The Safety to Humans of *Bacillus thuringiensis* Insecticidal Sprays: A Reassessment

CLAUDE GINSBURG
Director, No Spray Zone, Seattle Washington, USA (info (at-sign) nosprayzone.org)

Summary

The aerial and ground spraying of insecticide formulations based on Bacillus thuringiensis (Bt) is an increasingly widespread practice throughout the world. In particular, Bt subspecies kurstaki (Btk) is used to eradicate various species of moth larvae, and subspecies israelensis (Bti) is used to kill mosquito larvae. The long-term safety to human populations that are exposed to these microbes and their crystalline protein byproducts is not well demonstrated.

Previous epidemiological studies of exposed human populations have been flawed in that they do not follow subjects for a sufficient length of time, look only for specific symptoms, or draw conclusions of safety from arguably inconclusive data. This review examines some evidence that casts doubt on the safety of Btk-based insecticides to humans.

Background

The bacteria Bacillus thurengiensis, found naturally at extremely low levels in soil, is actually part of a continuum of bacilli that include B. thuringiensis, B. cereus (a common cause of food-borne gastroenteritis), and to some extent B. anthracis (anthrax) [Carlson et al. 1993]. These organisms are generally distinguished only by a few cellular organelles called plasmids. Plasmids produce proteins that are responsible for part of the particular pathological behavior of each species. However, B. cereus *sensu lato* (as both B. cereus and B. thuringiensis species may be identified as a group) all exhibit characteristics that can cause illness in humans. Because the overall cell architecture of the various species is virtually identical except for the plasmids, differentiating B. cereus *sensu stricto* from B. thuringiensis requires a certain amount of genetic testing.

Btk (B. thuringiensis var. kurstaki) has been used in insecticidal formulations for at least fifty years, and has been in widespread use (applied to hundreds of thousands or millions of hectares) around the world for at least twenty years. Public authorities have consistently maintained that these insecticides are almost completely harmless to humans and cannot enter into a vegetative state in the human body, as well as being a “natural” or “organic” pest control found in soil. This declaration of safety has enabled repeated spraying in densely populated regions. It is instructive to examine the basis of this declaration.

Previous epidemiological studies of exposed human populations have all been flawed or inconclusive in that they do not follow cohorts for a sufficient length of time [Green et al 1990], look only for specific signs or symptoms [Petrie et al. 2003 and Aer’Aqua 2001], or draw conclusions of safety from arguably inconclusive data [Pearce et al. 2002].

Exposure from Spraying

Humans can be exposed to Btk spores, cells, and proteins either by inhalation during a spraying episode or by ingestion (either from food or placing sprayed surfaces in the mouth).

To date, studies have been inadequate to infer the exact particle and droplet spectra during aerial sprayings [Hales et al.2004]. Droplet spectra from spray nozzles of various types necessarily include a significant portion of droplets below 50 μm in diameter, because the peak spray droplet size must be less than 100 μm to not penetrate foliage and end up on the ground. There is also the unknown influence of turbulence created by the fixed or rotating wings on the aircraft. Even considering weather conditions with higher humidity, a substantial portion of these droplets will evaporate, leaving spore and crystalline protein particles of less than 10μm diameter in the air [Ware 1983].

From the existing evidence from Btk-based pesticide sprayings [Teschke et al.2001] it is clear that exposure can be high (possibly much greater than the detection limit of 1600 CFU/m^3) during and immediately after spraying, and remains at a significant percentage of this level for 24 hours. Sealed buildings in the spray zone appear to maintain viable spore levels at significant levels (>500 CFU/m^3) for a number of hours after the spraying, suggesting that remaining indoors during a spraying is inadequate protection against inhalation. In addition, a significant amount of drift was detected at least 1 km outside the spray zone, so that a larger than anticipated population was exposed. No measurements of exposure levels to the crystalline protein byproducts of Btk (such as δ-endotoxin) or to other substances in the spray formulation have been made.

Viability in Humans

There is mounting evidence that Btk spores are able to enter the vegetative state in immunocompetent individuals. In several studies, viable Btk bacilli in the vegetative state were recovered from the nasal passages [Valadares et al. 2001] and feces [Jensen et al. 2000] of exposed populations, some weeks or months after exposure [Bernstein et al. 1999]. This fact, coupled with the conditions in the nares and human gut being similar to cultural requirements for B. cereus *sensu lato*, is strong evidence that reproducing populations were established *in vivo*. There has been no recorded evidence of illness caused by the vegetative cells in these studies; the infections, if present, remained asymptomatic. However, an immune response (serum IgE and IgG) can be detected in a number of exposed individuals – a response which may last up to three years in 25% of the test subjects [Doekes et al. 2004]. The IgG response, in particular, suggests an immune response to a reproducing population.

Bt, B. cereus and Enterotoxins

B. cereus *sensu stricto* has been thought to cause emetic or diarrhoeal forms of gastroenteritis due to its production of an enterotoxin, as well as a toxic nonspecific β-exotoxin. Btk insecticidal strains are selected to not produce β-exotoxin, but testing of commercial Btk-based pesticides has shown that enterotoxins are still present at significant levels[1] [Damgaard 1995, Tayabali and Seligy 2000] and it may be difficult or impossible to find a natural Btk strain that has appropriate insecticidal behavior while not producing enterotoxin or β-exotoxin [Perani et al.1998].

There is one documented case of Bt causing a gastroenteritis outbreak [Jackson et al. 1995] but it is quite possible that many cases of food-borne illness attributed to B. cereus are actually caused by Bt. B. cereus is currently the listed cause for 1-5% of food-borne illness in several Western countries. Hospitals almost never positively identify Bt because of the difficulty of distinguishing the two organisms – instead, if the etiology appears to be B. cereus *sensu lato*, it is labeled as B. cereus [Carlson op cit.]. Bt residual spray in many common raw and prepared foods can be 10^6 to 10^7/g, which is 1000 times the contamination level set by food industry for B. cereus levels [Rosenquist et al. 2005]. Enterotoxin production occurs in the vegetative state, and it is likely that Bt can actually enter this state in the human body, as described above.

Recent research [Yang et al. 2003] has identified a method for using genetic engineering (forming a recombinant plasmid) to prevent enterotoxin production in Bt, but this presents an entirely different sort of problem of testing the new organism sufficiently so that safety to humans and the environment can be established.

Other Bt Infections

Although rare, there are documented cases of fulminant Bt infections in humans [Samples and Buettner 1983, Hernandez et al. 1998]. While these sorts of overwhelming infections would not be expected in immunocompetent individuals except after overwhelming exposures, others may be at risk. B. cereus *sensu stricto* is known to have caused fulminant infections in immunocompromised persons and persons with depressed immune systems from steroidal therapy [Arnaout et al. 1999]. Bt superinfections have caused death in immunosuppressed mice [Hernandez 1998 and 1999] and mice infected with influenza-A virus [Hernandez et al. 2000].

Bioaerosols

The health effects of bioaerosols of various types have been documented (for example, see Hauswirth and Sundy 2004). In particular, endotoxins, which are produced by Bt, may play a key role in sensitization and disease. Particles of less than 10 μm diameter are difficult to expel and particularly liable to lodge in the lungs. Common reports of symptoms after sprayings with Btk-based pesticides include the same symptoms as seen with other bioaerosols [Hales 2004]. The potential for other bioaerosols to cause later chronic and serious disease has also been documented, and it is plausible to suggest the same could be true for Bt.

[1] Foray 48B, a commonly used Btk-based pesticide, produced enterotoxin at a level about 1/30th of known B. cereus pathogens.

Asymptomatic or Short-Duration Infections and Later Disease

There is a large body of evidence suggesting the role of bacterial infections in the later development of autoimmune diseases such as SLE (systemic lupus erythematosus) [Zandeman-Goddard and Schoenfeld 2005], MS (multiple sclerosis), psoriasis [Boehncke et al. 1997], scleroderma [Buechner et al 1993] and other skin diseases, and rheumatoid arthritis [Stiernstedt and Granstrom 1985]. It appears possible that the actual infection may be cleared or present in other parts of the body when the ultimate autoimmune reaction occurs [Wolfson and Talbot 2002]. These diseases may be triggered by the infection in combination with a genetic susceptibility and/or some kind of autoimmune mechanism or immunomodulation [Wedi and Knapp 2002] or a combination of other environmental factors [Molina et al. 2005]. It is worth mentioning the particularly strong apparent link between ALS (amyotrophic lateral sclerosis or "motor neurone disease") and mycoplasmal infection [Nicholson et al. 2002]. There has been no link established to date between B. cereus *sensu lato* and autoimmune disease, but this (at least possible) connection has not been explored.

Bt Still an Unknown

Not enough is known about the multiplicity of substances produced by Bt and B. cereus *sensu lato* to absolutely predict the behavior of these organisms. For example, a recent study [Ito et al. 2004] details a newly discovered class of proteins produced by some Bt organisms. These proteins are preferentially cytotoxic to some human cancer cells, but are still relatively toxic to normal cells in the range of 0.01 μg/ml. Products from the fermentation production stage may also be present in commercial spray mixtures, and could be toxic [Tayabali and Seligy 2000].

To complicate matters more, there is good evidence that members of the B. cereus *sensu lato* group can exchange plasmids, thus transferring the virulence of one organism to another that was not virulent. The plasmid exchange can and probably does happen in the gut of earthworms in soil, where Bt can be found in the vegetative state (it remains as a spore in soil) [Jensen et al. 2003].

Conclusions

Considerable questions remain as to the pathology of B. cereus *sensu lato*. In keeping with the Precautionary Principle, it is doubtful that there is sufficient evidence of safety to humans and the environment to continue to expose large populations to Btk and Bti based pesticides.

The public health problem is exacerbated in large-scale insect eradication and control programs because these programs are designed and administered primarily by entomologists. Entomologists can consult with health professionals but they are not generally knowledgeable about current microbiological and health science research . Bt has also been presented for years as a safer alternative to chemical pesticides. This may explain why so many entomologists are still of the opinion Bt is a "magic bullet" that has absolutely no potential for adverse human health or environmental effects [for example, Gara 2005].

There is some information about allergic reactions and sensitization, but the data, mostly concerning asthma, is mixed and inconclusive. Most larger studies show a marked increase in asthmatic complications after sprayings, but no long-term information is available. The evidence of IgE immune response suggests possible IgE modulated allergic reactions to repeated exposures (some of which might have been via food). This has not been fully explored.

Further research needs to be completed to accurately identify droplet spectra and sizes at the ground during and after sprayings, both inside and outside spray zones. In addition, the long-term risks from inhalation of the bioaerosols produced by spraying must be quantified. Larger cohorts must be followed for a number of years to try to identify health problems caused by the spraying. This will be difficult because the development of later disease, if found, must be linked with the initial exposures, but it is necessary to ensure safety.

References

Aer'aqua R Medicine Ltd. Health Surveillance following Operation Ever Green: A programme to eradicate the white-spotted tussock moth from the eastern suburbs of Auckland Report to the Ministry of Agriculture and Forestry. NZ Ministry of Ag. and Forestry, May 2001.

Arnaout, M.K., Tamburro, R.F., Bodner, S.M., Sandlund, J.T., Rivera, G.K., Pui, C.H and Ribeiro, R.C. Bacillus cereus causing fulminant sepsis and hemolysis in two patients with acute leukemia. J. Pediatr. Hematol. Oncol. 1999 Sep-Oct, 21(5):431-5.

Bernstein I.L., Bernstein J.A., Miller M., Tierzieva S., Bernstein D.I., Lummus Z., Selgrade M.K., Doerfler D.L., and Seligy V.L. Immune responses in farm workers after exposure to Bacillus thuringiensis pesticides. Environ. Health Perspect. 1999 Jul;107(7):575-82

Boehncke W.H., Zollner, T.M., Dressel, D. and Kaufmann R. Induction of psoriasiform inflammation by a bacterial superantigen in the SCID-hu xenogeneic transplantation model. J Cutan. Pathol. 1997 Jan;24(1):1-7.

Buechner, S.A., Winkelmann, R.K., Lautenschlager, S., Gilli, L. and Rufli, T. Localized scleroderma associated with Borrelia burgdorferi infection. Clinical, histologic, and immunohistochemical observations. J. Am. Acad. Dermatol. 1993 Aug;29(2 Pt 1):190-6.

Carlson, C.R. and Kolsto, A. B. A complete physical map of a Bacillus thuringiensis chromosome. Jour. Bacteriol. 1993 Feb, 175(4): 1053-60.

Damgaard, P.H. Diarrhoeal enterotoxin production by strains of Bacillus thuringiensis isolated from commercial Bacillus thuringiensis-based pesticides. FEMS Imun. and Med. Microb. 12, 1995. pp245-250.

Damgaard, P.H., Perani, M., Bishop, A. and Vaid, A. Prevalence of beta-exotoxin, diarrhoeal toxin and specific delta-endotoxin in natural isolates of Bacillus thuringiensis. FEMS Microb. Letters, 160, pp 55-60, 1998.

Doekes G., Larsen P., Sigsgaard T. and Baelum J. IgE sensitization to bacterial and fungal biopesticides in a cohort of Danish greenhouse workers: the BIOGART study. Am J. Ind. Med. 2004 Oct;46(4):404-7.

Gara, R. I., Prof. of Forest Entomology, University of Washington, email garar@u.washington.edu. "Of Insects and Ecosystems." Lecture presented at University of Washington, Feb. 23, 2005.

Green, M., Heumann, M., Sokolow, R., Foster, L.R., Bryant, R., and Skeels, M. Public health implications of the microbial pesticide Bacillus thuringiensis: an epidemiological study, Oregon, 1985-86. Am-J-Public-Health. 1990 Jul; 80(7):848-52

Hales, S. Precautionary Health Risk Assessment: Case Study of Biological Insecticides. EcoHealth 1, 399–403, 2004.

Hales, S., Baker, V., Dew, K., Moata'ane, L., Martin, J., Rochford, T., Slaney, D. and Woodward, A. Assessment of the potential health impacts of the 'Painted Apple Moth' aerial spraying programme, Auckland for the New Zealand Ministry of Health. Feb. 2004.

Hauswirth, D.W. and Sundy, S. Bioaerosols and Innate Immune Responses in Airway Diseases. Current Opin. Allergy Clin. Immunol. 4(5):361-366, 2004.

Hernandez, E., Ramisse, F., Ducoureau, J.P., Cruel, T., and Cavallo, J.D. Bacillus thuringiensis subsp. konkukian (serotype H34) superinfection: case report and experimental evidence of pathogenicity in immunosuppressed mice. J. Clin. Microbiol. 1998 Jul;36(7):2138-9.

Hernandez, E., Ramisse, F.. Cruel, T., Le Vagueresse, R. and Cavallo, J.D. Bacillus thuringiensis serotype H34 isolated from human and insecticidal strains serotypes 3a3b and H14 can lead to death of immunocompetent mice after pulmonary infection. FEMS-Immunol-Med-Microbiol. 1999 May; 24(1): 43-7

Hernandez, E., Ramisse, F., Gros, P. and Cavallo, J.D. Super-infection by Bacillus thuringiensis H34 or 3a3b can lead to death in mice infected with the influenza A virus. FEMS Immunol Med Microbiol. 2000 Nov;29(3):177-81.

Ito, A., Sasaguri, Y., Kitada, S., Kusaka, Y., Kuwano, K., Masutomi, K., Mizuki, E., Akao, T. and Ohba, M. A Bacillus thuringiensis Crystal Protein with Selective Cytocidal Action to Human Cells. J. Biol. Chem. Vol. 279, No. 20, May 14, pp. 21282–21286, 2004.

Jackson, S.G., Goodbrand, R.B., Ahmed, R. and Kasatiya, S. Bacillus cerues and Bacillus thuringiensis isolated in a gastroenteritis outbreak investigation. Letters in Appl. Microbiology, Vol. 21, 1995, pp103-105.

Jensen, G., Larsen, P., Jacobsen, B.L., Madsen, B., Smidt, L. and Andrup, L. Bacillus thuringiensis in Fecal Samples from Greenhouse Workers after Exposure to B. thuringiensis-Based Pesticides Appl. and Envir. Microbiology, Oct. 2002, p. 4900-4905, Vol. 68, No. 10.

Jensen, G.B., Hansen, B.M., Eilenberg, J. and Mahillon, J. The hidden lifestyles of *Bacillus cereus* and relatives. Environ. Microbiology, 2003, 5(8), 631–640.

Molina V. and Shoenfeld, Y. Infection, vaccines and other environmental triggers of autoimmunity. Autoimmunity. 2005 May;38(3):235-45.

Nicolson, G.L., Nasralla, M.Y., Haier, J. and Pomfret, J. High frequency of systemic mycoplasmal infections in Gulf War veterans and civilians with Amyotrophic Lateral Sclerosis (ALS). J Clin Neurosci. 2002 Sep;9(5):525-9.

Pearce, M, Habbick, B., Williams, J, Eastman, M. and Newman, N. The effects of aerial spraying with Bacillus thuringiensis Kurstaki on children with asthma. Can. Jour. of Public Health, Jan/Feb 2002; 93, 1.

Petrie K., Thomas M. and Broadbent E. Symptom complaints following aerial spraying with biological insecticide Foray 48B. N Z Med J. 2003 March 14, 116(1170):U354.

Perani, M., Bishop, A. and Vaid, A.Prevalence of b-exotoxin, diarrhoeal toxin and specific d-endotoxin in natural isolates of Bacillus thuringiensis. FEMS Microbiology Letters, 160 (1998) pp55-60.

Rosenquist, H., Smidt, L., Andersen, S.R., Jensen, G.B., and Wilcks, A. Occurrence and significance of Bacillus cereus and Bacillus thuringiensis in ready-to-eat food. FEMS Microbiology Letters Vol. 250, Issue 1, 1 September 2005, pp 129-136.

Samples, J.R. and Buettner, H. Ocular infection caused by a boil;ogical insecticide. J. Infect. Dis. 1983, 146:614.

Stiernstedt G. and Granstrom, M. Ixodes ricinus spirochete infection as the cause of postinfectious arthritis in Sweden. Scand. J. Rheumatol. 1985;14(4):336-42.

Tayabali, F.A. and Seligy, V.L. Human Cell Exposure Assays of Bacillus thuringiensis Commercial Insecticides: Production of Bacillus cereus-Like Cytolytic Effects from Outgrowth of Spores. Environ. Health Perspect. 108, N.10, Oct. 2000.

Teschke, K., Chow, Y., Bartlett, K., Ross, A., amd van Netten, C. Spatial and Temporal Distribution of Airborne Bacillus thuringiensis var. kurstaki during an Aerial Spray Program for Gypsy Moth Eradication. Environ. Health Perspect. 109:47-54 (2001).

Valdares de Amorim, G., Whittome, B., Shore, B. and Levin, D. Identification of Bacillus thuringiensis subsp. kurstaki Strain HD1-Like Bacteria from Environmental and Human Samples after Aerial Spraying of Victoria, British Columbia, Canada, with Foray 48B. Apll. and Envir. Microbiology, Mar. 2001 p1035-1043.

Ware, G.W. Reducing Pesticide Application Drift Losses. Coop. Ext. Service, Univ. of Ariz., Jan. 1983.

Wedi B. and Kapp A. Helicobacter pylori infection in skin diseases: a critical appraisal. Am. J. Clin. Dermatol. 2002;3(4):273-82.

Wolfson, C. and Talbot, P. Bacterial infection as a cause of multiple sclerosis. The Lancet, Volume 360, Issue 9330, 3 Aug. 2002, pp352-353.

Yang, C., Pang, J., Kao, S. and Tsen, H. Enterotoxigenicity and Cytotoxicity of Bacillus thuringiensis Strains and Development of a Process for Cry1Ac Production. J. Agric. Food Chem. 2003, 51, 100-105.

Zandman-Goddard, G. and Shoenfeld, Y. Infections and SLE. Autoimmunity. 2005 Nov. 38(7):473-85.