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A review of the environmental impacts of the microbial insecticide *Bacillus thuringiensis*

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SUMMARY

The use of chemical insecticides for the control of insect pests has proven very effective at increasing today's agriculture and forestry productivities. However, increased public concern regarding the potential adverse environmental effects of chemical insecticides has prompted the examination of an alternative methods for insect pest control. *Bacillus thuringiensis* (Bt) accounts for 95% of the world market of microbial pest control agents.

This document focuses on the environmental impacts of the use of conventional Bt-based commercial products as a microbial insect pests control agent. This includes ecological, economical, and social impacts. Ecological impacts, that is biotic and abiotic effects, of Bt are reviewed in chapter I. Direct and indirect possible economical benefits of the use of Bt are discussed in chapter II. Socio-cultural impacts, the consumers reactions regarding the use of pesticides in agriculture and public response to Bt spraying program, are discussed in chapter III.

INTRODUCTION

The introduction of DDT in 1945 and the following move towards the use of synthetic chemical insecticides has played a key role in the increase of agricultural productivity, protection of crops and forests and in the control of insect vectors of human diseases. However the heavy use of chemical insecticides has not been without drawbacks. Let's mention contamination of water and food sources, poisoning of non-target fauna and flora, concentration in the food chain and selection of insect pest populations resistant to the chemical insecticides. Increased public concern of the potential adverse environmental effects associated with the heavy use of chemical insecticides has prompted the examination of alternative methods for insect pest control. One of the promising alternatives is the use of entomopathogenic microorganisms such as *Bacillus thuringiensis* Berliner (Bt).

A limitation to the rate of progress in the introduction of many potential entomopathogenic microorganisms in the field is the relative lack of knowledge about their ecology and about the fate and effects of the applied micro-organisms in the field. The mode of application, the persistence of the introduced micro-organisms, their reproductive rate (multiplication), the rate of gene transfer to indigenous organisms, their movement away from the site of application (dissemination) and the effects on the balance and functioning of the exposed ecosystem (safety, benefit and harm) are of major importance and must be assessed before a release can be considered (Trevors et al., 1987).

There is a well-documented history of the safe application of Bt in the environment: a small number of wild-type strains, formulated as commercial products, have been applied in increasingly large quantities as insecticides for over three decades to food crops, ornamentals, forest trees and stored grains without incident or harm (Meadows, 1993). In addition, many fundamental and applied researches that have accompanied the exploitation and study of Bt have provided some limited knowledge of its behavior in the environment.

1. Advantages of microbial insect pest control

An important benefit of microbial control agents is that they can be used to replace, at least in part, some more hazardous chemical pest control agents. At the present time, chemical controls are far more commonly used in the world than microbial controls. It is unclear whether or not all chemical pesticides are environmentally harmful, so replacing all of them with microbial agents would not necessarily guarantee fewer environmental risks. Nonetheless, for the numerous chemical pesticides known to have toxic effects beyond their target pests—including toxic effects to animals and human—the opportunity to substitute safer, more selective, and biodegradable biocontrol agents can provide important ecological benefits. One of the ecological advantages of microbial control agents is that they tend to be highly selective, infecting or killing a very narrow range of target pests.

2. Early history of microbial insect pest control

The study of diseases of insects, mites and other invertebrates, the science of invertebrate pathology, provides the scientific foundations of microbial control. Invertebrate pathology has its origin in the study of diseases in beneficial organisms such as silkworm and honey bee. The first observations of diseased silkworms were made in China as early as 2700 BC and by Aristotle on honey bees in 335 BC. It was not until the work of Bassi in 1834 that a microorganism, the fungus *Beauveria bassiana*, was associated with the production of disease in an animal, the silkworm. Thirty years later, Louis Pasteur followed up with a more thorough study of the various diseases of the silkworm. Both of these 19th century pioneers suggested that microorganisms could be used to control insect pests (Steinhaus, 1956).

Although several developments in invertebrate pathology took place in the late 19th and the first half of this century, it wasn't until the discovery, development and subsequent commercial production of *Bacillus thuringiensis* Berliner that practical use of microbial control began on a large scale (Lacey and Goettel, 1995). So far, even though more than 100 bacterial species have been identified as insect pathogens, only certain *Bacillus* species have met with some commercial success, especially Bt (Starnes et al., 1993).

3. What is *Bacillus thuringiensis*

Bt is a gram-positive, rod-shape, aerobic, and spore-forming bacterium closely related to the omnipresent soil bacteria *Bacillus cereus*. The vegetative cells are 1 µm in width, 5 µm long, and have short hair-like flagellae. Bt is ubiquitous in the environment and can be isolated from soil, foliage, water and air. The species is distinguished from *B. cereus* by its ability to produce a protein crystal during sporulation (Höfte and Whiteley, 1989; Martin, 1994).

Bt was first isolated by the Japanese bacteriologist S. Ishiwata from diseased silkworm *Bombyx mori* (L.) larvae in 1901. In 1911, E. Berliner in Germany recorded the first scientific description of the bacterium. In 1916, Aoki and Chigasaki found that its activity was due to a toxin present in sporulated cultures, but not in young cultures of vegetative cells (Beegle and Yamamoto, 1992).

3.1. Bt δ-endotoxins and mode of action

Bt produces a parasporal inclusion body during sporulation usually referred to as a crystal. This crystal is made of proteins. A large number of related crystal proteins are known and more than one protein type can co-assemble in one crystal. In an effort to overcome a somewhat confused situation, a classification of crystal proteins and their genes was proposed (Höfte and Whitely, 1989). This classification is based on the crystal protein structure and on the host range. More than 14 distinct crystal protein (*cry*) genes are described, and recently additional insecticidal proteins have been identified (Lereclus et al., 1993).

The genes specify a family of related insecticidal (Cry) proteins, and are divided into four major classes: Lepidoptera-specific (I), Lepidoptera- and Diptera-specific (II), Coleoptera-specific (III), and Diptera-specific (IV) genes. A number of subclasses, based on insecticidal and structural properties, are also recognized within each class. More recently a newer classification system based solely on amino acid identity was proposed (Crickmore et al., 1998). The new classification allows closely related toxins to be ranked together and removes the necessity for researches to bioassay each new toxin against a growing series of organisms.

The crystal proteins exert their effect on the host by causing lysis of midgut epithelial cells, which leads to gut paralysis. The insect stops feeding and if it does not recover eventually dies. Upon ingestion, the crystals dissolve in the alkaline environment of the host insect midgut. The solubilized crystal protein or protoxin is proteolytically processed to produce the actual toxic fragment (toxin). The toxin binds to specific receptors present on the membranes of epithelial midgut cells. Finally, the membrane-bound toxin induces the formation of pores in the midgut epithelial cell membrane. As a result of pore formation the cells die, eventually leading to death of the larvae (Aronson et al., 1986; Höfte and Whiteley, 1989; Lereclus et al., 1989; Adang, 1991; Gill et al., 1992; Bauer, 1995).

From the Bt crystal protein's mode of action, it can be inferred that at least four parameters are involved in crystal protein function: 1) effectiveness of solubilization, 2) efficiency of protoxin-toxin conversion, 3) specific membrane receptor binding, and 4) membrane pore formation. All these parameters determine the specificity of a crystal protein (insecticidal spectrum).

3.2. Commercial use of Bt products

Bt has been applied to the environment since 1933, and first commercialized in France in 1938. However, it was not successfully commercialized until the 1950s, when the new technology of deep tank aerobic liquid fermentation was used to produce spore and crystal preparations. Major applications of Bt have taken place in North America for the control of over 40 pest species in field, forest, orchard, vineyard, park and gardens (Burges and Daoust, 1986). The first commercial Bt formulations were available for field testing in the United States in 1958 (Faust, 1974). In 1961, Bt subsp. *kurstaki* (Btk) was used as a biopesticide for the control of susceptible lepidopteran pests. Most Bt-based insecticides are formulated mixtures of δ-endotoxin crystals and Bt spores, which are known to synergize the toxicity of the crystals. Although the effectiveness of these early Bt-based insecticides was often erratic, progress was slow in research and development of improved Bt formulation, delivery, and application technologies, as well as in the discovery of more active strains. The market was dominated by products based on the HD-1 isolate of the *kurstaki* subspecies for the control of lepidopteran pests in forestry and agriculture. Until the mid-1970s, it was generally accepted that lepidopterans were the only targets of Bt.

New markets were opened by the discovery in 1976 of the *israelensis* subspecies, which is toxic to larval mosquitoes and black flies (Goldberg and Margalit, 1977), and the discovery of Bt subsp. *tenebrionis*, which is toxic to several beetle species (Krieg et al., 1983). These discoveries stimulated sudden and dramatic commercial interest in Bt (Van Frankenhuyzen, 1993). Lambert and Perferoen (1992) estimated that 40,000 strains of Bt are now stored, mainly in private collections, worldwide.

Currently, registered subspecies include Bt subsp. *kurstaki*, Bt subsp. *aizawai*, Bt subsp. *israelensis*, and Bt subsp. *tenebrionis* (equivalent to subsp. *san diego*), which display predominantly subspecies-related insecticidal activity against lepidopteran, dipteran, and coleopteran insect species, respectively (Table 1, page 11). The short half-life of Bt, due to ultraviolet inactivation when topically applied, has stimulated considerable research into alternative delivery strategies. By far the most controversial strategy is the use of insect-resistant transgenic crops expressing Bt δ-endotoxin genes.

This review paper focuses on sprayable Bt products commercially available. Consequently transgenic plants, plants that have been genetically transformed with a Bt δ-endotoxin genes, are not included in this study. Likewise Bt varieties producing ß-exotoxin are not included. Since 1971, Bt varieties producing ß-exotoxin are no longer registered for the control of insect pests.

CHAPTER I
ECOLOGICAL IMPACTS

1. Environmental fate of Bt

Persistance of Bt in the environment is important from both an ecological and economical points of view. This is reviewed by Otvos and Vanderveen (1993).

1.1. On foliage

Persistence of Bt on foliage is dependent on many environmental factors. Leong et al. (1980) conclude that sunlight exposure, leaf temperature and vapor pressure deficit contribute most to endospore decay. Sunlight, particularly ultra-violet radiation, inactivates 50% of Bt spores within 30 minutes and 80% within 60 minutes (Krieg, 1975). The inactivation of both spores and crystals is believed to be due to the production of peroxide or peroxide radicals following UV irradiation of the amino acids (Ignoffo and Garcia, 1978). Spores in absence of moist rapidly decay by exposure to sunlight, and thus are very susceptible in very dry conditions (Pinnock et al., 1971). In general, Bt loses 50% of its insecticidal activity in 1-3 days, often necessitating a second spray application for insects such as the Gypsy moth (*Lymantria dispar* Linnaeus), spruce budworm (*Choristoneura fumiferana* Clemens and *Choristoneura occidentalis* Freeman) and jackpine budworm (*Choristoneura pinus pinus* Freeman). In some studies (McLeod et al., 1983, Beckwith and Stelzer, 1987) longer residual activities (10 days) have been reported. Some viable endospores of Btk have been recovered from foliage one year after ground application of Btk (1 Billion International Units (BIU)/tree) (Reardon and Haissig, 1984).

1.2. In soil

Bt is an indigenous soil bacterium with a worldwide distribution (Martin and Travers, 1989). The vegetative form of Bt is generally not well adapted to soil, and it requires the specialized habitat of vulnerable insects to persist. Bt endospores, however, can survive in most types of soils although at pH below 4.8, Bt will not grow (Saleh et al., 1970). Repeated application of Bt results in no increased accumulation of the organism (Dulmage and Aizawa, 1982). The fate of Bt in soil is likely dependent on microbial competitions. The abundance of Bt rapidly diminishes in unsterilized soils but may increase in sterilized soils (Akiba et al., 1977). When soil was treated at 10^5 cells per gram, Bt persisted at 10^3 cells per gram for 12-16 months. However, the proportion of Bt compared to other soil bacilli was reduced from 20-40% to about 10% indicating that Bt is not well-adapted to soil environments. Pruett et al. (1980) have shown that in soil, although 38% of the endospores remained viable after 63 days, only 3% of the insecticidal activity remained. After 135 days, there were 23% of the original spores and no insecticidal activity.

The movement of Bt away from the site of application (dissemination) is an important factor that may influence possible impact. Martin and Reichelderfer (1989) studied dispersal of Bt using antibiotic-resistant, marked strains. No vertical movement through soil deeper than 6 cm was observed and movement outside the plot were less than 10 m. DeLucca et al. (1981) also showed that Bt does not move in the soil, as two serotypes sprayed in close proximity did not become cross-contaminated. No evidence of genetic exchange was noted, although potential for exchange might be limited in soil under circumstances where Bt can multiply (Meadows, 1993).

1.3. In water

In Nova Scotia, operational spray programs (4.7-9.4 l/ha of Thuricide 16B) showed that Btk could be found in stream and reservoir water for 8-12 days after spraying (Menon and De Mestral, 1985). They also tested the survival patterns of Btk in laboratory in four different types of water conditions: distilled-deionized water, tap water, lake and sea water. Approximately 30% of Btk survived after 70 days in distilled and tap water. In lake water, approximately 50% of cells remained viable after 70 days. In contrast, approximately 90% of Btk population died off after 30 days exposure to sea water and less than 10% of Btk cells survived after 40 days. The prolonged survival of Btk in lake water is believed to be due to the presence of higher concentrations of available nutrients.

2. Effects of Bt on soil

Changes in soil productivity and fertility due to Bt are not likely because of Bt's natural occurrence in soil, lack of accumulation, and relatively short persistence (USDA, 1995).

3. Effects of Bt on water

Bt may enter water (streams, rivers, lakes, seas) through direct application to surface water, runoff, or through the feces of animals who have ingested Bt. Water quality should not be directly affected by Bt, as it is not likely to affect most aquatic organisms. Decreases in detritus decomposition rates demonstrated at high doses of Btk in the laboratory are unlikely in the environment, given the lower doses used and the purification processes in natural systems (Kreutzweiser et al., 1993). Btk spraying program in Nova Scotia detected Btk in a municipal water system during the application. This implies that chlorination of water supply was not sufficient to kill Btk (Menon and De Mestral, 1985). Therefore, it is possible that a small amount of Bt may enter public water supplies as a result of aerial spraying programs in areas that contain water catchments or water supply reservoirs. Bt contamination may also occur if spray enters air vents of local drinking water distribution reservoirs. However, the presence of Bt in water is not considered to cause any adverse effects on human health as it is not a human pathogen. Nevertheless, people may be concerned about the presence of bacteria in water supplies.

4. Effects of Bt on plants

Phytotoxicity from Bt (adverse effects on plant health) has never been observed at field rates of application (USDA, 1995). In order for Bt to have toxic effects, it must be ingested by an organism and exposed to appropriate digestive enzymes at pH of 9.0 to 10.5 (Falcon, 1971). Therefore, plants (terrestrial and aquatic) could not be affected by Bt since they have no mechanism for ingesting the bacteria and for processing the crystal or the δ-endotoxins..

Plants that are pollinated exclusively or mainly by moth and butterflies may experience a temporary drop in seed set. However, plants are unlikely to be affected by reduced pollination, as even the plant which were solely reliant on moth or butterfly pollination would be able to be pollinated by species which were not at vulnerable instar stages when spraying took place (Operation Ever Green, 1997).

Applying Bt reduces damage to trees from leaf-eating caterpillars soon after ingestion of crystal. If the trees do not have to produce a new set of leaves to replace those eaten by caterpillars, tree can produce more products of photosynthesis to be used in tree growth and reproduction rather than forming a new set of leaves. Bt use, therefore, is likely to maintain the plant condition.

5. Effects on non-target invertebrates

Non-target organisms may be exposed to Bt either directly by encountering it in the environment (e.g. by eating leaves, litter, or the uppermost layer of the soil) or indirectly, by eating caterpillars which have been infected with Bt. Although Bt has a half-life in the environment of 12-32 hours, it has an insecticidal activity of a week or longer (USDA, 1995). Bt is therefore not expected to affect caterpillars other than the generation that is present when it is sprayed.

5.1. Soil microorganisms

Research concerning the effects of Bt on the soil microflora is limited, and results from the few studies that have been conducted are contradictory (Addison,1993). For instance, Pruett et al. (1980) reported increases in soil microbial numbers 2-4 weeks after using formulated product containing Bt subsp. *galleriae.* Petras and Casida (1985) reported similar results using Btk. On the other hand, Atlavinyté et al. (1982) measured a reduction in bacterial and actinomycete numbers and a rise in fungal numbers following the addition of Bt subsp. *galleriae* in a formulated product. Visser et al. (1994) cite a paper by Krieg (1983) indicating no significant effects of Btk on indigenous soil bacteria populations. From the experiments with DiPel® 176 in simple microcosms, Visser et al (1994) concluded that it is doubtful that Bt would have a significant impact on the non-target microflora under field condition.

5.2. Non-target Lepidoptera

Laboratory studies indicate that Bt is active against many micro- and macro-lepidopterans (Krieg and Langenbruch, 1981; Navon, 1993). There have been relatively few attempts to document the effects of Bt on native lepidopteran communities under field conditions. Morris et al. (1975) found that Bt applications against spruce budworm, *Choristoneura fumiferana* Clemens, caused a 70% reduction in populations of the lepidopteran defoliator, *Dioryctria reniculelloides* Mutuura & Munroe. Miller (1990) demonstrated that both richness and diversity of native Lepidoptera associated with Garry oak, *Quercus garryana* Dougal., were reduced after three aerial applications of Bt in Oregon. Johnson et al. (1995) found that early instar swallowtail butterflies in the genus *Papilio* were sensitive to Bt-treated foliage up to 30 days after application. Recently, Wagner et al. (1996) reported similar result and they concluded that although the effects to most lepidopteran species were transparent, the lepidopteran fauna was not eliminated by a single spring application of Bt at 90 BIU/ha. Miller (1990) noted significant reductions in species richness of Lepidoptera larvae collected in sprayed areas the year of treatment and one year after treatment, but not two years after. Factors which influenced the ability of non-target species to recover included the stage of larval development at the time of spraying, the number of generations in a year, and the insect's ability to disperse (Miller,1990). Permanent changes in non-target lepidopteran populations do not appear likely except, possibly, in habitats that support small isolated populations of Lepidoptera that are highly vulnerable to Bt. This is particularly so if there may be physical or biological barriers which prevent the insect from moving back into the sprayed area (USDA, 1995).

5.3. Honey bees

Many studies have been carried out on the effect of Bt on honey bees (Cantwell et al., 1972; Davidson et al., 1977; Lehnert and Cantwell, 1978; Vandenberg and Shimanuki, 1986; Vandenberg, 1990). No investigator has reported adverse effects on the bee colony when Bt was sprayed on foliage and bees were exposed to it under field or simulated field conditions. Very high concentrations (10^8spores/ml sucrose syrup) of Bt subsp. *tenebrionis*, which is used against beetles such as the Colorado potato beetle, reduced longevity of honey bee adults but did not cause disease (Vandenberg, 1990).

5.4. Other invertebrates

Addison (1993) indicated some soil invertebrates (nematodes, ground beetles) might also be at risk. All tested strains of Bt were toxic to eggs of the nematode, *Trichostrongylus colubriformis.* However, populations of other nematode species were increased following the field application of Bt. Bt applications were found to reduce populations of a species of predatory mite that is closely related to soil-dwelling species (Addison,1993).

In a review of the effects of Bt on other soil organisms, some species of earthworms were unaffected by Btk (Dipel) applied to ash-maple forest soils at 6000 mg/m^2, 100 times more than the recommended rate (Benz and Altweg, 1975).

Invertebrate parasites and predators that feed on Bt-infected insects may experience temporary drops in population numbers. However, this drop would be due to lack of food supply, rather than Bt toxicity. Giroux et al. (1994a, and 1994b) indicated no direct effects on the adult and larval instars of *Coleomegilla maculata lengi* Timberlake (Coccinellidae) from the use of Bt subsp. *san diego* commercial product (M-One™), in laboratory. Meanwhile, Flexner et al. (1986) cited the direct mortality of *Coccinella septempunctata* Linnaeus in laboratory, using Bitoxibacillin®, Entobakterin®, and Exotoxin®, however, other Bt formulations (e.g., Dipel® and Thuricide®) tested against *C. septempunctata* and other coccinellids have had little or no effect. Direct mortality of parasitoids has been observed by Muck et al. (1981). They tested the ichneumonid parasite *Pimpla turionellae* Linnaeus and the braconid parasite *Cotesia glomeratus* Linnaeus with Btk (Dipel®). They found that high concentrations applied orally (e.g., 10^8 and 10^9 spores/ml) significantly increased the mortality of *C. glomeratus* after 2 weeks. *Pimpla turionellae* was less affected than *C. glomeratus.* However, the rate at which Bt formulations are used in the field is lower than most of those used to initiate high mortality of adult parasites in the laboratory research. Thus, the probability that adult parasites would consume lethal doses of Bt while searching for hosts and nectar feeding within Bt-sprayed field might be very low.

No negative impacts were observed with invertebrate predators (Plecoptera, Odonata, Megaloptera, Trichoptera, Diptera) from the test of Bt subsp. *israelensis* (Bti) over a three year period in the field and laboratory by Merritt and Wipfli (1994). A summary of Bti safety tests on vertebrate and invertebrate non-target organisms compiled by the company Biochem Products (anonymous, undated) showed that, other than producing mortality in some species of flies and midges, no ill effects were detected in close to 100 different non-target invertebrates. Similar results were obtained by Garcia et al.(1980).

5.5. Aquatic invertebrates

Eidt (1985) evaluated the toxicity of Bt subsp. *kurstaki* (Btk) (Thuricide® 32) to aquatic insects and concluded that there was no hazard to fish-food organisms in streams from Btk aerial spray at rates adequate for spruce budworm control. In a monitoring program of a stream in Algonquin Park, Ontario, Buckner et al. (1974) found that Btk had no measurable effect on Trichoptera (caddisflies), Ephemeroptera (mayflies), Plecoptera (stoneflies), Odonata (dragonflies), Coleoptera (water beetles), Diptera (flies), Turbellaria (planaria, flatworms), Nematoda (nematodes, roundworms), Oligochaeta (earthworms), Hirudinea (leeches), Amphipoda (crustaceans), Decapoda (crayfish), Hydracarina (water mites), Gastropoda (snails) and Pelecypoda (clams, mussels). The effects of Btk on aquatic invertebrates were also summarized by Otvos and Vanderveen (1993). They concluded that Btk does not adversely affect the abundance and composition of benthic insects.

Bt subsp. *israelensis* (Bti) is highly toxic to certain Diptera (mosquitoes and blackflies) larvae upon consumption. Before large-scale use of Bti against mosquitoes, such as in the Upper Rhine Valley in the early 1980s, numerous safety tests on aquatic organisms were carried out. None of the tested taxa appeared to be affected when exposed in water containing large amounts of Bti (Merritt, 1989; Becker and Margalit, 1993). Within the Diptera, the toxicity exhibited by Bti is restricted to a few nematocerous families. Apart from larval mosquitoes and blackflies, only the closely related dixids are similarly sensitive to Bti. Larval psychodids, chironomids, sciarids and tipulids are generally far less sensitive. Other flies, such as the housefly, *Musca domestica* Linnaeus (Muscidae) and syrphids, such as *Helophilus pendulus* Linnaeus, are insensitive to Bti (Ali, 1981; Mulla et al., 1982; Back et al., 1985; Becker and Margalit, 1993). A trial conducted with a Bti product, Vectobac® 1200L, in Nova Scotia showed that there was no significant difference between pretreatment and post-treatment abundance of chironomid and simulid in the streams (McCracken and Matthews, 1997).

Merritt and Wipfli (1991) assessed indirect effects of Bti treatment with two black fly predators, *Nigronia serricornis* Say and *Acroneuria lycorias* Newman, and a detritivore, *Prostoia completa* Walker. They found that, although non-target organisms are not directly affected by Bti treatment, there are secondary consequences for non-target organisms, including predators and detritivores. They concluded that, since these insects are generalists, the effects of population suppression may be minor and the space vacated by black fly larvae may be taken up by other taxa that are suitable preys.

6. Effects on vertebrates

The effects of Bt formulations on animal health include direct effects of contact with Bt as well as indirect effects as a result of feeding on insects treated with Bt. Animals could be exposed to Bt-based insecticides through ingesting Bt on plants, ingesting insects infected with Bt, inhaling Bt spray, or through skin(dermal) contact with the spray. However, the mode of action of Bt indicates that there are no concerns about dermal contact and inhalation in animal (USDA, 1995).

The USDA (1995) provides a tabular summary on laboratory studies of toxicity of Btk to fishes, birds and mammals (Table 2, page 12).

Meanwhile, the changes in the food chain due to Bt applications could apply some environmental stresses on some animals that rely mainly on insects for food source.

6.1. Fishes

There has been no documented evidence of fishes killed as a result of the many forestry, agricultural and urban Bt spraying programs in Canada and US in the last 20 years (USDA, 1995). In reviewing the toxicity of Bt to fish, Forsberg et al. (1976) cite several studies conducted with older formulations of Bt and give toxicity values. It was unclear, however, whether or not these older Bt formulations contained ß-exotoxin. Presence of ß-exotoxin was not assessed at that time. Consequently reports of toxicities detected in these earlier studies must be analysed with caution. For the formulated product Thuricide, known at that time to contain ß-exotoxin, the studies reported no mortalities at 600 ppm or lower. Since this formulation contained ß-exotoxin, now known to be toxic to vertebrates, they represented toxicities significantly greater than those of modern Bt formulations which are ß-exotoxin-free.

In addition to the direct toxicity to fishes, indirect effects, such as what happens to fishes after they consume the cadavers of Bt-infected insects, are to be considered (Forsberg et al., 1976). No evidence exists that consumption of Bt-infected insects have adversely affected fishes to any noticeable degree (Surgeoner and Farkas, 1990)

6.2. Amphibians and reptiles

No references were found which indicated any direct adverse effect on amphibians and reptiles. These animals eat a combination of insect species and small invertebrates. Lepidoptera would form a rather small portion of their daily diet. Therefore most amphibians and reptiles are unlikely to be affected by Bt (USDA,1995).

6.3 Birds

There have been no significant reductions in bird populations (74 species representing 21 families) noted in areas treated with Bt in Canadian spruce-fir forest treatment plots, located in Manitoba and Ontario (Buckner et al. 1974). Weber (1993) indicated that there would be negligible mortality of adult birds and the most severe potential effect would be a localized decrease in breeding success of a few species which are most highly dependent on Lepidoptera for food.

Several field studies have examined the effects on insectivorous birds, including neotropical migrants, when food resources were reduced by the application of Bt (Rodenhouse and Holmes, 1992; Gaddis, 1987; Gaddis and Corkran, 1986). Rodenhouse and Holmes (1992) observed a significant reduction in the number of nesting attempts per bird per year, and in the number of caterpillars in the diets of black-throated blue warblers (*Dendroica caerulescens*, a neotropical migrant) in sprayed plots. There was no significant difference in the number of production of young per territory per season per pair despite fewer nesting attempts by birds on treated plots. No difference was noted between treated and control areas in caterpillar abundance 2 years after treatment. Gaddis (1987) and Gaddis and Corkran (1986) tested the reproductive success and feeding activities of chestnut-backed and black-capped chickadees near Portland, Oregon. They found no difference between treated and control sites in reproductive success or nestling growth measures in the first year. In the second year, however, they found a significantly lower fledgling success at treatment areas. Although significantly smaller proportion of caterpillars brought as food on treatment sites both years, the provision rate was not different. Therefore, the relationship between the application of Bt and the nest failures was uncertain. The USDA concluded that field studies show the effects of Bt spraying on insectivorous birds to be “subtle” (USDA, 1995).

6.4. Mammals

Some of the early safety studies to mammals were complicated by the presence of ß-exotoxin in the Bt preparations: strains containing ß-exotoxin have been banned in the United States since 1971 (Ignoffo, 1973). There are many references dealing with the possibility of toxicity or pathogenicity of Bt products, showing that these entomopathogens were virtually non-toxic to mammals provided that high dose levels were not used.

According to the World Health Organization (WHO)’s guidelines for assessing the safety of bacterial agents (Anonymous, 1981), in addition to the aforementioned tests (e.g. oral, intraperitoneal, respiratory, dermal and allergenicity and hypersensitivity), the recommended safety tests that have to be passed by bacterial pest control agents also include eye exposure (with rabbits) and in vitro mutagenicity tests. Bt has passed these as well, in addition to a subcutaneous and 3-week feeding study. Rogoff (1982) provides a history of regulatory safety data required for Bt to satisfy the needs of the Environmental Protection Agency (EPA).

One field study in Canada (Buckner et al., 1974) indicated that small mammals (woodland jumping mice, deer mice, short-tailed shrews, common shrews, red-backed voles and eastern chipmunks) continued breeding through the treatment periods, and trapping data indicated that the application of Bt treatments did not harm the small mammal complex inhabiting treatment areas.

Using commercially available preparations (Abbott Laboratories, North Chicago, Ill.), Siegel et al. (1987, 1990) demonstrated the safety of formulated products containing Bti in rats, mice and rabbits. There was no evidence that Bti infected rats and mice because recovery of colony-forming unit (CFU) decreased rather than increased over time. An intact immune system was not requisite to prevent infection because recovery of Bti decreased with time in both corticosteroid-treated euthymic mice and athymic mice. They concluded that Bti is avirulent and noninvasive to mammals, and that it can be used safely in environments in which human exposure is likely to occur.

Although highly toxic to Colorado potato beetle, acute toxicology tests have demonstrated that Bt subsp. *tenebrionis* is not toxic to mammals (Mycogen Corporation, Tier I toxicology tests)

7. Effects on human beings

Bt has been the subject of a very limited number of experiments on humans (Otvos and Vanderveen, 1993). In one study, 18 human subjects ingested one gram of a commercial Bt subsp. *thuringiensis* product in capsules daily for five days. Five subjects also inhaled 100 mg of the powder daily for five days. No adverse health effects were noted on physical, laboratory, or x-ray examination (Fisher and Rosner, 1959). In this experiment, humans have been exposed to 10^9 spores Btk/kg by inhalation and 10^{10} spores Btk/kg orally. These levels of exposure are many orders of magnitude higher than the levels to which members of the public could be exposed during an aerial spraying program.

There is only one case of human disease associated with Btk recorded in the medical literature. This occurred after a previously healthy 18-year-old farmer splashed a commercial product (DiPel®) into his eye. The patient was treated with antibiotic ointment. Three days later, when the eye was still irritated, he was treated with a corticosteroid ointment. Ten days after the accident, a corneal ulcer was discovered and was successfully treated with subconjunctival injections of gentamicin and cefazolin sodium. Bt was cultured from the corneal ulcer. After two weeks of topical treatment with gentamicin the ulcer had healed (Samples and Buettner, 1983). The authors attributed the ulceration to Btk infection, but did not entertain the alternate hypothesis that the Btk only inhabited the corneal ulcer that could have been caused by other factors.

In case of Bti, Warren et al. (1984) report on a graduate student who developed infection after accidental injection of Bti and *Actinobacter calcoaceticus* into his finger. In this case also antibiotic treatment resulted in complete recovery within a few days.

Two detailed epidemiological studies have been carried out on the exposure of the general public to Btk in Canada and USA. The most likely routes of exposure for the general public are oral, dermal, and inhalation. In addition to these routes of exposure, accidental parenteral or ocular exposures may occur in workers. In fact, a major study of workers in the Vancouver urban area spray program found that some people working on Btk ground spray programs, without protective clothing, developed minor irritations of skin, eyes and respiratory tract. These health effects tended to be transient and irritant in nature: dry skin, chapped lips, itchy, red and burning eyes, runny nose and nasal stuffiness. The symptoms were reported two to three times more frequently among ground spray workers than among the control group during the trial period. However, ground workers are likely to have greater levels of exposure to Btk than aerial workers or the general public. The exposure rates of the ground workers were up to 500 times the amount of Btk that a general public standing outside during the spray operation would be exposed to. Consequently, these effects are less likely to be observed in aerial workers or members of the general public after exposure to Btk (Nobel et al., 1992). The study also found that Btk persists in the nasal cavities of workers for up to four weeks (or longer in a minority of cases). No significant or serious health problems in spray workers resulted from Btk exposure and no loss of workdays could be attributed to Btk. A US study (Green et al., 1990) found that ground workers using spraying equipment had low levels of cumulative exposure to Btk during the spray period, and aerial workers had levels of exposure only slightly higher than those of the general public (USDA, 1995). Finally, US authorities have concluded that "on the basis of both the available epidemiological studies as well as the long history of use, no hazard has been identified for members of the public exposed to Btk formulations" (USDA, 1995).

8. Evolution of resistance

The continued efficacy of Bt-based insecticide for many years, until the mid-1980s, without any resistance being reported led to considerable skepticism that resistance to Bt was possible (Burges, 1971; Krieg and Langenbruch, 1981; Boman, 1981). It was suggested that multiple effects on the host insects or evolutionary advantages of the pathogen might preclude or greatly reduce the likelihood of insects becoming resistant to Bt. However, as the last decade has shown, resistance to Bt δ-endotoxins develops readily in many species of insect pests, both in the laboratory and in the field (McGaughey, 1985; McGaughey and Beeman, 1988). Tabashnik et al. (1990) presented the first well documented instance of resistance occurring against Btk in the field, although earlier reports had suggested the possibility of Btk resistance in populations of *Plutella xylostella,* in the Philippines (Kirsch and Schmutterer, 1988). Statistically significant resistance to Bti has been reported in mosquitoes *Culex quinquefaciatus* Say and *Aedes aegypti* Linnaeus (Georghiou et al., 1983; Goldman et al., 1986). At least one strain of Colorado potato beetle *Leptinotarsa decemlineata* Say has been selected for resistance to a Coleoptera-active strain of Bt subsp. *tenebrionis* (Miller et al., 1990).

The genetic capacity of insect populations to evolve resistance to Bt δ-endotoxins is now well recognized from the study of the many insect populations selected for resistance to Bt in laboratory (Van Rie et al., 1990; McGaughey and Whalon, 1992; Whalon and McGaughey, 1993; Tabashnik, 1992 and 1994; Tabashnik and McGaughey, 1994; Estada and Ferré, 1994; McGaughey and Johnson, 1994; Van Frankenhuyzen et al., 1995, etc). The summarized results are 1) Bt resistance alleles are present at varying levels in different insect species and populations, 2) within a single species, the genetics, mechanisms, level, and stability of resistance vary between selected populations, 3) selection with a blend of toxins can select for resistance to each toxin in the blend, 4) resistance occurs more rapidly with purified toxins than with spore/crystal preparations, 5) cross-resistance to δ-endotoxins is almost ubiquitous and often unpredictable, and 6) reselection of revertant populations is rapid (Bauer, 1995).

Presently, much of the effort toward resistance management or avoidance seems to focus on the presumption that there is an almost unlimited number of different Bt toxins available in nature and that resistance can be managed by using these in various mixture, mosaic, rotational, or sequential systems (Tabashnik, 1994). The implementation of integrated pest management (IPM) strategies that optimize the goals of resistance management involves 1) diversifying the sources of mortality to avoid selection for a single mechanism, 2) reducing selection pressure for the major mortality factor, 3) maintaining susceptible individuals by providing refuges and encouraging immigration, 4) monitoring for increasing resistance to any one of the mortality agents, and 5) responding to resistance through management strategies designed to reduce the frequency of the resistance trait (Whalon and McGaughey, 1993).

CHAPTER II
ECONOMICAL IMPACTS

Accurate economic information on the benefits of specific pesticide-use plans is needed by both growers and consumers to ensure that the maximum benefit is being achieved with the minimum risk. This information can take the following two forms: 1) proof that the standard commercial programs are maximizing yield while minimizing unnecessary use and 2) clear economic information on the benefit of use of alternative programs relying on pesticides with low mammalian toxicity that have broad legislative or public acceptance, or both.

Investment in pest control by pesticides has been shown to provide significant economic benefits. Pesticides may cost 4 to 11% of the gross cash value of a crop. Without crop protection chemicals, it was estimated that crop production would decline 15%, marginal land would be converted to row crops, and food costs would increase $228 per household annually (Smith and Lacewell, 1996). Money returns for the direct benefits to farmers have been estimated to range from $3 to $5 for every $1 invested in the use of pesticides (Headley, 1968; Pimentel et al., 1978). However, these figures do not reflect the indirect costs of pesticide chemical use such as human pesticide poisonings, reduction of fish and wildlife populations, livestock losses, destruction of susceptible crops and natural vegetation, honey bee losses, destruction of natural enemies, evolved pesticide resistance, and creation of secondary pest problems (Pimentel et al., 1980).

Bt-based formulations have been proven environmentally-friendly and safe for human beings as evidences in the previous chapter. In addition, Bt has low development costs compared with classical chemical pesticides. Traditionally, less than 1% of the cost of developing chemical pesticides has been spent on insect pathogens. The discovery rate of novel Bt strains of insecticidal crystal proteins is rather higher (1 in 1000 screens) than synthetic pesticides (1 in 20,000 screen compounds) (Lambert and Perferoen, 1992).

Trumble et al, (1997) studies on celery plantings in 1992 and 1993 (experimental plantings) and 1995 (commercial field) compared the benefit of current chemical standard pesticide practice with an integrated pest management program based on Bt. In 1992 and 1993, net profits with the use of Bt were $1,485 and $614/ha higher, respectively, than the standard chemical treatment. On a commercial celery operation, in 1995, the use of Bt generated a net profit more than $410/ha higher than that of the grower's chemical treatment. It also reduced pesticides use by 40% compared to most celery producers' use. A similar result was obtained from a study on fresh-market tomatoes. The net profits were higher by $500 to $1000/ha with the use of Bt as compared with the standard chemical treatment.

In the early 90s, Rigby (1991) estimated the global market for Bt-based bioinsecticide to be in the range of $100 million (North America: $57.2 million, Far East: $24 million, Middle East/Africa: $12.9 million, South and Central America: $8.1 million, Australasia: $2.1 million, Western Europe: $0.7 million). It did not include the use of Bt in former USSR and Eastern Europe. At present, a wide range of conventional Bt-based products are available for control of insect pests and the use of these products is still growing. In 1996 and 1997, Mycogen Corp. revenues from Bt-based formulations sales were $45.9 million and $40.5 million respectively. In 1997, the European biopesticide market had an estimated value of $102 million, expected to reach $167.2 million by the year 2004 (Anonymous, 1997). At present, the global insecticide market is estimated at 8 billion dollars annually and there is a very strong indication that biological insecticides will account for almost $500 million by the year 2000 (Georgis, 1996).

CHAPTER III
SOCIO-CULTURAL IMPACTS

1. Consumers response

Consumers have come to depend on inexpensive food, largely as a result of highly efficient production in agriculture. However, consumers also expect safe food and have become concerned about freedom from residues, the safety of their water, and the general bio-sustainability of their environment. Many advocates have raised major questions about these and related issues, which were reflected in the content and focus of recent congressional legislation in the United States. A recent U.S. poll (Morris et al., 1993) found that 91% of consumers are concerned about pesticides and chemicals used to grow food products, and 95% are concerned about pesticides and fertilizers getting in their water supply. Nine out of ten consumers believe it is important that U.S. farmers switch to low-chemical production strategies that rely primarily on natural methods and use chemicals only as a last resort. Most consumers say they would be willing to pay somewhat more for food grown with reduced levels of chemicals, and 79% would like to see more signs in supermarkets labeling food grown with less chemicals.

Bt is frequently used by the food industry to control insect pests. In Vancouver, operational spray program (Foray 48B, Btk) analyzed food samples for Btk. The researchers could cultivate Btk from a variety of vegetables during and after the spray program. They concluded that it was most unlikely that all of the Btk on the food came from the aerial spraying program (Noble et al., 1992). In addition, effects of the presence of Bt in food on human health appear to be negligible as Bt does not grow in warm blooded organisms and passes through the digestive system without producing any toxic effect. Because of these characters and non-toxicity to environment, Bt-based formulations have been a main component in integrated pest management programs.

A recent study (Anderson et al., 1996) concluded that a high percentage (85%) of customers would rather buy integrated pest management program-based grown food, and would be willing to support reduced pesticide chemical use in hopes of positive consequences for human health and environmental quality.

2. Public response to spraying

People who live near trees or farms could be exposed to Bt spraying. Other people who work in the woods or with trees, mix or apply insecticides, or work in laboratories with the Bt commercial products could have frequent exposure to the Bt commercial products. People's perceptions and behaviors in response to the insect treatment with Bt may vary depending on where people live. People in suburban and rural areas are more likely to encounter and be alarmed by insect pests. In general, people in rural agricultural areas are less likely to be concerned about spraying for pest control because of their familiarity with spraying of agricultural crops. Surveys of public opinion carried out as part of the US environmental impact assessments on the management of Gypsy moth (USDA, 1995) identified that people were anxious and fearful about the appearance of helicopters and planes used to spray insecticides. Some people have fear or anxiety about the safety of insecticides and distrust government actions to control insects. Some people were concerned about possible risks of spray projects, namely insecticide spills, airplane accidents, automobile accidents, and the exposure of spray workers to insecticides, traffics, powerlines, aggressive dogs, and other neighborhood or woodland conditions. Finally, the document emphasized the importance of communication, education and advanced notices to the public during spray operations to reduce people's anxieties.

CONCLUSIONS and PROSPECTS

The advantages of Bt are numerous and include: fast larvicidal activity, production on relatively inexpensive media, long shelf life, application using conventional equipment and minimal to no effects on beneficial insects and other non-target organisms. Numerous studies gave informations on the successful use of Bt in crop and forestry protection. The possibilities of integrating Bt with other biological control agents, culture practices and conventional pesticides, have increased considerably with the availability of novel toxins with broader host ranges, improved formulations and various options for their application. Indeed, Bt has become the cornerstone of several integrated pest management programs particularly in vegetable crops where chemical pesticides residues are a major concern.

Several factors may significantly increase the use of Bt in the future, in particular, the increase in social costs associated with the heavy use of chemical pesticides and the rapid escalation of direct costs of development and production of newer chemical insecticides. In the future, certainly, there will be a continuing interest in discovering novel Bt strains. Ongoing novel Bt screening programs will reveal new activities in terms of increased toxicity as well as new spectra of activity. In addition, the most effective way of treatment will continue to be a subject of research. Undoubtedly, improvements are also expected from the discovery of novel strains carrying novel, more efficient combinations of insecticidal toxins or from the development of novel combinations via recombinant DNA techniques. For example, companies in the Bt arena (e.g. Ecogen, Mycogen, and PGS, etc.) boast large collections of several thousand Bt isolates. Many new crystal types have been discovered with activity on mites, corn rootworms (*Diabrotica* spp.), nematodes, adult flies and ants. Many of these strains are still being characterized, and the potential for use of these new Bt in the near future is promising.

Table 1. Commercial products based on *Bacillus thuringiensis.*

Subspecies	Manufacturer	Product
kurstaki	Abbott Laboratories	Boibit, DiPel, Foray
	Bactec Corp.	Bactec Bernan
	Becker Microbial Products, Inc.	BMP 123
	Ecogen Inc.	Condor, Crymax, Cutlass, Raven
	Forward International Ltd.	Forwabit
	Thermo Trilogy	Costar, Javelin, Thuricide, Vault
	Sanex Inc.	Bactosid K
	Tecomag	Agrobac
	Troy Biosciences Inc.	Troy-BT
	Mycogen Corp.	MVP, MVP II, M-Peril
israelensis	Abbott Laboratories	Bactimos, Gnatrol, Skeetal, VectoBac
	Becker Microbial Products, Inc	Aquabac, Aquabac Primary Powder
	Sanex Inc.	Vectocide
tenebrionis	Abbott Laboratories	Novodor
	Mycogen Corp.	M-trak
aizawai	Abbott Laboratories	Xen Tari
	Thermo Trilogy	Agree, Design
	Mycogen Corp.	Mattch
morrisoni	Bactec Corp.	Bactec Bernan

(Adapted from Farm Chemicals Handbook, 1997)

Table 2. Toxicity data of *Bacillus thuringiensis* subsp. *kurstaki*

Species	Exposure/Dose	Effect
Mammals		
Human	Acute oral dose of 1 g/day for 3 consecutive days	No effect/infectivity
Rat	Acute oral dose	$LD_{50} \geq 4.7 \text{ X } 10^{11}$ spores/kg
Rat	Acute oral dose	$LD_{50} \geq 2.67$ g/kg
Mice	Acute oral dose of 10,000 mg/kg	no effect
Rabbit	Acute oral dose	$LD_{50} \geq 2.0 \text{ X } 10^{9}$ spores/animal
Dog	Acute oral dose of 10,000 mg/kg	no effect
Rabbit	Acute dermal dose (Dipel 6AF®)	$LD_{50} > 2,000$ mg/kg
Rat	Acute inoculation dose	$LD_{50} \geq 3.4 \text{ X } 10^{11}$ spores/kg
Rabbit	Acute inoculation dose	$LD_{50} \geq 6.9 \text{ X } 10^{7}$ spores/kg
Rat	Acute inhalation dose	$LD_{50} \geq 8 \text{ X } 10^{11}$ spores/animals
Sheep	Oral diet of 500 mg/kg/day of Dipel D® or Thuricide-HP® (approx. 10^{12} spores per day) for 5 months	No toxicity or significant treatment-related effect (physical or clinical)
Birds		
Birds	Acute oral dose	$LD_{50} = 178$ ppm, NOEL= 1ppm
Bobwhite quail	Acute oral dose	$LD_{50} > 10,000$ g/kg
Mallard	Acute oral dose	$LD_{50} > 2,000$ mg/kg
Fishes		
Rainbow trout	96-hour exposure	NOEL > 1,000 ppm
Rainbow trout	96-hour exposure	$LC_{50} > 10$ mg/L
Bluegill sunfish	96-hour exposure	$LC_{50} > 95$ mg/L
Rainbow trout, bluegill sunfish, sheepshead minnow	30-day at 100 X maximum expected environmental concentration (MEEC) for label rates of Dipel	No adverse effects
Eel	2,000 X MEEC for label rates of Dipel	

(Adapted from USDA, 1995)

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