Spatial and Temporal Distribution of Airborne *Bacillus thuringiensis* var. *kurstaki* during an Aerial Spray Program for Gypsy Moth Eradication

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We measured airborne exposures to the biological insecticide *Bacillus thuringiensis* var. *kurstaki* (*Btk*) during an aerial spray program to eradicate gypsy moths on the west coast of Canada. We aimed to determine whether staying indoors during spraying reduced exposures, to determine the rate of temporal decay of airborne concentrations, and to determine whether drift occurred outside the spray zone. During spraying, the average culturable airborne *Btk* concentration measured outdoors within the spray zone was 739 colony-forming units (CFU)/m^3 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 outdoors, but at 5–6 hr after spraying began, indoor concentrations exceeded those outdoors, with an average of 244 CFU/m^3 vs. 77 CFU/m^3 outdoors, suggesting that the initial benefits of remaining indoors during spraying may not persist as outside air moves indoors with normal daily activities. There was drift of culturable *Btk* 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* concentrations outside the spray zone were related to wind speed and direction, but not to distance from the spray zone. *Key words*: environmental exposure, environmental monitoring, insecticides, particle size, pesticide drift, pesticides. *Environ Health Perspect* 109:47–54 (2001). [Online 12 December 2000]
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The European gypsy moth (*Lymantria dispar*) was introduced to North America by Ettiene Trouvelot in the 1860s, and since that time has become an endemic defoliating pest in Canadian and American deciduous forests, mainly east of the Great Lakes (*1*). The western provinces and states have had occasional infestations, but the importance of the forest industry to the economies of Oregon, Washington State, and British Columbia has made local authorities quick to implement eradication programs to protect the forest resource. Historically, eradication programs have used insecticidal control methods that follow the pattern of pesticide use in North America, beginning with Paris green in the late 1800s, lead arsenate during the first half of the twentieth century, DDT in the post-World War II era, and then carbaryl and trichlorfon until the late 1980s (*1*). Most recently, a bacterial insecticide, *Bacillus thuringiensis* var. *kurstaki* (*Btk*) has been used widely against the gypsy moth. It is one of the few insecticides whose use is permitted on foodstuffs designated "organic," and was mentioned in Rachel Carson's seminal book *Silent Spring* as an "important answer to the problems of such forest insects as the budworms and the gypsy moth" (*2*).

In spring 1999, the government of British Columbia conducted an aerial insecticide spray program to eradicate gypsy moths in a populated area of southern Vancouver Island (greater Victoria) in the province of British Columbia. They used *Btk* in a formulation trade-named Foray 48B, which is registered by the Pest Management Regulatory Agency of Health Canada and the U.S. Environmental Protection Agency for aerial spraying over urban populations. The active ingredients of Foray 48B are the spores and proteinaceous delta-endotoxin crystal of *Btk* strain HD1, suspended in water at a concentration of 2.1% (*3*,*4*). The insecticidal activity is produced by the endotoxin when it is released in the alkaline environment of the gut of certain lepidopterans. It affects the gut permeability, eventually killing the insect (*3*).

Few published studies have examined human health outcomes or *Btk* exposure levels associated with spray programs (*5*,*6*). Thus, although *Btk* is a member of a new generation of highly specific biological pesticides expected to have few human health consequences, members of the public remain concerned. Therefore the Medical Health Officer responsible for the Victoria region set up a Human Health Surveillance Committee with membership from the Capital Health Region, the British Columbia Ministry of Health, Health Canada, the University of Victoria, the University of British Columbia, the British Columbia Medical Association, and the public. The Committee posed questions that guided the design of a series of studies to allow policy makers, health care providers, and members of the public to make decisions about risks that might accompany exposure to the aerial spray (*7*–*9*).

We report the results of one of these studies, an exposure study whose aims were to determine whether there was a difference in airborne concentrations of *Btk* between indoor and outdoor locations within the aerial spray zone; to examine the temporal gradient of airborne *Btk* concentrations after spraying; to determine whether there was drift of airborne *Btk* outside the aerial spray zone; and to characterize the size distribution of *Btk* aerosol within the aerial spray zone. The goal was to provide data that would allow public health authorities to advise the public about methods to minimize exposures.

Methods

Btk *air concentration measurements.* Samples for estimating "total" airborne culturable *Btk* counts were taken by pulling air through 2-µm pore-size Teflon filters (Teflo Membrane; Pall Gelman Laboratory, Ann Arbor, MI) mounted in 37-mm closed-face cassettes, using constant-flow, battery-powered, air sampling pumps (Aircheck 224-PCXR8 and 224-52; SKC Inc, Eighty-Four, PA). The pumps were calibrated to a flow rate of 2 L/min ± 5% before and, whenever possible, after sampling using a rotameter (FM-1052B-NV; Matheson Gas Products, Parsippany, NJ), calibrated in the laboratory using an automated soap-film flow meter (Gilibrator; Gillian Instrument Group, West Caldwell, NJ) as the primary standard. Approximately one field blank was included with every three field samples.

We took air samples to characterize the size distributions of the *Btk*-containing aerosols using size-selective, six-stage, microbial, cascade impactors (Graseby Andersen

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Instruments, Atlanta, GA) mounted with trypticase-soy-agar-filled plates (BBL; Becton Dickinson and Co., Cockeysville, MD). Air was drawn with a high volume pump (either a battery-powered Gillian AirCon2, Gillian Instrument Corp., or an AC-powered GE, model SKH33DN16GX, GE Motors, USA) at a flow rate of 28.3 L/min, maintained by a critical orifice placed after the sixth stage of the Andersen sampler. The Andersen viable sampler separates the following size ranges of aerosol: stage 1, ≥ 7 µm; stage 2, 4.7–7 µm; stage 3, 3.3–4.7 µm; stage 4, 2.1–3.3 µm; stage 5, 1.1–2.1 µm; and stage 6, 0.65–1.1 µm.

All air-sampling equipment was set on small portable tables 0.76 m above the ground.

Bacterial counts on the Teflon filters were quantified at the University of Victoria Molecular Microbiology Laboratory as described in detail elsewhere (*8*). In brief, the filters were placed face down on nutrient agar (NA; Difco Laboratories, Detroit, MI) and colonies were counted after 16–24 hr of incubation at 28°C. The Andersen sampler plates were also incubated for 16–24 hr at 28°C before counting. Colonies were picked and identified as belonging to the *Bacillus cereus* group by standard microbiological protocols and further speciated and confirmed as *Btk* HD1 by Random Amplified Polymorphic DNA (RAPD) analysis. Colonies identified as *Btk* HD1 were cultured in 96-well microtiter plates and analyzed for the presence of *cry*1A(a), *cry*1A(b), and *cry*1A(c) genes. These genes are characteristic of *Btk* HD1 and are found only rarely in other *B. thuringiensis* strains. Confirmation of the RAPD analysis and *cry*-gene specific polymerase chain reaction (PCR) was conducted by Dot-blot analysis with standard DNA hybridization techniques, using *Btk* HD1 and *B. cereus* as positive and negative controls, respectively.

We did not apply positive hole correction factors to the Andersen sampler counts because these were bacterial, not fungal, colonies and therefore less susceptible to overgrowth. Data analyses on a sample of the data, using positive hole correction factors, did not substantially alter the estimates of count median aerodynamic diameter.

We calculated concentrations of *Btk* colonies in air by dividing the colony count by the volume of air sampled, giving concentrations in colony-forming units (CFU)/m^3 of air.

Air sampling protocol. Aerial spraying was planned for three pairs of days approximately 10 days apart.

To assess whether there was a difference in concentrations of *Btk* between indoor and outdoor locations in the aerial spray zone, we randomly selected approximately 30 locations inside the spray zone from the Victoria telephone directory (as described below), and randomly designated each location for sampling during one of the three spray periods. At each location, one filter sample was taken inside the residence and one outdoors, during the aerial spraying. Residents were asked to keep their windows closed for the duration of indoor sampling. Air sampling began 2–3 min before spraying began, and lasted for 1 hr.

To assess whether there was drift of *Btk* outside the aerial spray zone, we selected approximately 30 locations outside the spray zone, and randomly designated each for sampling during one of the three spray periods. At each location, one filter sample was taken outdoors during spraying of the nearest area within the spray zone. Eighteen of the 30 samples were in locations downwind of the spray zone (based on the prevailing wind direction), 6 were in downtown Victoria (also downwind), and 6 were in other locations outside the aerial spray zone. Samples other than those from downtown Victoria were taken outside homes randomly selected from the Victoria telephone directory (as described below). All samples were located 125–1,000 m outside the aerial spray zone. Air sampling began 2–3 min before spraying began, and lasted for 1 hr.

We selected random sites inside and outside the spray zone using the following method: The 1999 Greater Victoria area telephone directory (white pages) was divided into sets of pages (1–530), columns (1–4), and rows (1–95). We randomly selected 478 number sets (page/column/row). Each such location was checked against a list of postal codes to identify whether or not it was in the spray zone. We identified 106 randomly selected sites as within the spray zone, and verified them by plotting each location on a map of the spray zone. The remaining 362 sites were also plotted on the map, to identify 50 sites within the defined area for measurements outside the spray zone. We sent residents at all randomly selected locations a letter of introduction to the study, and then phoned them within 2 weeks of the spray period to verify the location and the willingness of the subject to participate in the study. Informed consent was obtained from all subjects whose homes were entered.

Characteristics of the homes and the areas around the homes were recorded during the sampling period. For indoor samples, the data included whether windows and doors were open during spraying, the type of residence (house, apartment, townhouse), type of entry to the home (direct to outdoors, or through indoor hallway), the number of stories, the room in which measurements were taken, and the indoor temperature. For outdoor locations, the data included the distance to the nearest trees and buildings, their heights, the types of trees (conifers or deciduous), and the type of ground cover (pavement, grass, other). Outdoor temperature, relative humidity, wind speed, and wind direction on each sampling day were collected from the nearest of three meteorologic stations (maximum 10.8 km away).

To characterize the size distribution of *Btk* aerosol, 12 air samples were taken outdoors using the six-stage Andersen sampler, randomly distributed over the three spray periods. The sites were selected at random from the original measurement sites within the spray zone. Air sampling began when spraying began (i.e., plane spotted overhead), and lasted for 15 min. The Andersen samplers were placed adjacent to the filter samples. To determine the duration of sampling, we took test samples during spray trials on 9 April 1999. A series of three six-stage Andersen samples were taken for 5 min each. The first sample started at the time the airplane passed overhead (T0–T5), the second 2.5 min later (T2.5–T7.5), and the third 7.5 min later (T7.5–T12.5). Eighty percent of the bacterial colonies appeared in the upper two stages of the sampler in the T0–T5 sampling period; however, in the later samples 62% of the colonies were in the lowest four stages, representing aerosols < 5 µm, which settle very slowly. We estimated that 15-min samples could be conducted without overloading the plates, while allowing as much time as possible for the smaller aerosols to be captured.

To examine the temporal gradient of exposure, we randomly selected locations from the original measurement sites within the aerial spray zone for resampling at selected times. Twelve filter samples were taken outdoors at each of 6 times after spraying (beginning 2 hr, 5 hr, 9 hr, 1 day, 4 days, and 9 days after the start of spraying). Twelve samples were taken indoors at each of 2 times after spraying began (2 hr and 5 hr). The duration of air sampling was 1 hr.

To ensure insecticidal efficacy, aerial spray personnel normally measure spray deposition densities by counting droplets deposited on cards made of a glossy white thin cardboard, 11 cm x 11 cm (Kromecote cards; Champion International Corp., Stamford, CT). To examine the association between airborne *Btk* concentrations and aerosol sizes and Kromecote card droplet densities and sizes, we placed Kromecote cards at each of the 60 outdoor monitoring sites (inside and outside the spray zone) adjacent to the filter samples. The cards were left open to the air for the duration of the filter air sampling during aerial spraying (about 60 min). Droplet sizes and counts were

determined using a Meiji Techno SKC dissecting light microscope at 30 times magnification (Meiji Techno America, San Jose, CA).

Data analysis. We examined frequency distributions of the bacterial exposure concentrations, untransformed and log-transformed (base e), to determine whether the data approximated normal or log-normal functions. They indicated that the data were positively skewed, and were distributed more symmetrically when log-transformed; therefore we performed inferential analyses with the data so transformed.

Not all data were quantifiable; some samples had no growth (less than the concentration detection limit; left-censored) and others had too many colonies to count (right-censored). The distribution of the subset of data with quantitative measurements became the basis for imputing values for samples that were left- and right-censored. Values for samples less than the concentration limit of detection were randomly generated from the censored section of the distribution to the left of the limit of detection (~ 10 CFU/m^3), and values for samples too numerous to count were randomly generated from the censored section of the distribution to the right of the highest quantifiable concentrations (~ 1,600 CFU/m^3).

We calculated descriptive statistics (arithmetic and geometric means, geometric standard deviations, ranges, percent censored data) for the exposure measurements, stratified by location and time of sampling. Paired *t*-tests compared indoor and outdoor concentrations of *Btk* colonies at the same sites within the spray zone. Student's *t*-tests compared concentrations measured inside and outside the aerial spray zone. Multiple linear regression analyses examined the effect of time of sampling on the bacterial concentrations, while controlling for other factors (such as concurrent spraying in another area). Time of sampling was offered (in separate analyses) as a categorical variable and as a continuous variable.

We also examined other characteristics of the measurement locations (e.g., type of residence, type of landscaping, weather conditions) for their association with exposure levels. Correlations between independent variables were examined, and where Pearson r was > 0.7, only one variable was included in the analysis—the variable considered likely to be most directly related to exposure. Univariate relationships were examined using one-way analysis of variance (ANOVA) for categorical variables, and simple linear regression for continuous variables. Variables with $p < 0.25$ in univariate modelling were offered in multiple regression models, using a backward stepwise procedure with $p < 0.25$ to enter and $p < 0.10$ to remain in the model.

We estimated the size distributions of the *Btk* aerosol for each Andersen sample by plotting the cumulative *Btk* colony count against the upper aerodynamic diameter cutoff for each stage of the sampler. The 50th percentile, the "count median aerodynamic diameter," was read from the linear regression line for the relationship between the cumulative count and the aerodynamic diameter. We calculated the standard deviation (SD) of the distribution by subtracting the 50th percentile aerodynamic diameter from the 84th percentile (i.e., 1 SD in a normal distribution). It is reasonable to expect that the size distributions were log-normally distributed, so log-probability plots of the cumulative distributions were also done. This method gave nearly identical count median diameters. We calculated the geometric SDs of the distributions by dividing the 84th percentile aerodynamic diameter by the 50th percentile, read from the log-probability plot.

Simple linear regression, with Kromecote card densities as the independent variable and airborne *Btk* concentrations as the dependent variable, examined the association between these two variables.

All data analyses were conducted using SPSS (version 9.0; SPSS Inc., Chicago, IL).

Results

Aerial spraying of the Victoria area took place 3 times, spread over 2–3 days in each period: 8–9 May, 19–21 May, and 8–9 June 1999. Spraying was conducted before 0730 hr during wind-calm conditions with no precipitation. On most spray days, two DC-6 aircraft flying at 370 km/hr 61 m above the ground delivered the insecticide at 590 L/min with a target droplet size of 110–125 µm, at a target density of 35 droplets/cm^2, in spray swaths 228 m wide (Figure 1).

Participation. Table 1 indicates the participation rates inside and outside the spray zone. Several sites were considered ineligible because they were businesses or because the person identified in the telephone book no longer lived at the site or was moving. In addition, at about 10% of sites, no one could be contacted during the spray period. Of the residents at eligible and contacted sites, a higher proportion (74%) agreed to participate outside the spray zone than inside the spray zone (42%). We believe the reason is that outside the spray zone, only outdoor measurements were made, so the residents were not disrupted. Inside the spray zone, measurements were made indoors during spraying, and study personnel needed to enter homes between 0430 and 0530 hr. Other demands were made on these residents, including follow-up measurements at selected times after spraying. Despite the low participation rate in the spray zone, the sites sampled, both inside and outside the spray zone, were distributed according to the distribution of the Victoria population, as planned (Figure 1).

Description of air sample data. We had planned 186 filter samples, but took 193 due to changes in the scheduling of the aerial spraying. Of the 193 filter air samples taken, 8 were lost, and 10 were not used in the analyses because they were taken on days when spraying was called off at the last minute due to adverse weather conditions. Of 12 Andersen samples taken, 4 were excluded from data analysis because the appearance of the growth on the agar plates suggested that the impactor plates had not been properly seated, allowing air to bypass the sampler. Of 57 field blanks, 52 showed no *Btk* growth, three grew one or two colonies, one grew 11 colonies, and one had too many colonies to count. The laboratory reported that the overgrown plate resulted from laboratory contamination. No blank corrections were made.

Airborne concentrations outdoors and indoors in the aerial spray zone. Table 2 describes the concentrations of *Btk* colonies in the spray zone during spraying, outdoors and indoors. Concentrations outdoors ranged from below the detection limit to too numerous to count, with an arithmetic mean of 739 *Btk* CFU/m^3 and a geometric mean of 157 *Btk* CFU/m^3. Indoors the concentrations of *Btk* were 2.3–4.6 times lower than outdoors (depending on whether arithmetic or geometric means are compared). None of the indoor samples were too numerous to count, whereas 18% of the outdoor samples were. Paired comparisons of the log-transformed data at sites where measurements both indoors and outdoors were available (n = 27) indicated that outdoor concentrations were statistically significantly higher than indoor concentrations. *Btk* concentrations indoors and outdoors were correlated (Pearson r = 0.65): locations with higher outdoor concentrations tended to have higher indoor concentrations.

Temporal gradient of airborne concentrations in the aerial spray zone. Table 2 and Figure 2 indicate the temporal trends in airborne concentrations during and after spraying within the spray zone, outdoors and indoors. The data indicate that the highest exposures occurred from the start of spraying up to 3 hr later. Both outdoors and indoors, exposures tended to be higher during the 2–3 hr after the start of spraying than during spraying, though this was not statistically significant (p = 0.23, 0.15, respectively). After that, the outdoor concentrations decreased exponentially with time. Indoors, there was no clear pattern; in fact at 5–6 hr after spraying, the concentrations indoors exceeded those outdoors.

The outdoor data indicated that exposures increased again one day after the spraying. This was likely a result of the fact that in 9 of the 10 locations sampled at this time, spraying occurred concurrently in an adjacent zone.

A multiple linear regression analysis indicated the following temporal relationship for the outdoor data within the spray zone, over the 9 days after spraying:

$$\ln b = 4.63 - 0.27t + 1.38s, \quad [1]$$

where b = *Btk* concentration (CFU/m^3), t = time since spraying (days), and s = concurrent spraying in adjacent zone (1 = yes, 0 = no). This model explained 21% of the variance in outdoor exposures within the spray zone (R^2 = 0.21; $p < 0.001$). Because the pattern of temporal degradation outdoors appeared to have two phases, we also separately modeled the data in the first 10 hr after spraying, producing the following relationship:

$$\ln b = 5.29 - 5.01t, \quad [2]$$

confirming a much quicker decrease in concentration immediately after spraying. This model explained 11% of the variance in outdoor exposures within the spray zone in this time period (R^2 = 0.11; $p < 0.007$). Modeling of the indoor data did not produce any significant patterns, whether the time since spraying was used as a continuous or a categorical variable.

Drift outside the aerial spray zone. Comparisons of *Btk* concentrations inside and outside the aerial spray zone during spraying indicate no significant differences, suggesting that there was drift outside the spray zone, at least into the area 125–1,000 m away from the planes' aerial spray (Table 3). Although arithmetic mean concentrations were slightly higher inside the spray zone (739 vs. 484 CFU/m^3), geometric mean concentrations were slightly higher outside the spray zone (174 vs. 157 CFU/m^3). The differences were not statistically significant.

To further examine the issue of drift, we used linear regression analyses to examine the relationship between distance from the aerial spray zone and *Btk* concentrations, first using only data outside the spray zone and then all outdoor data, in two separate analyses. We found no relationship between the bacterial concentrations and distance ($p > 0.70$). Additional factors relevant to drift, particularly wind speed and direction, are discussed below.

Other characteristics related to Btk concentrations. We also examined the relationships between *Btk* concentrations and characteristics such as the type of residence (house, townhouse, apartment), the environment surrounding the residence, the location sampled inside the home, and weather conditions. These relationships were examined only for samples taken during aerial spraying.

Outdoor *Btk* concentrations inside and outside the spray zone during spraying were related to outdoor temperature and relative humidity and, outside the spray zone only, the wind speed vector [equivalent to wind speed for sites downwind and –(wind speed) for sites upwind of the spray zone]. A multiple linear regression analysis indicated the following relationship:

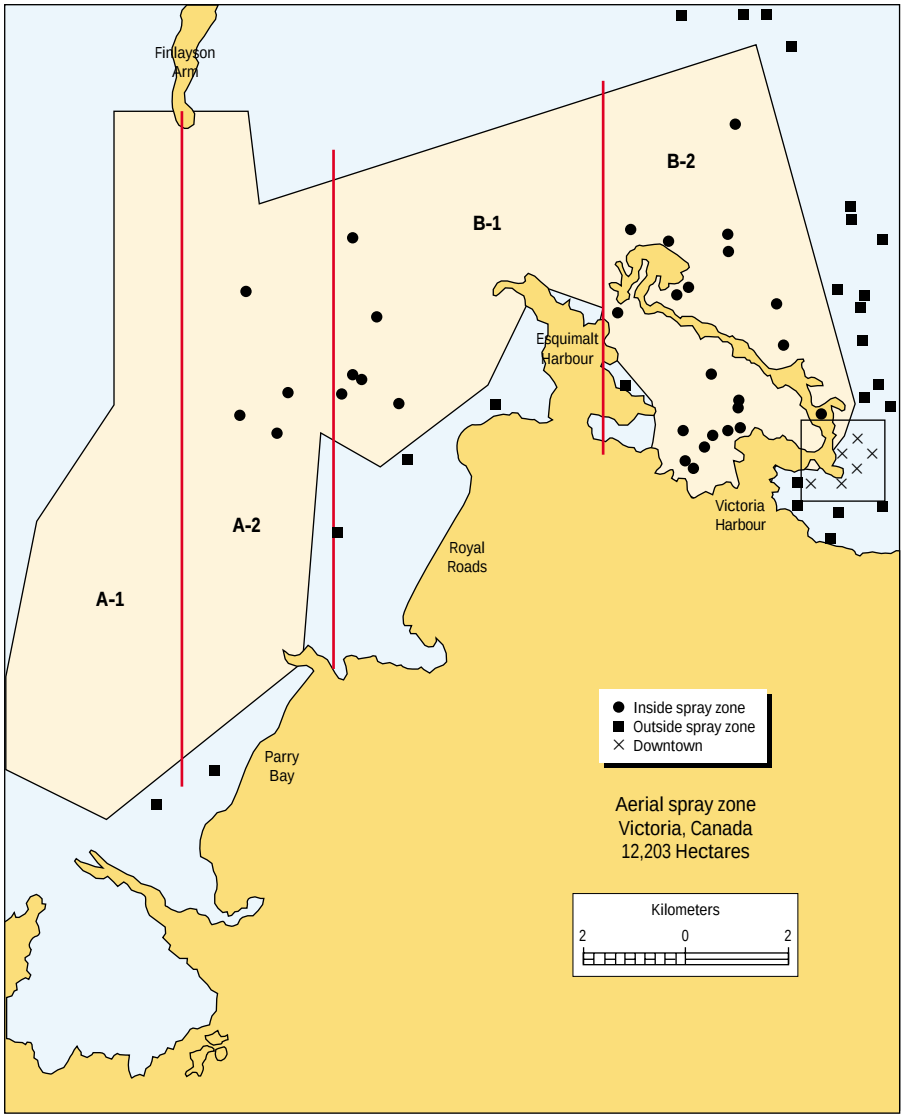

Figure 1. Map indicating sites where air samples were taken inside and outside the aerial spray zone (scale is approximate). The spray zone is the pale yellow area. North is at the top of the figure; the prevailing winds are to the northeast. A-1, A-2, B-1, and B-2 indicate subzones covered by a single plane in a single morning's aerial spraying. On most spray days, two planes operated, with one subzone separating them.

Table 1. Participation in the study.

	Inside spray zone		Outside spray zone	
	No.	(%)	No.	(%)
Randomly selected sites	106		50	
Not a residence	1		2	
Phone number incorrect or not in service	6		3	
Moved/moving	10		2	
Unable to contact	11		5	
Eligible and contacted	78		38	
Participated in study	33	(42)	28	(74)
Unwilling to participate	45	(58)	10	(26)

$$\ln b = 23.1 + 0.34c - 0.24h + 0.056v, \quad [3]$$

where c =outdoor temperature (°C), h = relative humidity (%), and v = wind speed vector (km/hr; nested variable, outside the spray zone only). This model explained 18% of the variance in outdoor exposures during aerial spraying ($R^2 = 0.18$; $p < 0.01$). It indicates that when the weather was warmer and drier, *Btk* concentrations were higher. The mean temperature during spraying was 8.6°C (SD = 2.8°C). The mean relative humidity during spraying was 79% (SD = 10%). In addition, locations outside the spray zone upwind of the aerial spraying (n = 5) had lower *Btk* concentrations on average than did those in the spray zone, and those downwind (n = 22) had higher concentrations. This trend increased with increasing wind speed. The mean wind speed during spraying was 10 km/hr (SD = 5.8 km/hr).

No other characteristics of the outdoor locations were related to airborne *Btk* concentrations: distance to and heights of nearest trees or buildings; whether nearby trees were deciduous or coniferous; type of ground surface; barometric pressure; distance vector from the spray zone [equivalent to distance for sites downwind and –(distance) for sites upwind of the spray zone]; or wind speed within the spray zone. None of the measured characteristics of the indoor environment were related to indoor *Btk* concentrations: type of residence; type of entry; story on which sampling was conducted; whether any window or doors were open; room where sampling was done; indoor temperature.

Size distribution of the Btk aerosol. The data from the size-selective Andersen samplers are presented in Table 4. *Btk* aerosols that were present in the air for 15 min after the start of spraying had count median aerodynamic diameters of 4.3–7.3 µm, with SDs of 2.6–3.8 µm. These aerosol sizes are not visible to the human eye, and are tiny enough to reach the small airways of the respiratory tract.

The aerodynamic diameters are consistent within days, suggesting that nozzle sizing, flight speeds, or weather conditions altered the size distributions between days. There were too few data for statistical analyses, but weather data for those days suggest that smaller aerosol diameters may be associated with higher wind speeds and lower relative humidities.

The diameters of droplets deposited on the surfaces of Kromecote cards ranged from 60 to 120 µm at the same sites (and from 50 to 150 µm over all study sites), much larger than the aerodynamic diameters measured by the Andersen samplers. The reasons for the differences are likely several-fold. The Kromecote cards passively received aerosols that fell from the air and were deposited on surfaces within 1 hr of the beginning of spraying (i.e., ~ 25 µm or greater; Table 5). In addition, the diameters measured on the Kromecote cards were not aerodynamic diameters but visual diameters of droplets. These diameters would be highly dependent on the interaction between the surface tension of the Foray 48B aerosols and the card's coating and humidity. This sizing method would not be expected to give uniform results under all conditions. In contrast, Andersen samplers actively drew aerosols from the air over 15 min and aspirated aerosols that did not settle quickly (i.e., ~ < 50 µm; Table 5) (*10*). Theoretically, all large aerosols should be captured on the top stage of the Andersen sampler, but because its sampling characteristics at sizes above 10 µm are not known, we cannot be certain that the sampling efficiency for larger particles was as good as for finer aerosols.

In a linear regression between the airborne *Btk* concentrations on filter samples and the density of the Foray 48B formulation deposited on the surface of Kromecote cards outdoors during spraying, *Btk* concentrations in air increased when Kromecote

Table 2. Concentrations of *Btk* in the aerial spray zone, outdoors and indoors, during spraying and at various times after spraying.

Time	Outdoors	Indoors
During spraying		
No.	33	27
Minimum to maximum (CFU/m^3)	< LOD– > TNTC	< LOD–627
percent samples < LOD	15	11
percent samples > TNTC	18	0
Arithmetic mean (CFU/m^3)	739	159
Geometric mean (CFU/m^3)	157[a]	69.7[a]
Geometric SD	10	4.6
2–3 hr after spraying began		
No.	12	11
Minimum to maximum (CFU/m^3)	24– > TNTC	16– > TNTC
percent samples > TNTC	8	9
Arithmetic mean (CFU/m^3)	501	395
Geometric mean (CFU/m^3)	239	155
Geometric SD	4.5	4.6
5–6 hr after spraying began		
No.	11	10
Minimum to maximum (CFU/m^3)	< LOD–277	24–814
percent samples < LOD	9	0
Arithmetic mean (CFU/m^3)	77.7	245
Geometric mean (CFU/m^3)	38.6	131
Geometric SD	3.5	3.4
9–10 hr after spraying began		
No.	10	
Minimum to maximum (CFU/m^3)	< LOD– > TNTC	
percent samples < LOD	20	
percent samples > TNTC	10	
Arithmetic mean (CFU/m^3)	162	
Geometric mean (CFU/m^3)	28.8	
Geometric SD	6.1	
1 day after spraying		
No.	10	
Minimum to maximum (CFU/m^3)	25– > TNTC	
percent samples > TNTC	30	
Arithmetic mean (CFU/m^3)	532	
Geometric mean (CFU/m^3)	243	
Geometric SD	4.8	
4 days after spraying		
No.	12	
Minimum to maximum (CFU/m^3)	< LOD–57	
percent samples < LOD	25	
Arithmetic mean (CFU/m^3)	27.1	
Geometric mean (CFU/m^3)	19.2	
Geometric SD	2.6	
9 days after spraying		
No.	12	
Minimum to maximum (CFU/m^3)	< LOD–94	
percent samples < LOD	42	
Arithmetic mean (CFU/m^3)	22.3	
Geometric mean (CFU/m^3)	11.3	
Geometric SD	3.3	

Abbreviations: LOD, concentration limit of detection (< 10 CFU/m^3); TNTC, too numerous to count (> 1,600 CFU/m^3).
[a]Paired *t*-test for differences, $p = 0.029$; paired correlation = 0.65.

card densities increased, but the relationship was very weak and nonsignificant ($n = 56$, $R^2 = 0.014$, $p > 0.10$).

$$\ln b = 4.9 + 0.017k, \quad [4]$$

where k = Kromecote card density (drops/cm^2). Examination of scattergrams showed that there were many "zero" Kromecote card densities with high concentrations of airborne *Btk* outside the spray zone, indicating that the large aerosols captured by the Kromecote cards were less likely to drift outside the spray zone (only 9 of 27 sites outside the spray zone had Kromecote card densities > 0), yet the smaller aerosols captured by the air samples did drift (24 of 27 air samples had detectable *Btk* concentrations).

Discussion

The range of culturable *Btk* concentrations measured outside residences in Victoria during aerial spraying ranged from < 10 CFU/m^3 to > 1,600 CFU/m^3, with an arithmetic mean of 739 CFU/m^3 and a geometric mean of 157 CFU/m^3. Inside residences, concentrations during aerial spraying ranged from < 10 CFU/m^3 to 627 CFU/m^3, with an arithmetic mean of 159 CFU/m^3 and a geometric mean of 70 CFU/m^3. In the only other study of airborne exposures to *Btk* published in the scientific literature to date, Elliott et al. (5) reported levels ranging from "zero" to 11,000 CFU/m^3, with a median value of 300 CFU/m^3. Their study was also conducted during aerial spraying, and used sampling and analytical methods similar to those in this study. The highest levels reported were among "card checkers" in the aerial spray zone.

Staying indoors during spraying effectively lowered exposures during aerial spraying; however, this benefit was not sustained over time. Data from air samples taken 2–3 hr after spraying began indicated that the concentrations indoors soon approached those outdoors, with arithmetic means of 395 CFU/m^3 indoors versus 501 CFU/m^3 outdoors, and geometric means of 155 CFU/m^3 indoors versus 239 CFU/m^3 outdoors. The migration of outside air to the indoors may have resulted from the movement of family members and study personnel in and out of the residences in the intervening period. Study personnel in particular may have transported entrained *Btk*, because they remained outdoors during the spray period. Natural air infiltration would also have contributed to migration of *Btk* indoors, but we do not know to what extent. In investigations examining passive infiltration into typical homes and office buildings, air exchange rates of 0.07–0.39/hr have been reported, representing complete exchange of air with the outside environment in 2.5–14 hr (*11–13*).

At 5–6 hr after spraying began, concentrations had diminished, but indoor concentrations now exceeded those outdoors, with arithmetic means of 244 CFU/m^3 indoors versus 77 CFU/m^3 outdoors, and geometric means of 131 CFU/m^3 indoors versus 39 CFU/m^3 outdoors. This result may reflect the potential for *Btk* spores to be killed by ultraviolet (UV) light from the sun outdoors, whereas little UV light is present inside homes. It may also reflect the possibility that aerosols infiltrating homes may be the smallest ones and therefore settle out of the air very slowly.

Sampling outdoors continued at several additional periods after spraying began: at 9–10 hr and 1, 4, and 9 days. These data indicated that airborne concentrations outdoors diminished quickly with time, with average concentrations less than one-fifth of the highest mean levels within 5–10 hr after spraying began, and less than one-tenth of the highest mean levels within 4 days of spraying. Using all 9 days of temporal data suggested the half-time of airborne *Btk* outdoors was 2.4 days (95% confidence interval:

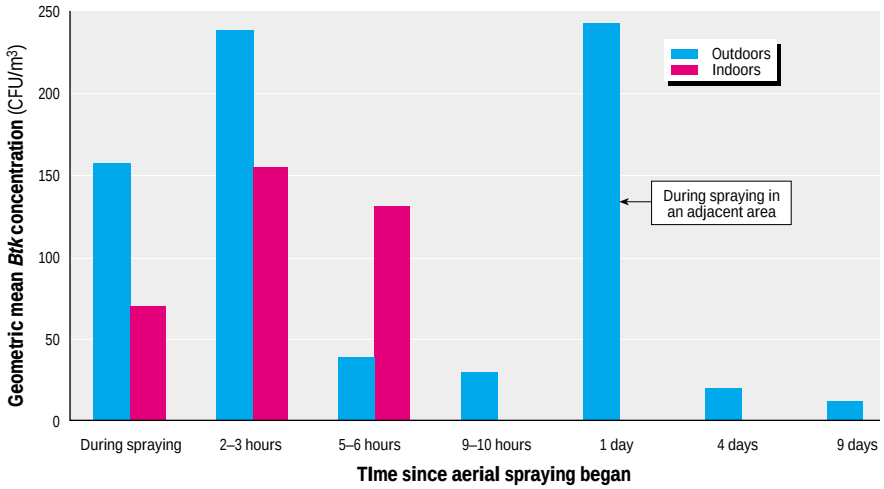

Figure 2. Geometric mean *Btk* concentrations indoors and outdoors within the aerial spray zone, at various times during and after the spraying.

Table 3. Concentrations of *Btk* inside and outside (125–1,000 m) the aerial spray zone, outdoors during spraying.

Measures	Inside spray zone	Outside spray zone
	($n = 33$)	($n = 27$)
Minimum to maximum (CFU/m^3)	< LOD–> TNTC	< LOD–> TNTC
Percent samples < LOD	15	7
Percent samples > TNTC	18	25
Arithmetic mean (CFU/m^3)	739	484
Geometric mean (CFU/m^3)	157[a]	175[a]
Geometric SD	10	5.8

Abbreviations: LOD, concentration limit of detection (< 10 CFU/m^3); TNTC, too numerous to count (> 1,600 CFU/m^3).
[a]*t*-Test for differences, $p = 0.84$.

Table 4. Size distributions of aerosol at Andersen sampler measurement locations, as measured by *Btk* count distributions on Andersen sampler stages, and by reading droplet sizes on adjacent Kromecote cards.

Date sampled	Andersen sampler CMAD (SD) (µm)	Kromecote card droplet sizes (µm)	Temperature (°C)	Relative humidity (%)	Wind speed (km/hr)
May 8th	5.8 (2.9)	80-110	5	85	7.4
May 9th	4.3 (2.9)	ND	3	84	9.5
May 9th	4.6 (2.8)	NS	3	84	9.5
May 20th	7.2 (3.7)	60-120	7	92	2.6
May 20th	7.3 (3.8)	ND	7	92	2.6
June 8th	5.4 (2.8)	80–120	10	87	11.1
June 8th	5.3 (2.6)	80–120	10	87	11.1
June 9th	5.3 (2.8)	60–120	8	87	6.4

Abbreviations: CMAD, count median aerodynamic diameter; ND, no droplets on card; NS, no sample taken. Note that estimated geometric SDs fell within a narrow range: ~ 1.65–1.75.

1.8–4.5 days). However, if data from only the initial 10 hr from the time of aerial spraying was used, the predicted half-time of airborne *Btk* outdoors was much shorter: 3.3 hr (95% confidence interval: 1.9–11.4 hr). The initial quick diminution of *Btk* may have resulted from settling of the larger aerosols and the killing of spores by UV light. The contrast in the two half-time predictions suggests that the airborne *Btk* concentrations diminish in two phases: a quick initial phase, and then a slower phase in which UV-resistant spores are diluted by uncontaminated air or removed by slow settling of the fine aerosols. Half-times may also appear slower if surface-deposited Foray 48B dries over time, allowing resistant spores to be reentrained into the air by the wind. UV-resistance of some spores could result from better coverage by sunscreens in the Foray 48B matrix.

Reviews of the *Btk* literature have reported losses in viability consistent with the shorter half-time calculated in this study. Ellis (*14*) described a laboratory study that found that 50% of the bacterial cells were inactivated within 30 min of UV light exposure. Otvos and Vanderveen (*3*) described a study that found that spores and crystals were almost completely inactivated after 12 hr of UV exposure. The *Btk* used in the reported studies was not protected within a Foray 48B formulation.

The data from sites outside the spray zone indicated that there was drift of culturable *Btk* throughout a 125–1,000-m band around the spray zone. Airborne concentrations in this zone increased in the downwind direction with increasing windspeed. Further evidence of drift outside spray areas came from the temporal samples taken one day after spraying. Nine locations sprayed on the first of two spray days were resampled one day later as spraying took place in adjacent zones. These samples had geometric mean *Btk* concentrations of 307 CFU/m^3, higher than the geometric means inside the spray zone during spraying (157 CFU/m^3).

Drift of culturable *Btk* outside the spray zone is expected, given the size distributions of the aerosols measured by the Andersen sampler in the 15 min following the start of spraying. These aerosols had count median aerodynamic diameters of 4.3–7.2 µm, so would be expected to remain suspended in the air for hours to days and be available to follow wind currents in the area. The extent of drift was not anticipated given the target droplet size of 110–125 µm, large enough to settle to the ground in < 5 min. Why were some *Btk* aerosols so much smaller than planned? The most likely explanation is that large aerosols produced by the nozzles were broken up immediately after their release by air pressures created by the speed of the planes. It is also possible that water evaporated from the aerosols during their descent decreasing the droplet sizes; this idea is supported by evidence that airborne *Btk* concentrations were higher with lower relative humidities and higher temperatures. Finally, it is possible that the applicator nozzles produced different size distributions than planned. Evidence from the Kromecote cards showed that the size range of the fast-settling droplets was 50–150 µm visual diameter, suggesting that droplet sizes occurred that were not only smaller, but larger than planned.

A difficulty with this study is that, as yet, no standard method exists for air sampling of bacterial spores in a pesticide formulation. We compared the airborne *Btk* concentrations measured by the Andersen sampler at eight locations to those measured by filter samplers at the same locations. The Andersen sampler and filter sampler concentrations were moderately correlated (Pearson r = 0.56, R^2 = 0.31), but on average the filter sample concentrations were 4.4 times lower. Reasons for this difference could be 2-fold: filter sampling may allow desiccation of the sample, and kill spores that would remain viable on the agar plates in the Andersen sampler; and the duration of sampling for the Andersen sampler was only the first 15 min after spraying began—a time when concentrations may be highest—whereas the filter samples were taken for 1 hr.

The one previously published exposure study used the same filter sampling and plating method as this study, and found similar average concentrations but a higher quantified range of exposures (to a maximum of 11,000 CFU/m^3) (*5*). To quantify these high concentrations, the investigators used lower air flow rates and shorter sampling durations, but they did not report a concentration limit of detection. In our study, the sampling and microbial analysis methods resulted in 10% of plates having too many colonies to count and 12% of samples below detection limits. Quantitative values for these samples were imputed allowing statistical analyses to include the censored samples, with values in their likely range. The random selection of values would randomly misclassify exposures within the censored regions. Imputing values would increase the power of the study to detect true differences by allowing analysis of more data; however, the misclassification would produce lower power than true quantitative measurements.

Table 5. Estimates of terminal settling velocities and times to settle from plane height of aerosols with various aerodynamic diameters [adapted from Hinds (*10*)].

Aerodynamic diameter (µm)	Approximate terminal settling velocity (cm/sec)	Approximate time to settle from plane height of ~ 61 m	Comments
1	0.0035	20 days	May not settle because normal air movement is faster than settling velocities; not visible to the naked eye
5	0.074	1 day	
10	0.31	5 hr	
25	1.9	1 hr	
50	7.4	14 min	
75	17	6 min	
100	25	4 min	Design size range, i.e., sizes meant to be delivered in aerosol spraying
150	47	2 min	

Conclusions

The results of this study indicate that drift outside the spray zone occurred because fine aerosols were produced during aerial spraying, despite target droplet sizes > 110 µm. Studies examining fine aerosol sizes and drift do not appear to have been reported elsewhere in the pesticide or agricultural literature. Detailed investigation of drift in other aerial pesticide application programs is warranted, with a specific focus on possible determinants of fine aerosol production such as plane speed, wind speed, humidity, and temperature. In spray operations where fine aerosols are produced, it would be valuable to investigate how far beyond the spray area the pesticide can drift, and what factors influence the drift distance.

Although this study provided evidence that exposure levels differed indoors and outdoors, the pattern of these differences was unexpectedly complex. The initial protection offered by indoor environments was not maintained within several hours after spraying, because indoor concentrations did not appear to dissipate as quickly as those outdoors. Factors that may influence indoor *Btk* concentrations and their longevity should also be investigated, e.g., UV light intensity, aerosol size distributions, mechanisms of air infiltration, and weather conditions.

References and Notes

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