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Toxicity to *Daphnia pulex* and QSAR Predictions for Polycyclic Hydrocarbons Representative of Great Lakes Contaminants

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The Laurentian Great Lakes contain the world's largest supply of freshwater; however, the quality of water of the Great Lakes to support aquatic life is impaired by the presence of chemical wastes from the surrounding industrialized regions of the United States and Canada (Baumann et al 1996). A wide array of polycyclic aromatic hydrocarbon compounds has been identified in sediment, water, and biota of the Great Lakes and its tributaries (Fabacher et al 1991; Passino and Smith 1987). The sources of polycyclic aromatic hydrocarbons are largely from fossil fuels; whereas related polycyclic hydrocarbons, especially those with bridged structures characteristic of sesquiterpenes, may be from natural plant products and from pulp mills. Extracts of environmental samples containing these compounds have been shown to be associated with formation of neoplasms in fish (Fabacher et al 1991). Relatively little is known about the toxicity to fish and invertebrates of many of these compounds that are detected in Great Lakes samples.

The objectives of this study were (1) to determine the toxicity of several types of polycyclic hydrocarbons characteristic of Great Lakes samples to Daphnia pulex, a Great Lakes zooplankter, (2) to investigate the influence of different structural characteristics on toxicity, and (3) to determine the linear solvation energy relationship (LSER) parameters and model that describe these compounds. These results will be related to comparative toxicity of other Great Lakes environmental compounds and to their application in site specific risk assessment.

MATERIALS AND METHODS

Chemicals used in acute bioassays were purchased from Aldrich, Milwaukee, Wisconsin; Fluka, Ronkonkoma, New York; Lancaster Synthesis, Windham, New Hampshire; Pfalz & Bauer, Waterbury, Connecticut; and Wiley Organics, Coshocton, Ohio, and had 94 to >99% purity (Table 1). The compounds tested had been either tentatively identified in Great Lakes fish or sediments or were surrogates for these compounds. In many cases the alkylated compounds from environmental samples were commercially unavailable, so parent structures were tested, e.g., dihydronaphthalene was tested in place of alkylated dihydronaphthalene. Stock solutions were prepared in pesticide grade acetone.

Daphnia pulex, from long-term cultures at the Great Lakes Science Center were reared and tested at 20 C according to standard procedures (ASTM 1980) as adapted by Passino

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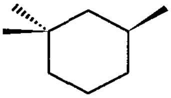
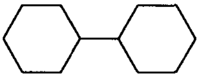
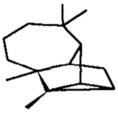
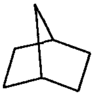
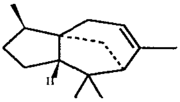
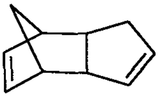
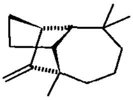
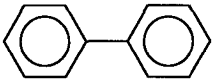
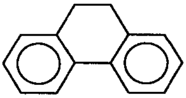
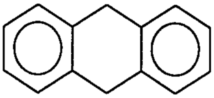
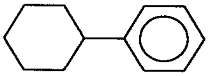
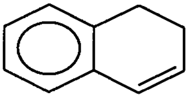
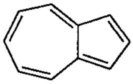
Table 1. Properties of Chemicals.

CAS #	Name	MW	Log K_{ow}	STRUCTURE
3073-66-3	**1,1,3-Trimethyl cyclohexane**	126	4.91	
92-51-3	**Bicyclohexyl**	166	5.9	
1137-12-8	**Longicyclene**	204	5.81	
508-32-7	**Tricyclene**	136	3.86	
279-23-2	**Norbornane**	96	3.11	
469-61-4	**(-)-∝-Cedrene**	204	6.07	
77-73-6	**Dicyclopenta-diene**	132	2.89	
475-20-7	**Longifolene**	204	6.07	
92-52-4	**Biphenyl**	154	4.03	
776-35-2	**9,10-Dihydro-phenanthrene**	180	4.78	

CAS #	Name	MW	Log K_{ow}	STRUCTURE
613-31-0	**9,10-Dihydroanthracene**	180	4.67	
827-52-1	**Cyclohexylbenzene**	160	4.91	
447-53-0	**1,2-Dihydronaphthalene**	130	3.56	
275-51-4	**Azulene**	128	3.32	

and Smith (1987) and Smith et al (1988). Reconstituted hard water was prepared from reverse osmosis water with reagent grade chemicals and had the following properties: total hardness = 160 - 200 mg/L as $CaCO_3$, alkalinity = 120-125 mg/L as $CaCO_3$, and dissolved oxygen 8-9 mg/L. Daphnids were fed four species of green algae plus Cerophyl® (Passino and Smith 1987). Neonates (< 24 hr old) were used for 48-hr bioassays without feeding. Each test included a solvent control containing a maximum of 0.5 mL/L acetone and five toxicant concentrations in a geometric progression. Range finding tests were conducted followed by bioassays until at least three valid bioassays were obtained (see criteria in ASTM 1980). Immobilization of daphnids was used as the endpoint and the 48-hr EC_{50} calculated by probit (SAS 1994) using nominal concentrations of the chemicals. In addition, the condition of the daphnids at 48 hr was evaluated for subacute response by categorizing their activity levels into six categories, where category “one” was normal activity and category “six” was dead. The data in these categories were condensed into a single value, which we termed an “activity index” by weighting the number of daphnids in a category and summing the numbers in the six categories. The “activity indices” were plotted as a function of the concentrations tested and also as a function of the concentration divided by the 48-hr EC50 to normalize the concentrations.

The Log K_{ow} values were obtained from the U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, QSAR data base by on-line searches and were based on CLOGP calculations (Leo and Weininger 1984). To compare measured toxicity with predicted toxicity, we estimated the toxicity to D. pulex with the LSER model (Passino et al 1988; Hickey and Passino-Reader 1991; Hickey 1996). The LSER equation developed for the expert system (Hickey et al 1992) uses a general narcosis model for a range of classes of compounds. Measured toxicity was compared with predicted toxicity by regression analysis (SAS 1992) with $p \le 0.05$. Measured toxicity falling outside the 95% confidence intervals of the predicted toxicity were considered outside the general narcosis model.

RESULTS AND DISCUSSION

The toxicities, 48-hr EC_{50}, (mg/L, mean ± SD), of these cyclic hydrocarbons to Daphnia pulex, using immobilization as the endpoint (Table 2) ranged from 0.035 (±0.0026) for bicyclohexyl to 5.2 (±0.66) for norbornane. The toxicity appeared related to the size, shape, and aromaticity of the molecule. Progressing from biphenyl, which is fully aromatic, to cyclohexyl benzene, with one benzene ring, to bicyclohexyl, which is fully reduced, the toxicity increased from 1.6 (±0.10) to 0.55 (±0.21) to 0.035 (±0.0026) mg/L, respectively. However, toxicity values for the naphthalene series were 4.7 (±0.14), 4.3 (±0.25), 2.4 (±0.92), and 2.5 (±1.2) mg/L for naphthalene; 1,2-dihydronaphthalene; 1,2,3,4-tetrahydronaphthalene; and decahydronaphthalene, respectively, where values other than 1,2-dihydronaphthalene are from Smith et al (1988). Toxicity increased with increasing number of rings for the partially reduced polyaromatic hydrocarbons, i.e., from 4.3 (±0.25) for 1,2-dihydronaphthalene to 0.56 (±0.080) and 0.42 (±0.036) mg/L for 9,10-dihydroanthracene and 9,10-dihydrophenanthrene, respectively. The sesquiterpene compounds with molecular weight (MW 204) and containing the bridging methano group (Table 1) were notably more toxic (Table 2) and also are frequently detected in Great Lakes fish and sediment samples (Larry Schmidt, per. commun). A related (MW 206) octahydrotetramethylmethanoazulene compound was the most toxic compound tested with D. pulex in an earlier series of bioassays on several classes of compounds (Smith et al 1988). An examination of the plots of “activity index” as a function of normalized concentration also showed a sharper reduction in activity relative to concentration for the most toxic compounds, i.e., bicyclohexyl and the three sesquiterpenes--cedrene, longicyclene, and longifolene, suggesting a mechanism of action other than narcosis. For the less toxic compounds, the “activity index” increased gradually with increasing toxicity, suggesting a general narcotizing of the daphnids. The four most toxic compounds had Log K_{ow} values of 5.8 to 6.1, whereas the least toxic compound, norbornane, had a Log K_{ow} value of 3.1 (Table 1).

Few data are available for aquatic toxicity of most of these compounds. Sweet and Meier (1997) reported 48-hr EC_{50}’s of azulene and longifolene to Daphnia magna (22 C) of 11.5 ± 2.7 SD) and 0.44 ±0.04 mg/L, compared with our values of for D. pulex of 1.6 ± 0.094 and 0.080 ± 0.0065 mg/L, respectively. Bobra et al (1983) reported 48-hr EC50 of biphenyl to D. magna as 3.1 mg/L, compared with our value of 1.6 ± 0.10 mg/L. Gersich et al (1989) reported a 48-hr LC50 at 20 C of biphenyl to D. magna of 0.36 [0.28 - 0.47 (95% C.I.)] mg/L and a 21-d MATC for both mortality and reproduction of 0.23 mg/L. No aquatic invertebrate data were available for bicyclohexyl, but Geiger et al (1990) reported that no mortality was observed when Pimephales promelas were exposed for 4 d to 0.103 mg/L. We report the 48-hr EC50 to D. pulex of 0.035 mg/L bicyclohexyl, supporting the hypothesis of greater sensitivity of invertebrates than fish to these compounds (Passino-Reader et al 1995).

The linear solvation energy parameters (Table 2) calculated by methods of Hickey and Passino-Reader (1991) and Hickey (1996) were used to predict toxicities to D. pulex with the following regression equation, modified from Hickey et al (1992):

Table 2. LSER Values (see text), Predicted and Observed Toxicity to Daphnia pulex.

Name	Vi/100	π*	β	α	Pred EC50 log (μM)	Pred EC50 (mg/L)	Obs EC50 log(μM)	Obs EC50 (mg/L) $\bar{x}$	Obs EC50 (mg/L) S.D.
1,1,3-Trimethylcyclohexane	0.884	0.00	0.00	0.00	1.1	1.6	0.92	1.0	0.12
Bicyclohexyl	1.126	0.01	0.00	0.00	0.14	0.23	-0.68	0.035	0.0026
Longicyclene	1.330	-0.03	0.00	0.00	-0.68	0.043	-0.50	0.065	0.0096
Tricyclene	1.035	-0.03	0.00	0.00	0.51	0.45	1.0	1.4	0.35
Norbornane	0.791	0.00	0.00	0.00	1.5	3.0	1.7	5.2	0.66
(-)-α-Cedrene	1.324	0.10	0.10	0.05	-0.67	0.044	-0.67	0.044	0.033
Dicyclopentadiene	0.908	0.20	0.20	0.10	1.0	1.3	1.5	4.2	1.0
Longifolene	1.354	0.09	0.10	0.05	-0.79	0.033	-0.40	0.080	0.0065
Biphenyl	0.920	0.40	0.20	0.00	0.88	1.2	1.0	1.6	0.10
9,10-Dihydrophenanthrene	1.094	0.26	0.20	0.00	0.21	0.29	0.37	0.42	0.036
9,10-Dihydroanthracene	1.178	1.18	0.3	0	-0.33	0.084	0.50	0.56	0.079
Cyclohexylbenzene	0.991	0.59	0.14	0.00	0.56	0.58	0.54	0.55	0.21
1,2-Dihydronaphthalene	0.857	0.60	0.22	0.05	1.1	1.7	1.5	4.3	0.25
Azulene	0.75	0.50	0.35	0.00	1.5	4.3	1.1	1.6	0.094

$$\log \mathrm{EC50}\ (\mu M) = 4.69 - 4.04\ V_i/100 - 0.20\ \pi^* - 0.06\ \beta_m + 0.29\ \alpha_m \qquad \text{(Eqn. 1)}$$
$$N = 36,\ R^2 = 0.853,\ SD = 0.422$$

where V_i is the intrinsic (van der Waals) molecular volume, π^* scales the solute electrostatic stabilization of solvent charge or dipole, and β_m and α_m scale solute ability to accept or donate a hydrogen (or donate or accept an electron pair) in a hydrogen bond (Hickey and Passino-Reader 1991; Hickey 1996). By comparing the measured toxicity (48-hr EC50) in this study with the 95% confidence intervals for a predicted value of toxicity, we observed that none of the measured values were outliers to Equation 1. This indicates that these compounds fit the model for general narcosis. The compounds used in developing Equation 1 were both narcosis I and narcosis II type compounds (Passino et al 1988; Veith and Broderius 1990). We added observed toxicity values and calculated LSER variables from this study (Table 2) to the original compounds in Equation 1 (Passino et al 1988) and calculated the following regression (SAS 1992):

$$\mathrm{Log\ EC50}\ (\mu M) = 4.62 - 3.94\ V_i/100 - 0.14\ \pi^* - 0.12\ \beta_m + 0.31\ \alpha_m \qquad \text{(Eqn. 2)}$$
$$N = 50,\ R^2 = 0.833,\ SD = 0.420$$

which utilizes a larger data set than Equation 1. There is no significant difference between Equations 1 and 2 ($p \leq 0.05$). The only significant independent variable in Equations 1 and 2 is $V_i/100$ ($p \leq 0.05$), which supports the general narcosis mode of action for these compounds. With a wider range of compounds, π^*, β_m and α_m would be expected to be significant (Hickey 1996).

Our measured toxicities of cyclic and polycyclic hydrocarbons to D. pulex will be useful in conducting risk assessments to aquatic biota. Toxicity data for invertebrates can serve as threshold toxicity values protective for fish for these compounds (Passino-Reader et al 1995). In lieu of chronic data, acute data can be adjusted by an application factor to estimate safe levels such as MATC's (Gersich et al 1989) for risk assessments.

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REFERENCES

ASTM (1980) Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. Annual Book of ASTM Standards. E728-80. American Society for Testing and Materials, Philadelphia, Pennsylvania

Baumann PC, Smith IR, Metcalfe CD (1996) Linkages between chemical contaminants and tumors in benthic Great Lakes fish. J Great Lakes Res 22: 131-152

Bentley RE, LeBlanc GA, Hollister TA, Sleight BH (1976) Acute toxicity of diisopropyl phosphate and dicyclopentadiene to aquatic organisms. Contract No DAMD-17-75-C-5073, US Army Medical Res Develop Command, Washington, DC, 98 p

Bobra AM, Shiu WY, MacKay D (1983) A predictive correlation for the acute toxicity of hydrocarbons and chlorinated hydrocarbons to the water flea Daphnia magna. Chemosphere 12:1121-1129

Fabacher DL, Besser JM, Schmitt CJ, Harshbarger JC, Peterman PH, Lebo JA (1991) Contaminated sediments from tributaries of the Great Lakes: Chemical characterization and carcinogenic effects in medaka (Oryzias latipes). Arch Environ Contam Toxicol 21:17-34

Geiger DL, Brooke LT, Call DJ (1990) Acute toxicities of organic chemicals to fathead minnow (Pimephales promelas). Center for Lake Superior Environmental Studies, University of Wisconsin-Lake Superior, vol 5, 332 p

Gersich FM, Bartlett EA, Murphy PG, Milazzo DP (1989) Chronic toxicity of biphenyl to Daphnia magna Straus. Bull Environ Contam Toxicol 43:355-362

Hickey, JP (1996) Linear solvation energy relationships (LSER): "Rules of thumb" for $V_i/100$, π^*, β_m, α_m estimation and use in aquatic toxicology. In Ostrander GK (ed) Techniques in Aquatic Toxicology. Lewis Publishers, Boca Raton, Florida, pp 435-457

Hickey JP, Passino-Reader DR (1991) Linear solvation energy relationships: "Rules of thumb" for estimation of variable values. Environ Sci Technol 25:1753-1760

Hickey JP, Aldridge AJ IV, Passino-Reader DR, Frank AM (1992) An expert system for prediction of chemical toxicity. Drug Inform J 26:487-495

Leo A, Weininger D (1984) CLOGP User Reference Manual, ver 3.2. Medicinal Chemistry Project, Pomona College, Claremont, California

Passino DRM, Smith SB (1987) Acute bioassays and hazard evaluation of representative contaminants detected in Great Lakes fish. Environ Toxicol Chem 6:901-907

Passino DRM, Hickey JP, Frank AM (1988) Linear solvation energy relationships for toxicity of selected organic chemicals to Daphnia pulex and Daphnia magna. In: Turner JE, Williams MW, Schultz TW, Kwaak NJ (eds) Proceedings of QSAR 88: Third International Workshop on Quantitative Structure-Activity Relationships in Environmental Toxicology. US Department of Energy, Oak Ridge, Tennessee, p 131-146

Passino-Reader DR, Berlin WH, Hickey JP (1995) Chronic bioassays of rainbow trout fry with compounds representative of contaminants in Great Lakes fish. J Great Lakes Res 21:373-383

SAS Institute Inc (1992, 1994) SAS user's guide: statistics releases 6.08, 6.10. SAS Institute, Inc, Cary, North Carolina

Smith SB, Savino JF, Blouin MA (1988) Acute toxicity to Daphnia pulex of six classes of chemical compounds potentially hazardous to Great Lakes aquatic biota. J Great Lakes Res 14:394-404

Sweet LI and Meier PG (1997) Lethal and sublethal effects of azulene and longifolene to Microtox R, Ceriodaphnia dubia, Daphnia magna, and Pimephales promelas. Bull Environ Contam Toxicol 58:268-274

Veith GD, Broderius (1990) Rules for distinguishing toxicants that cause Type I and Type II narcosis syndromes. Environ Health Perspect 87:207-211