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Acute Toxicities to Larval Rainbow Trout of Representative Compounds Detected in Great Lakes Fish[1]

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In recent years the National Fisheries Research Center-Great Lakes has ranked the potential hazard to fish and invertebrates of various chemical compounds detected in two Great Lakes fishes--lake trout, Salvelinus namaycush, and walleye, Stizostedion vitreum vitreum (Hesselberg and Seelye 1982). This hazard assessment has included the identification of the potential sources of the compounds, determination of the occurrence and abundance of the compounds in Great Lakes fish, and the determination of acute toxicities of representative compounds of 19 chemical classes (Passino and Smith 1987a). In further studies Smith et al. (1988) focused on 6 of the 19 classes of compounds using the zooplankter Daphnia pulex as the test organism. They ranked the six classes as follows (in decreasing order of toxicity): polycyclic aromatic hydrocarbons (PAHs), alkyl halides, nitrogen-containing compounds, cyclic alkanes, heterocyclic nitrogen compounds, and silicon-containing compounds.

I here focus on four of the classes; omitting the nitrogen- and silicon-containing compounds because they are less abundant or as prevalent as the other four classes in the Great Lakes. The PAHs are products of fuel combustion and components of fossil fuels (Eadie 1984). As noted by Passino and Smith (1987a), the other three classes principally originate from industrial applications (alkyl halides), as fossil fuels, insecticides, solvents, and in perfumes (cyclic alkanes); and as herbicides and insecticides (heterocyclic nitrogen compounds).

My purpose is to report results of static acute toxicity tests in which larval rainbow trout (Oncorhynchus mykiss) were used as the test fish and to compare results of acute toxicity tests with those obtained by Smith et al. (1988).

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MATERIALS AND METHODS

Eyed eggs of rainbow trout (RBT) were received from Ennis National Fish Hatchery (Ennis, Montana) or from Erwin National Fish Hatchery (Erwin, Tennessee). Upon arrival, the eggs were transferred to Heath[1] incubator trays (Heath Techna Corp., Kent, Washington) and held at 8.0 ± 1.0°C in well water (hardness averaged 450 mg/L as $CaCO_3$; Seelye et al. 1982) until testing. Daily maintenance included recording temperature and removing dead eggs and fry. All of the five strains of RBT used for testing--Arlee, Erwin, Redband, Shasta, and Wytheville--were tested with the PAH compound naphthalene to determine if strain differences existed. Fry 13-21 days old (post-hatch), in the yolk-sac stage just before swim-up, were used in the toxicity tests. Before fry were tested, they were acclimated for 24 hr to a mixture of deionized water and well water (hardness ≈ 250 mg/L as $CaCO_3$) and to temperature. Tests were conducted at 12 ± 1.0°C in glass battery jars (3.78 L) filled with 3 L of the mixed water (160-190 mg/L as $CaCO_3$) and placed in a 12°C water bath.

Compounds tested were commercially available and had a minimum purity of 95%. Acetone was used as carrier solvent in all stock solutions of test compounds, except that nicotine was dissolved in deionized water. The test compound was added to the dilution water (≤ 0.5 mL stock/L water), stirred, and 10 fish were added. Each toxicity test consisted of a control and five different concentrations; each nominal test concentration was at least 50% of the next higher concentration. Two or three replicate tests with the same compound were conducted concurrently. Preliminary or range-finding tests were sometimes required to obtain an estimate of the concentration needed to calculate a median lethal concentration (LC50).

Temperature, dissolved oxygen (mg/L), and mortality were monitored and recorded every 24 hr for 96 hr. Dead fry were removed and discarded. The following conditions were necessary for a valid test: temperature fluctuation of less then 1°C, dissolved oxygen concentration greater than 60% of saturation for the first 48 hr of the test and not less than 40% of saturation for the final 48 hr, and mortality in the control treatment not exceeding 10% (Parrish, 1985; ASTM, 1980).

The 96-hr LC50 was calculated by using a probit analysis (Finney 1971) program. The Aquatox program (Stephan 1977) was used to calculate approximate LC50s when standard probit analysis could not be used. Significant differences between compounds ($P \leq 0.05$) were determined by analysis of variance (ANOVA).

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[1] Use of trade names does not imply U. S. Government endorsement of commercial products.

RESULTS AND DISCUSSION

To determine the effect of strain of RBT fry on the results, I tested all strains with the PAH compound-naphthalene. Napthalene was not found in fish tissue most likely because it was metabolized (Hallett and Brecher 1984), but its occurrence is widespread in the Great Lakes ecosystem due to atmospheric transport (Eadie 1984). The LC50s for naphthalene with the different strains of RBT averaged 4.5 mg/L (± 0.5 SE) (Table 1). Statistical analysis (ANOVA) indicated statistically significant differences among the strains but the ecological significance is uncertain, considering the overlapping confidence intervals and the relatively narrow range of LC50s calculated. Therefore I used results from valid toxicity tests, regardless of the strain of fry.

Table 1. Acute toxicity of naphthalene to five strains of larval rainbow trout; mean 96-hr LC50 and 95% confidence intervals (C.I.).

Strain	n	Naphthalene concentration, mg/L 96-hr LC50	
		Mean	95% C.I.
Arlee	2	1.8	0.91 - 2.82
Erwin	6	6.1	3.68 - 9.84
Redband	3	2.6	1.50 - 4.05
Shasta	2	4.4	3.30 - 5.40
Wytheville	2	5.5	4.07 - 6.70

No significant differences in toxicity were found (ANOVA) between the PAH compounds tested (Table 2). Although several valid tests were completed with 2,6-dimethylnaphthalene, an LC50 could not be calculated because mortality was not dose-dependent. All organisms were stressed over the range of 0.5 mg/L to 10.0 mg/L; the fry lay on their sides, almost immobilized, on the bottom of the test jars. A precipitate that formed on the water surface indicated a solubility problem; the calculated solubility in water for 2,6-dimethylnaphthalene is 636 µg/L (Passino and Smith 1987b).

Two compounds from the alkyl halide class were tested, 1-bromodecane and iodohexadecane, but an LC50 was calculated only for 1-bromodecane (Table 2). The solubility of both compounds in water is low--196 µg/L for 1-bromodecane and 0.02 µg/L for iodohexadecane (Passino and Smith 1987b)--and the compounds formed a precipitate. For 1-bromodecane the remaining live fry at the conclusion of the 96-h test were stressed, showing symptoms similar to those of fry tested with 2,6-dimethylnaphthalene. For iodohexadecane, no mortality occurred over the ranges tested (1.0 to 30 mg/L) but all fry showed the symptoms of stress previously described.

No significant differences in toxicity were found between cis-1,2-dimethylcyclohexane and negative trans-pinane [(1S,2S)-2,6,6-trimethylbicyclo [3.1.1] heptane]. Only approximate LC50s could be calculated for cis-1,2-dimethylcyclohexane because there were no partial kills within the test range (1.0 to 10.0 mg/L); mortality was 100% at 7.3 mg/L and nil at 5.0 mg/L. Probit analysis required at least two concentrations with partial kills to calculate an LC50.

Again, only approximate LC50s could be calculated for the heterocyclic nitrogen compounds nicotine and 1-methylpyrrolidine; for nicotine, mortality was total from 5.0 to 10.0 mg/L and nil from 1.0 to 2.7 mg/L. The live fry showed no signs of stress. The approximate LC50 of 36.5 mg/L for 1-methylpyrrolidine was based on a range of concentrations from 20 mg/L to 100 mg/L, within which no mortality occurred below 50 mg/L. Both compounds are very soluble in water, 22.9 g/L for nicotine and 48.3 g/L for 1-methylpyrrolidine (Passino and Smith 1987b).

Table 2. Acute toxicity of four classes of compounds to larval rainbow trout and daphnia. Mean 96-hr LC50 and standard error, and mean EC50.

Class and Compound	n	RBT 96-hr LC50 (mg/L) Mean	SE	Daphnia[c] 48-hr EC50 (mg/L) Mean	%[d]
PAH					
Naphthalene	15	4.5	0.5	4.66	100
1,3-Dimethylnaphthalene	6	1.7	0.2	0.767	45
Phenanthrene	5	3.2	1.1	0.350	11
Alkyl Halide					
1-Bromodecane	4	18.7	2.0	0.074	0.40
Iodohexadecane		>30		0.25	<0.82
Cyclic Alkane					
Cis-1,2-Dimethylcyclohexane	3	7[a]		3.24	46
(-)trans-Pinane	3	5.7	0.5	3.35	58
Heterocyclic Nitrogen					
Nicotine	5	4[a]		0.24	6
1-Methylpyrrolidine	3	36.5[b]	-	2.08	5.7

[a] Approximate LC50's because no partial kills occurred over range of concentrations tested.
[b] Wide range of concentrations tested (20-100 mg/L).
[c] Smith et al. 1988.
[d] (EC50 for Daphnia/LC50 for RBT) x (100).

The following scale according to Multer (Passino and Smith 1987a) was used to describe the toxicity of these compounds to RBT fry (LC50 in mg/L): less than 0.01, super toxic; 0.01 to 0.1,

extremely toxic; 0.1 to 1, highly toxic; 1 to 10, moderately toxic; 10 to 100, slightly toxic; 100 to 1000 practically harmless; greater than 1000, relatively harmless. The median lethal concentrations calculated for the series of compounds listed (Table 2) were predominantly in the moderately toxic range; the calculated LC50 of the alkyl halide 1-bromodecane (18.7 ± 2.0 mg/L) would place the compound in the slightly toxic range.

Statistical analysis (ANOVA) indicated that 1-bromodecane was significantly less toxic than the other compounds ($P \leq 0.05$). The analysis also indicated that the cyclic alkane cis-1,2-dimethylcyclohexane (LC50 = 7.1 ± 0.7) was less toxic than the PAH compound 1,3-dimethylnaphthalene (LC50 = 1.7 ± 0.2), but there were no differences between any of the other compounds (1-methylpyrrolidine was not included because the approximate LC50 calculated was based on a wide range of concentrations; Table 2). Since all the compounds, except 1-bromodecane, fell within the moderate range (1.0 - 10.0 mg/L) on a biological toxicity scale (Passino and Smith 1987a), the statistical differences indicated are probably not ecologically significant. Solubility of the compounds in water is also a factor to be considered when LC50s are compared. The solubility of 1-bromodecane in water (196 µg/L) was significantly lower than that of the other compounds tested (1.17 mg/L - 29.4 g/L; Passino and Smith 1987b). Due to the low solubility of 1-bromodecane in water, a wider range of concentrations (1.0 to 25 mg/L) was tested before the LC50 of 18.7 mg/L was calculated and all the fry that did not die were physically stressed (immobilized, lying on their sides on the bottom of the jars). Therefore 1-bromodecane was more toxic to RBT fry than the LC50 results indicated.

The acute toxicities reported by Smith et al. for Daphnia pulex were measured as immobilization after 48-hr (EC50) as compared to mortality after 96-hr (LC50) for RBT (Table 2). I calculated ratios by using actual EC50 and LC50 values that could be used to readily see how well Daphnia EC50s predicted the RBT LC50s (Table 2). The Daphnia EC50 values from the PAH and cyclic alkane classes differed from the RBT LC50 values by a factor of 2.5 or less, with one exception--phenanthrene (factor of 10). The EC50 values for the heterocyclic nitrogen class differed from the RBT LC50 values by a factor of 17.5 and the alkyl halide values varied by a factor greater than 100 (Table 2). The Daphnia EC50 values calculated for 1-bromodecane and iodohexadecane were more indicative of the toxicity of alkyl halides than the RBT LC50 values because the fish showed symptoms of stress at 20 times lower concentrations than the LC50 value. Longer term chronic toxicity bioassays with RBT in which compounds from the alkyl halide and heterocyclic classes were used would give toxicity values more in agreement with the results of Smith et. al. (1988).

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