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Safety of *Bacillus thuringiensis* var. *kurstaki* Applications for Insect Control to Humans and Large Mammals

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This minireview discusses the risks to humans and large mammals associated with the use of *Bacillus thuringiensis* var. *kurstaki* (Btk) in forest, agricultural and urban environments. The first subspecies used for insect control was *Bacillus thuringiensis* subsp. *thuringiensis* (*Btt*), known at the time as *Bt* Berliner. Much of the early work done with *Bt* does not identify the subspecies. *Btk* is registered for use against many species that are pests in agriculture, forestry and horticulture. *Btk* is currently the most widely used insecticide in forestry in Canada. Review of the literature indicates that *Btk* is safe for the environment and its various components. Since its introduction in the 1960s, no scientifically documented case of human infection has been reported as a result of its use in forestry or agriculture against various defoliators or in urban environments during gypsy moth eradication programs. No human health problems have been proven to be attributable to the application of any *Bt* product on crops used for human consumption. Based on all available information, *Btk* is considered by most people to be the safest bioinsecticide available at present.

Introduction

This chapter discusses the risks to humans and large mammals associated with the use of *Bacillus thuringiensis* var. *kurstaki* (*Btk*) in forest, agricultural and urban environments. The safety of *Bt* toxins expressed in transgenic agricultural crops or forest trees is a seperate issue from aerial or ground applications for insect control, and will not be discussed in this chapter.

A brief review of the history of use of this bacterial pathogen in North America will be useful to better understand the risks. The variety or subspecies used in early insect control programs was *Bacillus thuringiensis* var. *thuringiensis* (*Btt*), known at the time as *Bt* Berliner (24). *Btt*[1] was first introduced for insect control in North America in 1958, when it received partial registration in the United States (US) for use on food and forage crops (25). Full exemption (i.e., there is no waiting period between the time of application and when the crop is sold on the market) was granted in 1960 (10). *Btt* was registered in Canada in 1961 (41), and in Germany in 1964 (44). *Btk* was first used in a commercial product (Dipel®) in 1970 (4). At least 84 subspecies of *Bt* have been identified by the Institut Pasteur, Paris, France (J.-F. Charles, personal communication), and most of these strains affect specific orders of insects. The most widely used strains are: *Btk* and *Bt* var. *aizawaii* (*Bta*), that affect Lepidoptera, *Bt* var. *israelensis* (*Bti*) affects Diptera, and *Bt* biovar. *tenebrionis* (= *Bt* subsp. *san diego*) affects Coleoptera.

Btk is active against over 200 species of Lepidoptera and registered for use against many of these species that are pests in agriculture, forestry and horticulture. Since the mid-1980s, *Btk* has been the most widely used insecticide in Canadian forests and has been applied against several species of defoliators (13, 36, 81).

In the US, the office of Pesticide Programs of the Environmental Protection Agency (US-EPA) "… has the authority [and obligation] to ensure that pesticide use in commerce will not result in unreasonable adverse effects to humans and environment …" (69). In Canada, the Pest Management Regulatory Agency (PMRA), under the responsibility of Health Canada, is charged with the same responsibilities (35, 36). Although both agencies require considerable data on the safety of any product they register, concerns are still expressed by some groups over the possible side effects of *Btk* applications, especially to humans, particularly when applied near water, highways or over populated areas.

This review, which is an update of part of an earlier report (60), brings together both published and unpublished information on the safety and toxicology of *Btk* and its potential environmental impact on humans and large mammals.

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[1] To avoid confusion, *Bt* var. *thuringiensis* will be abbreviated to *Btt*, and *Bt* var. *tenebrionis* will not be abbreviated.

Exposure of Humans to *Bt*

The potential effects of *Bt* on humans were investigated from the time that *Bt* was considered as a tool in insect control in North America. Early investigations on the safety of *Bt* involved experimental exposure of human volunteers "satisfied government officials and the manufacturer as to the safety of Thuricide [*Btt*]" (26), and these data were probably used to support registration. Later, when *Btk* sprays were used operationally (or experimentally to determine the efficacy of the various products becoming available commercially), human-exposure monitoring was conducted in the field (i.e. realistic conditions).

Exposure of humans in laboratory studies. The possible effects of *Btt* on humans were studied during early experimental exposure of human volunteers under controlled laboratory conditions in the late 1950s. Eighteen volunteers consumed 1 g of Thuricide® (active ingredient *Btt*, 9 x 10^9 spores/g, supplied by Bioferm Corp.) daily for 5 days, and five of these volunteers also inhaled 100 mg of the powder (containing 3 x 10^9 spores) daily for 5 days (25). Complete physical examinations measuring weight, height, blood pressure, respiratory rate, and pulse rate, as well as evaluations of genitourinary, gastrointestinal, cardiorespiratory and nervous systems, were conducted before, immediately after the 5-day administration of the insecticide and 4 or 5 weeks after administration. All volunteers remained well throughout the test period (p. 687). Despite the presence of β-exotoxin in the formulation, the volunteers showed no adverse effects resulting from either ingestion or inhalation of *Btt* over a 5-day period. In addition, 8 employees of Bioferm Corp. continuously exposed to *Btt* during the manufacturing process were observed over a 7-month period. No health problems arose in any of the employees, and "comprehensive" medical examinations (type of tests not specified) of these individuals did not reveal any adverse effects resulting from their exposure to *Btt* (25).

The only other laboratory study we found is mentioned in a review of human and laboratory animal toxicological data submitted to the US-EPA prior to 1989 to support registration of *Bt*-based microbial insecticides (52). Their data refer to a study in which five male and five female volunteers each ingested 1g ($1x10^{10}$) viable spores daily for 3 consecutive days. All blood cultures of the test subjects were negative, even though five of the 10 subjects showed viable spores of *Bt* 30 days post-ingestion. The study concluded that the large volume of mammalian toxicology data on *Btk* and *Bti* show that both are non-toxic or pathogenic to mammals, including humans (52). No further laboratory experiments were conducted with human volunteers, and the effects of human exposure to *Btk* were monitored during field applications to control native insects or to eradicate exotic insects.

Exposure of humans in field studies. Epidemiological studies have been conducted in association with 11 *Btk* applications near or over areas of human habitation in North America (Tables 1 and 2). Four of these studies were conducted during *Btk* treatments of the native spruce budworm (*Choristoneura fumiferana*) in eastern Canada, and seven studies during gypsy moth (*Lymantria dispar*) eradication programs in western North America.

Human exposure during the operational and experimental applications of *Btk* to control the native spruce budworm will be discussed first for two reasons. First, *Btk* treatment was only applied once per year to each area (Table 1). Second, because these monitoring studies also show that forest managers (or people responsible for making the control decisions) proactively monitored human exposure early on to detect any possible health complications arising from the *Btk* treatments. Eradication programs conducted against the gypsy moth, *Lymantria dispar*, will be discussed later because during these projects *Btk* was applied at higher doses and multiple times to each area in a single year.

Operational and experimental aerial spraying of Btk against spruce budworm. During the operational treatments conducted in Quebec, different *Btk* products were applied at dosages ranging from 20-30 BIU/ha in 2.3 to 5.9 L/ha (50). The results of part of the Quebec study on immunological responses will be discussed here; the sections dealing with spore density counts monitored in two communities, will be discussed later in the section "Airborne *Btk* following aerial sprays".

Province of Quebec, Canada – 1984 to 1987. *Btk* is the most widely used insecticide in the forests in Canada, and was used in the past mainly to suppress spruce budworm populations. Human health was monitored during spruce budworm suppression projects over 4 years using several different formulations containing *Btk*. Other products were also used experimentally (Table 1).

TABLE 1. Summary of *Btk* applications against *Choristoneura fumiferana* during which human health studies were conducted.

Location	Year	Area treated (ha)	Product	Dose and Rate	Est. human pop. in or near treatment area	Sources
Québec	1984	383,009	Dipel 88	20 BIU/ha in 5.85 L/ha	3,500[a]	50
			Dipel 132	30 BIU/ha in 2.37 L/ha		
			Futura	20 BIU/ha in 2.50 L/ha		
			Thuricide 32LV	20 BIU/ha in 4.68 or 2.34 L/ha		
			Thuricide 48LV	30 BIU/ha in 5.85 L/ha		
Québec	1985	479,293	Futura FC	20 BIU/ha in 2.50 L/ha	28,500[a]	2, 51
			Novabac 3	30 BIU/ha in 3.55 L/ha		
			Thuricide 48LV	30 BIU/ha in 2.37 L/ha		
			San 415	Not given - experimental		
			Thuricide 64B	Not given - experimental		
			Futura XLV	Not given - experimental		
			Biobit 64B	Not given - experimental		
			Dipel 176	Not given - experimental		
Québec	1986	18,160	Futura FC	20 BIU/ha in 2.50 L/ha	35,300[a]	19
			Thuricide 48LV	30 BIU/ha in 2.37 L/ha		
Québec	1987	197,992	Dipel 132	30 BIU/ha in 2.37 L/ha	2,900[a]	18
			Dipel 176	30 BIU/ha in 1.77 L/ha		
			Futura	20 BIU/ha in 2.50 L/ha		
New Brunswick[b]	1983 - 1989	645,088	Not given	Not given	Not given	22
Total					**Est. 70,200[a]**	

[a] Number of individuals in spray area during the operational sprays in Quebec were estimated from population of the communities (2-7 each year) in which the sampling devices were placed during the operational sprays to monitor aerial drift of *Bt* spores. These communities were located 1-13 km from the actual treatment blocks.
[b] Based on one anecdotal account from a 7-year period.

Human health monitoring was conducted on workers directly involved with the spray program during operational and experimental sprays of various formulations, dosages and volumes of *Btk* against spruce budworm in southeastern Quebec (Table 1). No communities were within the treated areas, although over 70,000 people lived within 13 km of the treated stands at the time of the study. These communities, located near the treated stands, were monitored for spray drift (see section on "Airborne *Btk* Following Aerial Spray Program Activities"). While most of the studies dealt with occupational exposure or aerial distribution of spores, one study (2) examined local residents for the presence of spores or immune response to *Btk* sprays. During this study, 484 nasal samples were obtained from primary school students (attending schools 3-15 km from treated stands), of which only 16 (3.3%) cultured positively for *Bt.* In addition, of 110 blood samples obtained from residents living within 20.5 km of the spray blocks, only one had an immunological response to *Bt* (2).

Quebec – 1995 to 2000. During another monitoring program, initiated in 1996 (but also examining samples obtained in 1995), a total of 89 cultures of *Bacillus cereus*-like bacteria grown from various sources (blood, pus, biopsies, eye, etc.) obtained from hospitals throughout Quebec were examined for the presence of *Btk* or *Bti* (48). Of the 89 cultures examined, 86 were identified as *B. cereus*, and three were identified as *Btk*; two from blood samples and one from abdominal fluid. However, in all three cases it was concluded that the *Btk* was present as a result of laboratory contamination of the samples (48). None of the reports we obtained from Quebec indicated any medical problems amongst either the workers involved in the spray program or the general populace (2, 18, 19, 20, 46, 48, 50, 51).

Province of New Brunswick, Canada (year not given). In a poorly documented incident (which took place sometime between 1983 and 1989) of overspraying of two elderly individuals in New Brunswick, concerns were raised because of reported post-exposure non-specific health effects (dermal rash, hive-like wheals, increased incidence and respiratory infections and general malaise). The negative symptoms may or may not have been associated with an aerial application of *Bt* against the spruce budworm (22). Considering that human volunteers were exposed to higher doses of *Btt* (25), it is likely the negative symptoms experienced by

TABLE 2. Summary of *Btk* applications to eradicate *Lymantria dispar* in western North America during which human health studies were conducted.

Location	Year	Area treated (ha)	Product	Dose and Rate	Est. human pop. in treatment area	Sources
Eugene, OR	1985	101,171	Dipel 8L	Not given	80,000	23, 29, 59
Eugene, OR	1986	109,265	Dipel 8L and Dipel 6AF	Not given Not given	40,000	23, 29, 59
Tacoma, WA	1992	47,128	Foray 48B	60 BIU/ha in 4.7 L/ha[a]	500,000[b]	82, 83
Seattle, WA	2000	Not given	Foray 48B	60 BIU/ha in 4.7 L/ha[a]	6,600	84
Vancouver, BC	1992	18,813	Foray 48B	50 BIU/ha in 4.0 L/ha (x4)	250,000[b]	56
Victoria, BC	1994	116	Foray 48B	50 BIU/ha in 4.0L/ha (x3)	5,250	5
Victoria, BC	1999	13,398	Foray 48B	50 BIU/ha in 4.0 L/ha (x3-5)	80,000	11, 61
Total					**Est. 961,850**	

[a] Approximate dose and volume applied, converted to metric.
[b] Estimated population in treated areas based on number of residential units or personal communication.

the two elderly people were likely coincidental and not related to *Btk* treatment.

Epidemiology studies of humans exposed to aerial spraying of *Btk* during gypsy moth eradication programs

Seven gypsy moth eradication programs were conducted against the gypsy moth (both European and Asian strains) in western North America between 1984 and 1999. During each of the eradication programs, *Btk* (in most cases Foray 48®B) was applied at 50-60 BIU in 4.0-4.7 L/ha, and treatments were applied three to five times, ca. 7-10 days apart (Table 2), depending on the location and estimated duration of larval hatch (the length of which can vary considerably and is affected by microclimatic conditions).

Eugene, Oregon State, United States – 1985 and 1986. Epidemiological studies were conducted as part of the gypsy moth eradication program in Eugene, Oregon. Bacterial isolates, cultured for routine clinical purposes from hospitals and outpatient clinics, were screened for possible human infections caused by *Btk* throughout the three sprays per year, and for 1 month after the last spray in both 1985 and 1986. About 80,000 residents lived in the treated areas in 1985, and 40,000 residents in 1986 (29). Samples collected from a non-treated community ca 100km from the treated area served as a control during the second year of the spray. In addition to the culture samples, telephone complaints made by the general public to the Lane County Health Department were tabulated and examined for observable patterns of clinical disease complaints.

A total of 56 *Bt*-positive cultures were obtained from patients, in the two spray years during and after spray: 55 from three hospitals and one from an outpatient clinic. Of the 56 cultures collected, 52 (92.9%) of the *Bt* isolates were assessed as probable contaminants, either of skin or tissue or of the laboratory plates, and not the result or cause of clinical illness. Among the four cultures of interest, one was collected from a spray project worker (not wearing a face mask) who received an accidental splash of *Btk* to his face, including his eyes. The worker developed dermatitis, pruritus, burning, swelling and erythema. Treatment, with a steroid cream to his eyelid and skin, resulted in complete recovery.

In the remaining three *Bt*-positive cultures (two from a hospital, one from outpatient clinic), it could not be proven conclusively whether or not the cultures were the result of an epidemiological infection by *Btk*. In-depth case studies revealed that all three patients were immunocompromised (29). The first patient was a 77-year-old male, admitted to hospital during the spray, with an underlying lung cancer. He was discharged about 2 weeks after the spray program ended, but was readmitted on July 14, 1985, when he developed a fever and pneumonia, and he died 13 days later. Because only one of the four samples taken from the patient's

lungs contained a *Bt*-like bacterium, and because the patient did not respond to antibiotics (to which *Bt* is susceptible), the pneumonia was most likely caused by a different organism. Unfortunately, this could not be confirmed because the family refused autopsy (29).

The second patient was a 31-year-old mentally challenged female who suffered from partial paralysis and subdural bleeding as the result of a car accident that occurred 10 years previously (29). She had surgery for gallstones and recovered "uneventfully". No *Bt* was cultured from gall bladder tissue, and only one of the eight fluid specimens showed *Bt* growth after 5 days of incubation. These observations, and the patient's lack of fever, indicate there was no infection by *Bt* (29), and the one sample showing *Bt* growth was probably the result of contamination.

The third culture was isolated from an abscess on the right forearm of a 25-year-old female who had a history of intravenous drug abuse. Twenty colonies of *Bt* grew from the abscess sample taken from the injection site in June 1985, some days after the completion of the spray program. Five days later, before she started treatment with antibiotics, the wound did not produce any *Bt* cultures. Another organism may have caused the infection and the *Bt* cultured from this area would likely be contaminants (29). Although it could not be proven conclusively that the cultures were the result of infection by *Btk*, circumstantial evidence indicates they were due to contamination of the culture media.

Many of the complaints made by the public during the epidemiological study were related to skin rashes and eye irritation. These symptoms may have been caused by the presence of the gypsy moth itself rather than by the *Btk* application (29). Both dermatitis and eye irritation have been documented by large numbers of people from the northeastern U.S., and this has been attributed to an allergic sensitivity to the "hairs" of gypsy moth larvae (75). Similar reactions are also caused by other caterpillars in the same family (16).

Btk and *Bta* are considered to be non-pathogenic to humans and other animals, although the increase in the proportion of immunocompromised people in the general population over the last 36 years has raised concerns that some people may become ill because of *Bt* sprays. Because of this, "the medical community has become more reluctant to label any bacterium as absolutely non-pathogenic to humans... These [pest control] microorganisms may have potential for causing disease in immunocompromised persons. Therefore, such individuals should be advised on how to use biopesticides and how to protect themselves from undue exposure in areas where they are used" (29). People with compromised immune systems or preexisting allergies may be particularly susceptible to the effects of *Bt* (72).

Tacoma, Washington State, United States – 1992. The Washington State Department of Health (DOH) monitored and evaluated the health effects associated with an aerial spray program to eradicate gypsy moth in 1992. A telephone hotline was established to receive health complaints related to the gypsy moth eradication spray program. There were 179 calls to the hotline involving 279 individuals, from an estimated population of about 500,000 (Table 2), with health complaints, but the reported illnesses were almost all relatively mild. Background illnesses in any community include hay fever-type symptoms, viral gastroenteritis (intestinal flu), rash illnesses and streptococcal throat infections, making it difficult to distinguish between such illnesses and health effects reported as a result of the *Btk* spray program. No reports of *Bacillus* infections associated with *Btk* applications were received by the DOH. The report concluded that there were "no demonstrated infectious complications" resulting from the *Btk* spray program (83).

Vancouver, British Columbia, Canada – 1992. Potential changes in human health during and after the combined ground and aerial spraying of Foray 48B to eradicate Asian gypsy moth in Vancouver (Table 2) were monitored during a multi-faceted study conducted by medical doctors at the University of British Columbia. The study included: food sampling during and after *Btk* spraying; monitoring health effects of *Btk* on workers occupationally exposed to the bioinsecticide; monitoring the frequency of visits to physician offices and hospital emergency wards during and after the spray; and examining cultures collected from patients visiting hospitals and physicians during the test period for the presence of *Btk* (56).

More than 26,000 telephone calls, 1140 family practice patients (visits) and 3500 admissions to hospital emergency departments were examined, and the health of 120 workers with occupational exposure to the

Btk spray was monitored during this study. In addition, the study examined more than 400 bacterial cultures submitted from 10 participating laboratories. The study also examined air samples for *Bt* spore concentrations to which both the general public and workers associated with the *Btk* spray were exposed, as well as samples of food from a variety of sources and times (56).

Complaints of respiratory and eye symptoms were no more frequent among those living within the spray zone than those living outside of the spray zone in the Vancouver study. Furthermore, complaints of such symptoms were no more frequent among individuals who had objective evidence of having been exposed to the spray than those who did not. "While symptoms may have been attributable to the spray, it is not possible to distinguish these from the identical complaints that regularly occur during spring due to environmental factors such as dust and pollen" (56). Furthermore, "…there was no evidence to suggest that the number of visits, and reasons for visits, to emergency departments were different as a result of the spray program…" (56). Although *Bt* (no serotyping, DNA analysis or bioassaying were done to confirm subspecies) was recovered from a broad range of body sites (blood, body fluids and tissues) from exposed individuals, the authors did not find a single case of *Btk*-caused infection. There was no significant difference in the percentage of *Bt*-positive cultures from nose samples of patients living within (57.8%) and outside (39.1%) of the spray zone (128 positive cultures total), the remaining 3.1% had no postal code information available and could not be slotted either in or outside the treated area. Positive *Bt* cultures did not result in a negative health outcome in any of the patients, even though 85% of the patients did not receive antibiotics. Moreover, examination of all significant cultures collected during the test period showed that there were no cases of infection in immunosuppressed people as a result of exposure to *Btk* spray. Results of this study clearly indicate that the large-scale *Btk* spray program for gypsy moth eradication in Vancouver did not cause any "measurable increase in serious community unwellness that could be attributed to the spray" (56).

Victoria, British Columbia, Canada – 1994. A health surveillance program was conducted in the Greater Victoria area when a 116 ha area with an estimated population of 5,250 residents, was treated with *Btk* during another gypsy moth eradication program (Table 2). Over 10,000 notification letters were sent to residences inside and within 30 m of the spray zone boundary, directing the public to report any symptoms believed to be associated with the spray to the Medical Health Officer (5). A total of 30 self-administered complaint questionnaires were requested by residents by calls to the office of the Medical Health Officer, but only 16 of the 30 questionnaires were completed and returned. Forty symptoms were reported associated with the *Btk* spray program, the three most frequently reported symptoms being headache (10%), dry sore throat (9%) and dry hacking cough (6%) (p. 43). It is of interest that of the 16 who completed the questionnaires, none were residents with addresses within the spray zone; five lived adjacent (within 1 km) to the spray zone boundary and 11 lived outside the 1km zone (5). Spray droplets could certainly have drifted this far, but the concentration of spray droplets would have been much lower than in the treated area (yet there were no complaints received from inside the treated area where these symptoms should have been more severe). Verification of exposure to spray droplets was not obtained, but the survey results were intended, in part, to serve as a documentation of the level of public concern and response over the *Btk* application in an urban area to eradicate the gypsy moth (p. 44). The small complaint response (16 or 0.15% of the notified residents) to a highly-publicized spray program, in which information leaflets were sent to 10,495 residents in and near the spray zone, is a noteworthy result and indirect evidence that people were not affected by the spray (5). The results of health monitoring during the 1999 gypsy moth eradication program in Victoria is reported in Levin (47). Reports of airborne concentrations of *Btk* during the 1999 spray program (74) will be reported in the next section.

Non-occupational exposure to airborne *Btk* following aerial spray program activities

Levels of *Btk* in the air were monitored for the first time in Canada at two municipalities in southeastern Quebec during the peak of the *Btk* application period. (50). *Btk* spores were collected by drawing air through a filter using a vacuum pump and the number of spores collected/minute/L (spores-min/L) of air sampled was calculated. Levels of *Btk* in the air ranged between 0 and 132.6 spores-min/L (94.0 spores/m^3), but most measurements were below 2 spores-min/L (1.4 spores/m^3), and were much lower than amounts inhaled and ingested by the human volunteers (25, 52). The highest level of *Btk* present in air samples following nearby spraying

was 132.6 spores-min/L. Assuming that this quantity of *Btk* in the air remained constant for a day [which is unlikely (74)], a person with an average respiration rate of 30 L/min could inhale a total of $4x10^4$ spores over a 24-hour period (50). Over a 25-day period, a person could maximally inhale a total of 10^5 *Btk* spores. In contrast, the human volunteers in Fisher and Rosner's experiment (1959) ingested 33,000 times ($3.3x10^9$ spores) this number of spores daily, and inhaled 3,300 times ($3.3x10^8$ spores) this number of spores daily for 5 days without experiencing any ill effects (50). In the treated areas in Quebec in 1985 spore concentrations ranged from $4.5x10^3$ to $2.8x10^5$ spores-min/L, and no human health problems were reported (20). The authors concluded that "*Bt* concentrations detected in the two municipalities monitored represent only a very minimal hazard for the populations concerned" (50). Several other similar studies (2, 18, 19, 51) conducted in the same area in 1985 and 1986, and in other municipalities in Quebec in 1987 during peak spray periods, confirmed these findings.

Airborne exposure to *Btk* during the 1999 aerial spray program to eradicate gypsy moth in the populated Greater Victoria, B.C., area was monitored to determine the rate of reduction of airborne concentrations following spraying, the occurrence of drift outside the spray area and whether staying indoors during spraying reduced exposures to *Btk* (74). Outdoor air concentrations of *Btk* were highest from the start of spraying and for up to 3 hours, then diminished exponentially over time. Within 8-13 hours after the spray, airborne concentrations were 20% of the highest mean spore concentrations at the time of the spray (74). Culturable airborne *Btk* concentrations measured outdoors after spraying ranged from below the detection limit to too numerous to count, with a mean of 739 *Btk* CFU/m^3 of air. *Btk* exposure inside residences in the spray zone initially averaged concentrations 2-5 times lower than that of outdoor concentrations, but at 2-3 hours after the start of the spray, indoor concentrations (395 CFU/m^3) approached outdoor concentrations (501 CFU/m^3) and then exceeded outdoor concentrations (244 CFU/m^3 versus 77 CFU/m^3) at 5-6 hrs after the start of spraying. Staying indoors during the spray, therefore, initially lowered exposure to *Btk*, but this benefit was not sustained within several hours as outside air moved indoors with normal daily activities and did not dissipate or degrade as quickly indoors as it did outdoors (74). None of the measured indoor characteristics (type of residence, type of entry, story of sampling, room sampled or indoor temperature, whether any windows or doors were open) were related to indoor concentrations. Drift occurred outside the spray zone by at least the sampled band of 125 to 1000 m (74). For additional information on the 1999 Victoria study (47).

Occupational exposure

Occupational exposure to one application of *Btk* was investigated for the first time in Quebec during spruce budworm control programs between 1984 and 1987. It was also investigated during eradication programs when *Btk* was applied several times in a ca. 2 month period and, in some cases, applied both from the air and from the ground.

Quebec – 1984 to 1987. During the operational and experimental sprays conducted against spruce budworm in southeastern Quebec, the effects of working in close and continual contact with *Btk* was studied. During 1984 and 1985, blood samples were collected from workers (loaders, mixers, etc.) who were exposed to *Btk* as part of their work. Samples were obtained from 28 workers three times during 1985: before the aerial spraying began, at the end of the spray program, and 10 weeks after the end of the spray. At the end of the spray program (second sample), six of the 26 workers (21.4%) had positive immunological responses to *Bt.* By the third sample, only four (14.3%) of these workers still had antibodies for *Bt*, and in all cases their immune response was 25-50% lower than in the second sample (2). It was also determined that the type of antibody produced was IgM, which is not involved in allergic responses. This is slightly different from the findings of Bernstein et al. (2000) (6), who found antibodies IgE and IgG produced by crop pickers exposed to *Btk.*

In a second study, conducted around the same time, blood samples were collected from field technicians and workers at the airports where the spray planes were loaded in 1984, 1985 and 1986. Samples collected in 1984 and 1985 were stored at -70°C until they were examined in 1986 for immunological response to the presence of *Btk* (46). Of the 136 workers tested, only five (all of whom worked closely with the *Btk*) reacted positively for the presence of anti-*Bt* antibodies; of these, four of the five had a positive response to vegetative cells only, spores and crystals elicited little or no response. In 1985, five of nine field technicians had positive antibody responses to spores and crystals, but did not react to vegetative cells (the other four tested negative). Of the airport workers (loaders and mixers) tested in 1985,

four of 12 had a positive reaction to vegetative cells, but only one of these workers also reacted positive to spores and crystals . However, these positive reactions were only temporary, lasting between 3 months to 1 year. Indeed, in 1986, no workers tested positive for anti-*Bt* antibodies. This may have been due to the lower volumes of *Btk* applied in 1986, or the fact that none of the formulations used required mixing (46).

Eugene, Oregon - 1985 and 1986. During the large-scale application of *Btk* in 1985 and 1986, personal exposure sampling was conducted to determine the occupational and general public's exposure to *Btk* sprays. In 1985, samples were collected from 22 individuals doing 15 different kinds of jobs, while in 1986, samples were collected from 19 individuals doing nine different jobs. General area air samples were collected at various locations within the spray boundary, as a reflection of public exposure potential (23). Breathing zone samples for a safety officer, helicopter pilot, aerial observer, card checkers and a security guard indicated *Btk* exposure ranged from 0 to 5,600 CFU/m^3, with one sample from a Kromecote card checker who was in brief direct contact with the spray recording 11,000 CFU/m^3 (23). General public exposure to *Btk* during the eradication program ranged from 0 to 1,600 CFU/m^3 (29). In comparison, during the spruce budworm programs in Quebec, where *Btk* was applied at lower rates (Table 1), the spore densities were ranged from 0 to 94.8 spores/m^3.

Vancouver, British Columbia – 1992. Occupational exposure to *Btk* was also investigated as part of an epidemiological study conducted in conjunction with the Asian gypsy moth eradication program (58). Within the study population of 120 occupationally exposed ground spray workers, almost two thirds of the workers reported eye, nose, and throat irritation, dry skin and chapped lips; complaints were most prevalent among workers who had a prior history of allergies (56). Symptoms were noted to occur only briefly at the beginning of each of the three treatments when the spray droplet concentrations of Foray 48 were at their maximum (average 2 x 10^6 to 5.9 x 10^6 spores/m^3; maximum recorded value 1.6 x 10^7 spores/m^3). Nearly all workers exposed to higher concentrations for 5-20 shifts retained *Btk* for at least 5-6 days, and most were culture positive for 14-30 days. There were, however, no days of work loss attributable to *Btk* exposure. The ground spray workers were exposed to *Btk* at rates as high as 500 times that of the general public living in the treated area, would have encountered during an aerial spray (56). For individuals who worked most shifts during the spray program, estimated cumulative *Btk* exposures ranged from a high of 7.2 x10^8 CFU/m^3 among workers applying the spray to a low exposure of 5.4 x10^6 CFU/m^3 among Kromecote card handlers (Kromecote cards are placed in the spray zone near ground level to estimate spray droplet size and density). No significant health problems resulted from Foray 48B exposure, and no differences were found with respect to gender or smoking status (56).

Miscellaneous studies. A health survey was conducted in farm workers before and after their exposure for about 4 months to *Btk* through the picking of sprayed vegetables. The investigation grouped workers into high, medium and low exposure groups and compared results of questionnaires, nasal and mouth lavages, ventilatory function assessments, and skin tests (6). As expected, the majority of positive skin-prick tests to *Btk* occurred in workers who had a higher degree of exposure. Specific IgG and IgE antibodies to vegetative cells were present in all groups of workers. Comparison between exposure groups in terms of the prevalence of IgG and IgE immune responses indicated that "exposure to *Btk* spray may lead to allergic sensitization, as indexed by both positive skin tests and specific IgE antibodies, induction of IgG antibodies, or both" (6). The authors suggested that allergenic effects of *Btk* in humans could be due in part to vegetative-derived allergens. They further suggested that the "immediate hypersensitivity developed in some workers indicates that adverse IgE mediated health effects could develop if repetitive exposure continue[s]". However, there was no evidence of occupationally related respiratory symptoms or clinical diseases in any of the workers (6). The antibodies detected in this study differ from those identified in a Quebec study (2), in which neither IgE nor IgG antibodies were detected, only IgM.

A *B. cereus*-like bacterium was reported in some stool samples obtained from Danish greenhouse workers where Dipel® was used (43). The isolate had the same RAPD pattern and gave the same results for PCR against *cry*I endotoxin and 16-23S rRNA, but analysis of the plasmid DNA showed that the plasmids differed from the *Btk* in the Dipel® used (43). This bacterium, isolated from the stool samples, was not positively identified (although most DNA testing indicated it probably was

Btk), therefore it was not ascertained if the strain was the same one used by the greenhouse workers, or whether they may have acquired the bacterium by some other means, including contaminated diet.

Dietary exposure

No human health problems have proven to be directly attributable to the use of *Btk* during the 35 years since its registration in 1970. *Bt* has been used extensively on fruit and vegetable crops, including maize, broccoli, cabbage, lettuce, apple and tomato (10). The U.S. Environmental Protection Agency approves the use of *Bt* products (β-exotoxin free) on food destined for human consumption up to and including the very day that these products are harvested, as well as for use on stored food products (79). The same also applies in Canada (66). The absence of a "waiting period" is an indication of the considered safety of *Btk* to consumers. Most likely, there have been instances where spores or crystals were present on treated produce sold at grocery stores, and the consumer did not wash, or inadequately washed, the purchased goods before consumption. For instance, *Bt* (possibly *Btk*) was repeatedly cultured from commercially available vegetables during and after the gypsy moth eradication program in Vancouver in 1992. As a result, the general public was “readily exposed to sources of *Btk* other than either the aerial or [local] ground sprays” (56). Despite this, the general health of individuals living in the spray area exposed to such produce was not adversely affected.

Another documented example was in which *Bt* was isolated from Red Tokay grapes imported from California, USA, and sold for human consumption in Saskatoon, Saskatchewan, Canada (8). No health problems were reported from eating these grapes.

The close taxonomic and molecular relationship between *B. thuringiensis* and *B. cereus* warrants close scrutiny of the published literature with respect to food safety. Due to a lack of a universally accepted method for conclusively differentiating *Bt* from *B. cereus* in public health laboratories, some researchers feel there may be an under-representation of food poisoning cases attributable to *Bt* (9), or, conversely, showing the safety of *Bt*. There is only one published case in the literature that implicates *Bt* with food poisoning. Investigation during a gastroenteritis outbreak in a chronic care institution in Ottawa, Ontario, recovered bacterial isolates presumptively identified as *B. cereus* from stool samples of four individuals, of which one sample was also positive for Norwalk virus, a known enteric pathogen (42). In the three other ill individuals, however, no other enteric pathogen was detected. The stool samples were subsequently stained for toxin crystal formation and identified as *B. thuringiensis*. *B. cereus* was isolated from spice (onion powder) samples submitted by the institution. However, these food isolates were determined, by phage typing, to be unrelated to the patient isolates (42). Since neither *Bt* nor *B. cereus* isolates from the stool samples matched isolates recovered from food samples, *Bt* cannot be directly attributed as the agent of the gastroenteritis (68). Furthermore, the suspected cases of food poisoning in the four individuals could not be traced to the use of *Btk* in the treatment of food crops or aerial applications against defoliators in the area (42).

Isolates of *Bt* originating from eight commercial *Bt*-based insecticidal products [*Btk* (Dipel®, Foray® 48B), *Bta* (Florbac® FC, Turex®, XenTari®), *Bti* (Bactimos®, VecTobac®) and *Bt* subsp. *tenebrionis* (Novodor® FC)] were all found to produce diarrhoeal enterotoxins when grown in the laboratory on brain heart infusium broth (14). However, it should be noted that it is highly unlikely that this type of substrate would be generally be present where any of these bioinsecticides would be used in the field. The quantity of diarrhoeal enterotoxin production varied by a factor of more than 100 among the different strains tested; *B. cereus* produced the highest amount of enterotoxin and *Btk* from Dipel® the lowest (0.86%). Although diarrhoeal enterotoxin production was low to moderate in most of the strains tested, the author warned that the results indicate that *Bt* is capable of causing food poisoning, and therefore, *Bt*-based insecticides with viable spores may, under the “right conditions” (these conditions were not specified), pose a potential risk for a gastroenteritis outbreak (14). Subsequent laboratory tests with six strains of *Bt* (including var. *kurstaki, israelensis* and *morrisoni*) also demonstrated enterotoxin production (9). The authors cautioned that “with current trends for increasing popularity of organically grown foods [on which biopesticides are commonly used] and decreasing cooking time for vegetables, a potential [non-lethal] food-poisoning risk exists if enterotoxin-producing strains of *B. thuringiensis* become employed as biopesticides” (9), and if the consumers do not properly wash the food before preparation and consumption.

As part of the same study, the isolate with the highest level of enterotoxin was fed to rats at a dosage of $5x10^{10}$ spores / day (total of 10^{12} spores) over 3 weeks, and 10^6 spores were injected subcutaneously. The rats suffered no ill-effects in terms of their condition or in the pathology of their internal organs despite the fact that the strain tested (*Bt* 13B) was capable of producing both β-exotoxin and enterotoxin. Dissection of sacrificed rats showed that the *Bt* spores did not germinate in the rat gut (9), which supports earlier work done on mice (70).

It has been demonstrated that washing vegetables (spinach leaves) in cold running water resulted in a reduction of only about 50% of the *Btk* spore load (9). Bactospeine® [*Btk* (HD-1)], applied in a greenhouse according to the manufacturer's instructions and sprayed until run-off on both surfaces of spinach (*Spinacia oleracea*) leaves, was investigated for spore load reduction after normal food preparation practices. Plants were harvested 24 hours following *Btk* application in attempt to show as high a residual load as possible. Boiling of the leaves effectively removed the spores (<10 CFU/g wet weight of leaves) due to dissolution of the spray formulation, not by thermal inactivation, since many viable spores were found in the boiled water (9). The residual spore load that can remain after washing vegetables shows that a potentially significant number of spores can be ingested after normal food preparation practices, sufficient for a strongly enterotoxic strain of *Bt* to cause non-lethal food poisoning in humans. However, the authors state that "the experimental work on humans [volunteers] and the paucity of positive reactions from humans after several decades of large scale use [of *Btk*] for pest control, suggest that [*Btk*] shows a very high degree of safety" (9). They also recommended that to ensure that biopesticides containing *Bt* continue to enjoy their excellent safety record, existing and newly registered *Bt* products should be thoroughly tested for their potential as enterotoxigenic food poisoning agents (9).

Another concern expressed by some is the effect of *Btk* spray on honey bees, honey, and subsequently on people. This concern may originate from a brief article published in the Pennsylvania Forest Pest Management News, outlining a possible case of human illness in Kansas City, Missouri, resulting from ingestion of honey that was ordered from a retail outlet in Maine and was received as a gift from a relative (39). Three of five members of the family who ate the honey suffered from diarrhea and vomiting approximately 5-10 hours after ingesting it, but recovered within 18 hours. The Centre for Disease Control (CDC) in Atlanta determined that *Bt*, and not *B. cereus*, was present in the honey. However, the epidemiologist at the CDC in Atlanta stated that there was no evidence that implicates *Bt* as the cause of the illness and no evidence to stop the use of *Btk* in the gypsy moth control program being conducted in Pennsylvania (39). Unfortunately, the reference does not specify if the retailer shipped the gift directly to the recipients, or how much honey the family members ingested before they became ill. It is somewhat unusual that only three of the five people from the same family became ill after eating the honey, and nobody else eating honey from the same distributor in Maine became ill.

It must be re-iterated that *Bt* has been widely and safely used as an insecticide for about 50 years without clinical incident, except for the two extreme cases reported as occupational accidents when people handling the *Btk* products were not wearing face or safety masks (29, 64, 65).

Although some *Bt* strains are capable of producing enterotoxins (9, 14, 63, 71), there has been no conclusive case reported in the literature associated with food-borne illness caused by *Btk* in humans. Why is this the case? One possibility already suggested is an under-representation of *Bt* in food poisoning cases due to misidentification of the causative agent as *B. cereus*. Laboratory differentiation between *B. cereus* and *B. thuringiensis* should be clear under light microscopy based on the presence of parasporal crystal toxins in *Bt*. However, misidentification could still occur if *Bt* strains lose their plasmids that encode δ-endotoxin (43, 63). Although the genes responsible for enterotoxin production have been identified in some strains of *Bt* (9, 31, 32, 40), there is no evidence that these strains produce enterotoxins under commercial fermentation conditions. No enterotoxins have been detected in commercially used formulations of *Bt*. Any entertoxins that may have been present may either be removed during processing or are degraded in the product by the end of the fermentation process (68). It should be noted that enterotoxins are produced during vegetative growth after spores have germinated, and at this time there is no evidence that spores germinate in humans after ingestion or inhalation.

Human cell exposure

It has been reported that at the human cell level, under certain conditions, *Btk* products can generate

B. cereus-like toxic effects (73). Human cell exposure assays of the commercial *Btk* insecticide product Foray 48B (and *Bti* insecticide Vectobac) generated nonspecific cytotoxicities, including cell lyses. However, the authors rightly stated that "to critically impact at the whole body level, the exposure outcome would have to be an uncontrolled infection arising from intake of *Btk* or *Bti* spores. For humans, such a condition would be rare, involving large doses of spores and individuals with weak or impaired microbe-clearance capacities and/or immune response systems" (73). To put this in perspective, it should be remembered that human volunteers consumed 1g of *Btt* for 5 days and also inhaled 100 mg of *Btt* for 5 days without detectable symptoms (25).

Activated δ-endotoxin of *Bt* subsp. *aizawai* did not cause any morphological changes to various mammalian cell lines (including HeLa and KB developed from human epithelial cancer cells and human erythrocytes), even when these cells were exposed for up to 5 hours. It was concluded that the dissolved δ-endotoxin of *Bt* had no cytotoxic effect on isolated mammalian cell lines *in vitro* (55). These results are supported by work done on bovine hepatocytes and human embryonic kidney cells using activated Cry1A δ-endotoxins (67, 75).

It should be noted that interpretation of all test results must take into account the dose administered, method of administration and the manner in which the safety concerns were evaluated (41). For example, mortality following intracerebral injection of rats with 2×10^8 CFU of *Btk* is not surprising, but would be cause for concern if it occurred after ingestion (45).

People, including researchers, should be careful in their work when studying the effects of *Btk on* mammalian, including human, cell lines. This type of experiment is highly unrealistic and far from representing what happens during and after ground and aerial applications of *Btk,* just as injecting *Btk* intraperitoneally into laboratory animals is highly unrealistic. Trying to extrapolate from the results of these unrealistic exposure experiments to possible effects of *Btk* exposure on humans is problematic, to say the least.

Reported cases of human infection by *Bacillus thuringiensis*

Over the past 46 years of widespread commercial use of *Bt* in general (36 years for *Btk*), there is only one clearly documented incident of human infection caused by *Btk* recorded in the medical literature (64), and another likely, but unproven, case. A healthy 18-year-old male agricultural worker (who was not wearing safety glasses) accidentally splashed the commercial product Dipel® into his right eye. This led to the development of a corneal ulcer that required medical treatment. Cultures from the corneal ulcer produced *Btk* cells, the same as those in the Dipel® formulation (64). Although no serotyping was done, cultures and cells of the bacterium were determined to be *Btk* because they had the same appearance as the *Btk* in Dipel®, and had the same activity against three insects: *Ephestia kuehniella*, *Galleria mellonella* and *Culex quinquefasciatus*. The ulcer healed after subconjunctival injections of gentamicin and cefazolin sodium (64). This case of a corneal ulcer is the first report of a *Bt* infection in a human caused by *Btk* (65).

The other case of a possible *Btk*-caused infection occurred in another spray worker (again not wearing protective face mask) in Oregon who also sustained a splash of commercial product containing *Btk* to his face and eyes (29). Although the bacterium isolated from the inner part of his infected eyelid were neither serotyped nor bioassayed, it is very likely it was *Btk*. However, the infection and other symptoms of irritation cleared up after the use of steroid cream (29). No other information available to us, published or unpublished, suggests any "harmful" effects of *Btk* on humans, even as a result of occupational exposure in manufacturing, mixing, or spraying this bioinsecticide (2, 46, 56). The absence of such information provides testimony to the safety of *Btk* use in pest management.

There are three other, more recent, unusual documented cases from the late 1990s in which subspecies of *Bt* not used in commercial products were recovered from humans. In the first case, four isolates of an unidentified strain of *Bt* were isolated from infections in two patients with severe burn wounds (30-70% of body) in an Italian hospital. The same strain was also isolated from the water used in the treatment of these burn wounds (15). The strain could not be serotyped, was non-flagellated and showed no insecticidal activity against larvae of *Pieris brassicae* (Lepidoptera) or *Aedes aegypti* (Diptera), in contrast to the commercially used *Btk* and *Bti* subspecies, respectively. This was the first report of an undetermined strain of *Bt* causing a non-gastrointestinal clinical infection in immunosuppressed patients (15). Patients with deep burn wounds covering

more than one-third of their body are considered to be highly immunosuppressed and consequently highly susceptible to bacterial infections, including *B. cereus* (15). However, *Bt* subspecies tested for dermal infectivity under normal conditions (on healthy individuals), as required by the US-EPA as part of the registration for commercial use, have tested negative in assays and are considered not to constitute a health hazard when used according to the label instructions as a microbial pesticide (17, 52, 85).

The second case involved a 28-year-old French soldier severely wounded by a land mine explosion in the former Yugoslavia in 1995 (38). Biopsy specimens of abscesses of the wounds (on the left thigh and knee) were obtained and cultured, and the bacterial colonies were identified as *Bt* var. *konkukian* (serotype H34). The potential pathogenicity of this subspecies was tested against mice by cutaneous injection of a bacterial suspension containing 10^5, 10^6 or 10^7 colony-forming units (CFU) *Bt* var*. konkukian* (38). For each concentration of inoculum, one group of mice was immunosuppressed (by intravenous injection of cyclophosphamide at 200 mg/kg of body weight) and one group that was not immunosuppressed served as control. Inflammatory lesions occurred cutaneously only at the highest dose in all test animals and healed after 48 hours, demonstrating the ability of this variety to produce myonecrosis in immunosuppressed mice via cutaneous infection. The authors suggest that the clinical data support the hypothesis that "disease may also be produced in patients with tissue devitalizations caused by massive tissue destruction" (38). Humans, particularly those who are immunocompromised, are more likely at risk from opportunistic infection from his/her own normal flora than from *Bt* applications (62).

The third report of *Bt* associated with infection in humans involved the recovery of *B. thuringiensis* and *B. cereus* isolates from dental patients in Norway and Brazil with periodontitis (37). The report is problematic, however, because the authors considered *B. cereus* and *B. thuringiensis* as belonging to the same taxonomic group, in spite of the fact that none of the 35 strains isolated from humans contained parasporal protein crystals, a diagnostic feature frequently used to distinguish between *B. cereus* and *Bt*.

There have been very few cases reported in the scientific literature of true clinical infections caused by *Bt* in humans, despite the use of commercial *Bt* products for pest control for nearly 50 years, and despite the natural occurrence of *Bt*, particularly in soil (15, 85). These four documented cases simply mean the absence of *B. thuringiensis* infections in humans and is supported by direct evidence (25) and the indirect evidence that no *Btk* or *Bt* infection was reported during the nearly 50 years during which *Bt* products (*Btt*, *Btk, Bta, Bti* and *Bt* subsp. *tenebrionis*) have been extensively used.

Tests on large mammals

Modern formulations of *Bt* are free of β-exotoxin, a substance that is toxic in various degrees to small mammals because it inhibits all RNA polymerase activity (3, 49). Tests done prior to 1971, and since that time, have provided satisfactory results in terms of the risk of *Bt* infection to small and large mammals. Several pre-1971 reports (21, 27, 28, 34, 58) and a more recent article (30) involved *Bt* feeding experiments with sheep and cows. The test results indicate that the spores and crystals survive passage through the gut of these animals and remain intact, even *Bt* varieties containing β-exotoxin.

A lengthy and detailed study of the effects of *Btk* on sheep was conducted using Dipel® and Thuricide®. Chronic feeding tests (500mg/kg or 10^{12} spores / day), involving rambouillet/merino sheep over a 5-month period, did not result in any serious adverse effects to the test animals (30). Several animals in both Dipel®- and Thuricide®-treated groups suffered diarrhea and occasional loose stools during the test, but symptoms did not persist for more than 1 week. This indicates that *Btk* is an avirulent bacterium in sheep when administered orally. The absence of lung lesions, which could have resulted because of the feeding habits of the animal, suggested that *Btk* does not cause disease in sheep when inhaled. Of all the cultures taken from abnormal tissue lesions, only one proved positive for *Bt* (strain not identified) and it was considered an aseptic lesion (30).

Several pre-1971 experiments showed that feeding various concentrations of *Btt* mixed with feed for various lengths of time can control house flies (*Musca domestica*), face flies (*Musca autumnalis*), horn flies (*Haematobia irritans*), and stable flies (*Stomyx calcitrans*) in cattle feces without any noticeable side effects on the test animals (21, 27, 28, 34, 58). These

experiments showed that *Btt* spores and/or crystals survived passage through the digestive tract of cattle and remained viable in the feces. *In vitro* tests on the survival of *Bt* (subspecies not identified) in the rumen environment of cows support this (1). No symptoms of toxicity were observed at any time in any of the test animals (28, 58).

Large game animals and hares were purposefully exposed to high doses (no conversion factor available for dose administered) of Dipel® in Bulgaria to determine the effect on health of wildlife. No treatment effects were found in the 25 one- and two-year-old deer that were kept in an enclosure in a hunting establishment . Wildlife was also monitored on the adjacent lands, and no mortality was recorded in the local hare populations (54).

The safety of *Bt* formulations containing the β-exotoxin (prior to 1971) demonstrates even more profoundly, although indirectly, the safety of current products that do not contain β-exotoxin (24). Other factors contributing to the safety of *Bt* towards mammals include an unfavorable acidic gut environment and the enzymes that can completely degrade the Cry toxin proteins into non-toxic fragments. Conversely, lepidopteran larvae possess the alkaline gut environment and appropriate enzymes necessary to convert the protoxin into the active toxin that lyses the gut wall cells (7, 12, 53).

Genetically Engineered Plants Expressing Cry toxins

Although genetically modified plants containing *Bt* toxins was not part of this review, a brief mention of this topic may be warranted. Only a few peer-reviewed studies have been published to date on the effects of transgenic plants expressing *Bt* genes producing the Cry δ–endotoxin proteins on mammals, including humans.

The main concern regarding plants containing Cry toxins grown for human consumption is that these proteins may cause allergic reactions. A study conducted with transgenic tomatoes expressing Cry1Ab showed it was safe to humans (57). In an unintended experiment, transgenic corn, grown as animal feed, found its way into the human food supply in September, 2000, resulting in food recalls. However, when the Center for Disease Control and Prevention investigated 51 reports of possible adverse reactions to the *Bt* corn, it did not confirm any allergic reactions (6). Nevertheless, in 2001, the US-EPA reassessed *Bt* crops registered for agricultural use (*Bt* corn, *Bt* cotton and *Bt* potato). This review determined that the toxins (Cry1Ab, Cry1Ac, Cry1F and Cry3A) did not show any of the characteristics of known toxins or food allergens, and do not pose unreasonable risks to human health, including infants and children, or to the environment (78).

Cry δ–endotoxin proteins have been sprayed on agricultural food and feed crops as a component of commercial biopesticides for over 40 years with a history of safe use throughout the world (7). Moreover, the few published studies available indicate, as did the US-EPA study in 2001, that Cry toxins do not fall within any of the categories of proteins known to induce allergic reactions in people (53), and is safe to humans (2, 7, 46, 47).

Conclusion

The general conclusions from the review of available literature indicates that *Btk* is safe for the environment and its various components. During the more than 35 years of *Btk* use, no scientifically documented cases of human infection have been reported as a result of its use in forestry or in urban environments during gypsy moth eradication programs (77). *Btk* has also been used in the so-called containment approach directed against the gypsy moth in the eastern United States without any reported ill effects on humans or large mammals.

The absence of reported human infection by *Bt* (*Btt, Btk or Bta*) during the last 46 years of its use on fruits and vegetables, as well as the alkaline condition required for *Bt* toxin activation, reinforces the safety of this pesticide during human ingestion (the human gut environment is acidic) (33). No human health problems have been proven to be attributable to the use of any of *Bt* product on crops.

Mammals, including man, not only lack the alkaline gut pH and enzymes necessary to activate δ-endotoxin, but will actually rapidly (in less than 1 hour) digest the toxin into non-toxic fragments (7, 12, 53). Spores and crystals that survive passage through the stomach are excreted, generally within a few days, without any harmful effects. Experimental evidence also shows mammalian cells do not possess the particular cell receptors to which the *Btk* toxin binds (26, 67, 75).

Human volunteers experimentally exposed to *Btk* in the 1950s, even when the product contained β–exotoxin, remained healthy. Subsequent studies with humans prior to 1987 confirmed these results. There is only one properly documented case of clinical non-lethal infection of a human by *Btk*, and even this could have been prevented by the simple expedient of wearing the appropriate safety gear (glasses or face mask) to protect the eyes. In the seven gypsy moth eradication programs that were examined, there were only three possible cases of infection where *Btk* spray might be implicated, all three were immunosuppressed individuals, out of almost 1 million people who lived in the treated areas (29). However, even in these three cases, clinical infection by *Bt* was not proven, and the isolates obtained from the patients were very likely the result of contamination. None of the other six studies conducted during gypsy moth eradications reported health problems, even with immunosuppressed individuals. Based on all the information reviewed, *Btk* is considered by most people to be the safest biopesticide available at present.

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