

Highlighted snapshots from:

**The Control/Eradication Agents for the Gypsy Moth - Human Health and Ecological Risk  
Assessment for *Bacillus thuringiensis* var. *kurstaki* (BtK)  
FINAL REPORT**

## **4. ECOLOGICAL RISK ASSESSMENT**

### **4.1. HAZARD IDENTIFICATION**

#### **4.1.1. Overview**

The mechanism of action of BtK in lepidoptera is relatively well characterized. BtK vegetative cells produce spores and crystals. After the insect consumes the crystals, toxins are formed that attach to the lining of the mid-gut of the insect and rupture the cell walls. The Btk spores germinate in the intestinal tract and enter the body cavity through the perforations made by the crystal toxins. The bacteria replicate in the body cavity, causing septicemia and eventual death. While various strains of Bt are often characterized as selective pesticides, Btk is toxic to several species of target and non-target lepidoptera. Sensitive non-target lepidoptera include larvae of the Karner blue butterfly, two species of swallowtail butterflies, a promethea moth, the cinnabar moth and various species of Nymphalidae, Lasiocampidae, and Saturniidae.

While some non-target lepidopteran species appear to be as sensitive as target species to BtK, most studies indicate that effects in other terrestrial insects are likely to be of minor significance.

The U.S. EPA classifies BtK as virtually non-toxic to fish, and this assessment is consistent with the bulk of experimental studies reporting few adverse effects in fish exposed BtK concentrations that exceed environmental concentrations associated with the use of BtK in USDA programs. Although there are no data regarding the toxicity of BtK or its formulations to amphibians, other strains of B.t. appear to have low toxicity to amphibians.

#### **4.1.2. Toxicity to Terrestrial Organisms.**

**4.1.2.1. Mammals** –The hazard identification for mammals is closely related to the hazard identification for the human health risk assessment (see Section 3.1) in that both are based, in part, on numerous standard toxicity studies in experimental mammals (Appendix 1). As discussed in Section 3.1 and summarized in Appendix 1, BtK may persistent—i.e., may survive and be recovered—in mammals for several weeks after exposure; however, there is little indication that oral or dermal exposure leads to serious adverse health effects. Most inhalation studies do not suggest a potential for adverse effects even at BtK concentrations much greater than those likely to be encountered in the environment. The lack of a positive hazard identification is supported by field studies in which no adverse effects were observed in populations of mammals exposed to BtK applications of (Belloq et al. 1992; Innes and Bendell 1989). Nonetheless, as discussed in the human health risk assessment (see Section 3.3.4), there are data to suggest that extremely high air concentrations of BtK in air might pose a hazard.

**4.1.2.2. Birds** – Toxicity studies in birds are limited to standard acute exposures required by U.S. EPA for product registration. The studies all involve either single-dose gavage

administration (Beavers et al. 1988a) or five daily-dose gavage administrations (Beavers 1991b; Lattin et al. 1990a,b,c,d,e,f,g), and none of the studies reports signs of toxicity or pathogenicity at single oral doses up to 3333 mg formulation/kg bw or at multiple oral doses up to 2857 mg formulation/kg bw (Appendix 2). Due to the lack of evidence regarding acute toxicity in birds exposed to *BtK* formulations or other *Bt* strains, the U.S. EPA did not require chronic or reproductive toxicity studies in birds.

The apparent lack of BtK toxicity to birds is supported by several field studies summarized in Appendix 2. BtK applied at rates sufficient to decrease the number of caterpillars had no substantial adverse effects on most bird species (Rodenhause and Holmes 1992; Nagy and Smith 1997; Sopuck et al. 2002). The relatively minor effects observed in some species were considered indirect and attributed to alterations in the availability of prey rather than to the direct toxicity of BtK (Gaddis 1987; Gaddis and Corkran 1986; Norton et al. 2001).

**4.1.2.3.2. Other Terrestrial Insects** – Some non-target lepidopteran species may be as sensitive as target species to BtK; however, most studies indicate that effects in other terrestrial insects are likely to be minor.

Honey bees are an important non-target insect for any pesticide, and bioassays on honey bees are required of all pesticides during the registration process. As noted by U.S. EPA (1998), the bioassays in honey bees submitted in support of the registration of *BtK* suggest: “minimal toxicity for *B. thuringiensis* subspecies *kurstaki*” (U.S. EPA 1998, p. 21). This conclusion is also consistent with numerous laboratory bioassays and field studies concerning the effects of *BtK* (Glare and O’Callaghan 2000; WHO 1999).

**4.1.2.4. Terrestrial Plants (Macrophytes)** – As indicated in the re-registration eligibility document on B.t. (U.S. EPA 1998), toxicity testing in non-target plant species was not required to support the re-registration of products containing B.t. because “...a review of the literature on *B. thuringiensis* and its byproducts indicate no known detrimental effects on plant life...” (U.S. EPA, 1998, p. 25). No information was found in the more recent literature regarding the toxicity of BtK or its formulations to plants, suggesting that effects on plants are not likely and that the phytotoxicity of BtK has not generated substantial interest.

#### **4.1.3. Aquatic Organisms.**

**4.1.3.1. Fish** – As summarized in the previous USDA (1995) risk assessment on BtK, field studies (Buckner et al., 1974; Otvos and Vanderveen 1993; Surgeoner and Farkas 1990) report no apparent fish kills or other adverse effects resulting from the use of BtK. Similarly, U.S. EPA (1998) classifies BtK as virtually non-toxic to fish, based on an assessment of several acute toxicity studies in trout and one study in bluegills. These conclusions are consistent with a relatively large number of experimental studies that report very few if any effects in fish at much higher concentrations than would be encountered in the environment after the use of BtK (Appendix 5). Acute exposure to BtK formulations at concentrations up to 1000 mg/L are not associated with fish mortality (e.g., Meher et al. 2002), and longer-term studies of formulated BtK in bluegills (Christensen 1990c), sheepshead minnow (Christensen 1991e) and trout (Christensen 1990d,i) report only decreased growth at concentrations up to 40,000X expected environmental concentrations.

## 4.2. EXPOSURE ASSESSMENT

### 4.2.1. Overview.

The exposure assessment for the ecological risk assessment on *BtK* are summarized in Table 4-3. Exposure assessments, based on the hazard identification, are presented for three groups: small mammals, terrestrial insects, and aquatic species. Although numerous exposure scenarios could be developed for terrestrial mammals, the only positive hazard identification for *BtK* involves inhalation exposures.

... As discussed in the hazard identification, there is no basis for concern about adverse effects in birds, plants, soil microorganisms or invertebrates, other than insects, exposed to *BtK*. Hence, explicit exposure assessments for these groups are not conducted.

## 4.4. RISK CHARACTERIZATION

### 4.4.1. Overview.

An overview of the risk characterization for *BtK* is presented in Table 4-6. The only organisms that are likely to be affected by *BtK* or *BtK* formulations are terrestrial insects. Separate dose response curves can be generated for both sensitive and tolerant terrestrial insects. At the application rates used to the control of the gypsy moth, the expected mortality rates for sensitive terrestrial insects range from about 80% to 94%. All sensitive terrestrial insects are comprised of lepidoptera, including some species of butterflies, like the endangered Karner blue, and some swallowtail butterflies and promethea moths. In some cases, lepidopteran sensitivity to *BtK* is highly dependent on developmental stage. This is particularly true for the cinnabar moth, with late instar larvae being as sensitive as target species to *BtK* and early instar larvae being among the most tolerant lepidoptera. Given the mode of action of *BtK*—i.e., it must be ingested in order to be highly toxic—effects on even the most sensitive species are anticipated only when species are in a sensitive larval stage at the time of or shortly after *BtK* application. Much lower mortality rates (on the order of less than 1% to about 4%) are anticipated in tolerant species, including non-lepidopteran insects and certain lepidoptera at a particular stage of development. The risk characterization for terrestrial mammals is unambiguous: under foreseeable conditions of exposure, adverse effects are unlikely. Similarly, based on a very conservative exposure assessment for aquatic species, effects in fish and aquatic invertebrates appear to be unlikely. As discussed in the hazard identification, effects in birds, plants, soil microorganisms or invertebrates other than insects appear to be of no plausible concern.

### 4.4.2. Terrestrial Organisms.

4.4.2.1. **Terrestrial Vertebrates** – The risk characterization for terrestrial mammals is unambiguous: under any foreseeable conditions of exposure, adverse effects are unlikely.

The identification of tolerant and sensitive organisms, however, is not always straightforward. As summarized in Table 4-5, target species like the gypsy moth and cabbage looper are clearly sensitive. In addition, some species of butterflies, including the endangered Karner blue and some swallowtail butterflies and promethea moths appear to be as sensitive as the target species to *BtK* exposure. For some lepidoptera, sensitivity to *BtK* depends primarily on developmental stage. This is particularly evident in the case of the cinnabar moth, with late instar larvae being as sensitive as target species to *BtK* exposure and early instar larvae being among the most

tolerant lepidoptera. All of the more sensitive organisms are lepidopteran larvae. Given the mode of action of *BtK*—i.e., it must be ingested in order to be highly toxic—effects on even the most sensitive species are anticipated only when the species is in a sensitive larval stage at the time of *BtK* application or shortly thereafter.

Tolerant species appear to be comprised of non-lepidopteran insects as well as certain larval stages of some lepidoptera. As noted above, early instar larvae of the cinnabar moth appear to be among the most tolerant lepidoptera. Based on the study by Peacock et al. (1998), owl moths and some looper butterflies also appear to be relatively tolerant to *BtK*. As illustrated in Figures 4-1 and 4-2, other lepidopteran species/instars display sensitivities that are intermediate between those of the most sensitive and most tolerant organisms, and the distribution of tolerances appears to be nearly uniform. As summarized in Appendix 3, the apparently wide variability of sensitivity among different lepidopteran species is supported by the recent field study of Rastall et al. (2003), who noted statistically significant decreases in three nontarget lepidopteran species but either no change or statistically significant increases in other nontarget lepidopteran species associated with the application of *BtK*.

Thus, the risk characterization for terrestrial insects is highly variable. Mortality rates are likely to be high among sensitive lepidopteran species after any *BtK* application that is effective for controlling the gypsy moth or other target species, whereas mortality rates are not likely to be detectable or biologically significant among non-lepidopteran insects or tolerant lepidoptera at certain stages of development. The response in other lepidopteran species will be intermediate between sensitive and tolerant species. As discussed in Section 4.1.2.3.2, an older oil-based formulation of *BtK*, Dipel 4L, decreased populations of *Collembola* as well as earthworms. Dipel 4L is not used in USDA programs. Nonetheless, any oil-based formulation of *BtK* (or any other pesticide) might be expected to cause adverse effects in some soil invertebrates.

As summarized in Table 4-5 and illustrated in Figure 4-1, the toxicity data on honeybees are encompassed by the dose-response curves for sensitive and tolerant insect species but the apparent slope of the mortality curve for honeybees is shallower than that for other insect species. This observation, however, is based on only a single study (Atkins 1991a) and should not be subject to over interpretation. Nonetheless, the data from Atkins (1991a) suggests that mortality rates in bees sprayed directly with *BtK* at application rates used to control the gypsy moth could be approximately 20%. In practice, applications of *BtK* to control the gypsy moth are not associated with substantial mortality in bees, which may be due to foliar interception of the applied *BtK*.