ADVERSE HEALTH CONSEQUENCES FOLLOWING AERIAL SPRAYING WITH BACILLUS THURINGIENSIS (VAR. KURSTAKI) (BTK), TO CONTROL THE GYPSY MOTH: FLAWS IN GOVERNMENT RISK ASSESSMENTS AND IN PUBLIC HEALTH OFFICIALS' ATTITUDES

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In the spring of 2009, forestry officials determined that an outbreak of gypsy moth larvae in wooded areas on the west side of the city of London, Ontario, Canada was severe enough to warrant a spraying program to control the moths (1). The areas of concern are in close proximity to residential suburbs. The gypsy moth *Lymantria dispar* (L.) is an invasive species introduced to North America in the 1860s from Europe. After mating the female moth lays her eggs in one, buff coloured mass that looks like a small sponge. These are placed in sheltered positions on trees rocks, stumps etc. A small caterpillar develops in the eggs and over-winters there. These larvae emerge the following spring and climb toward the treetops where they extrude a fine thread, like a spider web, and are carried by the wind to surrounding trees. There, they go through moulting stages called instars. The final instar yields a caterpillar with a voracious appetite for leaves, stripping the foliage in the process. While the moth has a preference for certain trees like oak, maple, cherry, willow and others there are over 300 known host trees that may be infested. In late summer the final stage pupates for about three weeks. When the moths emerge the female is so swollen with eggs that she can't fly. She gives off a pheromone, a sexual odour that attracts the mobile males. She mates once and then lays her egg mass and dies thereafter. Trees can generally stand up to three seasons of infestations without dying as long as they are not stressed by other factors such as drought (2).

The city elected to institute a spraying program to control the moths, using a biological agent, *Bacillus thuringiensis* (var. *kurstaki*) or Btk. This soil bacterium enters the digestive system of the moth larva as it feeds on vegetation and then releases toxins that kill it so the reproductive cycle is interrupted. A commercial preparation, Foray 48B, was used. It contains a number of "inert ingredients" designed to keep the product from clumping and to help it to adhere to tree leaves. Also some ingredients may be designed to prevent contamination with other organisms. The nature of these ingredients is secret due to patent protection.

On April 17 the city held a public meeting to discuss the proposed spray program. The meeting was held not far from the proposed spray zone. Two applications, about one week apart, were to be made from a helicopter flying about 15 meters above treetop height. Applications were to be made only in winds of 15 kilometres/hr (about 9 mph), or less. A notification zone was

established at 200 metres outside the spray zone. Residents within this zone received printed notices in advance of the spray date. On the city website the city published the following statement under “Preparation and Follow-up”. “To minimize potential risks associated *with the low-flying helicopter* (my italics), the public is requested to stay indoors for the duration of the spray and not travel in or out of the spray zone area during the treatment” (1). The city was apparently mostly concerned about helicopter crashes. The first spray application occurred on May 21. As one who has sailed in this area for decades I can attest to the fact that winds are rarely constant regarding either direction or velocity and this is especially true of light winds. Thus a 200 metre buffer zone could not be relied upon to provide adequate separation from spray drift. Attempts by residents of the area to reach and discuss their concerns with city officials met with little success. The medical officer of health was apparently absent from his office until shortly before the second spraying occurred. The individual in the Middlesex-London Health Unit who was in charge of the spraying program did not feel there was any cause for concern when called by residents and the city forester was unavailable for contact throughout the entire program. A meeting with some city councillors was unfruitful. Residents were told there was little they (the councillors) could do and apparently there was some concern about possible legal action should they interfere.

The Consequences: Following the initial application a number of residents experienced adverse health effects that caused them sufficient concern for them to hold a meeting of local residents and subsequently arrange the meeting with the city councillors to discuss their concerns. At least two pet dogs in the neighbourhood also were sufficiently ill to require the services of a veterinarian.

TABLE 1. LIST OF HEALTH PROBLEMS EXPERIENCED BY SOME MEMBERS OF ADJACENT NEIGHBOURHOODS FOLLOWING THE INITIAL SPRAYING WITH Btk (FORAY 48B)

SUBJECT #	SIGNS/SYMPTOMS
1.	STOMACH PAIN, TROUBLE BREATHING, SORE THROAT
2.	NAUSEA, VOMITING, SORE THROAT (SAME HOUSEHOLD AS #1)
3.	SORE THROAT, FUNNY TASTE IN MOUTH
4.	BAD COUGH, FEELING LETHARGIC
5.	SORE THROAT, STRANGE TASTE IN MOUTH
6.	NEWLY DEVELOPED MIGRAINES
7.	SORE THROAT
8.	BURNING EYES (SAME HOUSEHOLD AS #8)
9.	VOMITING
10.	DIARRHOEA (SAME HOUSEHOLD AS #9)
11.	SINUS CONGESTION, HEADACHE (SAME HOUSEHOLD AS #9)

12.	FATIGUE, MUSCLE CRAMPS
13.14.15.	SORE THROATS, COUGHS
16.17.18.	SORE THROATS
19.20.21	SORE THROATS
22.	SORE THROAT, SORE EYES, FLU-LIKE SYMPTOMS
23.	WEAKNESS, FATIGUE, HEADACHE, SORE THROAT, SORES IN MOUTH, DIZZY, MUSCLE ACHES
24.	LETHARGY, SORES IN MOUTH, SORE THROAT, SORE EYES, UPSET STOMACH, WEAK, DIZZY (SAME HOUSEHOLD AS # 23)
25.	SORE EYES, COUGH IN MORNING OF SPRAY

Evidence of Adverse Health Effects Associated with Btk Spraying: Very few scientific studies including adequate follow-up have been conducted after the use of Btk spray in urban areas. In fact, an internet search conducted through the science library of the University of Western Ontario revealed very few articles that attempted to look at health consequences of Btk spraying. This search surveys thousands of peer-reviewed journals in such data bases as Pubmed, Web of Science, Embase, SCOPUS, Cumulative Index to Nursing and Allied Health Literature (CINAHL) and Zoological Record to mention a few.

In 2002 Pearce *et al* (3) reported on a study conducted on Vancouver Island in association with aerial spraying with a commercial Btk preparation (Foray 48B^{R}). The spraying took place in early morning on dry days at a height of 61 metres. Three applications were made at 10 day intervals. A one kilometre buffer zone was established between the spray zone and the no spray zone. Children within the spray zone were age-matched with children outside the buffer zone. All had medically diagnosed asthma and were receiving medication. There were 29 subjects in each group with average ages of 9.8 years. Subjects were measured for Peak Expiratory Flow Rates (PEFRs) for one week before the first spraying and five days after the last. The group found no statistically significant differences between the groups with regard to PEFRs or reported asthmatic episodes in diaries. Nasal swabs were taken before the spray and two hours thereafter. Participants were advised to remain indoors during the spraying, with doors and windows shut, until the second swab was taken. They found that the Btk cell count on the ground could be as high up to 1000 meters from the spray zone as it was near the zone and was more dependent on wind speed and strength than on distance from the zone. While the outdoor cell count declined over several days the indoor count continued to increase for up to 5 days post spray. Cell counts from the nasal passages of people in and around the zone were several times higher after the spray.

This report seems to be at odds with the experiences of numerous other communities. It was a well-designed and apparently well-conducted study and thus merits additional discussion. The fact that the participants were being followed closely should have led to greater awareness of the potential hazard of the spray and hence to greater compliance with instructions to remain indoors for two hours after the spraying, until the second swab was taken. The fact that the participants were being closely monitored

for health effects could have allayed anxiety over the spraying and hence lessened or removed any psychological influence regarding the exposure. It must be noted, however, that the study dealt with a very specific and restricted population. It tells us nothing about the reactions of the general population, which might very well have been more typical of those reported from other areas. In fact, a post-spray site inspection by the Society Targeting Overuse of Pesticides (STOP) conducted for the Ecological Health Alliance yielded strikingly different results. They reported 290 incidents involving 51 symptoms including nausea, vomiting, cramps and diarrhoea and two incidents of "near death" (their words, no details). Excruciating itching was also a frequently reported symptom (www.bcn.ca/stop/part4.html). Their site inspection was conducted some time after the spraying, indicating that later follow-up could be an important and often neglected aspect of health and safety assessments. This will be noted again below.

Reported adverse reactions to aerial Btk spraying seem to vary greatly from locale to locale. In the Vancouver spraying program, nearly 250 people reported adverse reactions, mostly allergy-like and flu-like symptoms, and ground spraying personnel were frequently affected. During a Washington State program over250 people reported similar symptoms and six required treatment in emergency departments (4a). In neighbouring Oregon State post-spray cultures were taken from various body sites and fluids from people during routine clinical examination. Btk was cultured from 55 individuals and in three with pre-existing medical problems it was considered to be a possible infectious agent. The authors stated their belief that the role of Btk as a possible pathogen deserved further study (4b). Petrie *et al* (5) reported on the health effects of a spraying program with Foray 48B in Auckland, New Zealand. 292 residents within the Ministry of Agriculture and Forestry West Auckland spray zone were recruited into the study and completed a symptom checklist and a questionnaire measuring health perceptions 10 weeks before the first aerial spraying. Three months after the start of spraying 181 of them responded to a similar checklist and questionnaire. Symptom complaints increased significantly following the spraying. In particular, the following symptoms were often reported: sleep problems, dizziness, difficulty concentrating, irritated throat itchy nose, diarrhoea, stomach discomfort and gas discomfort. Subjects who suffered from hay fever reported a worsening of their condition after the spraying. The authors concluded that "aerial spraying with Foray 48B is associated with some adverse health consequences in terms of significant increases in upper airway, gastrointestinal, and neuropsychiatric symptoms, as well as a reduction in overall perception of health in the exposed population". These complaints, from individuals located thousands of kilometres away, are remarkably similar to those listed by London citizens in Table 1.

Prior to the proposed spraying program in Hamilton, New Zealand, the Auckland Regional Public Health Service published an extensive report after surveying the literature (6). Following a practice that appears to be the norm for government reports on this issue, their report is a

rather glowing endorsement of the safety of Btk aerial spraying. It does, however, concede that "Some people may experience minor eye, nose, throat and respiratory irritation. The (previous) HRAs (health risk assessments) raised the possibility of asthma aggravation which was considered biologically plausible". And "Some people find the odour of F48B unpleasant. Some people may experience nausea, headache and other symptoms if exposed to unpleasant smells". The report states that long term effects have not been reported but concedes that there is little reliable information available on this. Gastroenteritis was discounted as a problem, but the experience in both Auckland and here in London belies this claim as stomach cramps, nausea, vomiting and diarrhoea were often reported.

The Auckland experience differs from most similar ones in that spraying took place several times yearly over several years. Public outrage reached such a peak over concerns about adverse health effects that two events resulted. First, the New Zealand Government Ombudsman was convinced to conduct an investigation into the spray program and its possible health effects, and second, The People's Inquiry into the Impacts and Effects of Aerial Spraying Pesticide over Auckland, NZ was formed to conduct an independent investigation. Both reports became public in 2006-07. The Ombudsman's report (7) was very critical of the manner in which the Ministry of Agriculture and Forestry (MAF) had handled the situation. In essence he felt that the MAF trivialized the possible adverse health effects arising from exposure to the spray, did not adequately inform the public of these effects, and it and the Ministry of Health were dismissive of public concerns and complaints. In his recommendations he states (item 15) "I have reached the conclusions that insufficient attention was paid to the impact of these operations, and that, since there is the likelihood that the need to carry out similar operations may well arise in the future, it is important that a structure be established that will enable the worst features of these earlier operations to be avoided." Further, (item 16) "In particular, there needs to be a clear official acceptance that although the numbers of people may not be great as a proportion of the community in the spray zone, there will, in raw numbers, be a significant number who the evidence indicates will require medical attention, and in some cases removal from the area to be sprayed. It is no light thing to be sprayed, perhaps repeatedly, with some substance the ingredients of which are to some extent confidential, and to have one's life substantially disrupted for what may be quite a lengthy period of time." And "…..it was apparent that while the majority put up with the discomfort and inconvenience, there was a significant lack of public support, and mistrust of the Government agencies involved." The Ombudsman recommended that the spraying agency provide full and accurate information about the need for, and nature of, the spraying program and fully acknowledge that there may be adverse health consequences to some individuals.

The report of the People's Inquiry, unfettered as it was by political niceties was even blunter (8). The inquiry received hundreds of written and oral reports from individuals and families who

documented long-term health effects after the spraying. In addition to the symptoms noted above, people reported excruciating itching of the skin and other skin problems, muscle spasms, persistent fatigue and exhaustion. The MAF and its contracted physicians tended to dismiss these as “generic” or “work related allergies”. A persistent, racking cough was common enough to be dubbed “the moth cough”. Debilitating asthma developed in both adults and children. Serious illnesses were reported to be exacerbated by the spray. Numerous hospitalizations were reported. These findings are in stark contrast to those of health officials who reported no increase in visits to emergency departments or in hospitalizations. Perhaps they should have checked again at a later time. Admittedly it is very difficult to prove that these later developments were the result of the spraying, but in view of the MAF’s report stating that there is little information about long term effects they are at least an indication that much more research is required before sweeping statements regarding the safety of Btk sprays can be made.

The inquiry also noted that the concerns of several scientists raised when the spray program was first proposed, were ignored. Dr. Meriel Watts, who has served on numerous government bodies relating to pesticides found the related health risk assessments to be biased in favour of the spray application and that, where toxicological data were lacking, the assumption was made that the effects would not occur (e.g. Neurological effects). Dr. Simon Hales, an epidemiologist, noted that the spray is a bioaerosol with particle sizes capable of being inhaled and causing respiratory problems. Romeo *et al.* (9) conducted a retrospective study on the effects of aerosols with particle size of 10 microns (believed to be present in Foray 48B spray) and found that exposure to them led to an increase in hospitalizations for asthma and, in asthmatic children, to an increase in the frequency of asthmatic symptoms and the use of anti-asthma medications.

As noted by this author, the New Zealand Ombudsman and by Dr. Watts above, there is a powerful bias displayed by writers affiliated with government agencies against recognizing adverse health effects of Btk spraying. This is illustrated once again in the paper by Otvos *et al.* (10). In reference to two, elderly individuals in New Brunswick who reported post exposure dermal rash, hive-like wheals, increased incidence of respiratory infections and general malaise, the author dismissed the symptoms as being unrelated to the Btk exposure. This conclusion was based on two, very small human studies where volunteers were exposed mainly to oral ingestion without adverse effects. The conclusion ignores the fact that these symptoms are commonly reported following exposure to spray. During a spraying program in Eugene, Oregon in 1985-86, numerous people complained if skin rashes and eye irritation. Otvos *et al.* (10) felt this was likely due to an allergic reaction to the caterpillars rather than to the spray, and similar complaints occurring during the Vancouver ground and aerial spraying program were

discounted as being indistinguishable from common allergic symptoms due to other causes. These authors were all employed by Canadian government agencies at the time of writing.

Despite the claims of some government reports that Btk does not cause gastroenteritis, there is compelling evidence to the contrary, besides the fact that symptoms of gastroenteritis (GE) are commonly reported after exposure to Btk spray. In 1995 Jackson *et al.* (11) reported on an outbreak of GE in a chronic care facility. Norwalk virus, a common cause of GE, was isolated from two individuals and a bacillus, tentatively identified as *Bacillus cereus*, also a known cause of GE, was isolated from four people. *B. cereus* is closely related to *B. thuringiensis* and on more specific testing all clinical isolates was identified as *B. thuringiensis*. More importantly all bacillus isolates showed cytotoxic effects characteristic of the enterotoxin produced by *B. cereus.* It has long been known that *B. cereus* can cause two types of food poisoning, one characterized by stomach pain and diarrhoea, the other by nausea and vomiting (12). Each is caused by a specific bacterial toxin. These authors also examined 20 isolates each of *B. cereus* and *B. thuringiensis* and found these toxins in all but one. Of special interest is a study by Damgaard (13) who examined strains of *B. thuringiensis* from commercial insecticide preparations and found that all contained live spores, including Foray 48B, and tested positive for the diarrhoeal enterotoxin. The author concluded that there was a risk of an outbreak of gastroenteritis associated with the use of these insecticides. Abbott, the manufacturer of Foray 48B, claims that efforts are taken during its manufacture to exclude the presence of enterotoxins. However there is a phenomenon that may not make this totally possible. Bacteria contain little packets of DNA called plasmids that they are capable of passing from one cell to another by a process called conjugation. Conjugation is not constrained by either species or genus barriers so that the transfer of plasmids could occur between *B. cereus*, a very common organism, and *B. thuringiensis*. Plasmids are known to contain the genetic information for bacterial toxins (14). Btk cytotoxins from commercial insecticides have been shown to be toxic, causing death, in cultured human cells (15). This study was in fact conducted by Canadian government scientists from another branch of Health Canada, the Mutagenesis Section of the Environmental and Occupational Toxicology Division.

Conventional wisdom has always maintained that *B. thuringiensis* only rarely has caused outright infections in people and then only when there is either massive contamination or pre-existing serious medical problems (16). Recent evidence, however, indicates that the conventional wisdom is wrong. In 1983 a paper (17) reported that a farmer who splashed a Btk spray (DiPel) into his eye developed skin irritation, burning, swelling redness and conjunctivitis. Btk was cultured from his eye. Far from being an isolated incidence stemming from massive contamination, *B. thuringiensis* has emerged as a fairly common isolate from ocular infections. Callegan *et al.* (18) identified Bacillus species as causing one of the most rapidly blinding ocular infections, endophthalmitis. Bacillus species were isolated from ocular infections, identified,

and screened for virulence factors. *B.cereus* accounted for 53% of the isolates and *B. thuringiensis* for 26%. Toxins were identified in most isolates of both species and both species killed corneal and retinal cells within six hr. This group is the first to identify *B. thuringiensis* as "an important ocular pathogen". Helgason *et al.* (19) isolated *B. thuringiensis* as a frequent cause of periodontitis and other human oral infections and more recently *B. thuringiensis* bacteraemia was found in a patient with severe pulmonary disease and neutropenia (20). The authors injected the organism into the tracheas of mice, some rendered neutropenic with cyclophosphamide (an immunosuppressive drug), and found that the organism rapidly disseminated into deep organs and was always fatal. Immunosuppressed mice were much more susceptible than normal mice.

Another, very recent concern has emerged. *B. thuringiensis* was isolated from 21 patients with nosocomial bacteraemia in two hospitals. These patients all had serious, underlying disease. *B. thuringiensis* was isolated from catheter tips, gauze, and the hospital environment. The authors demonstrated the ability of this organism to form biofilms on hospital devices that constitute a threat to patient's health (24). There were earlier reports of *B. thuringiensis* causing wound infections in extensive war injuries and in burn victims (25, 26), again indicating that this organism is an opportunist that can cause problems in the hospital environment.

Of special interest is a report from Brazil that examined the genetic composition of *B. cereus* (a known pathogen) and *B. thuringiensis* and concluded that on the basis of genetic evidence they ought to be considered a single species (27). Given that they have been shown to share the endotoxins that cause gastroenteric food poisoning this would seem a reasonable conclusion. This observation also calls into question the assertion that specific sub-species such as *kurstaki* can be manipulated to avoid toxic effects. Indeed two Canadian government scientists from the Bureau of Chemical Hazards had earlier examined strains of Btk from commercial sources for toxic effects on a variety of human cell cultures and found evidence thereof including blebbing, degradation of immunodetectable proteins and cytolysis. The threshold dose equated to a single spore (15). The ready exchange of plasmids containing genetic codes for toxins further reduces confidence that a sub species can always be relied upon to be safe, especially since this can cross species barriers. In 2005 a Danish report examined the prevalence of *B. cereus* and *B. thuringiensis* in almost 49,000 ready-to-eat food products at a Danish retail market. They found that 0.5% had high counts and of 40 randomly selected strains, 31 could be classified as *B. thuringiensis* based on crystal production and/or cry genes. The 40 strains all had at least one component or gene related to diarrhoeal disease. Only one had the emetic toxin. This further illustrates the difficulty in separating the two species (28).

Ecological impact: Although beyond the scope of this review, there is ample evidence that Btk spray is not specific for the gypsy moth but can be fatal to any exposed moth or butterfly and

possibly other beneficial species such as bees, with negative consequences for any species dependent upon them as a food source (21,22) and in the post-spray survey of the Victoria B.C. area (see beginning of **Evidence of Adverse Health Effects** section) considerable evidence of negative environmental effects was noted (www.bcn.ca/stop/part4.html). Contrary to claims that Btk lasts only a few days in soil, a study conducted in Sardinia, where Foray 48B has been used for years to protect cork trees from the gypsy moth, found by immunological assay that Btk cell counts remained essentially unchanged for 28 months after spraying. The toxin was also detected at 28 months but at a reduced concentration. In some cases both were detected 88months after the last spraying (23). This has obvious environmental implications.

Summary and Conclusions

1. Government sponsored health and safety assessments (HSAs) of Btk aerial spraying habitually tend to minimize, even trivialize, its impact on human health. The same conclusion was reached by the National Ombudsman of New Zealand after his investigation of the spraying programs conducted in the Auckland and Hamilton areas. This investigation was precipitated by a massive public outcry over the impact of the spraying program.
2. The ombudsman also noted that health authorities were dismissive of the public's health concerns, an observation echoed by the experience locally in London, Ontario.
3. HSAs frequently state that *B. thuringiensis* does not cause gastroenteritis but there is considerable evidence to the contrary. Not only are symptoms of gastroenteritis frequently reported following exposure to Btk aerial spray but the bacterial toxins responsible for these symptoms are consistently found in Btk organisms. Moreover, an outbreak of gastroenteritis in a chronic care facility was attributed, at least in part, to *B. thuringiensis*.
4. It is also often noted in HSAs that *B. thuringiensis* only rarely causes infection in humans. In reality it is a frequent cause of a very serious and potentially blinding eye infection endophthalmitis and it is frequently found in oral infections such as periodontitis and apical abscesses. One recent paper reported that it caused a serious pulmonary infection and bacteraemia in a man whose white cell count was extremely low (neutropenia). Very recent evidence reveals that it can cause biofilms on hospital devices, such as catheters, that can result in nosocomial bacteraemias in severely ill patients. See also item 3 above.
5. Cells and toxins of Btk spray have been shown to persist in soil for months or even years following spraying, likely depending on soil and weather conditions. This is in contrast to claims that the effects disappear in a few days. This could have implications for persons who have become allergic through sensitization and creates the possibility of strains

becoming more virulent through exchange of genetic information (plasmids) coding for more potent toxins.

6. Follow-up studies conducted within a few days of spraying may be too soon to detect adverse health reactions. Several surveys conducted weeks or months later have revealed a much higher frequency of adverse effects.
7. New studies have emerged, and will likely continue to emerge, indicating that the adverse health effects of Btk spray are much greater than currently believed. One study suggests that *B. cereus* and *B. thuringiensis* are genetically indistinguishable. In light of this, its safety should be reassessed. Btk is no doubt an excellent product to treat vast areas of forest in sparsely populated locales, but in densely populated areas, some people will get sick. Officialdom needs to be more open and honest with the public and not conceal or trivialize the possibility of adverse health effects. It also needs to be more sensitive and responsive to public concerns over this issue.

In conclusion it seems that the question here is "How many people is it acceptable to make ill in the interest of protecting urban forests?"

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