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Human Health Impact Assessment after Exposures to *Bacillus thuringiensis* subspecies *kurstaki*

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Foray 48B is a biological insecticide, containing spores of *Bacillus thuringiensis* subspecies *kustaki*, commonly used to control European gypsy moth (Lymantria dispar) larvae. Short-term human health impacts of Foray 48B have been assessed in several epidemiological studies. This paper reviews data from studies conducted in the United States of America, New Zealand, and Canada. To date, no definitive evidence of adverse short-term health effects due to exposures to B. thuringiensis subsp. kurstaki HD1 have been reported.

B. thuringiensis is a spore-forming, gram-positive bacterium that produces intracellular, crystal proteins during sporulation. These parasporal proteins have insecticidal activity that are specific to certain groups of insects (24). The genus *Bacillus* has been divided into five groups (4,10). Group I contains a large number of soil-dwelling species, including *B. thuringiensis, B. sphaericus, B. subtilis, B. anthracis*, and *B. cereus*. Some of these bacilli are very closely related and form separate groups within the group I *Bacillus* species. The *B. cereus* group consists of *B. cereus, B. anthracis, B. mycoides*, and *B. thuringiensis*, with *B. cereus, B. mycoides*, and *B. thuringiensis* being so closely related that some systematists consider the three species to be subspecies of *B. cereus, sensu lato* (16). Analysis of the organization of the genomes of various strains of *B. cereus* and *B. thuringiensis* by pulsed-field electrophoresis revealed that some strains of *B. cereus* are more similar to certain strains of *B. thuringiensis* than they are to other strains of *B. cereus*, suggesting that these two species may actually be variants of the same species (6). At this time, however, *B. thuringiensis* stands as a separate species with distinct biochemical and genetic characteristics (15).

Because of the similarities between *B. thuringiensis* and *B. cereus*, and because *B. cereus* has been associated with food-borne outbreaks of gastrointestinal disease (8,14), *B. thuringiensis*-based products have been subjected to extensive evaluation both prior to and subsequent to becoming commercially available (17). McClintock *et al.* (17) reviewed both animal and human exposure data, and concluded that *B. thuringiensis* subspecies are neither toxic nor pathogenic to mammals, including humans. Animal experimentation, however, has shown that intraperitoneal injection of *B. thuringiensis* can cause death in guinea pigs (9) and that pulmonary infection can result in the deaths of immunocompromised mice (13). A corneal ulcer developed in a previously healthy 18-year-old farmer who accidentally splashed a commercial *B. thuringiensis* product into his eye (23). Multiple thigh and knee abscesses containing *B. thuringiensis* were found in a previously healthy soldier who was severely wounded by a landmine explosion (12).

Reports of human disease caused by *B. thuringiensis* subsp. *kurstaki*, however, are uncommon. Moreover, these reports do not reflect the types of exposure that human populations experience during applications of *B. thuringiensis* subsp. *kurstaki* (here after referred to as *Btk*) to control insect outbreaks in populated urban or suburban environments. Epidemiological studies have been conducted on several occasions after aerial applications of *Btk* over populated areas.

The Oregon State Health Division conducted a health surveillance study to assess the impacts of a spray program conducted in Lane County, OR., in 1985 to 1986. Fifty-five of 95 *Bacillus* isolates were identified as *Btk*. The bacteria were identified by microscopic examination, a technique which is currently not considered to be conclusive as it can result in a high proportion of misidentified bacteria. Upon further examination, 52 of the 55 isolates were assessed to be probable contaminants. Of the three remaining isolates, *Btk* could neither be ruled in nor be ruled out as a cause of the patient's disease (11).

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A health impact surveillance study conducted in Auckland, New Zealand, during a white-marked tussock moth control program recovered *Btk* from three clinical isolates (3). Clinicians concluded, however, that *Btk* was not causally associated with disease and that all three cases represented sample contamination. Petri *et al.* (22) investigated the effect of aerial *Btk* applications on the incidence of self-reported symptoms, health perceptions, and visits to health care professionals Some increases in upper respiratory, gastrointestinal, and neuropsychiatirc symptoms, as well as a reduced overall perception of health in the exposed population, were reported, but these data were not quantitatively correlated with exposure to *Btk*.

Health impact surveillance programs were conducted in British Columbia, Canada, and in the states of Washington and Oregon in response to a 1992 aerial spray campaign. *Btk* was isolated from none of the samples collected in Washington, from 1 sample collected in Oregon, and from 325 samples collected in the lower mainland of British Columbia (1,2,18,19). All *Btk* isolates were determined to be the result of sample contamination and not causal agents of infection (1,18,19).

A comprehensive, coordinated study of the short-term health effects of *Btk* strain HD-1 on human populations was undertaken during aerial applications of Foray 48B over the city of Victoria, British Columbia, Canada, in May and June, 1999 (5). The study included a survey of the health of asthmatic children in the region, a survey of the general health of the population, monitoring and analysis of visits to physician's offices and hospital emergency departments, a review of self-reported complaints of health symptoms made to telephone information and support lines (20), a study of severely asthmatic children exposed to *Btk* during the aerial spraying (21), clinical surveillance of patients with infections from which *Btk* HD1-like bacteria were isolated, and a measurement of the incidence and distribution of *Btk* HD1-like isolates, both in the environment and in the human population (7,25).

Environmental (air and water) and human (nasal swab) samples, collected before and after aerial applications of Foray 48B, both in the spray zone and outside of the spray zone, were analyzed for the presence of strain HD1-like bacteria. Random amplified polymorphic DNA analysis, *cry* gene-specific PCR, and dot blot DNA hybridization techniques were used to screen over 11,000 isolates of bacteria. Bacteria with genetic patterns consistent with those of *Btk* HD1 in 9,102 of 10,659 (85.4%) isolates obtained from the air samples, 13 of 440 (2.9%) isolates obtained from the water samples, and 131 of 171 (76.6%) isolates from the nasal swab samples. The data suggested that *Btk* HD1-like bacteria were present both in the environment and in the human population of Victoria prior to aerial applications of Foray 48B. The presence of *Btk* HD1-like bacteria in human nasal passages, however, increased significantly after the application of Foray 48B, both inside and outside the spray zone (7).

During spray applications, the average culturable airborne concentrations of Btk HD1 measured outdoors, within the spray zone, was 739 colony-forming units CFU)/m3 of air. Outdoor air concentrations decreased over time, quickly in an initial phase with a half time of 3.3 hr, and then more slowly over the following 9 days, with an overall half-time of about 2.4 days. Inside residences during spraying, average concentrations were initially 2-5 times lower than outdoor concentrations. Five to 6 hours later, however, indoor concentrations exceeded outdoor concentrations, with an average of 244 CFU/m3 vs. 77 CFU/m3, respectively. Spray drift containing culturable Btk was detected throughout a 125- to 1,000-meter band outside the spray zone where measurements were made, a consequence of the fine aerosol sizes that remained airborne (count median diameters of 4.3 to 7.2 µm). Btk HD1 concentrations outside the spray zone were related to wind speed and direction, but not to distance from the spray zone (25).

The short-term health impact assessment consisted of surveys to determine if aerial applications of Foray 48 B resulted in changes in mental and physical health status reported by residents of Victoria and southern Vancouver Island. A randomly selected population (1009 individuals) was interviewed pre- and post-spray utilizing a symptom survey and a health status survey tool called the Short Form 12 Health Status Profile (SF-12) provided by Medical Outcomes Trust. Analysis of symptom reporting showed no significant differences in symptom reporting following the aerial spray application either when comparing pre-and post-spraying timeframes or whether location of residence inside or outside the spray zone. Based on the SF-12, there were no significant changes in physical health scores and a small improvement in the average mental health score post-spray for residents both inside and outside spray zone. The population surveys suggested that exposures to Btk HD1 did not result in measurable acute health effects in the general adult population (20).

A pre/post matched pairs cohort design was used to determine if aerially spraying of *Btk* HD1 was associated with an increase in the symptoms or change in the Peak Expiratory Flow Rate of children with asthma. Children living in the spray zone were matched with children outside of the spray zone. Peak Expiratory Flow Rates, asthma symptoms and non-asthma symptoms were recorded in diaries. There were no differences in asthma symptom scores between subjects and controls, neither before nor after the spray; nor were there significant changes in Peak Expiratory Flow Rates for subjects after the spray period. No evidence of adverse effects from the use of the biological pesticide was found. We believe that this is the first paper to address the issue of whether or not aerial spraying with *Btk* HD1 has a harmful effect on children with asthma (21).

In summary, short-term human health impacts resulting from exposure to aerial applications of Foray 48B to control European gypsy moth (*Lymantria dispar*) larvae have been conducted in the United States of America, New Zealand, and Canada. To date, no definitive evidence of adverse short-term health effects due to exposures to *Btk* have been reported.

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