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Estimating Safe Concentrations of Fluoride for Three Species of Nearctic Freshwater Invertebrates: Multifactor Probit Analysis

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Environmental toxicology is a young scientific discipline that is concerned with the toxic effects of chemical and physical agents on living organisms, especially on populations and communities within defined ecosystems (Boudou and Ribeyre 1989). One of the most basic goals in environmental toxicology is to be able to predict concentrations of chemical compounds, pollutants primarily, that may be present in receiving waters without causing statistically significant impairment to the great variety of aquatic life. Traditionally, these safe concentrations (SCs), also known as no observable effect concentrations (NOECs) or maximum allowable toxicant concentrations (MATCs), are empirically determined by means of sublethal chronic toxicity bioassays or are theoretically derived through an application factor (AF) related to lethal acute toxicity bioassays (SC = AF x 96-hr LC50) (Buikema et al. 1982; Kenaga 1982). However, the performance of chronic toxicity bioassays is expensive in time, personnel and resources, and the use of application factors often is arbitrary. Thus, the development of innovative methodologies for estimating reliable safe concentrations of chemical compounds seems to be desirable.

At the present time, fluoride ion (F-) must be considered as a serious pollutant since its concentration in many aquatic ecosystems is significantly increasing as a consequence of man's activity (Oliveira et al. 1978; Martin and Salvadori 1983; Camargo 1991; Camargo et al. 1992) and owing to its detrimental effects upon aquatic biota (Connell and Airey 1982; Pimentel and Bulkley 1983; McClurg 1984; Dave 1984; Smith et al. 1985; Camargo 1991 Camargo et al. 1992). However, little is known about safe concentrations of F- for aquatic organisms. Connell and Airey (1982), McClurg (1984) and Dave (1984), on the basis of sublethal chronic toxicity bioassays, have respectively proposed SCs (mg F-/L) of 4.15 for the estuarine amphipod *Grandidierella lutosa*, 11.0 for the estuarine prawn *Penaeus indicus*, and 4.4 for the water flea *Daphnia magna*. In addition, Pimentel and Bulkley (1983), on the basis of an arbitrary application factor related to lethal acute toxicity bioassays, proposed SCs (mg F-/L) of 9.6 (hard water) and 2.5 (soft water) for the salmonid fish *Oncorhynhus mykiss*.

The main purpose of this work was to estimate safe concentrations of fluoride ion for last instar larvae of three species of Nearctic net-spinning caddisflies, *Cheumatopsyche pettiti* (Banks), *Hydropsyche bronta* Ross and *Hydropsyche occidentalis* Banks, using an innovative methodology, the multifactor probit analysis (MPA) software (US EPA 1991; Lee et al. 1995). Net-spinning caddisfly species (Hydropsychidae, Trichoptera, Insecta) are common benthic macroinvertebrates in running waters of North Americathat construct fixed silk

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retreat-nets on the bottom of rivers and streams to strain food particles from the current.

MATERIALS AND METHODS

Last instar larvae of net-spinning caddisfly species were hand-collected, one by one, from an unpolluted area of the Cache la Poudre River (Colorado). No larva died during transportation. In the laboratory, animals were randomly distributed into test aquaria and acclimatized to water quality conditions for 3 d prior to fluoride toxicity bioassays. All larvae built their retreat and capture nets within the early hours of their acclimatization. They were not fed throughout the investigation.

Static short-term toxicity bioassays were performed in duplicate for 6 d using glass aquaria, each containing 0.5 L of Fort Collins filtered tap water and a few granitic pebbles. The chlorine was removed during filtration. A control (average fluoride concentration of 0.6 mg F-/L) and five different fluoride concentrations were used per replicate (6.0, 12.3, 24.5, 49.0 and 94.8 mg F-/L for the first replicate, and 10.0, 18.5, 33.1, 59.0 and 105.0 mg F-/L for the second replicate), with ten larvae of each test species per concentration. Fluoride concentrations were made from sodium fluoride (NaF fluormetric, Matheson Coleman & Bell, USA) and monitored daily using a Hach model DR/2000 spectrophotometer (Hach 1990). During bioassays, dead larvae were removed every day.

Water oxygenation and turbulence were produced with air pumps and airstones. Chamber environmental temperature and a 12-hr photoperiod were utilized. Average water quality conditions during bioassays were 7.8 for pH, 9.5 mg/L for dissolved oxygen, 18 °C for temperature, 40.2 mg $CaCO_3$/L for hardness, and 31.7 mg $CaCO_3$L for alkalinity. These physicochemical parameters were analyzed in accordance with analytical methods described by APHA (1989). Toxicity bioassays were carried out in soft water to avoid the loss of fluoride ions by precipitationof CaF_2 and MgF_2 (Smith et al. 1985).

Safe concentrations (SCs) and their 95% confidence limits for each test species were estimated using the multifactor probit analysis (MPA) software (US EPA 1991; Lee et al. 1995). This original methodology utilizes data derived from short-term toxicity bioassays to predict the concentration of toxic substance that can exist in a laboratory environment for an extended exposure time (maximum average life time or infinite hours) causing quanta1 responses (e.g., mortality) at 0.01% population of test species. Lee *et al.* (1995) have recommended minimums of five different toxicant concentrations and four short-term exposure times to obtain estimations with small standard errors. In this investigation, short-term exposure times were 48, 72, 96, 120 and 144 hr. Because the maximum average life time of net-spinning caddisfly larvae seems to be a year or less (depending fundamentally upon temperature and resources). the extended exposure time was calculated as 8760 hr (= 365 x 24). Furthermore, because mortality appeared to be a more consistent endpoint than observed sublethal effects (e.g., net larva migration, protrusion of anal papillae) during acute toxicity testing (see Camargo et al. 1992), mortality was used instead of sublethality to estimate safe concentrations. Since mortality data have been previously published in Camargo et al. (1992), they are not included in the present work.

The MPA methodology solves the concentration-time-response equation simultaneously via the iterative reweighed least squares technique (multiple linear regression). The dependent variable is the probit of the proportion responding to each concentration, and the independent variables are exposure time and toxicant concentration. After evaluating several MPA models with regard to the heterogeneity factor (chi-squared variable divided by degrees of freedom), a parallel-regression-line model was selected as the best one since its heterogeneity factor was the smallest (US EPA 1991; Lee et al. 1995). This model assumes that the concentration-response relationship is a continuum in time, the mode of action of the toxicant being similar as the reciprocal of time varies. Since there is no probit value for 0% response (Finney 1971), the probit value of 1.281 for 0.01% response was chosen as the best approximation to estimate safe concentrations. In order to improve the calculation of SCs, all fluoride concentrations were $\log_{10}$ transformed before statistical analyses (US EPA 1991; Lee et al. 1995).

RESULTS AND DISCUSSION

In general, the mortality of net-spinning caddisfly larvae increased with increasing fluoride concentrations and exposure times. However, there was a differential response to fluoride toxicity between test species (see Camargo et al. 1992). As a consequence of that differential response, *H. bronta* exhibited the lowest 48, 72, 96, 120 and 144-hr LC0.01 values (Table 1). Safe concentrations (SCs or 8760-hr LC0.01 s), their 95% confidence limits, and application factors (AFs) for each test species are presented in Table 2. *C. pettiti* and *H. occidentalis* exhibited very similar SC values, these values being threefold higher than that for *H. bronta.* AF values (calculated as SC/96-hr LC50) are comparable, suggesting that the mode of action of F- ions is independent of the species factor. Overall, LC50 and LC0.01 values indicate that *H. bronta* would be the most sensitive species to fluoride pollution during short-term and long-term exposures.

From a simple comparison between estimated safe concentrations for Nearctic hydropsychid test species (Table 2) and proposed safe concentrations for other aquatic organisms, it may be inferred that net-spinning caddisfly larvae can be more sensitive to fluoride pollution than marine invertebrates (Connell and Airey 1982: McClrug 1984), water fleas (Dave 1984) and rainbow trouts (Pimentel and Bulkley 1983). This greater sensitivity would be due not only to physiological reasons but also to environmental causes. On the one hand, fluoride (F-) can form stable complexes with calcium (Ca^{2+}) in blood and bone of fish (Sigler and Neuhold 1972) whereas stable complexes could not be formed in aquatic insects; although some marine invertebrates exposed to fluoride compounds may accumulatefluoride in their exoskeleton during chronic exposures (Wright and Davidson 1975). On the other hand, aquatic organisms living in soft waters seem to be more sensitive to fluoride pollution than those living in hard (*e.g., Daphnia magna*) or sea (*e.g.,Grandidierella lutosa, Penaeus indicus*) waters because the toxicity of fluoride anions is decreased by high levels of water hardness. This would result from the formation of innocuous complexes with one or more cations, principally Ca^{2+} and Mg^{2+} (Oliveiraet al. 1978, Pimentel and Bulkley 1983, McClurg 1984, Smith et al. 1985). Furthermore, estimated safe concentrations for Nearctic hydropsychid test species seem to be similar, at least in magnitude, to those for Palearctic hydropsychid species (see Camargo and La Point 1995).

Table 1. LC0.01 values (expressed in mg F-/L) and their 95% approximate confidence limits estimated for each test species after fluoride short-term toxicity bioassays. These values were calculated using the multifactor probit analysis (MPA) software on mortality data from Camargo et al. (1992).

	48-hr	72-hr	96-hr	120-hr	144-hr
Hydropsyche bronta	2.6 (1.3-4.3)	1.1 (0.6-1.8)	0.7 (0.4-1.2)	0.6 (0.3-1.0)	0.5 (0.2-0.8)
Hydrosyche occidentalis	6.4 (3.6-9.8)	3.1 (1.7-4.7)	2.2 (1.2-3.3)	1.7 (1.0-2.6)	1.5 (0.8-2.3)
Cheumatopsyche pettiti	7.7 (4.3-12.0)	3.4 (1.9-5.1)	2.3 (1.2-3.5)	1.7 (0.9-2.8)	1.5 (0.8-2.4)

Table 2. Safe concentrations (SCs or 8760-hr LC0.01s expressed in mg F-/L) and their 95% approximate confidence limits estimated for each test species. Applications factors (AFs calculated as SC/96-hr LC50) and LC50s are also presented. SC and LC50 values were estimated using the multifactor probit analysis (MPA) software on mortality data from Camargo et al. (1992).

	SCs	96-hr LC50s	AFs
Hydropsyche bronta	0.2 (0.1-0.4)	15.8 (13.8-18.0)	0.013
Hydrosyche occidentalis	0.7 (0.4-1.2)	34.0 (30.4-38.2)	0.020
Cheumatopsyche pettiti	0.7 (0.3-1.3)	42.5 (37.8-48.2)	0.016

Predicting reliable safe concentrations of chemical compounds for aquatic life is priority in environmental toxicology, and the quickest way to achieve this goal is via short-term toxicity bioassays. Nevertheless, the traditional use of application factors to determine SCs (SC = AF x 96-hr LC50) seems to be inappropriate since this method does not take into consideration the sequence of the concentration-response relationship through time that is usually observed during acute toxicity testing; the 96-hr LC50 value represents only one point in time, whereas the progression of degree of response with duration of exposure is essential when one tries to predict chronic toxicity of chemical compounds from acute toxicity data (Lee et al. 1995). In this respect it is evident that the MPA methodology eludes that problem and, consequently, would be suitable for the quick estimation of safe concentrations of potential pollutants.

Because reliable safe concentrations estimated from chronic toxicity testing are usually based on sublethal endpoints (e.g., growth, reproduction), the MPA methodology should be used on sublethal data instead of lethal data when sublethality is shown to be a consistent endpoint during acute toxicity testing. This methodology, however, should not be viewed as a perfect alternative to

chronic toxicity testing. Long-term toxicity bioassays must be performed whenever time, personnel and resources are not limited. Likewise, laboratory investigations concerning the estimation of safe concentrations of chemical compounds should be conducted in water quality conditions of highest potential toxicity (e.g., soft water) and in those with characteristics of natural environments. It is concluded that further investigations on fluoride toxicity to aquatic life are required to improve fluoride safe criteria, and the data herein presented, particularly those regarding SC and AF values, may provide a practical background for future field and laboratory studies.

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