimens were judged to be prob-

able contaminants. The remaining 3 cultures came from blood (elderly immunocompromised person with underlying lung disease), gallbladder contents (1/8 cultures positive; it took 5 days for the positive culture to grow), and an antecubital abscess (intravenous drug user). The authors concluded that in these three cases Bt could not be ruled in or ruled out as the causative organism of infection. No Bt cultures were recovered from the control area (0/7). There were no increases in the number of telephone complaints nor was there a change in the normal pattern of complaints. The only health complaint that could be attributed to Bt occurred in a spray worker who was splashed in the face and eyes with a spray solution. This worker developed dermatitis, pruritis, burning, swelling, and erythema, with conjunctival injection. He was treated with steroid cream to the eyelid and skin, and the symptoms disappeared. The second study was conducted in Canada in an area with a population of 1,400,000 (Noble *et al.*, 1992). The researchers examined 429 bacterial isolates received from 10 participating laboratories, followed 1140 family practice patients (also analyzed nose cultures from these patients), followed 3500 admissions to hospital emergency departments, and examined the records of 26,000 telephone calls. This study also monitored 120 workers with occupational exposure to Bt serovar *kurstaki* spray and addressed the possibility that immunocompromised individuals might be at increased risk for Bt infections. Three hundred twenty-five isolates were characterized as Bt, of which 43 were pure cultures. Thirteen of the pure isolates came from genital-urinary cultures, 10 from cultures of eyes, and 9 from skin cultures. There was no difference in the percentage of Bt serovar *kurstaki* cultured from the noses of patients inside and outside of the spray zone (128 cultures total). None of the patients with a nose culture positive for Bt serovar *kurstaki* had a negative health outcome. There was no difference in emergency room visits between spray days and nonspray days, nor was there a significant change in the pattern of telephone calls. There was no evidence that Bt was associated with illness or infection and, in fact, there was a lower incidence of diarrhea in the spray area than in a control area. Some of the spray workers experienced dry skin, chapped lips, and/or minor irritation of their eyes, nose, and throat. Complaints were more likely to develop in individuals who had a prior history of allergies.

IMMUNE RESPONSE OF WORKERS EXPOSED TO Bt

Commercial Bt products consist of a mixture of spores, spores undergoing germination, vegetative cells, ICPs, and cell debris from lysed cells (Siegel and Shaddock, 1990a), all of which may trigger an immune response in mammals. Laferriere *et al.* (1987) reported that forest spray workers who used Bt serovar *kurstaki*

insecticides had a significant elevation in antibody titers and the titers were higher in workers who were exposed for 2 years. Vegetative cells caused the greatest reaction, but the antibodies disappeared 1 year after exposure. When the workers used a different product that did not have to be mixed before application, their antibody levels dropped, presumably because they had a decreased exposure to Bt. Bernstein *et al.* (1999) studied farm workers who picked vegetables treated with Bt serovar *kurstaki* products. The study divided the workers into high, medium, and low exposure groups and compared their skin test responses and humoral immunoglobulin G (IgG) and immunoglobulin E (IgE) antibody responses to spore and vegetative cell extracts of Bt. Workers in the high-exposure group had significantly elevated skin test responses to spore extracts compared to low- or medium-exposure workers. Specific IgG and IgE antibodies were present in more high-exposure than low-exposure workers. Specific IgE and IgG antibodies to vegetative cells were present in all groups. Only 2/123 workers sensitized by the respiratory route reacted to an ICP extract in a skin test. The authors stated that exposure to Bt sprays may lead to allergic skin sensitization and induction of IgE and IgG antibodies, or both. However, there was no increase in the incidence of asthma or other occupationally related clinical diseases in high-exposure workers.

The significance of elevated antibody levels, with regard to human health, following repeated administration of mixtures of Bt spores, vegetative cells, and ICPs is questionable. McClintock *et al.* (1995) state that dermal sensitization (hypersensitivity) studies were not required for registration of microbial insecticides in the United States because injection induction and challenge with foreign proteinaceous components into a laboratory animal would be expected to yield a positive response. Topical induction and challenge would probably lead to a negative response. In their review they noted that only two incidents of possible allergic reaction had been reported to the U.S. Environmental Protection Agency by 1995. One individual was suffering from a previously diagnosed disease unrelated to Bt exposure and the other individual was sensitive to materials used in formulating Bt products.

The development of transgenic plants incorporating Bt Cry toxins and molecular techniques that allow the production of a single Cry toxin has raised new issues regarding the potential of Cry proteins to become human allergens. Vazquez-Padrón *et al.* (1999), in a study designed to examine the mucosal and systemic immunogenicity of Cry1Ac protoxin, injected mice intraperitoneally with both the crystalline and the soluble forms of the protoxin (0.1–100 µg; 3 doses total) and reported that this protein induced a high systemic antibody response. Soluble Cry1Ac induced a mucosal response when administered intragastrically (accompanied by

TABLE 1

Route of Exposure and Maximum Dose with No Effect of *B. thuringiensis* (Primarily Serovars *kurstaki* and *israelensis*)

Route	Dose	Species	Reference
Oral	3×10^9 cfu ^a	Human	Fisher and Rosner (1959)
Inhalation	3×10^8 cfu	Human	Fisher and Rosner (1959)
Inhalation	5.6×10^3 cfu m ³	Human	Elliott <i>et al.</i> (1988)
Inhalation	5.9×10^6 cfu m ³	Human	Noble <i>et al.</i> (1992)
Inhalation	2×10^6 cfu per ml	Mouse	Siegel <i>et al.</i> (1987)
Intranasal	1×10^7 cfu	Mouse	Hernandez <i>et al.</i> (1999)
Intraperitoneal	3×10^7 cfu	Mouse	Siegel <i>et al.</i> (1987)
Dietary	7.3×10^{11} cfu ^b	Rat	Ignoffo (1973)
Dietary	1×10^{12} cfu	Rat	Bishop <i>et al.</i> (1999)
Intratracheal	1×10^8 cfu	Rat	Tsai <i>et al.</i> (1997)
Subcutaneous	1×10^6 cfu	Rat	Bishop <i>et al.</i> (1999)
Intracerebral	$1-3 \times 10^5$ cfu	Rat	Siegel <i>et al.</i> (1987)
Ocular	5×10^6 cfu	Rabbit	Siegel and Shadduck (1990)
Oral	1.5×10^{14} cfu ^b	Sheep	Hadley <i>et al.</i> (1987)

^a Colony forming units.^b Cumulative dose.

0.1 ml Maalox). It is difficult to determine the applicability of this study to the issue of human sensitization following Bt or protoxin exposure for several reasons. First, the goal of this study was to evaluate the use of a Bt protoxin as a carrier antigen in an oral or parenteral vaccination. Consequently, the study did not state the amount of either sprayed food products or transgenic plants that must be ingested to approximate the doses used. Second, the gastrointestinal tract was bypassed when intraperitoneal injection was used; consequently, Cry1Ac was not degraded by gastric juices. Although gastric exposure was also used in this study, the protoxin was administered in a solution with 0.1 ml of Maalox, which served as a buffer (the equivalent human dosage on a ml/kg basis would be 500 ml Maalox for a 100-kg man). Normally, the Cry1A proteins are rapidly degraded in the human digestive tract; therefore, the results of this study should not have implications for human health. However, some Cry proteins are more stable than Cry1A proteins (there are 73 holotype crystal toxins, http://www.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/index.html). Aventis Crop Science (formed by the merger of Rhone-Poulenc and Hoechst) is currently trying to register a transgenic corn hybrid containing Cry9C protein in the United States. This registration has been delayed because in the current model this protein is resistant to gastric digestion and is heat stable; these are two criteria that the United States Environmental Protection Agency uses to predict the potential of proteins to cause food allergies. Other criteria used are amino acid sequence, homology to known allergens, and molecular size (Hart, 2000). Aventis Crop Science researchers in their petition have noted that there are a number of relatively stable nonallergenic dietary proteins such as colostrum, and these researchers recently presented unpublished experimental data suggesting that the protein is partially digestible in rats and

fully digestible in simulated gastric fluid. This issue has not been resolved and may ultimately lead to a new model for assessing the allergenicity potential of Cry proteins. Further industry research regarding the safety and environmental impact of transgenic plants is available at <http://www.whylbiotech.com>.

SUMMARY AND CONCLUSIONS

In summary, numerous laboratory studies have been conducted on the infectivity and toxicity of Bt isolates and these studies have demonstrated that the isolates of Bt used in commercial products are safe. These studies have been validated by 40 years of Bt usage in the field. Mortality has occurred in some laboratory studies when extremely high doses were administered by invasive routes (intranasal, intracerebral, or intraperitoneal administration) but mortality was eliminated when the dose was decreased. Unfortunately, the language used in some studies may mislead the reader into believing that Bt is pathogenic. For example, Salamitou *et al.* (2000) state that "both Bt and *B. cereus* are opportunistic pathogens" but do not specify what "opportunistic" means in the context of their study. To reiterate, when mortality is taken out of context it is misleading. It is essential to convert the doses used in laboratory studies to cfu/kg before their applicability to human safety can be assessed. When the doses used in the intranasal instillation studies are converted to their human equivalents and the data from Table 1 are also considered, it is unwarranted to state that Bt is an opportunistic pathogen. In the two literature reports of Bt infection that seem straightforward (although Koch's postulates were not met), infection occurred only when there was severe tissue injury at the site of infection. These isolates did not belong to serovars used in insecticides. Although both reports

speculated that immunocompromised individuals were at increased risk for Bt infections, there are no published laboratory data or epidemiology studies that support this. In fact, the publicity surrounding spray campaigns would have been expected to increase the perceived linkage of Bt with human infection due to reporting bias (the public would have increased awareness of Bt being sprayed and been more likely to associate Bt with illness). Recently, researchers have expressed concern about the production of *B. cereus* enterotoxins by Bt isolates. It seems reasonable that current and future research on the close relationship between Bt and *B. cereus* will involve a search for other shared toxins and common mechanisms that regulate toxin production. However, despite the existence of enterotoxin genes in Bt, there are no laboratory safety studies that have documented illness in rats and sheep fed Bt products. Bishop *et al.* (1999) suggested that the rat may be an inappropriate model for enterotoxin detection in feeding trials, but the two epidemiology studies conducted found no increased incidence of diarrhea during Bt aerial spray campaigns. Ironically, in one study there was a decrease in the incidence of diarrhea in the spray area. Neither study found increased incidence of respiratory complaints; yet, it seems reasonable to predict that the report of Bernstein *et al.* (1999) may cause further regulatory scrutiny of spray products. Based on the results from the epidemiology studies, it seems prudent for workers handling Bt insecticides to follow the precautions listed on the product label. Several publications have appeared in the year 2000 that have a wider scope than this review, and those interested are referred to: Environmental Health Criteria 217: Microbial Pest Control Agent *Bacillus thuringiensis*, World Health Organization, Geneva; Entomopathogenic Bacteria: From Laboratory to Field Application (J. F. Charles, A. Delecuse, and C. Nielsen-LeRoux, Eds.), Kluwer Academic, Dordrecht; and *Bacillus thuringiensis*: Biology, Ecology & Safety (T. R. Glare and M. O'Callaghan, Eds.), Wiley, London.

ACKNOWLEDGMENTS

I thank the World Health Organization Special Programme for Research and Training in Tropical Diseases for funding my research on *Bacillus thuringiensis* serovar *israelensis* and *Bacillus sphaericus* and thank the World Health Organization Special Programme on Chemical Safety. I thank Dr. John Shaddock for sharing his philosophy of safety testing with me and Drs. H. Denis Burges, Robert Cibulsky, Terry Couch, Elizabeth Davidson, Brian Federici, Allan Felstot, Laura Hungerford, Lawrence Lacey, Brian Melin, Charles Scanlan, Robert Sherwood, A. Ray Smith, Isabel Thiery, and Brian Federici, Allan Yousten, as well as Robert Smith for their comments and insights over the years. I thank Drs. H. Denis Burges, Lawrence Lacey, Leellen Solter, and Patrick Vail for reviewing the manuscript.

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