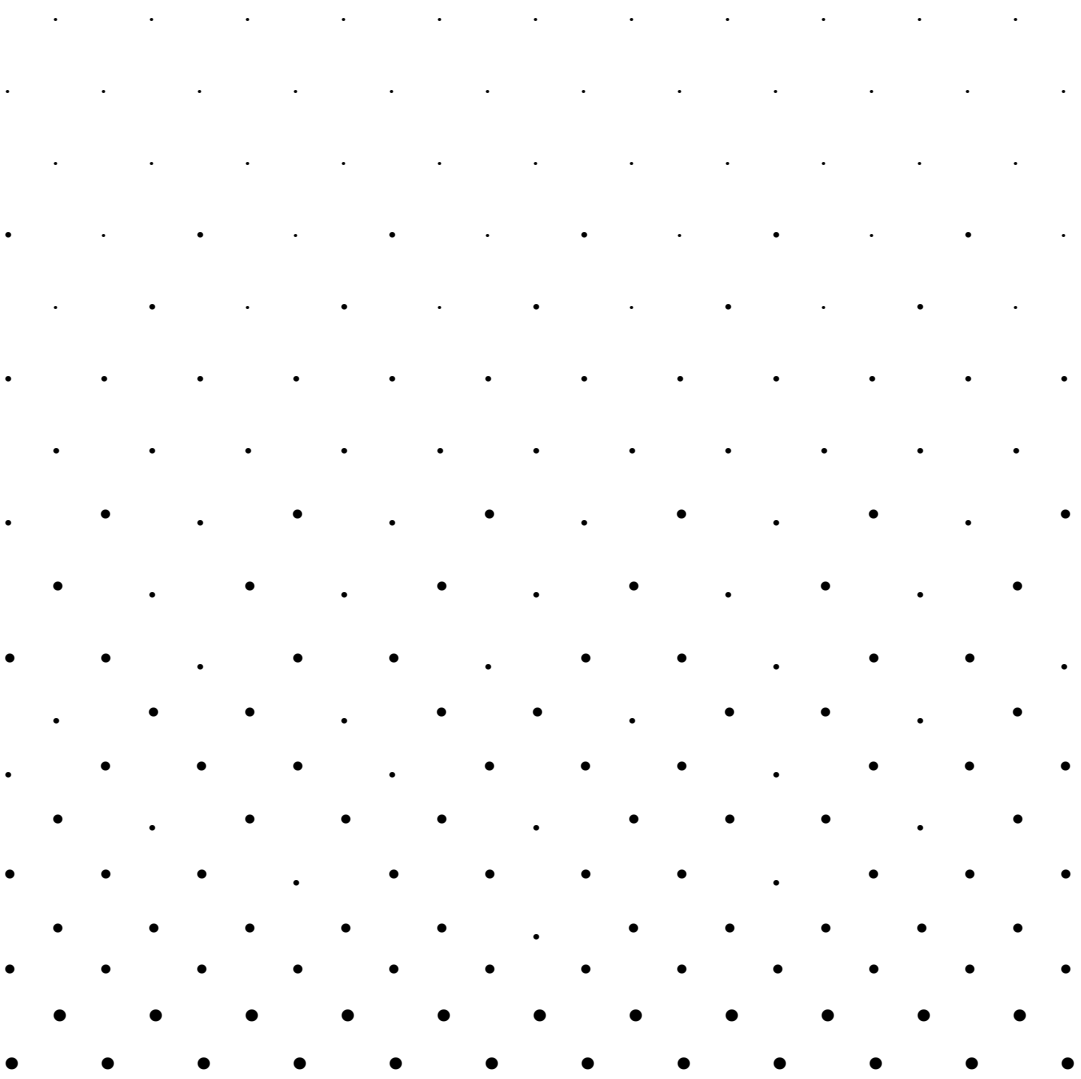
Airborne Exposures to *Bacillus thuringiensis* var. *kurstaki* During Gypsy Moth Eradication



Airborne Exposures to *Bacillus thuringiensis* var. *kurstaki* During Gypsy Moth Eradication

Final Report to the Capital Health Region

May 2000

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Summary

In the spring of 1999, the Ministry of Forests conducted an aerial spray program to eradicate gypsy moths in the southern region of Vancouver Island, using Foray 48B, a biological insecticide. This report presents the results of an exposure study which was part of a larger health study undertaken to gather data which would be of use for policy makers, health care providers and members of the public to make decisions about risks which may accompany exposure to the aerial spray.

In one part of the exposure study, we examined the undiluted insecticide in the laboratory to determine whether there were ingredients that could be measured in future field studies, and to attempt to answer questions posed by the public regarding chemicals in the insecticide formulation. In the other part of the exposure study, we measured airborne constituents of the Foray 48B during and after spray runs conducted in the Capital Regional District.

The questions asked in the laboratory study were:

- Could the inert ingredients in the formulation easily be identified?
- Were any of the inert ingredients volatile compounds which could be used as surrogate indicators of exposure?

The questions asked in the field exposure study were:

- Was exposure reduced if one stayed indoors with the doors and windows closed during spraying?
- Was there drift of insecticide droplets outside the spray zones?
- What was the size distribution of the spray droplets, and were the droplets small enough to enter the respiratory tract of people who might be in the area at the time of spraying?

Ingredients of Foray 48B

Foray 48B is registered for aerial spraying over urban populations by the Pest Management Regulatory Agency of Health Canada. The Material Safety Data Sheet for Foray 48B lists as the active ingredients the spores and delta endotoxin crystal produced by the bacteria, *Bacillus thuringiensis* var. *kurstaki* (*Btk*) HD1 strain. Only the spores and endotoxin crystal, not the bacterial cells, are present in the insecticide. In addition to the *Btk* there are other, inactive (so-called "inert") ingredients, not further identified by the manufacturer.

The inert ingredients of Foray 48B were examined to determine whether any of the constituents were volatile, that is, whether the liquid chemical would act more like a gas (like air) than a liquid (like water) at outdoor and indoor temperatures. Samples of the air immediately above the surface of the insecticide liquid in a closed container were analyzed by gas chromatography/mass spectrometry (GC/MS). Thirty-eight of the chemicals isolated by this method were identified using standardized, computer-assisted libraries of chemical compounds. Of potential interest were sydnones which may give colour to the product, trimethyl phosphine which may be a contributor to the characteristic unpleasant odour of

Foray 48B, and butylated hydroxy toluene and benzoic acid which may be used as preservatives in the *Btk* suspension media.

None of the volatile chemicals identified in the bulk sample were measurable in the field trials at detection limits in the parts per million range (ppm), the smallest concentration which would be detected using available sampling and analysis techniques.

Analyses for non-volatile components of the insecticide were conducted using high performance liquid chromatography (HPLC). These analyses indicated a complex spectrum of constituents. This is to be expected from Foray 48B's suspension of spores, spore components, endotoxin, additives, wetting agents, sunscreens, and culture media. An extensive research program would be required to identify the compounds in the HPLC analyses.

Air concentrations of *Btk* during and after aerial spraying

The air concentrations of *Btk* during the aerial spraying were measured by collecting airborne droplets containing *Btk* spores, and counting the colonies of bacteria which grew from the spores when cultured under appropriate conditions. During spraying, the average culturable airborne *Btk* concentration measured outside residences in the Victoria spray zone was 739 colony forming units per cubic meter of air (CFU/m^3). We expect that these concentrations may be underestimated because of some limitations of the methods used for quantification of the colonies.

Additional outdoor samples were taken up to 9 days after spraying. Outdoor air concentrations decreased over time. These outdoor samples suggested that airborne *Btk* concentrations diminish in two phases. There is an initial phase in which the concentration declined to only half of the original concentration in a few hours (during which time larger droplets may have settled and susceptible *Btk* may have been killed by UV light). This was followed by a slower decrease in concentration (in which spores may have been diluted by uncontaminated air or removed by slow settling of the smaller droplets).

Inside residences during spraying, average concentrations were initially 2.3 to 4.6 times lower than outdoors, but at 5-6 hours after spraying began, indoor concentrations exceeded those outdoors, with an average of 244 CFU/m^3, possibly because droplets which travelled indoors were smaller and settled more slowly or because killing of spores by UV light is less likely inside homes. The presence of *Btk* indoors may have resulted from the movement of family members and study personnel in and out of the residences after the end of the spray period. Indoor measurements were discontinued 6 hours after spraying began.

Drift of *Btk* outside the spray zone

Measurements of airborne *Btk* were made up to 1,000 meters (1 kilometer) outside the spray area. There was drift of culturable *Btk* throughout the 125- to 1000-meter band where measurements were made. Upwind sites usually had *Btk* concentrations lower than those within the spray zone, but sites downwind of the zone usually had higher concentrations than those in the spray area. This effect increased as wind speeds increased.

Drift of culturable *Btk* outside the spray zone is expected given the presence of very fine spray droplets measured in the 15 minutes following the start of spraying. The average sizes of the droplets that remained airborne were 4.3 to 7.2 microns ($1/1000^{th}$ of a meter), much smaller than the 50 to 150 micron droplets which quickly deposited on surfaces inside the spray zone (measured on "Kromecote cards"). These small airborne droplets are not visible to the human eye and can be inhaled into the small airways of the respiratory tract. Factors which may produce smaller droplet sizes may include higher plane speeds, higher wind speeds, and lower relative humidities.

Recommendations

The results of this study suggest the following ideas for the future:

- The field exposure study indicated that not only volatile components of the Foray 48B are potentially inhaled. Fine spray droplets which stay suspended in air include all components of the insecticide formulation, and can be inhaled. Therefore identification of all agents in the formulation is important. A cost-effective method to ease public concerns about the constituents of the formulated Foray 48B would be release of this information by the manufacturer.
- During the spray period, staying indoors with all doors and windows closed resulted in exposures lower than those outdoors. However, exposures indoors increased within 3 hours after spraying and were higher than outdoor concentrations by 5 to 6 hours after spraying began. Indoor concentrations appeared to dissipate much more slowly than outdoor concentrations. In future exposure studies, indoor concentrations should be measured for up to 9 days after spraying to determine the half-time of the *Btk* in indoor environments. Studies should also attempt to assess how factors such as movement of people in and out of buildings affect indoor *Btk* concentrations.
- Drift of the *Btk* droplets was detected throughout a zone up to 1 km away from the spray area. Future studies should measure air concentrations much more distant than 1 km away from the spray zone, to allow estimation of the maximum drift distances. Some factors which contribute to drift, such as wind speed, temperature, and relative humidity, were detected in this study. Studies examining these and other potential explanatory factors (e.g., plane speed) would be valuable.
- Kromecote cards were not an effective indicator of airborne exposures to *Btk*. Future studies examining personal exposures should use air sampling techniques rather than surface deposition techniques to estimate air concentrations of *Btk*.

Acknowledgements

We would like to extend our appreciation to the residents of the study locations, for their kind participation and their willingness to welcome study personnel into their homes before dawn.

We are very grateful to Marty Pearce and Christine Bender for all the logistical arrangements they made on our behalf, and to the air sampling personnel, who had to rise at times when the rest of the world was sleeping and change their plans according to vicissitudes of the aerial spray schedules: Kathy Barilla, Sara Bates, Simon Cowell, Mark Crawford, Trina Demonye, Michael Dykstra, David Lawes, Brett Mawson, Michael McKinley, Dan Moreau, Vicky Naish, Marty Pearce, Katya Ponich, Cindy Ruttan, Casey Stamps, Erica Thorleifson, Simon Ting, Diane Tyler, Mary Wagner, Doug Wilde, and Ian Wong.

This study was funded in part by the Capital Health Region.

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1. Introduction

In the spring of 1999, the Ministry of Forests conducted an aerial insecticide spray program to eradicate gypsy moths (*Lymantria dispar*) in the southern region of Vancouver Island, using Foray 48B, a biological pesticide registered for specific uses by the Pest Management Regulatory Agency of Health Canada. The active ingredients of Foray 48B are the spores and proteinaceous delta-endotoxin crystal of *Bacillus thuringiensis* var. *kurstaki* (*Btk*) strain HD1, suspended in water at a concentration of 2.1% [Otvos and Vanderveen, 1993; Abbott Laboratories, 1999]. 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 [Otvos and Vanderveen, 1993].

The formulated Foray 48B suspension includes a number of additives: thickening agents; wetting agents; phagostimulants (e.g., molasses and sugar, to enhance ingestion); anti-vapourants; stickers (used to enhance retention of spray deposits); and sun screens. The manufacturer considers the identity of these "inert" (non-insecticidal) ingredients proprietary, and has not released their identity in the Foray 48B Material Safety Data Sheet or in other public domain information [Abbott Laboratories, 1999].

To date there have been few studies of human exposures to *Btk* during application [Elliott et al., 1988; Thorogood et al., 1996; Noble et al., 1992]. To our knowledge, there have been no studies of exposures to the "inert" ingredients. Because the 1999 aerial spraying involved treating residential areas, members of the public were concerned about potential health effects of exposures. As a condition of a provincial Order in Council permitting the spraying, the Ministry of Forests was required to fund a series of studies, under the aegis of the Capital Region Health Board, to investigate health effects and potential exposures associated with the spraying.

This report presents the results of a laboratory study of the "inert" ingredients of Foray 48B and a field study of airborne concentrations of *Bacillus thuringiensis* var. *kurstaki* HD1 during the spray period. The specific aims of these studies were

- to determine whether there were compounds of concern present in the "inert" ingredients of the formulated insecticide Foray 48B;
- to determine whether any volatile "inerts" in Foray 48B could be used as indicators of exposure to volatile chemical components of the formulation;
- to determine whether there was a difference in airborne concentrations of *Btk* between indoor and outdoor locations within the aerial spray zone;
- to determine whether there was airborne drift of *Btk* outside the aerial spray zone;
- to examine the temporal gradient of airborne concentrations of *Btk* after spraying;
- to characterize the size distribution of *Btk* aerosol within the aerial spray zone;
- to estimate the levels of personal airborne exposure to *Btk* on the spray day;

- to determine the association between airborne concentrations of *Btk* and standard measurements of spray deposition density made by the Ministry of Forests; and
- to determine the association between airborne concentrations of *Btk* and the presence of *Btk* on nasal swabs of subjects in the aerial spray zone.

2. Methods

2.1 Identification of "inert" ingredients in Foray 48B

A bulk sample of about 100 mL of Foray 48B was sent from the Capital Health Region to the main laboratory of the School of Occupational and Environmental Hygiene of the University of British Columbia in late March 1999. An attempt was made to identify constituents of the formulation, using chemical analytical methods, in the 3 weeks prior to the training session for the field-study air-sampling technicians. The initial trials were followed by additional laboratory analyses after the aerial spraying was completed.

2.1.1 Analysis for volatile agents, field trial

The presence of volatile organic compounds in the air during aerial spraying was assessed using standard occupational exposure assessment methods during spray trials at the Abbottsford airport on April 9, 1999. These methods typically measure concentrations in the part per million range. 60-minute air samples were simultaneously collected using the following three sampling trains:

- an activated charcoal tube (Supelco ORBO32 No. 2-0228) attached to a constant-flow sampling pump (SKC Model 224-PCXR4) running at a calibrated flow rate of 2 L/min;
- a silica gel sorbent tube (SKC Catalog No. 226-10-04) attached to a constant-flow sampling pump running at a calibrated flow rate of 1 L/min; and
- a 0.5-micron pore-size Teflon® filter mounted in a 37-mm closed-face cassette attached to a constant-flow sampling pump running at a calibrated flow rate of 2 L/min.

The sampling started as the aircraft flew overhead. The same three sampling trains were positioned at four different locations downwind of the spray path. One field blank was collected for each sampling train.

The activated charcoal was desorbed by agitation in 3 mL of carbon disulphide for 30 minutes, in accordance with method 2901 of the Workers' Compensation Board of BC [WCB, 1989]. A 2-μL sample of the desorbed solution was analyzed using gas chromatography/mass spectrometry (GC/MS), using a Varian 3400 gas chromatograph equipped with a Supelco 30 m x 0.25 mm i.d. x 0.25 μm df column and a Varian Saturn II mass spectrometer. The GC was programmed to run at 40 °C for 0.5 minutes, then raised to 280 °C at 20 °C/min and kept at 280 °C for the remaining 3.5 minutes of the run. The Septum Programmable Injector was programmed at 38 °C for 0.25 minute, then raised to 280 °C at 300 °C/min and kept at 280 °C for the remainder of the run. The Saturn II mass spectrometer was tuned to achieve optimal sensitivity using the auto-tuning function of the Saturn software. Mass spectra of the compounds detected were compared to those in the NIST-92 mass spectra library (National Institute of Standards and Technology, USA).

The silica gel was desorbed by agitation in 3 mL of methanol for 30 minutes followed by high performance liquid chromatography (HPLC) analysis under conditions described in section 2.1.3 below. Material collected on the Teflon filters was extracted and analyzed the same way as the silica gel sorbent tube.

2.1.2 Analysis for volatile agents, laboratory trials

The presence of volatile compounds among the "inert" ingredients of Foray 48B was assessed in laboratory trials using four different techniques:

- Two extracts of 1 mL of the bulk Foray 48B sample were made, each using 1 mL of different solvents, one polar (95/5% ethanol/isopropanol mixture) and one non-polar (100% toluene). Each sample was shaken for one minute followed by a one-minute sonication. The samples were centrifuged for 5 minutes at 3000 rpm. The clear supernatant was removed and transferred to a vial awaiting analysis by GC/MS. The GC/MS conditions were as described above for the field trial, except that a split/splitless injector was used and the column temperatures were changed, as follows. The injector was set a splitless mode and operated at an isothermal temperature of 280 °C. The column was held at 40 °C for three minutes after which the temperature was raised to 280 °C at a rate of 12 °C per minute. At the end of the run, the column was held at 280 °C for an additional 15 minutes.

- Samples in the head space of a bulk Foray 48B sample were taken using a solid-phase micro-extraction portable field sampler (SPME; Supelco). The SPME sampler contains a 75-micron fibre coated with a polydimethyl siloxane/carboxen adsorbent. When the fibre is exposed to a contaminated environment, it collects the sample by diffusion. Two trials were conducted, identical in procedure, but at a different temperatures: one at 23 ºC, corresponding to room temperature; and one at 37 ºC, corresponding to likely worst case conditions on sprayed surfaces exposed to the sun. A trial consisted of inserting the sampler through a septum into the head space of a 4-mL vial containing 1 mL of Foray 48B. The SPME sampler was allowed to equilibrate for 30 minutes under these conditions. The volatile compounds adsorbed onto the sampler were desorbed by inserting it directly into the GC injector held at 280 °C for 3 minutes. The GC/MS analysis followed the same procedures as in the other laboratory trials, described above, except that the column temperature was held at the final temperature for 2 minutes instead of 15 minutes. Control samples were analyzed prior to each run by injecting the SPME sampler into the injection port of the GC after the sampler was reconditioned at 280 °C for 30 minutes.

- The presence of head space volatiles was also investigated using two impingers in series. Laboratory air was drawn for 10 minutes over the surface of a 10-mL Foray 48B sample in the first impinger (held in one trial at 37ºC and in another trial at 23ºC), then bubbled through a second impinger, filled with 10 mL of a 95/5% ethanol/isopropanol mixture in one trial and 100% toluene in another trial (both held at 0 ºC), for a total of four trials. A vacuum pump, with a calibrated flow rate of 1 L/min, was used to provide the air flow. Controls samples, with distilled water replacing the Foray 48B emulsion, were obtained prior to each trial and analyzed in the same way. All solutions were analyzed using GC/MS as in the other laboratory trials, as described above for the bulk sample extraction.

- A fourth technique was identical to the method described above, except that the impinger containing the trapping solvents was replaced with an activated charcoal tube (Supelco ORB 032). The exposed activated charcoal was extracted with carbon disulfide according to method 2901 of the Workers' Compensation Board of BC [WCB, 1989].

The extract was analyzed using GC/MS as in the other laboratory trials, as described above for the bulk sample extraction.

2.1.3 Analysis for non-volatile agents, laboratory trials

In order to examine the non-volatile constituents of the Foray 48B, two high performance liquid chromatographic (HPLC) methods were used:

- A 10-μL sample of bulk Foray 48B (diluted 1:50 (w:v) in methanol) was injected into a Varian HPLC system with a UV-Visible detector (model 9050, variable wavelength). The system was equipped with a model 9101 ternary pump, a Waters Nova-Pak C18 3.9 x 150 mm, 60A, 4-μm column (Waters Part No. 36975) and a Nova-Pak guard column (Waters Part No.152220) under a solvent gradient program with 18:82 (v/v) acetonitrile (A):0.1 % acetic acid (B) for 4 minutes. The solvent was raised linearly to 90:10 (v/v) A:B at 10 minutes and maintained until 15 minutes. It was finally reduced to 18:82 A:B at 17 minutes. The pump flow rate was set at 1.2 mL/min. Peaks were detected at a wavelength of 254 nm.
- The HPLC run above was repeated using a Hewlett Packard HPLC system with a mass spectrometer detector. Eluents from the HPLC run were first passed through the Varian UV-Visible detector mentioned above, then sent to a Micromass VG Quattro I mass spectrometer. The mass spectrometer was operated in the electrospray mode with 3.5 kV needle voltage for positive ionization and 2.5 kV for negative ionization respectively. The HPLC column and UV-Visible detector used were identical to those used in the HPLC/UV-Visible run described above. The solvent mixtures were modified to meet the needs of the HPLC/MS: acetonitrile (A):0.1% formic acid (B) in water for positive ionization mode; and acetonitrile (A):10 mM ammonium acetate (B) in water for negative ionization mode. The solvent gradient programs were the same as in the HPLC/UV-Visible operation, described above. The collision energy was 30 eV and the argon pressure was 4.5E-7 bar for both modes.

2.2 Airborne concentrations of *Btk* during aerial spraying

2.2.1 Measurement method

Samples for estimating the "total" airborne culturable colony counts and "total" airborne culturable ***Btk*** counts were taken by pulling air through 0.5-micron pore-size Teflon® filters mounted in 37-mm closed-face cassettes, using constant-flow, battery-powered, air sampling pumps (SKC Aircheck model 224). The pumps were calibrated to a flow rate of 2 L/min ± 5% before and, whenever possible, after sampling using a rotameter (Matheson). A calibration curve for the rotameter was established at the University of British Columbia School of Occupational and Environmental Hygiene laboratory using an automated soap-film flow meter (Gilibrator) as the primary standard. Approximately 1 field blank was included with every 3 field samples.

Air samples to characterize the size distributions of the ***Btk***-containing aerosols were taken using a size-selective, 6-stage, microbial, cascade impactor (Andersen) mounted with

trypticase-soy-agar-filled plates. Air was drawn with a high volume pump (either a battery-powered Gillian AirCon2 or an AC-powered GE) 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 sampler separates the following size ranges of aerosol: stage 1: ≥ 7 microns; stage 2: 4.7 to 7 microns; stage 3: 3.3 to 4.7 microns; stage 4: 2.1 to 3.3 microns; stage 5: 1.1 to 2.1 microns; and stage 6: 0.65 to 1.1 microns.

The laboratory of Dr. David Levin at the University of Victoria quantified total bacterial counts on the Teflon filters by placing the filters face down on nutrient agar and counting colonies after 16 to 24 hours of incubation at 28 °C. The Andersen sampler plates were also incubated for 16 to 24 hours at 28 °C prior to counting. Random amplified polymorphic DNA analysis, *cry* gene specific polymerase chain reaction (PCR), and Dot-blot DNA hybridization techniques were used to screen the colonies for ***Btk***, as described elsewhere [Levin and de Amorin, 1999]. Positive hole correction factors were not applied to the Andersen sampler counts. Data analyses on a sample of the data, using the positive hole correction factors, did not substantially alter the results.

Concentrations of total colony-forming units and ***Btk*** colonies in air were calculated by dividing the colony count by the volume of air sampled, giving concentrations in colony-forming units per cubic meter of air (CFU/m^3).

2.2.2 Field study design

Air sampling was conducted by students from the University of Victoria and Royal Roads University, and by Capital Health Region Environmental Health Officers, trained by University of British Columbia School of Occupational and Environmental Hygiene personnel (YC and DR). All sampling personnel signed affidavits assuring they would keep confidential all information about the sampled sites and the study participants.

Aerial spraying of the Victoria area 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, approximately 30 locations inside the spray zone were randomly selected from the Victoria telephone directory (as described below). Each location was randomly designated for sampling during one of the three spray periods. At each location, one sample was taken inside the residence, and one was taken outdoors, during the aerial spraying. Residents were asked to keep their windows closed for the duration of indoor sampling. Air sampling began when spraying began, and lasted for one hour.

To assess whether there was drift of ***Btk*** outside the aerial spray zone, approximately 30 locations outside the spray zone were selected. Each location was randomly designated for sampling during one of the three spray periods. At each location, one sample was taken outdoors during spraying of the nearest area within the spray zone. 18 of the 30 samples were selected to be in locations downwind of the spray zone (based on the prevailing wind direction), 6 were in downtown Victoria, and 6 were in other locations outside the aerial spray zone. Samples other than those taken in downtown Victoria were taken outside homes

randomly selected from the Victoria telephone directory (as described below). All samples were located from 125 to 1,000 meters outside the aerial spray zone. Air sampling began when spraying began, and lasted for one hour.

Random site selection inside and outside the spray zone was conducted 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). 478 random number sets (page/column/row) were generated. Each location so identified was checked against a list of postal codes to identify whether or not it was in the spray zone. 106 randomly selected sites were identified to be within the spray zone; this was verified 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. Residents at all randomly selected locations were sent a letter of introduction to the study (Appendix A), then phoned by study personnel within 2 weeks of the spray period to verify the location and the willingness of the subject to participate in the study.

Characteristics of the homes and the areas around the homes were recorded during the sampling period (Appendix B). 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 storeys, 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 meteorological station. Data from three stations were used: the Royal Roads University site, a maximum of 3.5 km away from any measurement site for which its data were used; Environment Canada's Esquimalt Harbour site, a maximum of 4.5 km away; and Environment Canada's Victoria Gonzales site, a maximum of 10.8 km away.

To characterize the size distribution of *Btk* aerosol, 12 air samples were taken using the 6-stage Andersen sampler, randomly distributed over the three spray periods. The sites were selected at random from the 30 outdoor locations sampled within the spray zone. Air sampling began when spraying began (i.e., plane spotted overhead), and lasted for 15 minutes. The Andersen samplers were placed adjacent to the filter samples. To determine the duration of sampling, test samples were taken during spray trials at the Abbottsford airport on April 9, 1999. A series of three 6-stage Andersen samples were taken for five minutes each. The first sample started at the time the airplane passed overhead (T0-T5), the second 2.5 minutes later (T2.5-T7.5), and the third 7.5 minutes later (T7.5-T12.5). 80% 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 4 stages, representing aerosols smaller than 5 microns which settle very slowly. It was estimated that 15-minute 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, locations were randomly selected from the original measurement sites inside the aerial spray zone for resampling at selected times. Twelve samples were taken outdoors at each of 6 times after spraying (beginning 2 hours, 5

hours, 9 hours, 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 hours and 5 hours). The duration of air sampling was one hour.

The Ministry of Forests measures spray deposition densities by counting droplets deposited on Kromecote cards, to determine whether spray coverage was adequate for insecticidal purposes. These cards are made of a glossy white thin cardboard, and are 11 by 11 cm in size. To examine the association between airborne *Btk* concentrations and Kromecote card droplet densities, Kromecote cards were obtained from the Ministry of Forests, and placed at each of the 60 outdoor monitoring sites (inside and outside the spray zone). The Kromecote cards were placed adjacent to the filter samples. The cards were left open to the air for the duration of the filter air sampling during the aerial spraying (about 60 minutes), then transferred spray side up to plastic CD holders until they were dry. The cards were then transferred to a CD rack and brought to the Ministry of Forests to be read by their personnel using a Meiji Techno SKC dissecting light microscope at 30 times magnification.

To examine the association between airborne *Btk* concentrations and the presence of *Btk* on nasal swabs, all residents of sampling locations within the spray zone were asked to submit nasal swabs immediately after aerial spraying. Subjects swabbed the entrance of each nostril before they left the house in the morning of the day when the airborne exposure samples were taken at their home. Swabs were replaced in cylindrical tubes containing a growth medium and returned to study personnel for transportation to the laboratory of Dr. David Levin at the University of Victoria. They were tested for the presence or absence of *Btk*, according to methods described elsewhere [Levin and de Amorin, 1999].

2.2.3 Data analysis

Frequency distributions of the Teflon filter samples, untransformed and log-transformed (base e), were examined to determine whether the data approximated normal or log-normal functions. Because examination of frequency histograms of the exposures variables suggested that the data were approximately log-normally distributed, all exposure data were log-transformed (base e) in inferential tests.

A short note on log-normal distributions. Many environmental and occupational distributions are approximately log-normally distributed, i.e., they are bounded by zero on the left, tend to have a single mode close to the lower bound, but long tails to the right. These distributions are approximately bell-shaped (normally distributed) when the exposure data are log-transformed. In addition to the usual arithmetic mean, another measure of central tendency used to describe such data is the geometric mean: the antilog of the mean of the log-transformed values. This is the same as the median when the data are exactly log-normal. Note that the arithmetic mean will be higher than the geometric mean, and this difference will increase the more skewed the data. Similarly the geometric standard deviation is the antilog of the standard deviation of the log-transformed values. It is unitless. Low geometric standard deviations ($< \sim 2$) indicate less skewed data, whereas higher geometric standard deviations ($> \sim 3.5$) indicate very skewed data.

Not all data was quantifiable; some samples had no growth (less than the detection limit; left-censored) and others had too many colonies to count (right-censored). The distribution of the subset of data with quantitative measurements was used as the basis for imputing values for samples that were left- and right-censored. Values for samples less than the limit of detection were randomly selected 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 selected from the censored section of the distribution to the right of the highest quantifiable concentrations (~ 1600 CFU/m^3). Imputing values allows quantitative analyses to include the censored samples, with values in their likely range. The random selection of values will have the effect of randomly misclassifying exposures within the censored regions. Imputing values increases the power of the study to detect true differences by allowing analysis of more data; however, the misclassification results in lower power than true quantitative measurements.

Descriptive statistics (arithmetic and geometric means, geometric standard deviations, ranges, % censored data) were calculated for the exposure measurements, stratified by location and time of sampling. Paired t-tests were used to compare indoor and outdoor concentrations of total colonies and *Btk* colonies at the same sites within the spray zone. Student's t-tests were used to compare concentrations measured inside and outside the aerial spray zone. Multiple linear regression analyses were used to examine 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.

Other characteristics of the measurement locations (e.g., type of residence, type of landscaping, weather conditions) were also examined for their association with exposure levels. Correlations between independent variables were examined, and where Pearson $r \geq 0.7$, only one variable was chosen for inclusion 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 backwards stepwise procedure with $p < 0.25$ to enter and $p < 0.10$ to remain in the model.

The size distributions of the *Btk* aerosol were estimated for each Andersen sample by plotting the cumulative *Btk* colony count against the upper aerodynamic diameter cut-off for each stage of the sampler. The 50^{th} percentile, the "count median aerodynamic diameter", was read from the linear regression line for the relationship between the cumulative count and the aerodynamic diameter. The standard deviation of the distribution was calculated by subtracting the 50^{th} percentile aerodynamic diameter from the 84^{th} percentile (i.e., one standard deviation in a normal distribution), both also read from the regression line. 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. The geometric standard deviations of the distributions were calculated by dividing the 84^{th} percentile aerodynamic diameter by the 50^{th} percentile, read from the log-probability plot.

To estimate the levels of personal airborne exposure to *Btk*, each study participant within the aerial spray zone was asked to complete a diary indicating the locations at which he/she spent the day of the spraying. This data was used in conjunction with the area sampling results inside and outside the spray zones to calculate time-weighted average personal exposure levels on the spray days.

Simple linear regressions, with Kromecote card densities as the independent variable and airborne *Btk* concentrations as the dependent variable, were used to examine the association between these two variables. Regressions examined all data combined, as well as subsets of the data stratified by location of air sampling.

Student's t-tests were used to examine whether positive and negative nasal swabs were associated with differing airborne *Btk* concentrations, using indoor and outdoor *Btk* data in separate analyses.

3. Results

3.1 Identification of "inert" ingredients in Foray 48B

3.1.1 Volatile agents, field trial

The analysis of the air samples collected during the aerial spray field trial at the Abbotsford airport did not indicate the presence of any volatile organic compounds at concentrations high enough to produce either GC or HPLC peaks above the background signal.

3.1.2 Volatile agents, laboratory trials

The results of the search for volatile compounds in the laboratory analyses of Foray 48B are summarized in Table 1. Only compounds not detected in blanks are listed in the table. Compounds identified are stratified by the capture technique used, then listed in order of their retention time in the gas chromatograph (specific to the GC program used). Compounds identified using solid-phase micro-extraction (SPME) at 37 °C are listed first, since this technique detected the greatest number of compounds. The table lists the chemical's name, its Chemical Abstract Service (CAS) number, and the "fit" of the extracted Foray 48B compound's mass spectrum to the matched compound identified by the mass spectra library. A fit of 1000 indicates a perfect match. When fit numbers are less than 1000, other compounds with similar mass spectra may have been present instead. The degree of certainty is therefore indicated by the fit number. The table also indicates whether the compound is included on any of the US Environmental Protection Agency's lists of "inert" ingredients [EPA, 2000].

It can be observed from the results in Table 1 that the SPME technique was far superior in trapping volatile agents from the Foray 48B samples than the other techniques used. This procedure allowed the identification of 19 compounds with sampling at room temperature and of 23 compounds with sampling at 37 °C. Examples include the propenyl ester of acetic acid; 2-methyl 2,3-pentanediol; trimethyl phosphine; benzoic acid; butylated hydroxy toluene; and a number of siloxane derivatives.

The carbon disulfide extract of the charcoal tube sample only captured siloxane derivatives, one of which was also found in the SPME trials. The alcohol and toluene extracts of the bulk Foray 48B samples found a number of compounds at high concentrations. In order to obtain peak separation, the extracts had to be diluted by a factor of 10. Benzoic acid, also found using the SPME technique, was trapped in both extracts of the bulk samples.

The impinger samples using either 95/5% ethanol/isopropanol or toluene as the trapping solutions did not detect the presence of any compounds at concentrations high enough to produce GC peaks above the background signal; consequently this technique is not listed in Table 1.

Table 1: Summary of volatile compounds in Foray 48B, identified by GC/MS

Retention Time	Compound Identified	Fit	CAS number[1]	EPA List of "Inerts"[2]
Solid-phase micro-extraction sampler at 37ºC				
86	thietane	772	287-27-4	-
90	acetic acid, 2-propenyl ester	869	591-87-7	-
118	2-butanone, 4-acetyloxy)-	830	10150-87-5	-
141	acetic acid, anhydride	821	108-24-7	4B
221	1,5-hexanediene-3,4-diol, 2,5-dimethy	919	4723-10-8	-
230	sydnone, 3-(phenylmethyl)	801	16844-42-1	-
246	2 methyl-2,3-pentanediol	846	7795-80-44	-
248	phosphine, trimethyl	694	594-09-2	-
270	2 methyl-2,3-pentanediol	826	7795-80-44	-
280	2 methyl-2,3-pentanediol	882	7795-80-44	-
283	phosphine, trimethyl	673	594-09-2	-
330	cyclotrisiloxane, hexamethyl	968	541-05-9	-
496	disiloxane derivative	841	18420-09-2	-
560	cyclotetrasiloxane, octamethyl	979	556-67-2	3
663	5-hexen-2-one, 5-methyl	869	3240-09-3	-
674	2,4-hexadienedioic acid	849	505-70-4	-
726	cyclopentasiloxane, decamethyl	965	541-02-6	3
768	benzoic acid	973	65-85-0	4B
885	cyclohexasiloxane, dodecamethyl	972	540-76-6	-
1027	trisiloxane	903	3555-47-3	3
1042	butylated hydroxy toluene	984	128-37-0	3
1154	phenyl amine – silane derivative	949	10538-85-9	-
1264	cyclohexasiloxane, dodecamethyl	974	540-97-6	-
Solid-phase micro-extraction sampler at 23ºC				
88	acetic acid, mercapto -,methyl ester	788	236-48-2	-
*92	acetic acid, 2-propenyl ester	869	591-87-7	-
104	ethylene diamine	879	107-15-3	3
121	ethanol, 2-(1methylethoxy)-	731	109-59-1	-
227	2-heptanone, 3-hydroxy-3-methyl	915	13757-91-0	-
*240	2 methyl-2,3-pentanediol	846	7795-80-44	-
*243	phosphine, trimethyl	689	594-09-2	-
277	ether, sec-butyl isopropyl	867	18641-81-1	-
*335	cyclotrisiloxane, hexamethyl	977	541-05-9	-
502	disiloxane derivative	834	1438-82-0	-
*566	cyclotetrasiloxane, octamethyl	990	556-67-2	3
668	1-propanesulfonyl chloride	776	10147-36-1	-
*726	cyclopentasiloxane, decamethyl	985	541-02-6	3
*768	benzoic acid	973	65-85-0	4B
*885	cyclohexasiloxane, dodecamethyl	972	540-76-6	-
*1034	trisiloxane	916	3555-47-3	3
*1049	butylated hydroxy toluene	956	128-37-0	3
*1154	phenyl amine – silane derivative	944	10538-85-9	-
*1270	cyclohexasiloxane, dodecamethyl	980	540-97-6	-
Charcoal tube				
1030	penta siloxane, dodecamethyl	950	141-63-9	-
1158	benzoic acid, siloxane derivative	973	10586-16-0	-
*1268	cyclohexasiloxane, dodecamethyl	986	540-97-6	-
1366	cyclohexasiloxane, dodecamethyl	977	540-97-6	-
*1455	trisiloxane derivative	985	3555-47-3	3

> 1455	additional siloxane derivatives			
Alcohol extract of bulk Foray 48B sample				
284	ethanol, 1-methoxy-,acetate	879	4382-77-8	-
*749	benzoic acid	963	65-85-0	4B
1478	galacticol	838	608-66-2	-
Toluene extract of bulk Foray 48B sample				
118	chloroform	667	67-66-3	-
691	2-hydroxy pyridine	893	142-08-5	-
*793	benzoic acid	984	65-85-0	4B
1005	benzoic acid, 2-hydroxy-,phenyl ester	929	118-55-8	-

[1] CAS = Chemical Abstract Service

[2] The US Environmental Protection Agency lists "inert" ingredients which might be used in pesticides. The lists have the following meanings:

- 3 Inerts of unknown toxicity.
- 4B Inerts with sufficient data to substantiate that they can be used safely in pesticide products.
- \- Compound not found on EPA lists

* identical to compound identified in the solid-phase micro-extraction at 37 ºC

3.1.3 Non-volatile agents, laboratory trials

HPLC/UV-Visible analysis of the diluted bulk Foray 48B sample showed a number of peaks with retention times between 0.70 and 2.20 minutes and four stronger peaks with retention times of 6.59, 7.00, 8.28, and 8.43 minutes respectively (Figure 1). This method does not allow identification of the constituents without ***a priori*** information which would allow comparison of retention times to those of standard samples.

The results of the HPLC/MS analysis of a bulk Foray 48B sample are shown in Figure 2. It can be observed from the high background of the baseline that a clean separation between the many constituents could not be achieved. This background makes the identification of each of the peaks shown virtually impossible. The large number of peaks do indicate however that many constituents are present. These peaks do not necessarily correspond to the various peaks that were found using a UV-Visible detector at 254 nm (Figure 1).

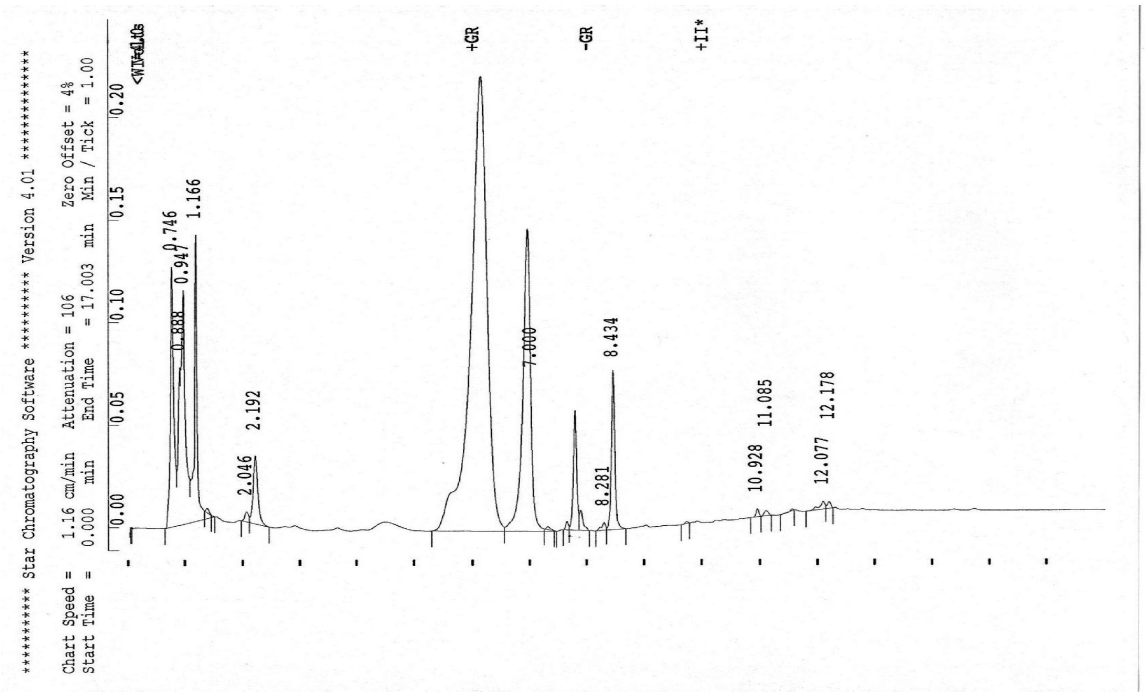

Figure 1. Chromatogram of bulk Foray 48B sample from HPLC/UV-Visible run.

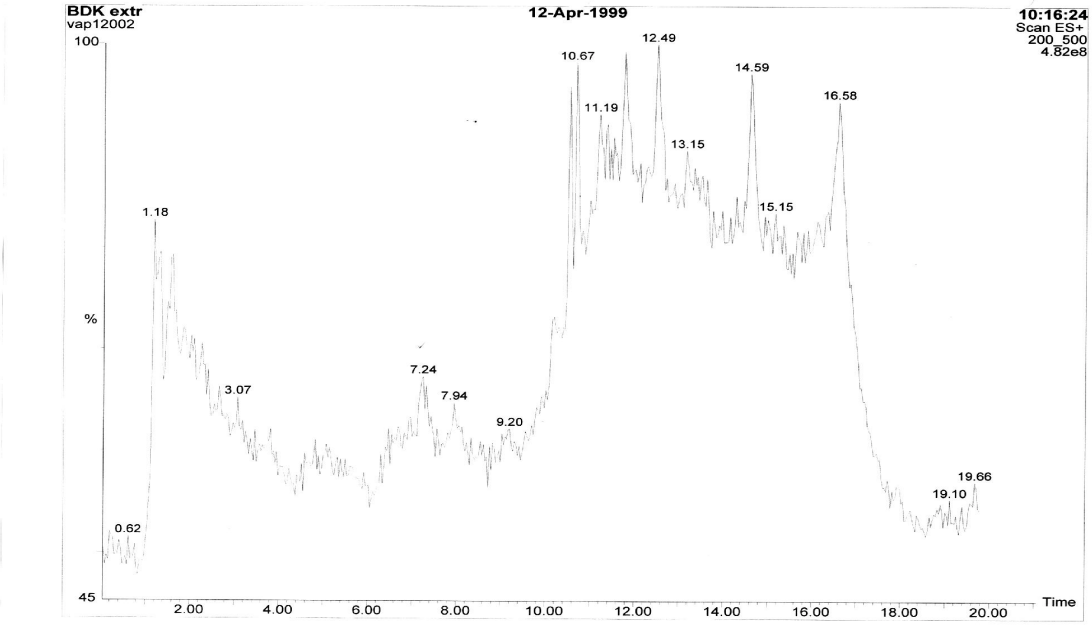

Figure 2. Chromatogram of bulk Foray 48B sample from HPLC/MS run

3.2 Airborne concentrations of *Btk* during aerial spraying

3.2.1 Participation

Table 2 indicates the participation rates inside and outside the spray zone. A number of 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 in the process of moving. In addition, at about 10% of sites, no one could be contacted. 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 expect that the reason for this difference is that outside the spray zone, only outdoor measurements were made, therefore the residents were not disrupted. Inside the spray zone, measurements were made indoors during spraying, and study personnel needed to enter homes between 4:30 and 5:30 a.m. Other demands were made on these residents, including completion of personal diaries, nasal swabs, and follow-up measurements at selected times post spraying.

Aerial spraying of the Victoria area was conducted three times, spread over 2 to 3 days in each period: May 8th to 10th, May 19th to 21st, and June 8th and 9th, 1999. 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 (Figure 3).

Table 2: Participation in the air measurement study, and reasons for non-participation

	Inside Spray Zone		Outside Spray Zone	
	N	(%)	N	(%)
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)
Reason for not participating				
No reason given	31		10	
Will be at work at that time	5		0	
Too busy	4		0	
Too early in morning	1		0	
Private apartment	1		0	
Have ill person in house	1		0	
Do not want stranger in house	1		0	
Do not speak English	1		0	

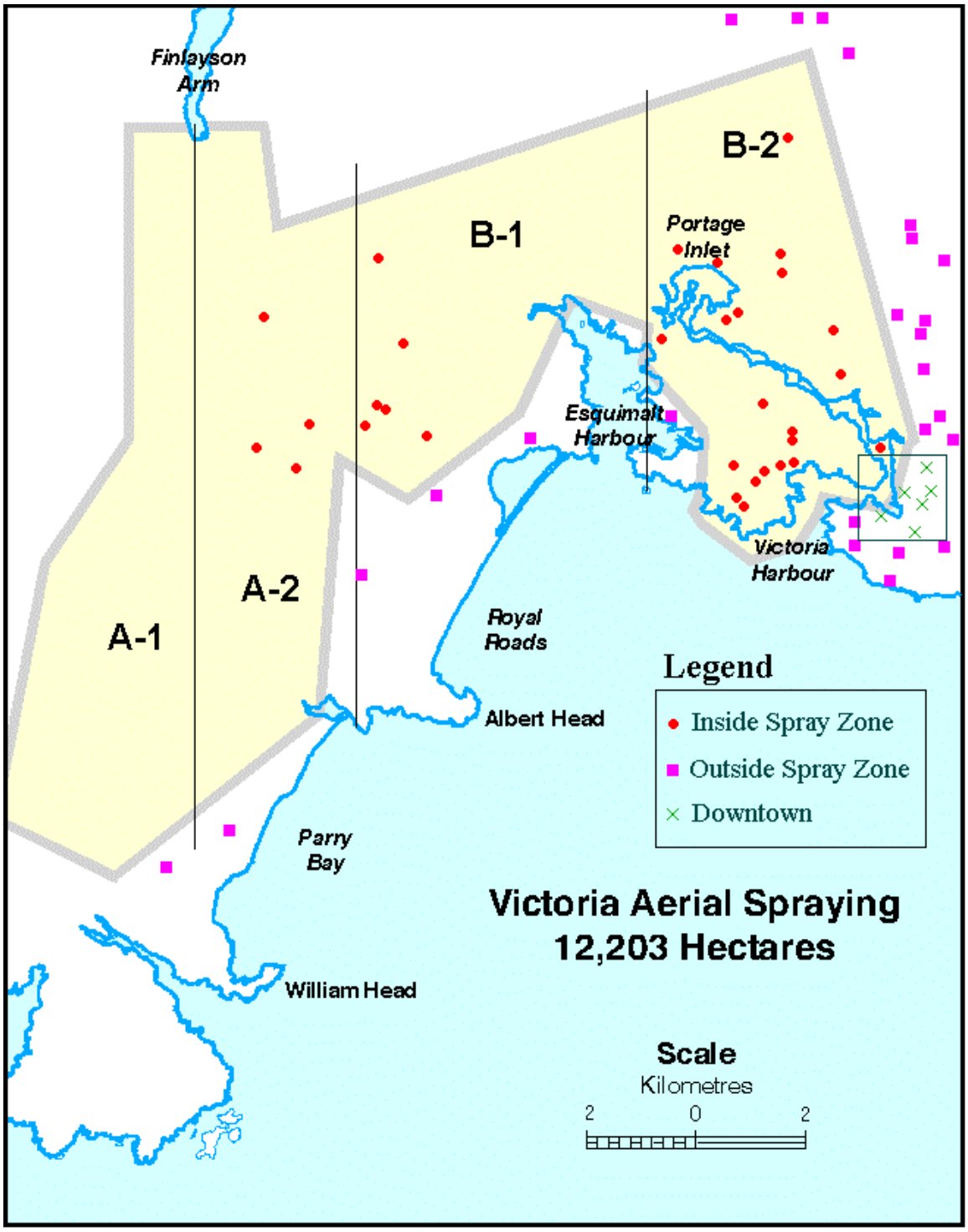

Figure 3. Map indicating sites where air samples were taken inside and outside the aerial spray zone. The spray zone is the lightest-shaded area outlined in mid-gray. North is at the top of the figure; the prevailing winds are to the northeast. A-1, A-2, B-1, and B-2 indicate sub-zones covered by a single plane in a single morning's aerial spraying. On most spray days, two planes operated with one sub-zone separating them.

3.2.2 Description of air sample data

186 filter samples were planned, and 193 were taken. The reasons for differences were primarily changes in the scheduling of the aerial spraying. On some occasions, samples were taken when no spraying occurred, requiring rescheduling of subjects for another day of sampling. 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 did not occur. 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 bacterial growth, three grew 1 or 2 colonies, one grew 11 colonies, and one had too many colonies to count. The laboratory reported that the overgrown plate likely resulted from laboratory contamination. No blank corrections were made.

Histograms of the bacterial exposure concentrations indicated that the data were positively skewed, and were more symmetrically distributed when log-transformed (as an illustration see Figures 4 and 5, summarizing the quantified *Btk* data). Subsequent inferential analyses were performed with the data so transformed.

12% of the plated filters grew no colonies, and thus gave air concentrations below a detection limit of ~ 10 colony forming units per cubic meter of air (CFU/m^3). 10% of the plated filters grew too many colonies to count, yielding concentrations greater than ~ 1600 CFU/m^3. Numerical values for these samples were imputed from randomly generated points within a log-normal distribution with geometric mean and geometric standard deviation based on the concentration data with quantified values.

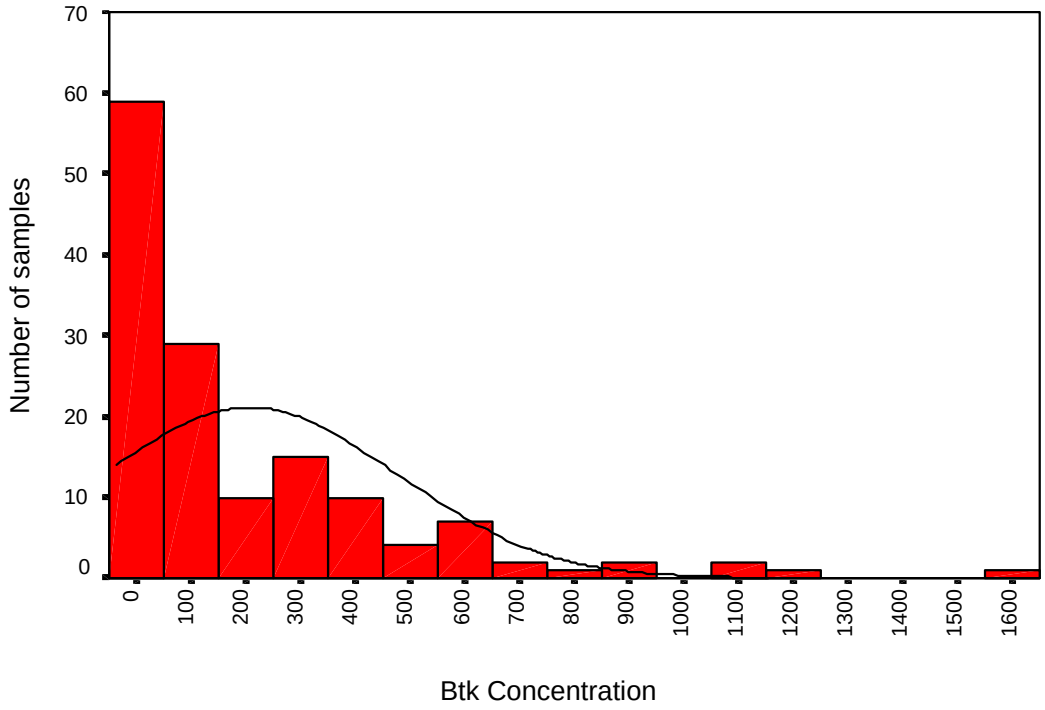

Figure 4. Frequency histogram of all quantifiable *Btk* concentrations, data untransformed. Solid line is standard normal curve with the same mean and standard deviation.

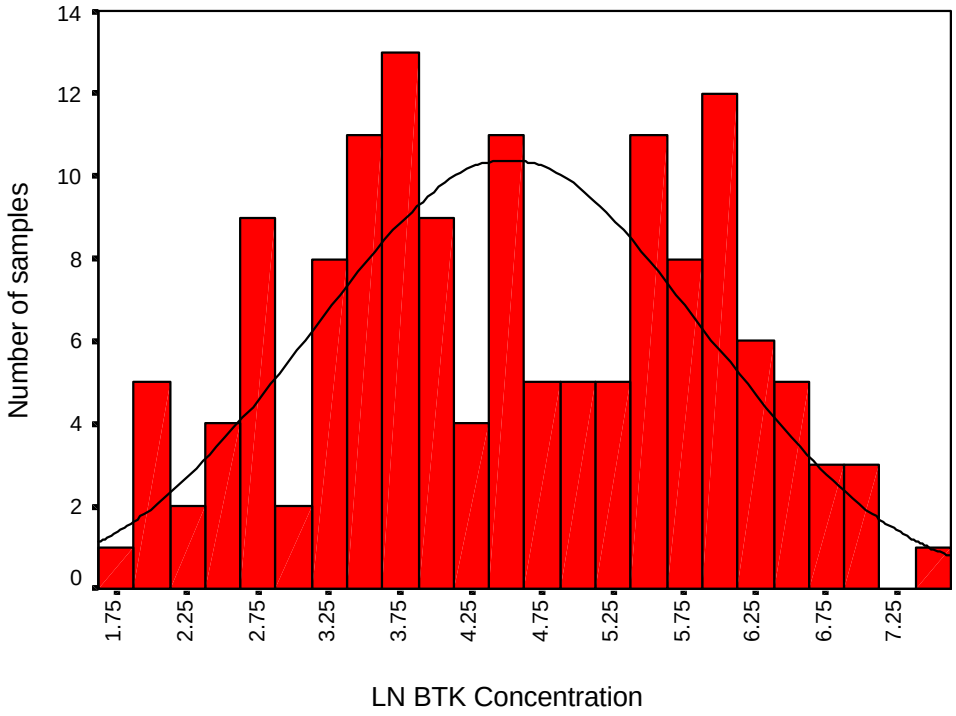

Figure 5. Frequency histogram of all quantifiable *Btk* concentrations, data log-transformed (base e). Solid line is standard normal curve with the same mean and standard deviation.

3.2.3 Airborne concentrations outdoors and indoors in the aerial spray zone

Table 3 describes the concentrations of total colonies and ***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 arithmetic means of 770 total CFU/m^3 and 739 ***Btk*** CFU/m^3 and geometric means of 227 total CFU/m^3 and 157 ***Btk*** CFU/m^3, respectively. Indoors the concentrations of ***Btk*** were 2.3 to 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.

Though ***Btk*** concentrations indoors were consistently lower than those outdoors during the spray, indoor and outdoor concentrations were correlated (Pearson r = 0.65); i.e., locations with higher outdoor concentrations tended to have higher indoor concentrations.

Table 3: Concentrations of total colony forming units and *Bacillus thuringiensis* var. *kurstaki* in the aerial spray zone, outdoors and indoors, during spraying

Location	Total Colonies	*Btk*
Outdoors (N=33)		
Samples < LOD (%)	9	15
Minimum > LOD (CFU/m^3)	13.2	11.2
Samples > TNTC (%)	18	18
Maximum < TNTC (CFU/m^3)	1660	1614
Arithmetic mean (CFU/m^3)	770	739
Geometric mean (CFU/m^3)	*[1]227*	*[2]157*
Geometric standard deviation	7.5	10
Indoors (N=27)		
Samples < LOD (%)	11	11
Minimum > LOD (CFU/m^3)	14.7	9.83
Samples > TNTC (%)	0	0
Maximum (CFU/m^3)	688	627
Arithmetic mean (CFU/m^3)	180	159
Geometric mean (CFU/m^3)	*[1]87.3*	*[2]69.7*
Geometric standard deviation	4.3	4.6

[1] paired t-test for differences, p=0.001; paired correlation = 0.76
[2] paired t-test for differences, p=0.029; paired correlation = 0.65
LOD = limit of detection; TNTC = too numerous to count

3.2.4 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 to 1000 meters away from the planes' aerial spray (Table 4). 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, linear regression analyses were used to examine the relationship between distance from the aerial spray zone and ***Btk*** concentrations, first using only data outside the spray zone, then all outdoor data, in two separate analyses. No relationship between the bacterial concentrations and distance was found ($p > 0.70$). Additional factors relevant to drift, in particular wind speed and direction, are discussed in section 3.2.6 below.

Table 4: Concentrations of total colony forming units and *Bacillus thuringiensis* var. *kurstaki* inside and outside the aerial spray zone, outdoors during spraying

Location	Total Colonies	*Btk*
Inside spray zone (N=33)		
Samples < LOD (%)	9	15
Minimum > LOD (CFU/m^3)	13.2	11.2
Samples > TNTC (%)	18	18
Maximum < TNTC (CFU/m^3)	1660	1614
Arithmetic mean (CFU/m^3)	770	739
Geometric mean (CFU/m^3)	*[1]227*	*[2]157*
Geometric standard deviation	7.5	10
Outside spray zone (N=27)		
Samples < LOD (%)	4	7
Minimum > LOD (CFU/m^3)	31.8	5.5
Samples > TNTC (%)	25	25
Maximum < TNTC (CFU/m^3)	681	651
Arithmetic mean (CFU/m^3)	511	484
Geometric mean (CFU/m^3)	*[1]249*	*[2]175*
Geometric standard deviation	4.0	5.8

[1] t-test for differences, p=0.84
[2] t-test for differences, p=0.84
LOD = limit of detection; TNTC = too numerous to count

3.2.5 Temporal gradient of airborne concentrations

Tables 5 and 6 indicate the temporal trends in airborne concentrations during and after spraying within the spray zone, outdoors and indoors respectively. Figure 6 is a plot of the geometric mean ***Btk*** concentrations, showing the temporal trends graphically. The data indicate that the highest exposures occur from the start of spraying up to 3 hours later. Both outdoors and indoors, there was a tendency for exposures to be higher in the period 2-3 hours after the start of spraying than during spraying, though this was not statistically significant ($p = 0.23$, 0.15 respectively), likely because the numbers of samples was small. After that, the outdoor concentrations decreased in an exponential pattern with time. Indoors, there was no clear pattern; in fact at 5-6 hours post-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 was occurring concurrently in an adjacent zone.

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

(1) Ln ***Btk*** concentration = 4.63 – 0.27 x time since spraying (in days, continuous) + 1.38 x concurrent spraying in adjacent zone (yes or 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 modelled the data in the first 10 hours after spraying. It gave the following relationship:

(2) Ln ***Btk*** concentration = 5.29 – 5.01 x time since spraying (in days, continuous)

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 ($R^2 = 0.11$; $p < 0.007$). Modelling of the indoor data did not find any significant patterns, whether using the time since spraying as a continuous or categorical variable. This is likely due to insufficient data to detect a relationship, rather than absence of a trend.

Table 5: Concentrations of total colony forming units and *Bacillus thuringiensis* var. *kurstaki* in the aerial spray zone, outdoors, at various times after spraying

Time	Total Colonies	*Btk*
During spraying (N=33)		
Samples < LOD (%)	9	15
Minimum > LOD (CFU/m^3)	13.2	11.2
Samples > TNTC (%)	18	18
Maximum < TNTC (CFU/m^3)	1660	1614
Arithmetic mean (CFU/m^3)	770	739
Geometric mean *(CFU/m^3)*	*227*	*157*
Geometric standard deviation	7.5	10

2-3 *hours after spraying began* (N=12)		
Samples < LOD (%)	0	0
Minimum (CFU/m³)	31.7	23.7
Samples > TNTC (%)	8	8
Maximum < TNTC (CFU/m³)	1352	1089
Arithmetic mean (CFU/m³)	541	501
Geometric mean *(CFU/m³)*	*264*	*239*
Geometric standard deviation	4.3	4.5
5-6 hours after spraying began (N=11)		
Samples < LOD (%)	9	9
Minimum > LOD (CFU/m³)	7.9	7.9
Samples > TNTC (%)	0	0
Maximum (CFU/m³)	301	277
Arithmetic mean (CFU/m³)	104	77.7
Geometric mean *(CFU/m³)*	*49.7*	*38.6*
Geometric standard deviation	4.1	3.5
9-10 hours after spraying began (N=10)		
Samples < LOD (%)	10	20
Minimum > LOD (CFU/m³)	13.1	13.1
Samples > TNTC (%)	10	10
Maximum < TNTC (CFU/m³)	116	116
Arithmetic mean (CFU/m³)	168	162
Geometric mean *(CFU/m³)*	*41.7*	*28.8*
Geometric standard deviation	4.4	6.1
1 day after spraying (N=10)		
Samples < LOD (%)	0	0
Minimum (CFU/m³)	33.4	24.5
Samples > TNTC (%)	30	30
Maximum < TNTC (CFU/m³)	771	690
Arithmetic mean (CFU/m³)	586	532
Geometric mean *(CFU/m³)*	*319*	*243*
Geometric standard deviation	4.0	4.8
4 days after spraying (N=12)		
Samples < LOD (%)	0	25
Minimum > LOD (CFU/m³)	15.9	7.9
Samples > TNTC (%)	0	0
Maximum (CFU/m³)	97.2	57.2
Arithmetic mean (CFU/m³)	45.0	27.1
Geometric mean *(CFU/m³)*	*37.4*	*19.2*
Geometric standard deviation	1.9	2.6
9 days after spraying (N=12)		
Samples < LOD (%)	8	42
Minimum > LOD (CFU/m³)	8.1	8.1
Samples > TNTC (%)	0	0
Maximum (CFU/m³)	123	94.3
Arithmetic mean (CFU/m³)	35.9	22.3
Geometric mean *(CFU/m³)*	*22.6*	*11.3*
Geometric standard deviation	2.8	3.3

LOD = limit of detection; TNTC = too numerous to count

Table 6: Concentrations of total colony forming units and *Bacillus thuringiensis* var. *kurstaki* in the aerial spray zone, indoors, at various times after spraying

Time	Total Colonies	*Btk*
During spraying (N=27)		
Samples < LOD (%)	11	11
Minimum > LOD (CFU/m³)	14.7	9.8
Samples > TNTC (%)	0	0
Maximum (CFU/m³)	688	627
Arithmetic mean (CFU/m³)	180	159
Geometric mean *(CFU/m³)*	*87.3*	*69.7*
Geometric standard deviation	4.3	4.6
2-3 hours after spraying began (N=11)		
Samples < LOD (%)	0	0
Minimum (CFU/m³)	16.5	16.2
Samples > TNTC (%)	9	9
Maximum < TNTC (CFU/m³)	897	896
Arithmetic mean (CFU/m³)	421	395
Geometric mean *(CFU/m³)*	*183*	*155*
Geometric standard deviation	4.3	4.6
5-6 hours after spraying began (N=10)		
Samples < LOD (%)	0	0
Minimum (CFU/m³)	23.8	23.8
Samples > TNTC (%)	0	0
Maximum (CFU/m³)	862	814
Arithmetic mean (CFU/m³)	275	245
Geometric mean *(CFU/m³)*	*163*	*131*
Geometric standard deviation	3.1	3.4

LOD = limit of detection; TNTC = too numerous to count

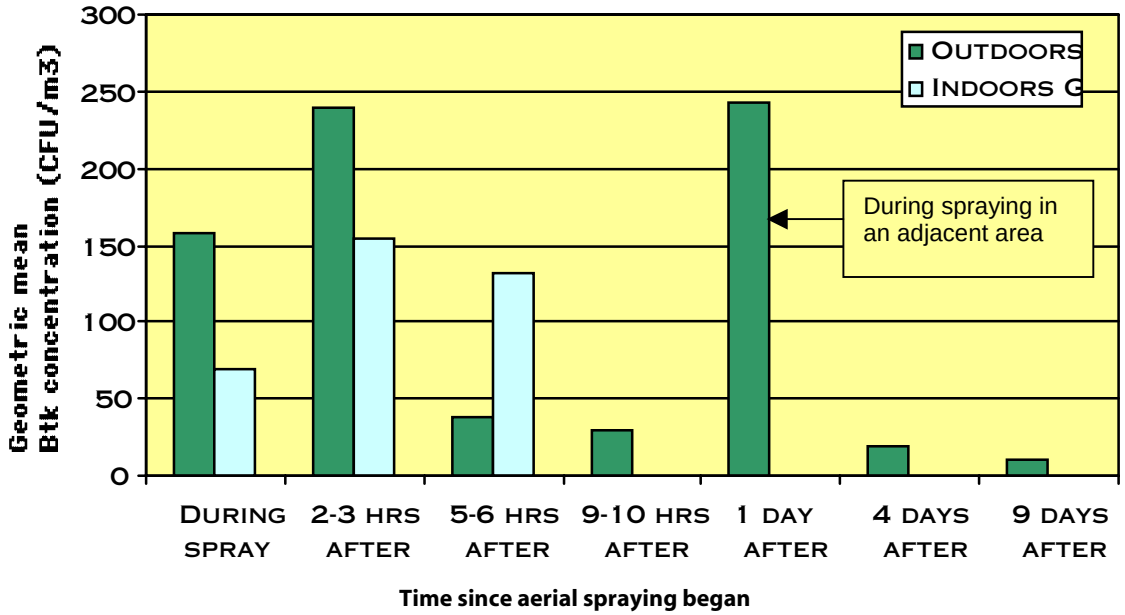

Figure 6. Geometric mean *Btk* concentrations indoors and outdoors within the aerial spray zone, at various times during and after the spraying

3.2.6 Other characteristics related to Btk concentrations

The relationships between *Btk* concentrations and characteristics such as the type of residence, the environment surrounding the residence, the location sampled inside the home, and weather conditions were also examined. 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 (wind speed times 1 for sites downwind and times -1 for sites upwind of the spray zone). A multiple linear regression analysis indicated the following relationship:

(3) Ln *Btk* concentration = 23.1 + 0.34 x outdoor temperature (°C) - 0.24 x relative humidity (%) + 0.056 x wind speed vector (nested variable, outside the spray zone only; km/hr)

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 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/hour (SD = 5.8 km/hour).

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, i.e., pavement, grass, garden; barometric pressure; distance vector from the spray zone (distance times 1 for sites downwind and times -1 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, i.e., house, duplex, townhouse, apartment; type of entrance, i.e., direct to outdoors or through an interior hallway; storey on which sampling was conducted; whether any window or doors were open; room where sampling was done; indoor temperature.

3.2.7 Size distribution of the Btk aerosol

The data from the size-selective Andersen samplers are presented in Table 7. *Btk* aerosols which were present in the air for a 15-minute period after the start of spraying had count median aerodynamic diameters of 4.3 to 7.3 microns, with standard deviations of 2.6 to 3.8 microns. 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. The 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 Kromecote cards ranged from 60 to 120 microns at the same sites (and from 50 to 150 microns over all study sites). The aerodynamic diameters measured by the Andersen samplers were considerably smaller than the droplet sizes measured on the Kromecote cards at the same locations. The reasons for the differences are likely several-fold. The Andersen samplers actively drew aerosols from the air over a 15-minute period and would include aerosols which did not settle quickly (i.e., less than 50 microns, Table 8)[Hinds, 1982]. This sampler should capture all large aerosols on its top stage, but its sampling characteristics at sizes above 10 microns are not known, so we cannot be certain that the sampling efficiency for larger particles is as good as for finer aerosols. In contrast to the Andersen samplers' active sampling of the air, the Kromecote cards passively received aerosols which fell from the air and were deposited on surfaces within one hour of the beginning of spraying (i.e., 25 microns or greater, Table 8). 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. Thus, one would expect that this sizing method would not give uniform results under all conditions.

Table 7: Size distributions of aerosol at Andersen sampler measurement locations, as measured by *Bacillus thuringiensis* var. *kurstaki* count distributions on Andersen sampler stages, and by reading droplet sizes on adjacent Kromecote cards

Date Sampled	Andersen Sampler CMAD (SD) (microns)		Kromecote Card Droplet Sizes (microns)	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)	-	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

CMAD = count median aerodynamic diameter
SD = standard deviation; note that estimated *geometric* standard deviations fell within a narrow range: ~ 1.65 to 1.75
- = no sample taken
nd = no droplets on card

Table 8. Estimates of terminal settling velocities and times to settle from plane height of aerosols with various aerodynamic diameters

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

3.2.8 Association between airborne Btk concentrations and surface deposition of Foray 48B on Kromecote cards

Table 9 shows the results of linear regressions between the airborne *Btk* concentrations and the density of the Foray 48B formulation deposited on the surface of Kromecote cards during spraying. The data were grouped in several ways: first, all the outdoor data, including those with imputed values; then only the outdoor data with quantified *Btk* values. Both of these were stratified by whether the data was from inside or outside the spray zone. Finally the airborne *Btk* exposure data indoors was compared to Kromecote card densities

outdoors, also considering first, all the *Btk* data, then only the data with actual quantified values.

Although the intercepts for the relationships were relatively constant, and the coefficients were always positive (i.e., *Btk* concentrations in air increased when Kromecote card densities increased), the relationships were very weak. The highest correlation coefficient was 0.39, indicating that 15% of the variance in *Btk* concentrations was explained by the Kromecote card densities (R^2). Overall, Kromecote card densities were not good predictors of airborne exposures to *Btk*.

Scattergrams of the outdoor data show that there were many "zero" Kromecote card densities with high concentrations of airborne *Btk* (Figures 7 and 8). This was particularly so 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 > 1), yet the smaller aerosols captured by the air samples did drift.

Table 9: Linear regression showing association between airborne concentrations of *Bacillus thuringiensis* var. *kurstaki* (CFU/m^3, ln-transformed, dependent variable) and Kromecote card densities (drops/cm^2, independent variable), during spraying

Data used	intercept	coefficient	unadjusted R^2	correlation coefficient
Outdoors Btk vs. outdoors Kromecote				
All data (N=56) (i.e., including LOD/TNTC estimates)	4.88	0.017	0.014	0.12
Inside spray zone (N=29)	4.37	0.030	0.033	0.18
Outside spray zone (N=27)	4.80	*0.18	0.14	0.37
All measured data only (N=36) (i.e., excluding samples < LOD or > TNTC)	4.63	*0.029	0.097	0.31
Inside spray zone (N=18)	4.25	0.040	0.15	0.39
Outside spray zone (N=18)	4.70	0.078	0.020	0.14
Indoors Btk vs. outdoors Kromecote, inside spray zone only				
All data (N=27) (i.e., including LOD/TNTC estimates)	3.87	0.017	0.029	0.17
All measured data only (N=24) (i.e., excluding samples < LOD or TNTC)	4.47	0.004	0.003	0.05

LOD = limit of detection; TNTC = too numerous to count
* $p < 0.10$
R^2 = proportion of variance in *Btk* concentrations explained by Kromecote card densities

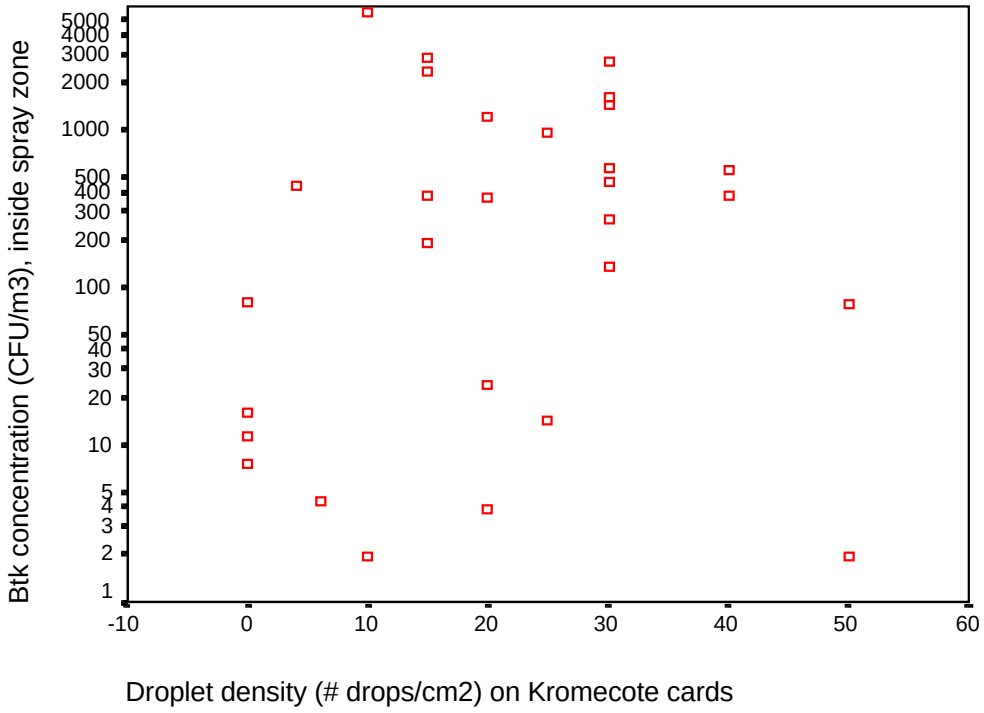

Figure 7. Relationship between airborne *Btk* concentrations (log-transformed, base e) and surface deposition of Foray 48B on Kromecote cards, outdoors, inside the spray zone

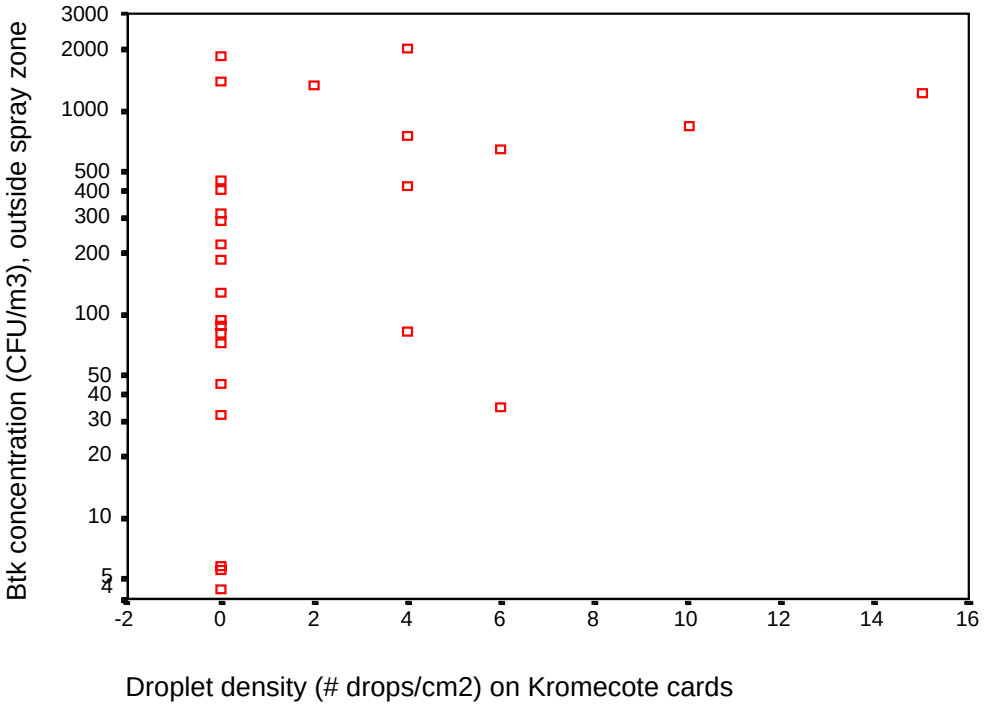

Figure 8. Relationship between airborne *Btk* concentrations (log-transformed, base e) and surface deposition of Foray 48B on Kromecote cards, outdoors, outside the spray zone

3.2.9 Association between airborne Btk concentrations and presence of Btk on nasal swabs

There was nasal swab data available for 16 subjects residing within the aerial spray zone. Table 10 shows the arithmetic and geometric mean *Btk* concentrations indoors and outdoors, stratified by whether subjects had nasal swabs positive or negative for *Btk*. No significant relationship between airborne *Btk* concentrations and the presence or absence of *Btk* on nasal swabs was detected. The trend, if any, was in the direction opposite to that expected. This suggests that personal or environmental factors not recorded in this study were more predictive of positive nasal swabs than airborne concentrations.

Table 10: Association between positive and negative nasal swabs among residents of the aerial spray zone and concentrations of *Bacillus thuringiensis* var. *kurstaki* indoors and outdoors at their residence during spraying

Location where *Btk* air samples taken	*Btk* Concentration (CFU/m³)	
	Negative nasal swab N = 6	Positive nasal swab N = 11
Indoors		
Minimum	24	2.5
Maximum	293	626
Arithmetic mean	138	152
Geometric mean	*[1]101*	*[1]65*
Geometric standard deviation	2.6	4.4
Outdoors		
Minimum	13	1.9
Maximum	1620	1416
Arithmetic mean	615	282
Geometric mean	*[2]240*	*[2]56*
Geometric standard deviation	6.3	9.8

[1] t-test for differences, p=0.52
[2] t-test for differences, p=0.20

3.2.10 Levels of personal exposure

32 residents inside the spray zone provided 24-hour diaries indicating where they spent their time on the day of aerial spraying. These diaries were meant to be used to estimate 24-hour time-weighted-average personal exposures to *Btk* aerosols, based on our analyses of the temporal and spatial distributions of airborne concentrations. Certain characteristics of the air sampling results made it impossible to complete estimates for all times and places. First, because the indoor sampling extended to only 6 hours after the initiation of spraying, and because *Btk* levels indoors at that time were still elevated, we could not estimate personal exposures for periods beyond 6 hours after spraying. In addition, because the air samples extended to only 1 km beyond the spray zone, and *Btk* levels at that distance were still elevated, we could not estimate exposures beyond the area where air concentrations were measured.

Estimates of 6-hour time-weighted-average exposures were made for all subjects who submitted diaries, and who spent all of this time period within the measured area (N=23). The estimated mean personal exposure over the six hours was 224 ***Btk*** CFU/m^3, with a range of 195 to 230 CFU/m^3 and a standard deviation of 9 CFU/m^3. These data were calculated by applying the results of temporal regression equation (2) to all time spent outdoors in the measured area. No distinction was made between areas inside and outside the spray zone, since these concentrations were not significantly different in samples made during aerial spraying. All indoor time was assigned values based on the measurements indoors, during spraying, 2-3 hours after spraying started, and 5-6 hours after spraying. Concentrations between these times were interpolated.

Table 11 shows the average temporal and spatial distribution of all 32 subjects who provided diaries. Most subjects spent the majority of their days indoors within the measured area.

Table 11: Mean times spent indoors and outdoors inside and outside the measured area, at various times in the 24 hours after spraying, by subjects residing within the spray zone who provided diaries (N = 32)

Time Since Start of Spraying	In the Measured Area		Outside Measured Area (minutes)
	Indoors (minutes)	Outdoors (minutes)	
0-1 hrs	60.0	0.0	*0.0*
1-2 hrs	59.3	0.7	*0.0*
2-3 hrs	52.1	6.9	*0.9*
3-4 hrs	44.9	6.5	*8.5*
4-5 hrs	40.9	7.9	*11.3*
5-6 hrs	33.7	13.7	*12.7*
6-7 hrs	*34.5*	*15.2*	*12.2*
7-8 hrs	*38.5*	*11.0*	*10.5*
8-9 hrs	*46.8*	*5.7*	*7.5*
9-10 hrs	*39.9*	*11.2*	*8.9*
10-24 hrs	*728.9*	*51.7*	*51.0*

Note: times and locations in italics could not be included in the personal ***Btk*** exposure estimation, because no suitable air concentration data were available.

4. Discussion

4.1 Identification of "inert" ingredients in Foray 48B

Solid-phase micro-extraction of the head space of Foray 48B bulk samples captured the greatest number of volatile compounds, allowing identification of an even wider spectrum of compounds than direct extraction of Foray 48B using either toluene or a mixture of alcohols. Even the combined results from both the polar and non-polar solvent extracts did not include a similar number of compounds. Head space sampling with a charcoal tube and carbon disulphide desorption, a standard technique for capturing volatile organic compounds in air, was also not capable of detecting the range of volatiles present. This method detected only siloxane derivatives with high retention times. Only one of these coincided with compounds found using solid-phase micro-extraction.

The volatile compounds identified were numerous. An in depth evaluation of each of these is beyond the scope of this report. A brief overview of some of the compounds follows:

- Sydnones are 5-membered heterocyclic nitroso compounds which can be formed by the reaction of acetic anhydride, present in the Foray 48B mixture, with N-nitrosophenyl-glycine. These compounds have photochromic properties [Budavari et al., 1996], and may be responsible for the colour of the Foray 48B.
- Trimethyl phosphine is a methylated derivative of phosphine gas (PH_3). Whether the smell of decaying fish that is associated with this gas is also associated with its trimethyl derivative is not known, but if so, this compound could be a contributor to the characteristic unpleasant odour of Foray 48B.
- Butylated hydroxy toluene is a well known antioxidant which is found in a variety of products including food, animal feeds, petroleum products, animal oils, and vegetable oils.
- Benzoic acid occurs in many natural and synthetic products. It is used as a food preservative, as an antifungal agent, and as the base compound for a family of herbicides [Budavari et al., 1996].
- The siloxanes identified should be interpreted with caution. Siloxane derivatives were detected using the charcoal tubes and to a lesser degree with some of the other techniques used. Laboratory solvent blanks also showed siloxane derivatives, though at much lower levels than in the samples. Siloxane derivatives are constituents of the GC column, the septum of the injection port, as well as the coating of the SPME fibre. In the presence of polar solvents, there may be reaction between the siloxanes in the GC and organic compounds in the sample, forming a range of derivatives.
- In order to consider the potential toxicity of the compounds identified, the Threshold Limit Values [ACGIH 2000], internationally recognized occupational exposure guidelines, were checked to determine whether there were guidelines for any of the identified compounds with fits greater than 800. Three compounds had occupational exposure guidelines: acetic acid, 10 parts per million (ppm) to protect against irritant

effects; ethylene diamine, 10 ppm to protect against irritant and asthma effects; and butylated hydroxy toluene, 10 mg/m^3 to protect against irritant effects.

Of the agents identified, no single one stands out for use as an exposure indicator in field applications. Only solid-phase micro-extraction in the head space of a bulk Foray 48B sample was sensitive enough to capture a range of volatiles. This technique has detection limits in the part per billion range or below, but does not allow quantification of concentrations. The volatiles identified using this sensitive method were demonstrated under laboratory conditions (head space sampling) which aimed to generate the highest possible concentrations. In addition, many of the compounds detected are only "volatile" in the high temperature conditions of the GC (up to 280 °C), not at ambient temperatures possible during the aerial spraying. The difficulty of measuring volatiles during aerial spraying is supported by the poor results of the charcoal tube and impinger sampling in the laboratory, as well as charcoal and silica gel tube sampling in the field. These are standard methods for capturing volatiles in air which should detect concentrations in the parts per million range.

The results of the field exposure study provide evidence that not only volatile components of the Foray 48B are potentially inhaled. The count median aerosol sizes of the Andersen samples were in the 4 to 7 micron range. When in such a fine aerosol form, liquid and solid components of the pesticide formulation could also be inhaled and transported into the lower portion of the respiratory tract. Therefore identification of these components is also important. The analyses for non-volatile agents using two HPLC methodologies produced a complex spectrum of constituents. This is to be expected from Foray 48B's suspension of spores, spore components, endotoxin, additives, wetting agents, sunscreens, culture media, etc. An extensive research program would be required to identify the peaks in the HPLC analyses. A more cost-effective method to ease public concerns about the constituents of the formulated Foray 48B would be release of this information by the manufacturer.

Abbott Laboratories indicated that their additives are included on the US Environmental Protection Agency's lists of "inert" ingredients: List 3 (1,775 agents, Inerts of unknown toxicity); List 4A (119 agents, Inerts of minimal concern); or List 4B (308 agents, Inerts with sufficient data to substantiate that they can be used safely in pesticide products) [EPA, 2000]. Of the 38 compounds we identified in our analyses of the Foray 48B bulk sample, 5 were found on List 3 (butylated hydroxy toluene, ethylene diamine, and three siloxanes), and 2 were found on List 4B (acetic acid anhydride and benzoic acid). Uncertainties in our identification procedure could be a partial explanation for the remaining 33 compounds not being located on the EPA lists. This should not be the case for the compounds identified with excellent fit scores. Some of remaining compounds may be constituents or by-products of the *Btk* itself or of its growth media, and therefore would not be listed as "inerts". Finally, it is possible that EPA's lists simply do not include some of the ingredients added to Foray 48B.

4.2 Airborne concentrations of *Btk* during aerial spraying

4.2.1 Airborne concentrations of Btk

The range of culturable *Btk* concentrations measured outside residences in Victoria during aerial spraying ranged from less than 10 CFU/m^3 to greater than 1600 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 less than 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 study of airborne exposures to *Btk* published in the scientific literature to date, Elliott et al. [1988] 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.

In the study of Thorogood et al. [1996, as reported in Public Health Protection Service, 1997], spore levels inside homes during and immediately after spraying were as high as 1000 spores/m^3. The sampling and analytical methods were not described in the source report.

Noble et al. [1992] conducted personal exposure monitoring of ground spray workers during Foray 48B application in the city of Vancouver, and found exposures ranging from 200,000 to 15,800,000 CFU/m^3. The much higher levels detected in this study could reflect the fact that the exposures were measured among the ground spray application team, some of whom were visibly wet from the spray. It is also possible that differences in measurement methods may have contributed to the higher measured levels. The air sampling technique was similar to that used in this study, but the colonies were counted after plating serial dilutions of a water suspension of the filter contents.

4.2.2 Spatial and temporal distribution of Btk

Staying indoors during spraying was an effective means of lowering exposures *during* aerial spraying. Results from this study indicate that *Btk* concentrations in indoor air were 2.3 to 4.6 times lower than outdoors. Study subjects were asked to keep their doors and windows closed during spraying. Although some did not comply with this request (N = 8, of 27 indoor sites), we were not able to detect a difference in indoor exposures with open windows and doors with this small sample size.

The lower concentrations indoors were not sustained over time. Data from air samples taken 2-3 hours after spraying began indicated that the concentrations indoors soon approached those outdoors, with arithmetic means of 395 CFU/m^3 indoors vs. 501 CFU/m^3 outdoors, and geometric means of 155 CFU/m^3 indoors vs. 239 CFU/m^3 outdoors. The migration of outside air to the indoors likely 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* since they remained outdoors during the spray period. At 5-6 hours after spraying began, concentrations had diminished, but indoor concentrations now exceeded those outdoors, with arithmetic means of 244 CFU/m^3 indoors vs. 77 CFU/m^3 outdoors, and geometric means of 131 CFU/m^3 indoors vs. 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 hours, and 1, 4, and 9 days. These data indicated that airborne concentrations outdoors diminished quickly with time, with average concentrations less than $1/5^{th}$ of the highest mean levels within 5-10 hours after spraying began, and less than $1/10^{th}$ of the highest mean levels within four days of spraying. Using all 9 days of temporal data, the regression analysis of *Btk* concentrations against time suggested that the half-time (time for the concentration to diminish to 50% of its original concentration) of airborne *Btk* outdoors is 2.4 days (95% confidence interval: 1.8 to 4.5 days). However, if data from only the initial 10 hours from the time of aerial spraying is used, the predicted half-time of airborne *Btk* outdoors is much shorter: 3.3 hours (95% confidence interval: 1.9 to 11.4 hours). 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, then a slower phase in which UV-resistant spores are diluted by uncontaminated air or removed by slow settling of the fine aerosols. 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 [1991] described a laboratory study which found that 50% of the bacterial cells were inactivated within 30 minutes of UV light exposure. Otvos and Vanderveen [1993] described a study which found that spores and crystals were almost completely inactivated after 12 hours of UV exposure. The *Btk* studied in these trials were 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- to 1000-m band around the spray zone. Airborne concentrations in this zone were determined by the speed and direction of the wind on the spray day. Upwind sites had *Btk* concentrations lower than within the spray zone, but sites downwind had higher concentrations than those in the spray area. This effect was exacerbated as wind speeds increased. 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 minutes following the start of spraying. These aerosols had count median aerodynamic diameters of 4.3 to 7.2 microns, with standard deviations of 2.6 to 3.8. They would be expected to remain suspended in the air for hours to days, and would be available to follow wind currents in the area. The extent of drift may be not have been expected by those who planned the insecticide application since their target droplet size was 110-125 microns, large enough to settle to the ground in less than 5 minutes. A question that arises is why some *Btk* aerosols were so much smaller than planned? There are several possible explanations. The most likely is that large aerosols produced by the nozzles were broken up immediately after their release by air pressures created by the speed of the plane (370 km/hour). It is also possible that water evaporated from the aerosols during their descent decreasing the droplet sizes; this idea is supported

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 to 150 microns visual diameter, suggesting that not only smaller, but larger than planned droplet sizes occurred.

4.2.3 Use of Kromecote cards as predictors of airborne Btk exposure

Surface deposition of Foray 48B on Kromecote cards appears to be a poor predictor of concentrations of inhalable *Btk* in the air. This finding is not surprising given the different functions of and different aerosol size ranges captured by the two sampling methods. Although increases in Kromecote card densities were related to increases in *Btk* concentrations, only a small proportion of the outdoor *Btk* variance (1-15%) was explained. Even less of the indoor *Btk* variance was explained by the Kromecote card data (0.3-3%).

4.2.4 Relationship between presence of Btk on nasal swabs and air samples

There was no relationship between the presence of *Btk* on nasal swabs and indoor or outdoor air concentrations at the subject's residence. This suggests that factors other than airborne exposures contribute to the presence of *Btk* in nasal swabs. Such factors might include hand contact with contaminated surfaces; rhinitis; individual susceptibility; and personal airborne exposure levels which may differ from residential airborne concentrations, especially if the individual is mobile during the day.

4.2.5 Limitations

The search for the "inert" constituents of Foray 48B was extremely difficult without access to more specific information about possible ingredients *a priori*. Verification of peaks with standard solutions is a necessary confirmatory step for the volatiles identified in the GC/MS analyses, and a requirement for identification of the non-volatile components assayed by HPLC/MS. We could not purchase standards for the 2,200 "inerts" on EPA's lists 3, 4A, and 4B.

Concentrations of volatile "inerts" were too low to be assessed by standard air sampling techniques; therefore no sampling of volatiles was conducted during the field study. Further research would be required to identify methods which would allow air sampling of the volatiles identified in the laboratory, during aerial spraying in the field.

Participation in the exposure measurement study within the spray zone was low (42%) because of the early start times of aerial spraying. Despite the low participation rate, the distribution of sampling sites throughout the spray zone reflected the distribution of the Victoria population, as originally planned.

The laboratory method for quantifying the numbers of bacterial colonies on the air filters differed from that planned. Filters were plated directly onto agar, instead of plating serial dilutions of a water suspension of the filter contents. The method used ensured the lowest possible limits of detection, but lowered the maximum counts possible. This resulted in 10% of plates having too many colonies to count. Another 18% were very difficult to count. The

latter plates were divided into 2 to 5 sections; one section was counted and its count multiplied by the number of sections. The outcome of this change in protocol would be underestimation of the high airborne *Btk* concentrations.

We examined this possibility by comparing the airborne *Btk* concentrations measured by the Anderson sampler at eight locations to those measured by filter samplers at the same locations (Table 12). On average, the filter sample concentrations were 4.4 times lower (note that the filter concentrations included 3 imputed values: two for samples below detection limits, one for a sample with colonies too numerous to count). Reasons other than the filter plating technique could contribute to the concentration differences in the two sampling methods: filter sampling allows desiccation of the sample, and may kill spores which 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 minutes after spraying began, the time when concentrations are likely to be highest, whereas the filter samples were taken for one hour. On the positive side, there was moderate correlation between the Andersen sampler and filter sampler concentrations (Pearson $r = 0.56$, $R^2 = 0.31$), much better than the correlation between the Andersen sampler and the Kromecote card data (Pearson $r= 0.19$, $R^2 = 0.04$).

The one previously published exposure study used the same filter plating method as this study, and found similar average concentrations, but a higher range of exposures (to a maximum of 11,000 CFU/m^3) [Elliott et al., 1988]. To quantify such high concentrations, the investigators used lower air flow rates and shorter sampling durations; they did not report a limit of detection. In the unpublished study of Noble et al. [1992] during an earlier Vancouver spray program, serial dilutions of the filter wash were used, and a much higher concentration range was measured (up to millions of CFU/m^3). It is likely that the main reason for the higher concentrations in that study was that measurements were made during ground spraying rather than aerial spraying, not the microbial counting technique.

Table 12: Comparison of air concentrations of *Bacillus thuringiensis* var. *kurstaki* measured using Andersen samplers and filter samplers, and of spray deposition densities measured on adjacent Kromecote cards, at 8 locations where Andersen samples were taken

	Btk concentrations		Spray Deposition Densities
Date Sampled	Andersen Sampler (CFU/m^3)	Filter Sampler (CFU/m^3)	Kromecote Card ($drops/cm^2$)
May 8th	1545	135	30
May 9th	2210	80	0
May 9th	2226	649	ns
May 20th	1668	*8	0
May 20th	2045	14	25
June 8th	3074	*2847	15
June 8th	2874	*4	20
June 9th	2982	465	30
Mean	*2328*	*525*	*17*
(SD)	*(590)*	*(968)*	*(13)*
Correlations (Pearson r above the diagonal, Spearman rank r below)			
Andersen Sampler	-	0.56	0.19
Filter Sampler	0.45	-	0.003
Kromecote Card	-0.05	0.25	-

SD = standard deviation
ns = no sample
* = imputed value
- = identical measurements, correlation = 1.0

4.2.6 Recommendations

The results of this study suggest the following:

- Small *Btk* aerosols, in a size range that can reach deep into the human respiratory tract, were measured during and after the aerial spraying. These aerosols likely contain all components of the Foray 48B formulation, yet most of these components remain unknown because of the difficulties of identifying specific chemicals in such complex mixtures. It would facilitate risk assessment if the pesticide manufacturers would identify the "inert" ingredients.
- During the spray period, staying indoors with all doors and windows closed resulted in exposures lower than those outdoors. However, exposures indoors increased within 3 hours after spraying and were higher than outdoor concentrations by 5 to 6 hours after spraying began. Indoor concentrations appeared to dissipate much more slowly than outdoor concentrations. In future exposure studies, indoor concentrations should be measured for up to 9 days after spraying to determine the half-time of the *Btk* in indoor environments.
- The pattern of airborne concentrations of *Btk* appeared to differ indoors and outdoors. Future studies should measure factors such as UV light intensity, aerosol size distributions, traffic into and out of homes, humidity, temperature, local wind speeds, presence of open windows, and natural air infiltration to determine which factors are associated with increased and reduced *Btk* concentrations in both indoor and outdoor environments.
- Drift of the *Btk* aerosol was detected throughout a zone up to 1 km away from the spray area. Future studies should measure air concentrations more distant than 1 km away from the spray zone (perhaps up to 25 km away), to allow estimation of the maximum drift distances. Some factors which contribute to drift, such as wind speed, temperature, and relative humidity, were detected in this study. Studies examining other potential explanatory variables (e.g., plane speed) would be valuable.
- Kromecote cards were not an effective indicator of airborne exposures to *Btk*. Future studies examining personal exposures should use filter or Andersen sampling techniques to directly measure air concentrations of *Btk*.
- No relationship was seen between air concentrations and positive or negative nasal swabs. Future studies should record factors such as hand contact with contaminated surfaces, rhinitis, and personal airborne exposure levels in order to investigate factors which contribute to the presence of *Btk* in nasal swabs.

5. References

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Appendix A: Sample Contact Letter

Dear Name:

You may be aware that several areas on Vancouver Island will be sprayed this spring with a biological pesticide to control the gypsy moth. I am writing to ask your help in an important scientific study that we are conducting during the spray period, "**Airborne Exposures During Gypsy Moth Spraying**". It is being conducted by the University of British Columbia, and aims to determine the potential for the general public to be exposed to the active and "inert" ingredients used in the spray program.

The study would involve taking samples of the air outside and inside your home during and after one of the spray days. In addition, we will ask you complete a diary of the street addresses where you spend your time on the sampling day. In order to take the air samples, we will use small air pumps and air-filtering devices which will be set up inside and outside your home. The pumps and filters can be set up in whichever location is most convenient to you. They will be turned on for about one hour at a time. We will take 2 indoor samples and two outdoor samples concurrent with the spray period. In addition, we will return up to 6 times over the next 9 days to take additional 1-hour samples outside your home. We will need you to be present for the duration of the sampling inside your house only (up to a total of 5 hours).The main inconvenience will be the early hour of the day: the spraying will start at about 6 a.m. and finish about 7 a.m.

Your participation in this study is voluntary. You may choose to withdraw at any time. In addition, your decision about whether or not to participate will be kept confidential. The general results of the study will be published in scientific journals, but information which identifies you or your home will remain strictly confidential. No data with results from your home will be stored together with your name or address. Once the study is complete, you will be sent a summary of the general results of the study. The Capital Health Region will also receive a report summarizing the results, but no information will be given which identifies specific results with you or any other person who participates.

We hope that you will help us in our efforts to assess the potential for exposure to the public. Our Study Coordinator, Yat Chow, will call you in a few days (in the evening) to ask you if you are willing to participate in this study, and to set up the air sampling time if you agree. You should feel free to ask any questions you may have at that time. If you have any questions before then, or at any other time, please don't hesitate to call Yat Chow at 604 822-0837 or me at 604 822-2041 (please feel free to call collect).

Yours sincerely,

Kay Teschke, PhD, CIH, ROH
Associate Professor

Appendix B: Sampling Data Form

IN vs. OUT

Research Assistant Name: _____ Date: _____

Name of Resident: _____

Address/Location: _____

Outdoors Cassette ID: _____

Outdoors Pump ID: _____

Time Pump Started: _____

Time Pump Stopped: _____

Pump Counter Reading: _____

Field Blank Cassette ID: _____

***Indoors** Cassette ID:_____

***Indoors** Pump ID: _____

*Time Pump Started: _____

*Time Pump Stopped: _____

*Pump Counter Reading: _____

Kromecote Card ID: _____

Time Card Put Out: _____

Time Card Put Back in CD Holder: _____

*__Andersen Sampler Plate__ ID: _____

*__Andersen__ Pump ID: _____

*Time Pump Started: _____

*Time Pump Stopped: _____

* *Indicate that these types of samples are not being given to every research assistant. You may not have one.*

(DO NOT FILL IN BELOW)

Pump Calibrations:

Pump ID	Flowrate (pre)	Flowrate (post)

Outdoor Environment

Approx. distance form sampling site to nearest trees (in metres) _____

Approx. height of these trees (in metres) _____

Type of these trees (circle as many as applicable) [conifer] [deciduous]

Approx. distance from sampling site to nearest buildings (in metres) _____

Height of these buildings (in metres) _____

Type of ground surrounding sampling site (circle as many as applicable):
[pavement] [grass] [other: specify: _____]

Weather conditions (circle as many as applicable):
[rain] [shower] [sunny] [cloudy] [other: specify: _____]

Make a diagram of pump location, below, if it is easier:

*INDOOR ENVIRONMENT

Resident type (circle one only) [house] [duplex] [townhouse] [apartment]

If apartment, townhouse, or duplex, is home entry (circle 1 only) [direct to outdoors]
[from indoor hallway]

storeys tall: _____

On which storey was sampling conducted? _____

All windows and door in home closed during sampling? [yes] [no]

Sampling location? (circle one) [kitchen] [living room] [family room] [other, specify: _____]

Indoor temperature: (^{o}C) _____