Bull. Environ. Contam. Toxicol. (1988) 41:604-608

Bulletin of Environmental Contamination and Toxicology

Toxicity of Six Heterocyclic Nitrogen Compounds to *Daphnia pulex*[1]

Cynthia M. Perry[2,4] and Stephen B. Smith[3]

[2]Department of Biology, Eastern Michigan University, Ypsilanti, Michigan 48197 and [3]National Fisheries Research Center—Great Lakes, 1451 Green Road, Ann Arbor, Michigan 48105

Samples of whole lake trout (Salvelinus namaycush) and walleyes (Stizostedion vitreum vitreum) collected from the open waters of the Great Lakes or Lake St. Clair yielded 476 organic compounds when analyzed by gas chromatography/mass spectrometry (GC/MS). In contrast, only eight compounds were identified in hatchery reared lake trout (Hesselberg and Seelye 1982). When the 476 compounds were grouped into chemical classes by toxicity, occurrence, and source (Passino and Smith 1987), the heterocyclic nitrogen compounds ranked ninth among twenty classes that were regarded as potentially hazardous to aquatic resources. Many heterocyclic nitrogen compounds are of anthropogenic origin, though some occur naturally in fish tissue (Welch et al. 1981).

We determined the relative toxicities to the aquatic crustacean Daphnia pulex of the six heterocyclic nitrogen compounds: 3-(1-methyl-2-pyrrolidinyl)pyridine (nicotine), 2-pyrrolidinone, 1-methylpyrrolidine, 2-(2-hydroxyethyl)pyridine, 2-amino-4,6-dimethylpyridine, and 2-amino-4,7(1H,8H)-pteridinedione(isoxanthopterin). These compounds were selected because they were detected in lake trout or walleyes and were commercially available. Daphnia pulex was used as the test organism because it is endemic to the Great Lakes, is easy to culture, has parthenogenic reproduction, constant genetic makeup over generations, and is sensitive to ecological stress.

[1]Contribution #700 of the National Fisheries Research Center-Great Lakes, US Fish and Wildlife Service, Ann Arbor, Michigan 48105.

[4]Present address: Maine Cooperative Fish and Wildlife Research Unit, Department of Zoology, University of Maine, Orono, Maine 04469.

Send reprint requests to Stephen B. Smith at the above address

MATERIALS AND METHODS

The culturing and bioassay methods used were those of the American Society for Testing and Materials (1980), the U. S. Environmental Protection Agency (1975), and the contaminant toxicology project at the National Fisheries Research Center - Great Lakes (Passino and Smith 1987).

All chemicals used for making contaminant test solutions were of 97% or higher purity; those used to prepare the culture media were of reagent grade or higher purity. All chemicals were soluble in water.

Daphnia pulex were cultured in 2-L jars of reconstituted hard water (20^{o}C; pH,7.6-8.0; dissolved oxygen, 60-100% saturation; hardness 160-180 mg/L as $CaCO_3$; alkalinity 110-120 mg/L as $CaCO_3$). To minimize leaching, dissolution and sorption of toxicants from the water we used only glassware and tubing made from perfluorocarbon plastic for culturing and testing.

The daphnid food was a mixture of the four algal species Ankistrodesmus falcatus, Chlamydomonas reinhartii, Chlorella pyrenoidosa and C. vulgaris, reared in Woods Hole media (Stein 1973) plus cerophyl, at a ratio of 1:1:1:1:4. The daphnids were fed five times a week with 3 mL of food per liter of culture water.

We conducted 48-h tests with 10 neonates (<24 h old) in five concentrations of each toxicant and the control. Toxicant concentrations (in 150 mL of reconstituted hard water) were at least 50% of the next concentration. The six test beakers, covered with parafilm, were placed in a constant temperature water bath at 20^{o}C with a photoperiod of 16 h light, 8 h dark. Test animals were not fed during the experiment. After 48 h the daphnids were pipetted into a watch glass and examined for immobilization.

Mean effective concentration (EC50) and standard error were calculated from the immobilization data for valid toxicity tests (American Society for Testing and Materials 1980). A mean was taken from three valid tests. To calculate EC10, EC50, and EC90 values, we used a computer modification (Peltier et al. 1985) of Finney's (1952) probit analysis. Statistical comparisons were made on logarithmically transformed EC50's using analysis of variance (ANOVA) and Tukey's HSD test (Steel and Torrie 1960).

RESULTS AND DISCUSSION

The most toxic heterocyclic nitrogen compound was nicotine, followed by 1-methylpyrrolidine, isoxanthopterin, 2-amino-4,6-dimethylpyridine, 2-pyrrolidinone, and 2-(2-hydroxyethyl)pyridine (Table 1). Statistical comparisons (ANOVA) indicated that nicotine was significantly more toxic than 1-methylpyrrolidine and isoxanthopterin. 1-methylpyrrolidine and isoxanthopterin were significantly more toxic than 2-amino-4,6-dimethylpyridine, 2-pyrrolidinone, and 2-(2-hydroxyethyl)pyridine. These last three compounds were significantly less toxic than all other compounds tested.

The range over which the compounds had toxic effects (EC10-EC90) on *Daphnia pulex* were narrower for nicotine, 1-methylpyrrolidine, and 2-amino-4,6-dimethylpyridine than for the others. The wide range of toxicity for 2-pyrrolidinone and 2-(2-hydroxyethyl) pyridine, suggested that these compounds were less specific in their modes of action in *D. pulex*. Effects of individual heterocyclic nitrogen compounds on *D. pulex* extended over two orders of magnitude.

Table 1. Ranked toxicity of certain heterocyclic compounds and some pesticides to *Daphnia pulex* or *D. magna* (where indicated) at 17° or 20°C. Compound means that share a group letter were not significantly different.

Compound	EC50 (mg/L)		
	Mean	SE	Group
DDT (*D. magna*)	0.0011[a]	0.0001	
DDT (17°C)	0.0019[b]	0.0001	A
Chlordane (*D. magna*)	0.097[a]	0.005	
Nicotine	0.242	0.02	B
Nicotine (17°C)	0.326[b]	0.074	B
Pentachlorophenol	2.00[c]	0.0	C
Pentachlorophenol (*D. magna*)	2.5[c]	0.1	C
1-methylpyrrolidine	2.08	0.20	C
Isoxanthopterin	2.97	0.47	C
2-amino-4,6-dimethylpyridine	9.19	1.85	D
2-pyrrolidinone	13.21	4.02	D
2-(2-hydroxyethyl)pyridine	13.82	3.60	D

[a] Randal et al. (1979).
[b] Passino and Smith (1987).
[c] Canton and Adema (1978).

Our results suggest that the potential threat of certain heterocyclic nitrogen compounds to aquatic organisms in the Great Lakes may be as great as that of organochlorine pesticides. Using Tukey's (HSD) procedure, we found no significant difference ($P>0.05$) in the toxicities of the heterocyclic nitrogen compounds 1-methylpyrrolidine and isoxanthopterin and that of the pesticide pentachlorophenol (Table 1).

Heterocyclic nitrogen compounds were detected in fish at five of eight Great Lakes and Lake St. Clair collection sites by Hesselberg and Seelye (1982). Of the compounds tested in the present study, isoxanthopterin was the most widely distributed, occurring at four sites. Because isoxanthopterin was not soluble in reconstituted water at 20^{o}C in our testing procedure (calculated solubilities were not available), the greater environmental threat may lie in its synergistic effect or potentiation. Isoxanthopterin may biodegrade quickly or be easily bound up in the sediments and not readily available to organisms in the water column. When either nicotine or 2-(2-hydroxyethyl)pyridine was detected, it was among the most prominent compounds. The other compounds (1-methylpyrrolidine, 2-amino-4,6-dimethylpyridine, and 2-pyrrolidinone) were detected in lower concentrations at one or more sites. Higher concentrations of a compound at one site could increase the toxicity risk at that site, whereas widespread occurrence of a compound would increase an organism's risk of exposure. Both factors influence a compound's potential as an environmental hazard.

Our data demonstrated that nicotine, 1-methylpyrrolidine, 2-amino-4,6-dimethylpyridine, 2-pyrrolidinone, and 2-(2-hydroxyethyl)pyridine ranged from highly to moderately toxic (Zucker 1985) to _Daphnia pulex_. These compounds may alter the distribution, density, and behavior of aquatic organisms by lethal or sublethal action, and indirectly affect a species' food or response to competition and predation (Maciorowski and Clarke 1980). Stress to the daphnid populations may affect forage fish populations that depend either directly or indirectly on zooplankton as a food source in the Great Lakes.

Acknowledgements. We thank Dr. Dora Passino of the National Fisheries Research Center - Great Lakes, for her initial counsel and original cultures of _Daphnia_ and algae. Ms. Perry acknowledges the assistance of Drs. Dale Wallace, William Fennel, Ronald Scott, and Howard Booth, Eastern Michigan University, for serving on her graduate committee.

REFERENCES

American Society for Testing and Materials (1980) Standard practice for conducting acute toxicity tests with fish, macroinvertebrates, and amphibians. Am Soc Test Mat, Philadelphia, E729-80

Finney DH (1952) Statistical methods in biological assay. C. Griffin and Co Ltd., London, 661 pp

Hesselberg RJ, Seelye JG. (1982) Identification of organic compounds in Great Lakes fish by gas chromatography/mass spectrometry: 1977. US Fish Wild Serv, Great Lakes Fishery Laboratory, Ann Arbor, Admin Rep No 82-1

Maciorowski HD, Clarke R (1980) Advantages and disadvantages of using invertebrates in toxicity testing. In: Buikeman AL and Cairns J (eds) Aquatic Invertebrate Bioassays, Am Soc Test Mat, STP 715, Philadelphia, p 36-47

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

Peltier WH, Weber CI(eds) (1985) Methods for measuring the acute toxicity of effluents to freshwater and marine organisms, 3rd ed Environ Monitor Support Lab, US Environ Protect Agency,Cincinnati, Rep no 600/4-85-013

Randall WF, Dennis WH, Warner MC (1979) Acute toxicity of dechlorinated DDT, chlordane and lindane to bluegill (Lepomis macrochirus) and Daphnia magna. Bull Environ Contam Toxicol 21:849-854

Steel RGD, Torrie JH (1960) Principles and Procedures of Statistics, McGraw Hill, New York

Stein JR (1973) Handbook of physiological methods: culture methods and growth measurements. Cambridge University Press, New York, 448pp

US Environmental Protection Agency (1975) Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. National Environmental Research Center-Corvallis, US Environ Protect Agency, Rep No 660/3-75-009, p 61

Welch KJ, Keuhl DW, Leonard EN, Weith DD, Schoenthal ND (1981) Background hydrocarbon residues in fish from the Great Lakes and eastern Montana. Bull Environ Contam Toxicol 26:724-728

Zucker E (1985) Standard evaluation procedure: acute toxicity test for freshwater invertebrates. Office of Pesticide Programs US Environ Protect Agency Washington, DC, Rep No 540/9-85-005

Received April 11, 1988, Accepted April 19, 1988.