Bull. Environ. Contam. Toxicol. (1992) 49:252–258

Bulletin of Environmental Contamination and Toxicology

Lethal and Sublethal Effects of *Bacillus thuringiensis* var. *kurstaki* on Aquatic Insects in Laboratory Bioassays and Outdoor Stream Channels

David P. Kreutzweiser,[1] Stephen B. Holmes,[1] Scott S. Capell,[1] and David C. Eichenberg[2]

[1]Forestry Canada, Forest Pest Management Institute, 1219 Queen St. East, P.O. Box 490, Sault Ste. Marie, Ontario, P6A 5M7, Canada and [2]Lake Superior State University, Sault Ste. Marie, Michigan 49783, USA

The microbial insecticide Bacillus thuringiensis is generally considered to be more environmentally acceptable than many broad-spectrum chemical insecticides (Laird et al. 1990), and Bacillus thuringinesis var. kurstaki (Btk) is now widely used in forest management for controlling defoliating insects (van Frankenhuyzen 1990). Broadscale applications of Btk to forested areas may result in contamination of watercourses, and may pose some hazard to aquatic organisms. Although the impact of B. thuringiensis var. israelensis (a dipteran-active serotype used for biting-fly control) on non-target aquatic fauna has been extensively investigated (e.g., Merritt et al. 1989), few published studies have reported the effects of Btk (the variety used in forest pest management) on aquatic invertebrates. Previous risk assessments of Btk to aquatic invertebrates have been largely based on static bioassays (Eidt 1985) and unpublished data from field investigations (Surgeoner and Farkas 1989).

Our objective was to improve the risk assessment for stream insects exposed to Btk by providing further toxicity data for an array of insect taxa under more natural exposure regimes and environmental conditions. We conducted experiments to measure the acute lethal response of aquatic insects to Btk (Dipel 8AF) in recirculating laboratory bioassays, and to determine lethal and behavioural (drift) effects of Btk on aquatic insects in outdoor stream channels.

MATERIALS AND METHODS

Test organisms were collected from streams with an electroshocker and acclimated at the test sites for 24 hr before the treatments. No attempt was made to determine specific stages of development for the specimens, but most (except Taeniopteryx nivalis and some groups of Lepidostoma sp.) were in late or final instar. Btk was applied as formulated product (Dipel 8AF, Abbott Laboratories, Chicago, IL) at a maximum concentration of 600 IU/mL. As a means

send reprint requests to D.P. Kreutzweiser at above address

of standardizing the expression of potency of Bt, the insecticidal activity of Bt formulations is expressed in international units (IU) per volume, as determined in bioassays against the cabbage looper, Trichoplusia ni (van Frankenhuyzen 1990). Dipel 8AF has a potency of 16.9 BIU (billions of international units)/L, and this was diluted with water to a test concentration of 600 IU/mL in our experiments. This was considered to be 100 X the expected environmental concentration in 50 cm of water as a result of direct overspray of Btk applied at a rate of 30 BIU/ha.

The laboratory bioassay apparatus was modified from Rodrigues and Kaushik (1984), and was operated at the Lake Superior State University aquatics laboratory in Sault Ste. Marie, Michigan. The bioassay system comprised 10 test units contained in a large water bath for temperature control. Each test unit consisted of a 20-L glass aquarium, a power aquarium pump, a glass mixing chamber, and a two-lane, glass dosing trough which contained the test specimens. The units could be held in recirculation mode (water pumped from the aquarium, into the mixing chamber, through the dosing troughs, and back to the aquarium), or in a by-pass mode with water diverted from the end of the dosing troughs into the water bath, through a filtering unit, and into the laboratory outflow. Water lost during the by-pass mode was replaced with ambient laboratory water (drawn from Lake Superior).

The Btk treatments and untreated controls were randomly assigned among test units. Test species were randomly assigned to lanes of the test units, with each species tested in duplicate (two separate lanes). Btk was injected from a micro-pipettor into the aquarium of each treated unit, mixed by hand, and allowed to recirculate through the test unit for 24 hr. There was no visible indication of formulation particles settling out in the aquarium during the dosing period. At the end of this period, the units were set to by-pass for an additional 24 hr to flush residual insecticide from the units (turnover time was about 5 min), then returned to recirculation mode for the observation period (daily observations for a total of 9 d). Percent mortality of test organisms in treated units was corrected for mortality in the control units by the method of Abbott (1925). In treated units where corrected mortality was greater than 0, the mortality in treated units was tested for difference from control by χ^2 analysis with significance at $\alpha = 0.05$.

Lethal and behavioral effects (drift response) of Btk on aquatic insects were determined in a stream-side test system at the Icewater Creek Research Area north of Sault Ste. Marie, Ontario. This system and procedures are described in detail by Kreutzweiser and Capell (1992). Briefly, stream water was diverted through channels containing natural substrate and was treated with Btk dripped into the lower portions of the channels from Mariotte bottles. The upper (control) and lower (treated) sections of the channels each contained a test unit in which

invertebrate response was monitored. Each test unit was constructed of Plexiglas and was 70 cm long, 30 cm wide, 10 cm high, screened on both ends (1-mm mesh stainless steel), open on top, and longitudinally divided into three lanes. A 35-cm section of each lane contained the substrate where test organisms were placed prior to the Btk applications. Insects that dislodged from the substrate of each lane, could be observed drifting through the next 25-cm section (no substrate) and into the lower end (collector) of the lane. The floor of the collectors was recessed to allow the insects to escape the current, and each collector contained an irregular-shaped stone to provide a site for re-attachment.

The Btk treatments in the stream channels were of 2½-hr duration. This short-term exposure more closely resembles the transient nature of pesticide contamination in streams than conventional 48 or 96-hr exposures (Muirhead-Thomson 1987). During the treatments, and for 1 hr after, observers recorded invertebrate drift (those individuals that dislodged from the substrate and drifted into the collectors). Total counts (alive and dead) of test specimens in the collectors were recorded at 1 hr after the treatment, and daily for 7 d. At the final observation times, the substrate of each lane was also searched, and the number of alive and dead specimens was recorded.

This design and response monitoring procedure allowed a quantitative analysis of 1) drift response (proportion of test specimens that drifted), 2) survival of insects that drifted, and 3) survival of insects remaining in the substrate. Differences in drift behavior and survival between control and treated groups were analyzed by χ^2 analysis with significance at $\alpha = 0.05$. Each lane of a test unit was considered to be a treatment replicate. The response in the three lanes of a test unit were tested for heterogeneity (Zar 1984), and when there were no differences among replicates (non-significant test for heterogeneity, $p>0.05$), the three replicate lanes were pooled to increase the power of the χ^2 test. A significant difference between treated and control units in terms of drift response or survival was considered a significant pesticide effect.

RESULTS AND DISCUSSION

During laboratory toxicity testing, dissolved oxygen (DO) ranged from 8-10 mg/L, pH was 6.6-7.5, and hardness ($CaCO_3$) was approximately 45 mg/L. Temperatures ranged from 8-10°C. In the stream channels, DO was 10-12 mg/L, pH was 6.2-7.0 and hardness was 20-30 mg/L $CaCO_3$. Average daily stream temperatures ranged from 9-14°C.

Twelve insect taxa (two instars of Lepidostoma sp. for a total of 13 tests) were exposed to Btk in the recirculating laboratory bioassays at 600 IU/mL for 24 h. Eleven species exhibited no significant mortality after 9 d (most were <5% when corrected for

control mortality), but Taeniopteryx nivalis showed an average mortality of 30% (χ^2 $p<0.01$, 27% and 33% mortality in two replicates) by the end of the 9-d observation period (Table 1).

Table 1. Mortality (mean of two replicates) of test organisms exposed to 600 IU/mL of Dipel 8AF (24-hr exposure, 9-d observation) in recirculating bioassays. Significant mortality (χ^2 analysis) is indicated by ** ($p<0.01$).

Test organisms	No. per rep.	Actual % mortality	Corrected % mortality[1]	LC50
Ephemeroptera				
Ephemerella sp.	15	20	0	>600 IU/mL
Heptagenia flavescens	15	26	15	>600 IU/mL
Isonychia sp.	15	0	0	>600 IU/mL
Paraleptophlebia ontario	15	7	0	>600 IU/mL
Rithrogena sp.	15	26	0	>600 IU/mL
Stenonema vicarium	15	7	7	>600 IU/mL
Plecoptera				
Acroneuria abnormis	13-15	31	10	>600 IU/mL
Isogenoides sp.	15-17	58	4	>600 IU/mL
Taeniopteryx nivalis	15	30**	30	>600 IU/mL
Trichoptera				
Hydropsyche sp.	15	10	0	>600 IU/mL
Hesperophylax designatus	15	0	0	>600 IU/mL
Lepidostoma sp.[2]	15	30	4	>600 IU/mL
Lepidostoma sp.[3]	15-17	25	0	>600 IU/mL

[1] Mortality in treated units corrected for mortality in control units (see methods).
[2] late-instar Lepidostoma
[3] early-instar Lepidostoma

T. nivalis was one of two species tested in the final bioassay just before the project was terminated, and no further testing could be conducted to determine a no-effect level. The 30% mortality at the maximum concentration indicates that the LC50 for T. nivalis is greater than 600 IU/mL.

The Btk treatments at 600 IU/mL in the outdoor stream channels did not affect invertebrate drift (Table 2). The % drift by 1 hr after the 2½-h applications was slightly higher than control in 5 of 10 species tested, but these were not significant ($p>0.05$). The survival of drifted insects by 1 hr after the applications was likewise unaffected. Very few died and none of the mortality was significantly different from controls ($p>0.05$).

By 7 d after the treatments in the stream channels, the survival of invertebrates in the collectors (those that drifted during the 7-d period) and in the substrate was not affected by the Btk applications (Table 3). Mortality in the treated units was low,

Table 2. Effects of Dipel 8AF on drift (mean % of 3 replicates and 1 SE), and survival of drifted insects in stream channels 1 hr after a 2.5-hr exposure at 600 IU/mL. None of the drift increases or mortalities were significantly different from controls (χ^2 p>0.05).

Test organisms	No. per rep.	% drift control	% drift treated	No. dead / total drift control	No. dead / total drift treated
Ephemeroptera					
Epeorus vitrea	15	15.6 ± 2.2	11.1 ± 4.4	0/7	1/5
Heptagenia flavescens	15	4.4 ± 2.2	0	0/2	0/0
Isonychia sp.	15	11.1 ± 5.9	15.6 ± 5.9	0/5	0/7
Stenonema vicarium	15	4.4 ± 2.2	6.7 ± 0	0/2	0/3
Plecoptera					
Acroneuria abnormis	12	8.3 ± 8.3	2.8 ± 2.8	1/3	0/1
Isogenoides sp.	15	2.2 ± 2.2	2.2 ± 2.2	0/1	1/1
Pteronarcys sp.	14	2.4 ± 2.4	4.8 ± 4.8	0/1	0/2
Trichoptera					
Hydropsyche sp.	15	0	0	0/0	0/0
Odonata					
Boyeria grafiana	15	26.7 ± 7.7	31.1 ± 5.9	0/12	0/14
Ophiogomphus carolus	15	2.2 ± 2.2	4.4 ± 2.2	0/1	0/2

Table 3. Effects of Dipel 8AF on survival of insects in the collector and substrate sections of test units in stream channels 7 d after a 2.5-hr exposure. Emerged or pupated insects were not included in mortality counts (except E. vitrea). None of the mortalities in treated units were significantly different from control units (χ^2 p>0.05).

Test organisms	Total in three replicates	No. dead/total in collectors control	No. dead/total in collectors treated	No. dead/total in substrate control	No. dead/total in substrate treated
Ephemeroptera					
Epeorus vitrea[1]	45	1/17	0/11	0/2	0/3
Heptagenia flavescens	45	0/32	0/41	0/9	0/1
Isonychia sp.	45	0/19	0/33	1/25	1/12
Stenonema vicarium	45	0/41	0/43	0/4	0/2
Plecoptera					
Acroneuria abnormis	36	4/11	1/8	0/25	0/25
Isogenoides sp.	45	8/36	4/32	1/7	1/9
Pteronarcys sp.	42	3/39	0/27	0/3	0/12
Trichoptera					
Hydropsyche sp.	45	3/16	1/29	7/25	1/13
Pycnopsyche guttifer	45	0/45	3/42	0/0	0/0
Odonata					
Boyeria grafiana	45	0/11	1/7	0/34	0/38
Ophiogomphus carolus	45	2/12	2/17	0/30	0/23

[1] Total of 25 emerged in control and 27 emerged in treated units

and the frequency of mortality was not significantly different from the rate of mortality in control units ($p>0.05$).

Results from the laboratory bioassays and the stream channel treatments indicated that contamination of watersheds with Btk is unlikely to directly affect aquatic insects, even at 100 X the expected environmental concentration in 50 cm of water (600 IU/mL). A significant effect (30% mortality) was observed in only 1 of 12 species exposed to 600 IU/mL, but this concentration represents a considerable margin of safety. Our results concur with those of Eidt (1985) who tested 9 taxa of aquatic invertebrates in static bioassays, and found effects only on Simulium sp. and only at the maximum test concentration of 430 IU/mL.

Aknowledgments. The technical assistance of John Graham and George Nixon is greatly appreciated. Dipel 8AF was supplied by Abbott Laboratories Limited, Chicago, Illinois. This project was supported in part by NAPIAP grant NA-16 provided by the Northeastern Area State and Private Forestry, USDA Forest Service, under a US Forest Service - Forestry Canada cooperative agreement.

REFERENCES

Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18:265-267

Eidt DC (1985) Toxicity of Bacillus thuringiensis var. kurstaki to aquatic insects. Can Entomol 117:829-837

Kreutzweiser DP, Capell SS (1992) A simple stream-side test system for determining acute lethal and behavioural effects of pesticides on aquatic insects. Environ Contam Toxicol (in press)

Laird M, Lacey LA, Davidson EW (1990) Safety of microbial insecticides. CRC Press, Inc., Boca Raton, Florida

Merritt RW, Walker ED, Wilzbach MA, Cummins KW, Morgan WT (1989) A broad evaluation of B.t.i. for black fly (Diptera:Simuliidae) control in a Michigan river: efficacy, carry and nontarget effects on invertebrates and fish. J Amer Mosq Control Assoc 5:397-415

Muirhead-Thomson RC (1987) Pesticide impact on stream fauna with special reference to macroinvertebrates. Cambridge University Press, Cambridge, UK

Rodrigues CS, Kaushik NK (1984) A bioassay apparatus for the evaluation of black fly (Diptera:Simuliidae) larvicides. Can Entomol 116:75-78

Surgeoner GA, Farkas MJ (1989) Review of Bacillus thuringiensis var. kurstaki (Btk) for use in forest pest management programs in Ontario with special emphasis on the aquatic environment. Report to the Water Resources Branch, Ontario Ministry of the Environment, Toronto, Canada

van Frankenhuyzen K (1990) Development and current status of *Bacillus thuringiensis* for control of defoliating forest insects. For Chron 66:498-507
Zar JH (1984) Biostatistical analysis, 2nd ed., Prentice-Hall Inc., Englewood Cliffs, New Jersey

Received October 16, 1991; accepted March 2, 1992.