Journal

WATER POLLUTION CONTROL FEDERATION

THE ACUTE TOXICITY OF SOME HEAVY METALS TO DIFFERENT SPECIES OF AQUATIC INSECTS

Stephen L. Warnick and Henry L. Bell

FEBRUARY 1969
TWO PARTS, PART 1

THE ACUTE TOXICITY OF SOME HEAVY METALS TO DIFFERENT SPECIES OF AQUATIC INSECTS

Stephen L. Warnick and Henry L. Bell

Various metal salts are constituents of mine effluents, brines from oil wells, and wastes from metal processing, chemical manufacturing, and many other industries (1) (2). Because of the toxic properties of these metals and their other adverse effects on water quality, their discharge is a serious water pollution problem.

Water quality criteria on which water pollution parameters are based are needed for the protection of aquatic life. Acute toxicity tests are a first step in determining these criteria. They indicate relative species sensitivity and lethal concentrations. This information then can be used as a basis for long-term tests to establish the requirements necessary for the well-being of aquatic life.

Information on the toxicity of metals to fish has been summarized by Doudoroff and Katz (3), McKee and Wolf (1), Rudolfs, Barnes, *et al.* (4), and others. However, information on the toxicity of metals to aquatic insects (Table I) is very limited.

The purpose of this study was to determine the acute toxicity of the salts of copper, zinc, cadmium, lead, iron, nickel, cobalt, chromium, and mercury to three species of aquatic insects as a first step in the development of criteria for protecting these important fish-food organisms.

Stephen L. Warnick and Henry L. Bell are Research Aquatic Biologists, Federal Water Pollution Control Administration, National Water Quality Laboratory, Duluth, Minnesota.

The metal compounds considered here are those simple inorganic salts, the toxicity of which appears to be due to the component metals. The wide range of concentrations at which metals are toxic to fish (Table II) indicates that chemical and physical factors and environmental variables have an important effect on the degree of toxicity. Recent workers have recognized this fact and have adopted a more uniform bioassay procedure. By reporting such important parameters as DO, pH, alkalinity, acidity, and hardness of the dilution water, they have improved greatly the significance and reproducibility of their work.

Methods

To evaluate the acute toxicity of the heavy metals to aquatic insects, static bioassays were conducted according to the methods recommended by the American Public Health Association (5), and a TL_m value (median tolerance limit) was determined on the basis of the calculated concentrations. In some cases a 96-hr TL_m was not determined, since the insects tested did not die in 96 hr at the maximum concentration (64 mg/l) that was used. In these cases results are reported in terms of survival times of the different insects in various metal concentrations. Higher concentrations were not used with insects, because fish seem to be more sensitive to metals. Therefore, concentrations that would protect fish also would pro-

TABLE I.—The Concentrations of Heavy Metals Having an Effect on Aquatic Organisms

Metal	Metal Concentration (mg/l)	Type of Water	Organism	Effect	Reference
Arsenic	3–14	—	mayfly	no harm	(5)
Arsenic	10–20	—	damsel flies	no harm	(5)
Barium	29	Lake Erie	daphnia magna	immobilized	(11)
Cadmium	0.10	River Havel	daphnia magna	threshold toxic	(12)
Chromium	0.70	River Havel	daphnia magna	threshold toxic	(12)
Cobalt	5.00	River Havel	daphnia magna	threshold toxic	(12)
Copper	0.027	Lake Erie	daphnia magna	immobilized	(12)
Lead	5.00	River Havel	daphnia magna	threshold toxic	(12)
Mercury	0.03	River Havel	daphnia magna	threshold toxic	(12)
Nickel	6.00	River Havel	daphnia magna	threshold toxic	(12)
Silver	0.03	River Havel	daphnia magna	threshold toxic	(12)
Zinc	1.80	River Havel	daphnia magna	threshold toxic	(12)
Zinc	0.30	—	mayfly nymphs	killed	(5)
Zinc	0.33	—	stoneflies, caddisflies	no effect	(5)

tect the aquatic insects. TL_m values are expressed in terms of the initial concentration (mg/l) of metal added to the dilution water. The concentrations tested for each metal were: 0.0, 0.1, 1.0, 4.0, 16.0, and 64.0 mg/l expressed in terms of the amount of metal added to the dilution water initially (Table III). The metal concentrations in the stock solutions and in the test dilutions were checked with a polarograph and were found to be within five percent of the calculated values. The concentrations in the test dilutions were determined again at the end of the two-week bioassay period and, in some cases, had decreased considerably (Table III).

TABLE II.—The Toxicity of Some Metals to Fish

Metal	Metal Concentration (mg/l)	Reference
Zn	0.78 – 33.4	Pickering (10)
Zn	0.01 – 10.1	Rudolfs, Barnes, *et al.* (5)
Zn	0.3 – 0.7	Jones (6)
Cu	0.015– 3.0	Rudolfs, Barnes, *et al.* (5)
Cu	0.022– 1.76	Pickering (10)
Cu	0.01 – 0.02	Jones (6)
Co	1.0 – 10.0	Schweiger (7)
Co	7.0 – 15.0	Jones (8)
Ni	0.08 – 1.0	Jones (8)
Ni	4.45 – 44.5	Pickering (10)
Pb	0.1 – 6.3	Anon. (9)
Pb	5.58 –482.0	Pickering (10)
Pb	0.1 – 0.4	Jones (6)
Cd	0.01 – 10.0	McKee and Wolf (1)
Cd	0.63 – 73.5	Pickering (10)
Cd	0.03	Jones (8)
Hg	0.01 – 0.02	Jones (8)

Ten individuals of each species were used in each metal concentration and control. One liter of carbon-filtered tap water from Lake Superior was set up in a two-liter Erlenmeyer flask for each metal concentration and control unit. A small piece of plastic screen was used for insect attachment, and a small stream of air from a glass tube was bubbled through the water to maintain the oxygen level and produce some circulation of the water.

The dilution water had the following physical and chemical characteristics: * temperature, 18°C ± 2°; DO, 9.2 mg/l; pH, 7.25; alkalinity, 40.0 mg/l acidity, 4.0 mg/l; and hardness, 44.0 mg/l. The values for these same parameters at the conclusion of the tests are given in Table III.

The three species of insects used were: a stonefly, (Plecoptera) *Acroneuria lycorias;* a mayfly, (Ephemeroptera) *Ephemerella subvaria;* and a caddisfly, (Trichoptera) *Hydropsyche betteni.* These were chosen because they are widespread, easily collected and maintained in the laboratory, and they are important as fish food organ-

* Characteristics were determined using Hach Water and Sewage Analysis Procedures, Hach Chemical Co., Ames, Iowa.

TABLE III.—Bioassay Data*

Metal	Insect	96-hr TL_m (mg/l)	50% Survival (days)	50% Survival (mg/l)	Physical and Chemical Data of the 16 mg/l Test Water after Two Weeks: C°	DO (mg/l)	pH	Alk. (mg/l)	Acid. (mg/l)	Hard. (mg/l)	N (mg/l)	Metal (mg/l)	Mg Metal Absorbed by Insects
Cu^{++} from $CuSO_4.5H_2O$	acroneuria	8.3			18.5	8.0	6.8	54	20	40	4.9	12.3	0.06
	ephemerella	0.32 (48-hr)			18.5	8.0	6.9	42	6	40	1.5	10.4	0.14
	hydropsyche		14	32.0	18.5	8.0	6.8	40	4	46	0.9	14.5	0.22
Zn^{++} from $ZnSO_4.7H_2O$	acroneuria		14	32.0	18.5	8.0	7.6	46	12	50	6.5	5.5	0.01
	ephemerella		10	16.0	18.5	8.0	7.6	30	8	54	1.7	7.9	0.12
	hydropsyche		11	32.0	18.5	8.0	7.6	30	8	52	1.1	4.7	0.18
Cd^{++} from $CdSO_4.8H_2O$	acroneuria		14	32.0	18.5	8.0	7.3	60	12	52	7.2	14.1	0.42
	ephemerella	2.0			18.5	8.0	7.0	56	8	54	1.6	15.2	0.05
	hydropsyche		10	32.0	18.5	8.0	7.0	54	6	56	1.5	14.0	0.13
Pb^{++} from $PbSO_4$	acroneuria		>14	64.0	18.5	8.0	7.3	60	12	54	7.6	2.2	0.05
	ephemerella		7	16.0	18.5	8.0	7.0	56	6	52	3.1	3.0	0.12
	hydropsyche		7	32.0	18.5	8.0	7.1	42	6	54	3.5	3.2	0.08
Fe^{++} from $FeSO_4$	acroneuria		9	16.0	18.5	8.0	7.7	72	14	48	14.1	1.2	0.02
	ephemerella	0.32			18.5	8.0	8.2	54	6	48	0.95	1.4	0.01
	hydropsyche		7	16.0	18.5	8.0	8.1	46	6	50	1.28	1.6	0.01
Ni^{++} from $NiSO_4.6H_2O$	acroneuria	33.5			18.5	8.0	7.0	54	10	40	6.0	6.4	0.04
	ephemerella	4.0			18.5	8.0	7.0	46	6	42	1.5	7.2	0.03
	hydropsyche		>14	64.0	18.5	8.0	7.0	42	6	48	1.1	13.0	0.02
Co^{++} from $CoSO_4.7H_2O$	acroneuria		8	32.0	18.5	8.0	7.2	66	12	50	12.3	9.5	0.01
	ephemerella	16.0			18.5	8.0	6.9	46	6	50	1.2	10.6	0.03
	hydropsyche		7	32.0	18.5	8.0	7.0	46	6	46	1.3	14.7	0.01
Cr^{+++} from $CrCl_3.6H_2O$	acroneuria		7	32.0	18.5	8.0	6.8	50	8	50	9.2	4.7	0.03
	ephemerella	2.0			18.5	8.0	6.0	46	14	50	4.7	7.7	0.01
	hydropsyche	64.0			18.5	8.0	6.4	46	14	42	2.2	5.2	0.01
Hg^{++} from $HgCl_2$	acroneuria	2.0			18.5	8.0	7.8	52	8	46	6.5	—	—
	ephemerella	2.0			18.5	8.0	7.6	46	6	42	1.6	—	—
	hydropsyche	2.0			18.5	8.0	7.6	46	6	42	1.4	—	—

* DO concentration of 8.0 mg/l maintained.

isms. They were obtained from streams near Duluth, Minnesota, and kept in holding tanks in the laboratory for at least a week before using. The insects were acclimatized carefully in the test aquaria for 24 hr before the metals were added.

Results

A summary of the acute toxicity of some of the heavy metals to the aquatic insects is given in Table III.

Discussion

In the dilutions up to 16 mg/l, the temperature, DO, pH, alkalinity, acidity, and hardness remained fairly constant, and good survival in the controls indicates these factors did not influence mortality. At 64 mg/l some of the metal salts did lower the pH, but this effect was not considered in this study. The metal concentration decreased considerably over the two-week period in several cases due to absorption, precipitation, complexing, etc., and this indicates a weakness of the static bioassay. However, for 48 or 96 hr the concentrations are fairly dependable. Analysis of the killed insects revealed significant amounts of metal had been absorbed by them.

Ephemerella was the most sensitive to all the metals, and copper was the most toxic to it at 0.32 mg/l (48-hr TL_m), followed by iron at 0.32 mg/l (96-hr TL_m); cadmium, chromium, and mercury at 2.0 mg/l, nickel at 4.0 mg/l, and cobalt at 16.0 mg/l, (all 96-hr TL_m). Mercury also was toxic to *Acroneuria* at 33.5 mg/l, and cobalt to *Hydropsyche* at 64.0 mg/l, (also 96-hr TL_m). In all other cases the test organisms lived beyond 96 hr, even at concentrations up to 64.0 mg/l, indicating that the aquatic insects may not be as sensitive to the heavy metals as are fish.

Summary

Two generalities might be drawn from these results: (*a*) the species of mayfly used was the most sensitive of the insects tested to all the metals that were checked; and (*b*) the insects seem to be less sensitive to the heavy metals than many fish that have been tested. As is the case with fish, relative toxicity of the heavy metals to aquatic insects varied widely with the test species. Therefore, statements comparing the toxicity of the metals to aquatic insects should indicate the test species and, conversely, the relative resistance of two species should be compared only for a specific toxicant. These TL_m values are a measure of the toxicity of some metals to certain aquatic insects under the experimental conditions specified. Such concentrations could be expected to cause mortality under most environmental conditions even for short exposure.

References

1. McKee, J. E., and Wolf, H. W., "Water Quality Criteria." A report prepared for the State of California Water Pollution Control Board (1963).
2. Ellis, M. M., "Detection and Measurement of Stream Pollution." *Bull. Bur. Fish.*, U. S. Bureau of Fish., **48**, 365 (1932).
3. Doudoroff, P., and Katz, M., "Critical Review of Literature on The Toxicity of Industrial Wastes and Their Components to Fish. II. The Metals, as Salts." *Sewage and Industrial Wastes*, **25**, 7, 802 (July 1953).
4. Rudolfs, W., Barnes, G. E., Edwards, G. P., Heukelekian, H., Hurwitz, E., Renn, C. E., Steinberg, S., and Vaughan, W. F., "Review of Literature on Toxic Materials Affecting Sewage Treatment Processes, Streams, and B.O.D. Determinations." *Sewage and Industrial Wastes*, **22**, 9, 1157 (Sept. 1950).
5. "Standard Methods for the Examination of Water and Wastewater." 12th Ed., Amer. Pub. Health Assn., New York, N. Y. (1965).
6. Jones, J. R. E., "The Relative Toxicity of Salts of Lead, Zinc, and Copper to the Stickleback (*Gasterosteus aculeatus L.*) and the Effect of Calcium on the Toxicity of Lead and Zinc Salts." *Jour. Exp. Biol.*, (Brit.), **15**, 394 (1938).

7. Schweiger, G., "The Toxic Action of Heavy Metal Salts on Fish and Organisms on Which Fish Feed." *Arch. Fischwiss.* (Germany), **8**, 54 (1957); Water Poll. Abs., **34**, 9, 1744 (1961).
8. Jones, J. R. E., "The Relation Between the Electrolytic Solution Pressures of the Metals and Their Toxicity to the Stickleback (*Gasterosteus aculeatus L.*)." *Jour. Exp. Biol.* (Brit.), **16**, 425 (1939).
9. Anon., "Report of the Water Pollution Research Board, with the Report of the Director of the Water Pollution Research Laboratory for the Year 1959." Dept. of Sci. Ind. Res., H. M. Stationery Office, London (1960).
10. Pickering, Q. H., and Henderson, C., "The Acute Toxicity of Some Heavy Metals to Different Species of Warm Water Fishes." *Proc. 19th Ind. Waste Conf.*, Purdue Univ., Ext. Ser. **117**, 578 (1964).
11. Anderson, B. G., "The Apparent Thresholds of Toxicity of *Daphnia magna* for Chlorides of Various Metals When Added to Lake Erie Water." *Trans. Amer. Fish. Soc.*, **78**, 96 (1948); Water Poll. Abs., **23** (Dec. 1950).
12. Bringmann, G., and Kuhn, R., "The Toxic Effects of Waste Water on Aquatic Bacteria, Algae, and Small Crustaceans." *Gesundh. Ingr.* (Germany), **80**, 115 (1959).