

EFFECT OF VARYING PESTICIDE EXPOSURE DURATION AND CONCENTRATION ON THE TOXICITY OF CARBARYL TO TWO FIELD-COLLECTED STREAM INVERTEBRATES, *CALINEURIA CALIFORNICA* (PLECOPTERA: PERLIDAE) AND *CINYGMA* SP. (EPHEMEROPTERA: HEPTAGENIIDAE)JENNIFER L. PETERSON,<sup>†</sup> PAUL C. JEPSON,<sup>\*†‡</sup> and JEFFREY J. JENKINS<sup>†</sup><sup>†</sup>Department of Environmental and Molecular Toxicology, 1007 ALS, Oregon State University, Corvallis, Oregon 97331-2907, USA<sup>‡</sup>Department of Entomology, Oregon State University, 2046 Cordley Hall, Corvallis, Oregon 97331-2907, USA

(Received 22 June 2000; Accepted 5 February 2001)

**Abstract**—The effect of exposure duration on the toxicity of a forest insecticide (carbaryl) was assessed under environmentally realistic exposure regimes against two stream invertebrates indigenous to the United States Pacific Northwest, *Calineuria californica* (Plecoptera: Perlidae) and *Cinygma* sp. (Ephemeroptera: Heptageniidae). Laboratory bioassays were conducted to evaluate the relationship between pulsed exposures of 15, 30, and 60 min and toxicity for a range of chemical concentrations (10.2–1,730 µg/L). For *Cinygma* sp., the 50% lethal concentration (LC50) values were calculated as 848 µg/L (15 min), 220 µg/L (30 min), and 165 µg/L (60 min). The *C. californica* consistently had lower mortality at a given concentration compared with *Cinygma* sp. Fifteen- and 30-min exposures did not elicit 50% mortality with *C. californica*, and it had a 60-min LC50 of 1,139 µg/L. Time to 50% mortality over 96 h after a 15-, 30-, or 60-min exposure, with the rest of the test period in freshwater (PLT50), was a function of exposure duration and concentration. Analysis of symptomology throughout the test period for *C. californica* gave evidence of recovery from the knockdown and moribund states, but this was not the case for *Cinygma* sp. The pulse duration resulting in 50% mortality was calculated as 43 min for *Cinygma* sp. exposed at 204 µg/L and 16 min at 408 µg/L. A three-dimensional probit plane model [ $Y = -10.86 + 4.83(\ln C) + 3.0(\ln T)$ ], where  $Y$  is probit mortality,  $C$  is concentration in µg/L and  $T$  is time in hours, was used to explain the interaction between concentration (µg/L) and duration of exposure (hours) for *Cinygma* sp.

**Keywords**—Pulsed exposure    Recovery    Macroinvertebrates    Postexposure effects    Uncertainty analysis

## INTRODUCTION

Pesticide spray drift or runoff from agriculture or forestry may result in pulsed inputs to waterways [1]. Stream organisms may be exposed to pulses of pesticides that cause toxic effects despite the fact that maximum concentrations are present for only a short time before attenuation [2]. Gradual dissipation of the pesticide pulse occurs as a result of stream flow, hydrological dilution, and habitat and streambed characteristics that determine the degree of partitioning from water to air or sediments [3,4]. As the chemical moves downstream, organisms may be exposed to progressively lower pesticide concentrations but for longer periods of time [1,3]. Pulses of widely used pesticides may, however, combine as stream channels merge, thus extending the duration of exposure of organisms in higher order streams that are downstream from the spray application.

Exposure assessment is a component of ecological risk assessment in which the contact between the pollutant and organisms in the environment is described and quantified [5]. Regulatory testing procedures that are designed to estimate the risks associated with exposure to pesticides in stream habitats should take into account the temporal dynamics of exposure that are unique to stream systems. The majority of standardized laboratory tests for aquatic macroinvertebrates, developed for regulatory purposes, use either a constant chemical concentration for a preset exposure duration or allow the pesticide to dissipate within a closed system over the duration

of the bioassay (i.e., 24, 48, or 96 h) [6,7]. Although these tests form the basis of tier-one testing, in which the primary goal is to determine if effects are possible, additional testing may be needed to evaluate whether effects could occur under more realistic conditions of exposure.

Tests with more realistic exposure regimes provide a more accurate exposure–response model, where the pattern of exposure (concentration vs time profile) is as similar as possible to that encountered in the field [8,9]. Pulse-exposure tests have been advocated for use in risk assessment in order to more accurately evaluate effects that may occur under natural exposure conditions while retaining the advantages of simplicity and repeatability associated with single-species laboratory tests [10–15].

The purpose of this study was to determine the relationship between exposure time, concentration, and toxicity using carbaryl, a carbamate insecticide, and two aquatic insects native to the Pacific Northwest, *Calineuria californica* (Banks, 1905) (Plecoptera: Perlidae) and *Cinygma* sp. (Eaton, 1885) (Ephemeroptera: Heptageniidae). These macroinvertebrates were selected as representatives of the Oregon, USA, stream fauna [16]. Analysis of community sensitivity statistics based on 96-h 1% lethal concentration (LC1) values for six indigenous macroinvertebrate species, including these two [16], revealed that the 95% protection level (HC5, hazardous concentration to 5% of the theoretical community based on the lower 95% confidence limit [HC5/95]) [17] fell within the range of carbaryl concentrations that are detected in Oregon streams [18,19]. This study aimed to evaluate toxic effects under more realistic exposure regimes to provide insight into the level of

\* To whom correspondence may be addressed (jepsonp@bcc.orst.edu).

Table 1. Treatment concentrations and durations for *Calineuria californica* and *Cinygma* sp., including tolerance values that set the dose range

Test organism	Body size (mm)	96-h LC50 ( $\mu\text{g/L}$ ) ( $\pm\text{SD}$ ) <sup>a</sup>	Test concentrations ( $\mu\text{g/L}$ )	Exposure times (min)
Plecoptera: Perlidae <i>Calineuria californica</i> (Banks, 1905)	8.4	17.3 (14.06–20.2)	17.3, 173, and 1,730	15, 30, 60
Ephemeroptera: Heptageniidae <i>Cinygma</i> sp. (Eaton, 1885)	8.8	11.1 (7.7–13.9)	10.2, 102, 204, 408, and 1,020	15, 30, 60

<sup>a</sup> LC50 value calculated in Peterson et al. [16]; SD = standard deviation.

uncertainty that is associated with risk assessments based on 96-h, continuous-exposure tests. The objective of this research was to vary both pesticide exposure time and concentration in order to investigate the toxicity of brief exposure events more characteristic of pesticide exposure in forest stream systems. To enable the data to be comparable with previous continuous-exposure tests [16], the experiments used the same flask bioassay system, running under identical conditions, with the same basic regime of observations and a 96-h endpoint.

## MATERIALS AND METHODS

### Test organisms

The test species were field-collected stonefly and mayfly nymphs, *C. californica* and *Cinygma* sp., respectively. These species were selected because they are representative of Oregon stream communities during the application season for pesticides in forestry. They are also highly abundant, facilitating their use in toxicity tests [16]. The organisms were collected in the autumn of 1998 from two different stream sites in western Oregon, USA. *Cinygma* sp. was collected from Gleason Creek, a first-order headwater stream, and *C. californica* was collected from the Alsea River. Every effort was undertaken to ensure the correct species was used; however, confirmation of identification was made for all organisms at the end of the test period.

Organisms were transported in chilled and aerated water to the laboratory, where they were transferred to holding tanks. The tank system provided the chilled (10°C), oxygenated groundwater required to maintain stream insects in the laboratory [20]. Organisms acclimated for at least 24 h prior to testing in order to eliminate individuals injured during collection and transport. No food was provided over the 24 h prior to testing.

### Chemicals used

Formulated carbaryl (Clean Crop®, Platte Chemical, Fremont, NE, USA, emulsifiable concentrate (EC), 43% active ingredient (a.i.), w/v) was obtained for this study. Stock solutions were prepared and stored according to a previously described methodology [16]. Stock solutions were subsequently diluted to the appropriate test concentrations (Table 1). Carbaryl has been found to be moderately toxic to aquatic organisms in acute tests [21] and has predictable dose-dependent symptomology and toxic effects, including hyperactivity, incoordination, convulsions, paralysis, and death [22]. It is also detected in stream waters in Oregon, and this investigation provided further quantitative analysis of the risks that it might pose to aquatic macroinvertebrates.

### Test methodology

The water in laboratory holding tanks and test systems (stock and test solutions) was obtained from a groundwater

source located at the U.S. Environmental Protection Agency's Western Research Station (Corvallis, OR). Water hardness, pH, and temperature were measured before and after testing according to American Society for Testing and Materials standards [6]. Hardness of the test water ranged from 30 to 40 mg/L and pH from 7.37 to 7.87.

A flask bioassay system [16] was used to expose organisms for a range of fixed times to carbaryl. The system consisted of a series of 250-ml Erlenmeyer flasks, chilled to  $10 \pm 0.5^\circ\text{C}$  in a water bath. Water in each flask was oxygenated using airstones (inflow), and the air outflow was routed through an activated charcoal filter to absorb pesticide vapor in the exhaust gases. Loss of carbaryl was unlikely to have been significant in the low water temperature and pH of the test system. This conclusion is supported by the Henry's Law constant ( $2.65 \times 10^{-7}$ ) [23], which falls in the midrange for pesticides, and particularly by the short durations used in this study.

The test concentrations were the statistically derived LC50, 10 times the LC50, and 100 times the LC50 (Table 1) for each organism obtained from a 96-h continuous-exposure study [16]. For *Cinygma* sp., two additional intermediate concentrations (204 and 408  $\mu\text{g/L}$ ) were tested because organism availability was not limiting. Availability was limited for *C. californica*, and numbers of test concentrations were therefore smaller. The test organisms were exposed for 15, 30, and 60 min at each concentration. Test concentrations were not replicated.

At the start of testing, organisms were removed from the holding tanks and distributed randomly into mesh exposure cages. These were designed to transfer stream organisms from exposure flasks containing insecticide to observation flasks containing fresh water without damaging or stressing the organisms excessively (Fig. 1). Up to 10 organisms were placed

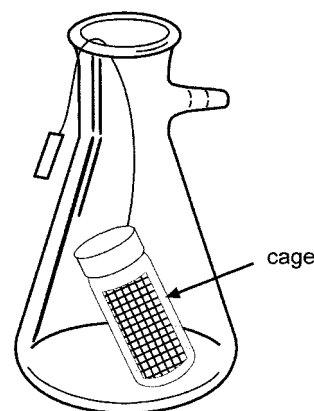


Fig. 1. Exposure cage used to expose aquatic organisms briefly to pesticides. Mesh cages allowed a transfer from a flask containing pesticide to one containing freshwater without damaging the organisms.

Table 2. Percentage mortality and estimates of lethality (LC1<sup>a</sup>, LC50<sup>b</sup>) at 96 h for *Calineuria californica* and *Cinygma* sp. exposed to carbaryl for periods of 15, 30, or 60 min and then transferred to clean water

Concentration ( $\mu\text{g/L}$ )	96-h % mortality and LC1 and LC50 estimates after the exposure period ( $\pm 95\%$ CL) <sup>c</sup>		
	15 min	30 min	60 min
<i>Calineuria californica</i>			
17.3	30.0	0	0
173	0	14.3	12.5
1,730	22.2	30	60.0
LC1 estimates ( $\mu\text{g/L}$ )	—	—	31.1 (0–152.3)
LC50 estimates ( $\mu\text{g/L}$ )	—	—	1,139.4 (370.0–15,410.0)
Slope	—	—	2.48
<i>Cinygma</i> sp.			
10.2	0	10.0	0
102	0	10.0	10.0
204	0	22.2	77.8
408	33.3	100	100
1,020	100	100	100
LC1 estimates ( $\mu\text{g/L}$ )	14.7 <sup>d</sup>	13.0 <sup>d</sup>	61.0 (15.6–91.0)
LC50 estimate ( $\mu\text{g/L}$ )	848 <sup>d</sup>	220 <sup>d</sup>	165 (124–232)
Slope	2.89	3.8	3.36

<sup>a</sup> Lethal concentration to 1% of the test population.

<sup>b</sup> Lethal concentration to 50% of the test population.

<sup>c</sup> Confidence limits included where calculable. Dashes indicate 50% mortality was not reached during test period; LC50 and slope values could not be calculated.

<sup>d</sup> Insufficient data for the calculation of confidence limits; significant heterogeneity at  $p < 0.05$  using the  $\chi^2$  test for heterogeneity.

in each cage, depending on organism availability. The cages were then lowered into exposure flasks, where they remained for the appropriate time period (15, 30, or 60 min). At the end of the exposure period, the cages were removed, rinsed three times in fresh water, and placed in flasks containing fresh water for the remainder of the 96-h test period. Control organisms for each test period were subjected to the same transfer process in order to evaluate handling effects. Assessments of knock-down, the moribund state, and mortality were made throughout the test observation period. Knockdown was defined as the inability of the insect to hold onto and maintain position within the test cages. The moribund state was characterized by a lack of significant movement with the exception of characteristic twitching of the legs and mouth parts. Mortality was determined by absence of movement of the body, mouth parts, or gills after stimulation. At 96 h, the organisms were removed from the cages, evaluated, and preserved in 80% ethanol for confirmation of identification.

#### Statistical treatment

Five analytical methods were used to explore the data. Method 1 (calculation of lethal estimates) enabled quantitative comparisons between the results of continuous-exposure bioassays and the pulsed-exposure assays in the present research. Lethal concentration estimates to 1% and 50% of the test population for the two test organisms were determined by probit analysis [24] using SPSS<sup>®</sup> software [25]. Method 2 (analysis of symptomology) enabled trends in recovery as a function of exposure duration to be calculated. Method 3 (analysis of lethal times in each dose/duration treatment [PLT50]) determined the time for lethal effects to take place in each treatment. Method 4 (duration of pulsed exposure to give 50% mortality at 96 h) enabled estimation of the time it would take for lethal doses to be accumulated. Finally, method 5 (probit plane analysis) was used to generate a model that predicted mortality at 96 h

for different combinations of dose and exposure. This final analytical step enables tentative extrapolation to field conditions.

## RESULTS

### LC50 analysis

Lethal concentrations (LC50) for exposure durations of 15, 30, and 60 min at 96 h were determined by probit regression analysis [24] using SPSS software [25]. Percent mortality values at 96 h after 15-, 30-, or 60-min exposures increased as exposure time increased for both organisms (Table 2). The proportion dead for *Cinygma* sp. was consistently greater than *C. californica* exposed for the same amount of time and at similar concentrations. This was despite the fact that LC50 values for 96-h continuous exposure were similar for both species (LC50 [95% confidence limits]: *Cinygma*, 11.1  $\mu\text{g/L}$  [7.7–13.9  $\mu\text{g/L}$ ], and *C. californica*, 17.3  $\mu\text{g/L}$  [14.1–20.2  $\mu\text{g/L}$ ]) [16]. Control mortality was 0% in all test runs for both organisms.

For *Cinygma* sp., the relationship between LC50 and the duration of exposure was curvilinear (Fig. 2). The *C. californica* LC50 values could not be calculated for 15- and 30-min exposures because 50% mortality was not reached in any test concentration (Fig. 3). We assume the 15-min 17.3  $\mu\text{g/L}$  mortality of 30% was an anomaly because no mortality occurred at the 30- and 60-min exposure times at the same concentration. Sixty-minute LC50 values were, however, significantly different between species ( $p < 0.05$ ), with *Cinygma* sp. being more sensitive than *C. californica* (Table 2).

### Analysis of recovery

Analysis of the symptomology of carbaryl intoxication showed that significant recovery did not occur after the appearance of effects for *Cinygma* sp. The majority of organisms

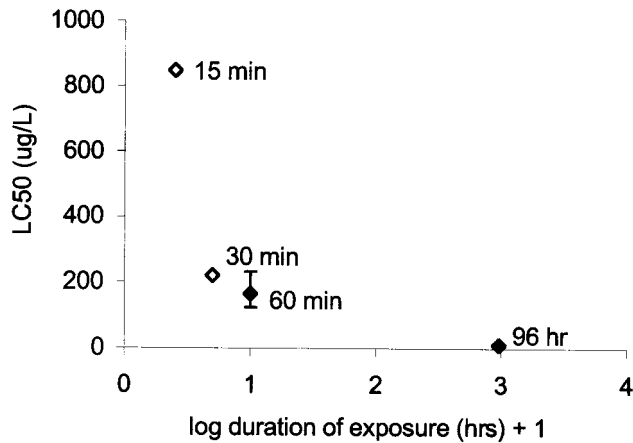


Fig. 2. *Cinygma* sp. lethal concentrations to 50% of the test population (LC50) for a 15-, 30-, and 60-min pulsed exposure to carbaryl. Open symbols indicate where confidence intervals could not be calculated. The 96-h value is from Peterson et al. [16].

exhibiting knockdown or the moribund state at the first assessment after exposure eventually died during the recovery period in fresh water. Symptomology analysis for *C. californica*, however, provided evidence for recovery 5 h after 15-, 30-, or 60-min pulsed exposures to high concentrations (e.g., for 1,730 µg/L; see Fig. 4A to C and Peterson [20]).

#### Lethal times for pulsed exposures: PLT50

Probit analysis was used to determine LT50 values (lethal time to 50% mortality over the 96-h test period) for all combinations of dose and duration of exposure that gave graded lethal responses spanning 50% mortality over the 96-h assessment period for each assay. The data for mortality at each assessment time over 96 h were analyzed by probit regression. This was termed the PLT50 to indicate pulsed exposure followed by a recovery period in uncontaminated water. For *Cinygma* sp., up to seven mortality values could be included in the analysis (i.e., data for assessments at 0.5, 1.0, 5, 24, 48, 72, and 96 h) (Table 3). For *C. californica*, there were only two assessment times (0.5 and 96 h) and PLT50 values were estimated graphically.

Lethal time (PLT50) values decreased with increasing concentration for both species tested. Values for *Cinygma* sp. were

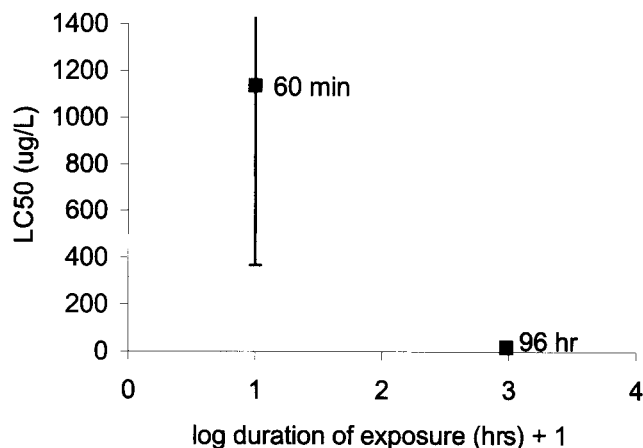


Fig. 3. *Calineuria californica* lethal concentrations to 50% of the test population (LC50) for a 15-, 30-, and 60-min pulsed exposure to carbaryl. The 96-h value is from Peterson et al. [16].

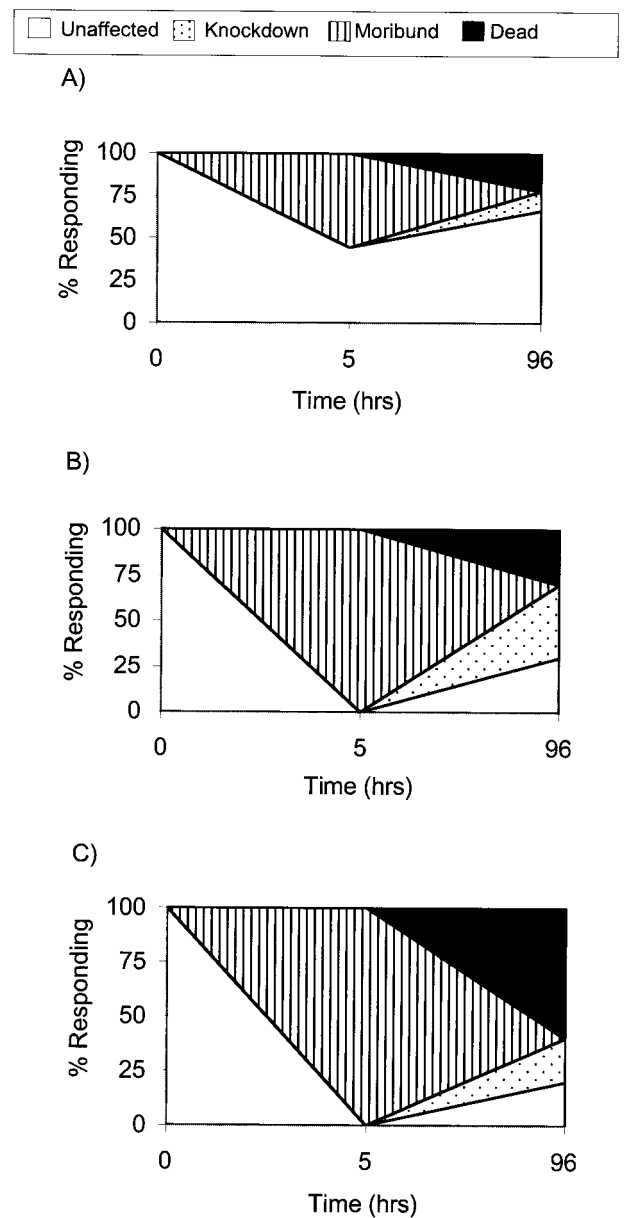


Fig. 4. *Calineuria californica* symptomology over the 96-h test period after a (A) 15-, (B) 30-, or (C) 60-min exposure to a carbaryl concentration of 1,730 µg/L.

lower than for *C. californica* exposed at similar concentrations. For example, the 60-min, 1,020-µg/L PLT50 for *Cinygma* was 1.62 h (97 min), while the 60-min, 1,730-µg/L PLT50 for *C. californica* was 81 h (Figs. 5C and 6C, respectively). The PLT50 values for *Cinygma* sp. at 1,020 µg/L and both 30- and 60-min pulses were significantly lower than the 408-µg/L PLT50 values at 30 and 60 min and the 204 µg/L PLT50 at 60 min.

Graphical analysis was used to compare trends in PLT50 values from the present study and LT50 values from 96-h continuous-exposure bioassays reported in Peterson et al. [16] (Fig. 5A to C for *Cinygma* sp. and Fig. 6A to C for *C. californica*). For both species, mortality values did not exceed 50% across a wide range of concentrations that yielded LT50 values in the 96-h continuous-exposure investigation. For *Cinygma* sp. with 15-min pulses, exposure was nonlethal at 10.2, 102, and 204 µg/L (Fig. 5A), and effects were limited at 408

Table 3. Time (hours) to 50% mortality for *Cinygma* sp. and *Calineuria californica* after an exposure duration of 15, 30, or 60 min, with the remainder of the 96-h test period in clean water (PLT50)

Concentration ( $\mu\text{g/L}$ )	PLT50 (hours) after the given exposure time (minutes) ( $\pm 95\%$ CL) <sup>a</sup>		
	15 min	30 min	60 min
<i>Calineuria californica</i>			
17.3	—	—	—
173	—	—	—
1,730	—	—	81.0 <sup>b</sup>
<i>Cinygma</i> sp.			
10.2	—	—	—
102	—	—	—
204	—	—	18.0 (6.1–30.2)
408	—	20.4 (9.0–29.7)	20 (8.5–28.6)
1,020	0.56 <sup>c</sup>	1.62 (0.003–4.2)	1.62 (0.003–4.2)

<sup>a</sup> Confidence limits included where calculable; dashes indicate values not calculated because organisms did not reach 50% mortality during test period.

<sup>b</sup> Value estimated graphically.

<sup>c</sup> Insufficient data for the calculation of confidence limits.

$\mu\text{g/L}$ . Lethal time (PLT50) values at 1,020  $\mu\text{g/L}$  for 30- and 60-min exposures fell below the LT50 value from continuous exposure; however, this difference was not statistically significant based on the regression model reported in [16] ( $p > 0.05$ ) (i.e., the LT50 at 1,020  $\mu\text{g/L}$ , predicted from the regression model for LT50 [h] vs log concentration [ $\mu\text{g/L}$ ] in [16], was 0.6 h [30 min] and the PLT50 at 1,020  $\mu\text{g/L}$  [ $\pm 95\%$  confidence limits] for both 30- and 60-min pulses was 1.62 h [0.003–4.2]).

Over 30- and 60-min pulsed exposures, the trends were similar, with a number of doses failing to elicit 50% mortality in pulsed tests within the range that would be lethal and generate an LT50 value over continuous exposure. Intermediate concentrations gave PLT50 values that exceeded the 96-h continuous values, and PLT50 values at higher concentrations were closer to the continuous-exposure LT50 values.

For *C. californica*, all combinations of dose (17.3, 173, and 1,730  $\mu\text{g/L}$ ) and exposure time (15, 30, and 60 min) were nonlethal or did not reach 50% mortality, with the exception of the 60-min 1,730  $\mu\text{g/L}$  (Fig. 6C). The PLT50 value in this treatment was greater than the LT50 value from continuous testing. Exposure over shorter periods, even at these high concentrations, failed to elicit significantly toxic effects, suggesting again that rates of uptake may be low for this species over short, pulsed exposures.

#### Duration of exposure to 50% mortality

Lethal response data for organisms exposed to specific concentrations for different periods allowed for calculation of the exposure time needed to elicit 50% mortality at a given concentration. The 96-h mortality data and pulse durations at each concentration were analyzed by probit analysis. This analysis was only possible for *Cinygma* sp. exposed at concentrations of 204 and 408  $\mu\text{g/L}$ , where responses spanned mortality ranges that could be analyzed over different exposure times. Concentrations higher than 408  $\mu\text{g/L}$  elicited 100% mortality at all exposure times, while some concentrations lower than 204  $\mu\text{g/L}$  failed to elicit 50% mortality. This is indicative of a steep dose–response curve.

In order to elicit a 50% response over 96 h at 204  $\mu\text{g/L}$ , *Cinygma* sp. would have to be exposed to carbaryl for 43 min.

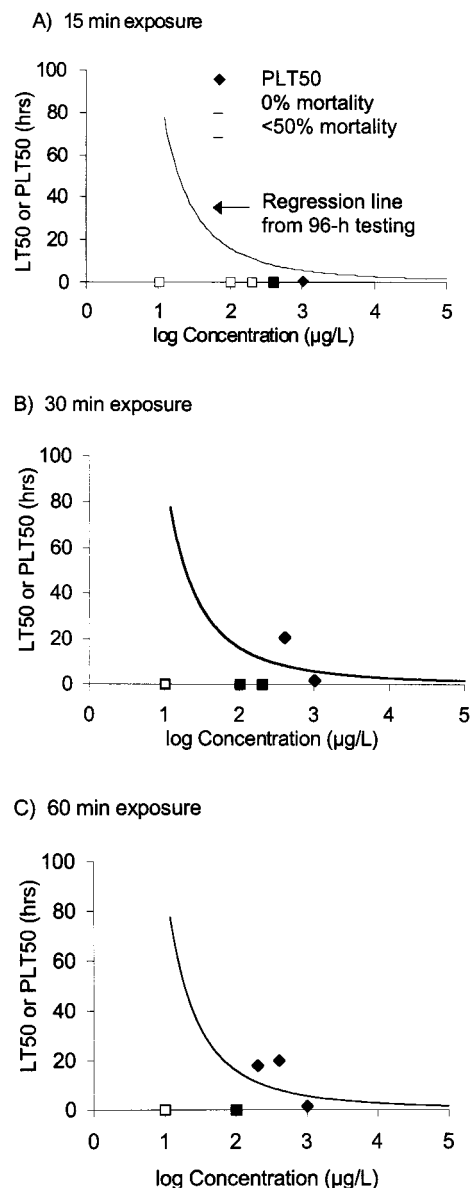


Fig. 5. Lethal time to 50% mortality after a pulsed exposure with the remainder of the test period in freshwater (PLT50) for *Cinygma* sp. after a (A) 15-, (B) 30-, or (C) 60-min exposure to carbaryl. As a basis for comparison with the trends observed in continuous testing, the solid line denotes lethal time to 50% mortality (LT50) values calculated in 96-h tests reported in [16]. Solid diamonds represent PLT50 values obtained in pulsed-exposure tests, solid squares indicate tests where 50% mortality was not reached, and open squares indicate tests where 0% mortality resulted during the 96-h test period. Symbols that occur within the range of the curve but that fall below it indicate effects that are lower than would have been found under continuous exposure at the same concentration.

Increasing the concentration to 408  $\mu\text{g/L}$  results in this exposure time falling to 16 min. The exposure duration required to elicit 50% mortality over 96 h at concentrations tested above 408  $\mu\text{g/L}$  was estimated to be less than 15 min. These estimates were consistent with measurements derived from sequential assessments during continuous 96-h exposure at these concentrations [20].

It is logical that the values for time to 50% effect are shorter in duration than the calculated PLT50 values. The times estimated by method 4 provide an estimate of how long it takes for a sufficient dose of pesticide to be accumulated for 50%

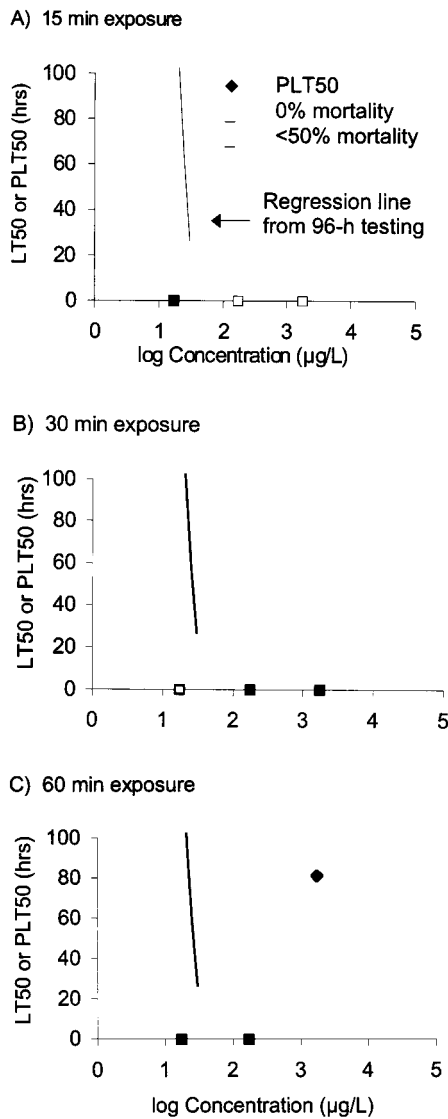


Fig. 6. Lethal time to 50% mortality after a pulsed exposure with the remainder of the test period in freshwater (PLT50) for *Calineuria californica* after a (A) 15-, (B) 30-, or (C) 60-min exposure to carbaryl. Symbols are the same as in Figure 5.

mortality to be recorded at 96 h. The PLT50 values report the time for lethal effects to evolve within the 96-h assessment period based on direct observations during the period in fresh water. The time to appearance of symptoms tends to be considerably longer than the time it takes to accumulate a lethal dose of pesticide.

#### Probit plane analysis

A three-dimensional probit plane model [24,26] was used to explore the interaction between pesticide concentration, duration of exposure, and mortality. Each point on this plane represents a particular combination of mortality, concentration, and time. The standard equation for the probit plane model is

$$P = a + b \ln(C) + d \ln(T)$$

where  $P$  is probit mortality,  $C$  is pesticide concentration in  $\mu\text{g/L}$ , and  $T$  is duration of exposure in hours.

A model was determined for the mayfly *Cinygma* sp. Concentrations used in the analysis included 10.2, 102, 204, 408, and 1,020  $\mu\text{g/L}$  with exposure durations of 15, 30, and 60

Table 4. Model parameter estimates for the probit plane model developed to explain the interaction of concentration ( $\mu\text{g/L}$ ) and duration of exposure (hours) on percent mortality

Parameter	Regression coefficient	Standard error	Pearson Chi square (df)
Concentration ( $\mu\text{g/L}$ )	4.83	0.942	6.78 (17)
Duration of exposure (hours)	3.0	0.605	

min. Estimates for mortality over 96 h of continuous exposure were made from the probit analysis reported in Peterson et al. [16].

The resulting probit plane model for *Cinygma* sp. exposed to carbaryl is

$$Y = -10.86 + 4.83 \ln(C) + 3.0 \ln(T)$$

(Chi-squared test for homogeneity not significant,  $p > 0.05$ ) (Table 4).

This model permits mortality to be predicted from exposure duration (hours) and carbaryl concentration ( $\mu\text{g/L}$ ) (Fig. 7). This approach could help quantify the level of uncertainty associated with predicting impacts in the field, where both parameters may vary. The zones of high risk (combinations of dose and time that would elicit >99% mortality), intermediate risk (combinations that elicit 1–99% mortality), and low risk (where <1% mortality is predicted) can be compared with known data for pulse duration and environmental concentration. In the case of Oregon, surface-water concentration estimates for carbaryl have not been found to exceed 2  $\mu\text{g/L}$  [18,19], and low risk is predicted over a wide range of exposure durations for *Cinygma* sp.

Model validation was conducted by plotting values predicted by the model against those observed in 96-h continuous exposure, which were not included in the probit plane analysis (Fig. 8). The model overestimates mortality at doses that elicit limited effects (below 60% lethal impacts) and underestimates mortality at high concentrations. These deviations are likely to be a result of the differing modes of exposure in the data sets used for model generation (pulsed exposures of 60 min or less in duration) and the data used for validation (obtained during 96-h exposure assays).

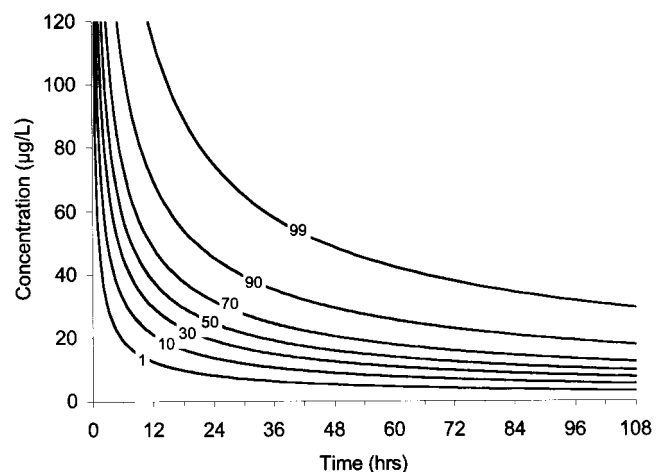


Fig. 7. Interaction of concentration ( $\mu\text{g/L}$ ) and time of exposure (hours) on percent mortality as predicted from the model  $Y = -10.86 + 4.83 \ln(C) + 3.0 \ln(T)$ . Lines represent percent mortality at various combinations of concentration and time.

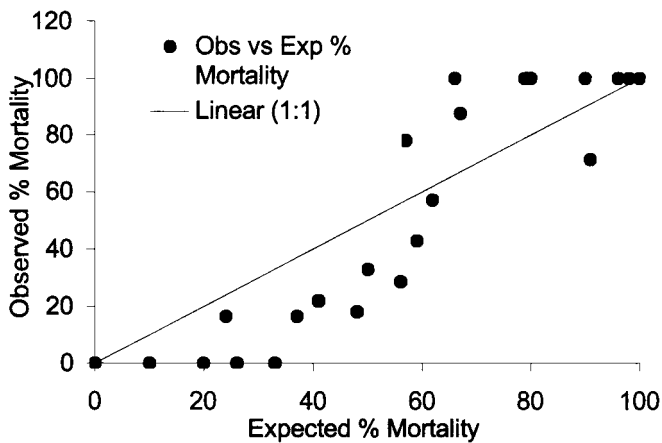


Fig. 8. Observed percent mortality for *Cinygma* sp. exposed to carbaryl from continuous [16] and pulsed exposure plotted against percent mortality expected from the model  $Y = -10.86 + 4.83 \ln(C) + 3.0 \ln(T)$ .

### CONCLUSIONS/DISCUSSION

The use of a postexposure observation period and a 96-h assessment endpoint allowed the toxicity of pulsed exposures to be compared with effects seen in 96-h continuous-exposure bioassays. Mortality decreased with decreasing exposure time for both organisms tested. This is consistent with other studies evaluating effects from brief exposures to carbaryl [13,27]. The use of test concentrations that were considerably higher than those that commonly occur in the environment enabled effects to be properly resolved and provided a basis for estimating impacts at the predicted environmental concentration.

Differences in the responses to pulsed exposures were observed in the two test species. *Calineuria californica* was approximately 1,000-fold less sensitive to carbaryl than *Cinygma* sp. during short, pulsed exposures, although the relative susceptibilities of these species after 96 h of continuous exposure were similar [16]. This may reveal significant differences in rates of uptake and time to equilibration between the two species. Differences of this form, if occurring widely among stream macroinvertebrates, could radically alter rankings of relative susceptibility based on continuous exposure and alter the findings of risk assessment procedures based on this standard methodology. Morphological differences, including cuticular thickness or gill surface area, could account for the differences detected in the present study. Increased rates of metabolism or excretion by *C. californica* could also explain these differences, although the similarity in 96-h LT50 values suggests that the difference between the two species may lie in rates of uptake.

Carbaryl toxicity results from inhibition of acetylcholinesterase in the nervous system. Carbamate insecticides, unlike the organophosphates, which share a similar mode of action, have poor complementarity with the active site and can be displaced after exposure to the chemical ceases [28]. Reactivation of the carbamylated enzyme occurs through hydrolysis. With the half-life of carbamylated enzymes reported as 30 to 40 min, nearly complete recovery of enzyme activity could occur several hours after removal from chemical exposure [22]. Mosquito larvae have been shown to recover from immobilization following short exposures to carbaryl (0.5–4 h) [13]. Ability to recover was shown to decrease with increasing exposure time, and after 8- to 24-h exposure, there was little or no recovery. Mosquito larvae have been shown to recover from

2-h exposures to carbaryl if 6 h in clean water is provided [27]. Black fly larvae exposed to carbaryl were found to recover from immobilization following a 5- to 20-min exposure to carbaryl [29].

There is evidence from this study that duration of exposure and chemical concentration both determine the degree of toxicity. Significant mortality occurred at high concentrations, even if exposure time was short (15 min). At lower concentrations, recovery was more likely and rates of mortality decreased. For concentrations lower than the apparent lethal threshold for the exposure durations tested (204 and 408  $\mu\text{g/L}$ ), reversal of acetylcholinesterase inhibition, in conjunction with metabolism and excretion, may have enabled recovery following exposure. The lowest concentrations tested (10.2 and 17.3  $\mu\text{g/L}$ ) elicited very little mortality at any exposure duration, indicating that insufficient amounts of toxin penetrated to the site of action to elicit lethal effects.

Sublethal effects such as knockdown that may have occurred directly after the exposure period (15, 30, and 60 min) were not assessed. The first observations were normally made about an hour following exposure. Some organisms that may have been initially affected by the chemical may have recovered in this time. Further analysis of short-term effects that may cause drift or a failure to locate favorable habitat conditions is required. Pulsed exposure to pesticides in stream systems has been associated with increases in invertebrate drift [30]. In addition to mortality, sublethal effects of pesticide poisoning including hyperactivity and knockdown symptomatology may result in an impaired ability to recolonize available habitat. For example, an increase in downstream displacement in the form of drifting and crawling organisms was attributed to increased locomotor activity in *Acroneturia lycorias* (Plecoptera) in response to methoxychlor exposure [31]. Drift can occur at lower concentrations than those required to elicit mortality, resulting in a loss of organisms from the system and possible shifts in community structure [31–33].

Lethal time (PLT50) analysis (method 3) determined the amount of time following pulsed exposure for lethal effects to appear. Short exposures can yield effects at 96 h, highlighting the importance of having an extended postexposure assessment period where effects may accumulate over days. The need for postexposure observation periods has been reported previously [11,14]. The PLT50 analyses are distinct from those obtained in the analysis of duration of exposure required to elicit 50% mortality (method 4). Method 4 provides an estimate of exposure time required for the organism to acquire a chemical dose that would elicit a 50% response at 96 h. Symptoms and lethal effects may occur, however, well after the uptake has taken place. The probit plane model analysis (method 5) also used 96-h toxicity data and therefore incorporated symptoms that were expressed over the full 96-h test period.

The duration of a pulsed exposure event in stream systems will be a function of the physical characteristics of the watershed, the pattern of the pesticide application, and the physical and hydrological characteristics of the stream. Peak pesticide concentrations have been found to be higher but present for shorter durations in small streams compared with larger ones [1]. However, duration–concentration relationships need to be established for stream systems of the Pacific Northwest. The LC50 values calculated in this research show that mortality is unlikely to occur as a result of the short, pulsed exposures that may be expected in high-order, high-gradient streams where the pesticide pulse would be expected to move

through the system quickly. Based on the LC50 values calculated in 96-h conventional tests [16] (11.1 for  $\mu\text{g/L}$  *Cinygma* sp. and 17.3 for  $\mu\text{g/L}$  *C. californica*) compared with the LC50 values for more realistic exposure regimes of 15 (848  $\mu\text{g/L}$  for *Cinygma* sp.), 30 (220  $\mu\text{g/L}$  for *Cinygma* sp.), and 60 min (165  $\mu\text{g/L}$  and 1,139  $\mu\text{g/L}$  for *Cinygma* sp. and *C. californica*, respectively), conventional tests may greatly overestimate the acute toxicity of carbaryl to stream insects exposed to short pesticide pulses. Invertebrates in lower gradient valley streams that integrate chemical inputs from throughout agricultural watersheds may be exposed for longer periods, and LC50 values from continuous testing may more closely approximate the risks that these organisms face. These data demonstrate the need to characterize the nature of the chemical pulse in pesticide risk assessment for aquatic macroinvertebrates.

Previous research [16] evaluated uncertainty associated with single-species standardized tests by assessing sensitivity across a representative assemblage of native macroinvertebrate species. The resulting statistical model of community sensitivity, which took into account the number of species tested, suggested that a proportion of the macroinvertebrate community could be at risk in Oregon streams contaminated with carbaryl. In the community sensitivity analysis, the main source of uncertainty was that associated with variation in susceptibility across the whole community of macroinvertebrates. The statistical correction used [34], reduced in direct proportion to the number of test species, and validation of the HC5 can only be obtained by undertaking a number of further 96-h bioassays and determining the change in HC5 relative to environmental concentration.

The pulsed-exposure analysis for two of the test species explored uncertainty that might derive from variation in the responses of organisms to short pulses, compared with continuous exposure, both of which could occur in the real world. Large differences in the form of the response of the two test species to pesticide pulses were observed, and this provides a strong case for more detailed analysis of pesticide exposure, uptake, and symptomology across a wide range of species. The probit plane model provides a statistical tool for the estimation of risk under realistic conditions of exposure. The model must, however, be developed for a number of species before the relative effectiveness of continuous versus pulsed assay regimes in risk assessment can be properly evaluated.

**Acknowledgement**—We would like to thank Liz Dent and Jennifer Walsh from the Oregon Department of Forestry for proposing this project. This research was funded by support from Oregon State University College of Agricultural Sciences and College of Science to Paul Jepson and the Department of Environmental and Molecular Toxicology at Oregon State University.

## REFERENCES

- Richards RP, Baker DB. 1993. Pesticide concentration patterns in agricultural drainage networks in the Lake Erie Basin. *Environ Toxicol Chem* 12:13–26.
- Crossland NO, Shires SW, Bennett D. 1982. Aquatic toxicology of cypermethrin. III. Fate and biological effects of spray drift deposits in freshwater adjacent to agricultural land. *Aquat Toxicol* 2:253–270.
- Bath TD, Vandegrift AE, Hermann TS. 1970. Concentration profiles downstream from instantaneous pollution loadings. *J Water Pollut Control Fed* 42:582–595.
- Wanner O, Egl T, Fleischmann T, Lanz K, Reichert P, Schwarzenbach RP. 1989. Behavior of the insecticides disulfoton and thiometon in the Rhine River: A chemodynamic study. *Environ Sci Technol* 23:1232–1241.
- Suter GW. 1993. *Ecological Risk Assessment*. Lewis, Boca Raton, FL, USA.
- American Society for Testing and Materials. 1993. Standard guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. E729-88. In *Annual Book of ASTM Standards*, Vol 11.04. Philadelphia, PA, pp 456–457.
- Webber CI. 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 4th ed. EPA 600/4-90-027F. U.S. Environmental Protection Agency, Cincinnati, OH.
- Clark JR, Borthwick PW, Goodman LR, Patrick JM Jr, Loes EM, Moore JC. 1987. Comparison of laboratory toxicity test results with responses of estuarine animals exposed to fenthion in the field. *Environ Toxicol Chem* 6:151–160.
- Poirier DG, Surgeoner GA. 1988. Evaluation of a field bioassay technique to predict the impact of aerial applications of forestry insecticides on stream invertebrates. *Can Entomol* 120:627–637.
- Abel PD. 1980. Toxicity of  $\gamma$ -hexachlorocyclohexane (Lindane) to *Gammarus pulex*: Mortality in relation to concentration and duration of exposure. *Freshw Biol* 10:251–259.
- Pascoe D, Shazili NAM. 1986. Episodic pollution: A comparison of brief and continuous exposure of rainbow trout to cadmium. *Ecotoxicol Environ Saf* 12:189–198.
- Holdway DA, Dixon DG. 1986. Impact of pulse exposure to methoxychlor on flagfish (*Jordanella floridae*) over one reproductive cycle. *Can J Fish Aquat Sci* 43:1410–1415.
- Parsons JT, Surgeoner GA. 1991. Effect of exposure time on the acute toxicities of permethrin, fenitrothion, carbaryl and carbofuran to mosquito larvae. *Environ Toxicol Chem* 10:1219–1227.
- Brent RN, Herricks EE. 1998. Postexposure effects of brief cadmium, zinc, and phenol exposures on freshwater organisms. *Environ Toxicol Chem* 17:2091–2099.
- Naddy RB, Johnson KA, Klaine SJ. 2000. Response of *Daphnia magna* to pulsed exposures of chlorpyrifos. *Environ Toxicol Chem* 19:423–431.
- Peterson JL, Jepson JP, Jenkins JJ. 2001. A test system to evaluate the susceptibility of Oregon, USA, native stream invertebrates to triclopyr and carbaryl. *Environ Toxicol Chem* 20:2205–2214.
- Van Straalen NM, Deeneman CAJ. 1989. Ecotoxicological evaluation of soil quality criteria. *Ecotoxicol Environ Saf* 18:241–251.
- Anderson CW, Rinella FA, Rounds SA. 1997. Occurrence of selected trace elements and organic compounds and their relation to land use in the Willamette River Basin, Oregon, 1992–94. Water-Resources Investigations Report 97-4234. U.S. Geological Survey, Portland, OR.
- Anderson CW, Wood TM, Morace JL. 1997. Distribution of dissolved pesticides and other water quality constituents in small streams and their relation to land use, in the Willamette River Basin, Oregon. Water-Resources Investigations Report 97-4268. U.S. Geological Survey, Portland, OR.
- Peterson JL. 2001. The use of native macroinvertebrates to assess pesticide risk to Oregon streams. PhD thesis. Oregon State University, Corvallis, OR, USA.
- Kamrin MA. 1997. *Pesticide Profiles: Toxicity, Environmental Impact, and Fate*. CRC, Boca Raton, FL, USA.
- Kuhr RJ, Dorough HW. 1976. *Carbamate Insecticides: Chemistry, Biochemistry, and Toxicology*. CRC, Boca Raton, FL, USA.
- U.S. Environmental Protection Agency. 1992. Environmental fate one line summaries. Office of Pesticide Programs, Environmental Fate and Effects Division, Arlington, VA.
- Finney DJ. 1971. *Probit Analysis*, 3rd ed. Cambridge University Press, Cambridge, UK.
- SPSS. 1998. *SPSS® for Windows*, Release 8.0.2. Chicago, IL, USA.
- Hewlett PS, Plackett RL. 1979. *An Introduction to the Interpretation of Quantal Responses in Biology*. Edward Arnold, London, UK.
- Kallander DB, Fisher SW, Lydy MJ. 1997. Recovery following pulsed exposure to organophosphorus and carbamate insecticides in the midge, *Chironomus riparius*. *Arch Environ Contam Toxicol* 33:29–33.
- Reiner E. 1971. Spontaneous reactivation of phosphorylated and carbamylated cholinesterases. *Bull World Health Organ* 44:109–112.
- Travis RV, Wilton DP. 1965. A progress report on simulated stream tests of blackfly larvicides. *Mosq News* 25:112–118.

30. Muirhead-Thompson RC. 1987. *Pesticide Impact on Stream Fauna with Special Reference to Macroinvertebrates*. Cambridge University Press, Cambridge, UK.
31. Scherer B, McNicol RE. 1986. Behavioural responses of stream-dwelling *Acronuria lycorias* (Ins., Plecopt.) larvae to methoxychlor and fenitrothion. *Aquat Toxicol* 8:251–263.
32. Cuffney TF, Wallace JB, Webster JR. 1984. Pesticide manipulation of a headwater stream: Invertebrate responses and their significance for ecosystem processes. *Freshw Invertebr Biol* 3:153–171.
33. Wallace JB, Lugthart GJ, Cuffney TF, Schurr GA. 1989. The impact of repeated insecticidal treatments on drift and benthos of a headwater stream. *Hydrobiologia* 179:135–147.
34. Aldenberg T, Slob W. 1993. Confidence limits for hazardous concentrations based on logistically distributed NOEC toxicity data. *Ecotoxicol Environ Saf* 25:48–63.