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1

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EFFECT OF PHYSICOCHEMICAL FORM ON COPPER
AVAILABILITY TO AQUATIC ORGANISMS

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EFFECT OF PHYSICOCHEMICAL FORM ON COPPER AVAILABILITY
TO AQUATIC ORGANISMS

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ABSTRACT: Copper concentration and speciation were determined in influent and effluent waters collected from eight power stations that used copper alloys in their cooling systems. Quantities of copper associated with particles, colloids, and organic and inorganic ligands differed with the site, season, and mode of operation of the station. Under normal operating conditions, the differences between influent and effluent waters were generally small, and most of the copper was in bound (complexed) species. However, copper was high in concentration and present in labile species during start-up of water circulation through some cooling systems and during changeover from an open- to closed-cycle operation.

Copper sensitivity of selected ecologically and economically important aquatic organisms was also evaluated. Our primary emphasis was on acute effects and most of the testing was performed under controlled laboratory conditions. However, sublethal effects of copper on a population of bluegills living in a power station cooling lake containing water of low pH were also assessed. The toxic response to copper differed with the species and life stage of the animal and with the chemical form of copper in the water.

KEY WORDS: copper, bioavailability, bioassays, physicochemical form, speciation, toxicity.

Introduction

An important goal of biogeochemistry is to understand the transport, fate, and effects of metals introduced into natural ecosystems. Copper is of special interest--its partitioning among abiotic compartments and availability to biota in aquatic ecosystems is dependent on its physicochemical form.

Not all physicochemical forms of copper are equally available to aquatic organisms [1-5]. Soluble copper is accumulated by most, if not all organisms, but the quantities accumulated are related to the chemical form (species) of copper in the water. Particulate copper in the water column is known to be effectively concentrated by some filter feeders and copper in bottom sediments is accumulated in varying amounts by deposit feeders.

Numerous attempts have been made to quantify the physicochemical forms of copper in water systems [4-8]. Field measurements have been made using a variety of techniques. The validity of some measurements is in question because of the sampling and analytical methods used. Furthermore, comparison of results from different sites is difficult because the techniques used by different investigators are rarely the same.

This study was initiated to determine the physicochemical forms and effects of copper in effluents from power stations adjacent to aquatic ecosystems with water that differed in salinity, pH, and concentrations of organic and inorganic constituents. In addition, responses of selected ecologically and economically important marine and freshwater organisms to increased concentrations of soluble copper were evaluated. The same parameters were measured and the same analytical techniques were used throughout the study.

Experimental Procedures

Water Sampling Location and Techniques

Water samples were collected in the intake and discharge zones of power stations situated in widely different locations (Table 1). At each site, a group of parameters was measured (Table 2). Water samples were collected through an all-plastic system that had been acid washed, rinsed with double-distilled water, and then rerinsed with double-distilled water immediately before use. Samples were filtered in situ through a 0.4- μm (pore size) Nuclepore, 293-mm (diam) membrane filter, and the filtrate was collected into specially cleaned containers. The filtrate was analyzed for total, labile, and bound copper, for dissolved organic carbon, and for apparent complexing capacity (ACC). The total, nonfilterable residue was analyzed for dry and wet weight, total copper content, copper distribution coefficient, and organic carbon. Detailed information on the sites, collection methods, and analytical techniques is available in a series of reports [13-16].

Copper Molecular-Size Fractionation

Two 2-litre aliquots of filtered water that had been stored at 4°C less than 24 h after collection were spiked with ^{64}Cu , allowed to equilibrate a minimum of 12 h, and then subjected to ultrafiltration (Fig. 1). One of the 2-litre aliquots was photooxidized with UV light to reduce the concentration of dissolved organic matter. Two drops of 35 percent H_2O_2 were added to each 100 ml of water to accelerate the oxidation. The reaction rate was also increased by the increase in temperature that occurs during irradiation (60°C). Ultrafiltration was performed with standard model 402 cells, which contained

TABLE 1--Power stations surveyed, sources of cooling water, number of field collections, and the years studied.

Power station	Nearest major city	Cooling water	Collections	
			Total	Years
<u>Marine ecosystems</u>				
Diablo Canyon	San Luis Obispo, Calif.	Pacific Ocean	3	1977-78
San Onofre	San Clemente, Calif.	Pacific Ocean	4	1977-78
<u>Estuarine ecosystems</u>				
Salem	Salem, N.J.	Delaware River	4	1978-80
Surry	Williamsburg, Va.	James River	3	1978-80
<u>Freshwater ecosystems</u>				
Fort St. Vrain	Denver, Colo.	Well water and St. Vrain Creek	3	1978
H.B. Robinson	Florence, S.C.	Pee Dee River	3	1978-80
Kewaunee	Manitowoc, Mich.	Lake Michigan	3	1978
Vermont Yankee	Battleboro, Vt.	Connecticut River	5	1978-79

TABLE 2--Parameters measured in water collected for field studies conducted at
power stations.

Compartment	Parameter	Instrument ^a
Water (<0.4 μm) ^b	Conductivity	Conductivity meter
	Temperature	Thermometer
	pH	pH meter
	Total dissolved copper	Atomic absorption spectrometer [9]
	Labile versus bound copper	Polarograph [10] Ion-exchange resin [11]
	Dissolved organic carbon	Carbon analyzer [12]
	Apparent complexing capacity	Polarograph [13]
Suspended particles (>0.4 μm)	Total, nonfilterable residue	Atomic absorption spectrometer [14]
	copper	
	Total nonfilterable residue	Filtration apparatus
	Copper distribution coefficient	Gamma-well radionuclide counter [11]
	Particulate organic carbon	Carbon analyzer [12]

^aReference for the method used is given in brackets.

^bSamples were filtered in situ through a 0.4- μm Nuclepore filter.

magnetic stirrers (Amicon Corporation, Lexington, Mass.). The nominal molecular weight retentions of the membranes used in this test were XM-100A, 100 000; UM-10, 10 000; and UM-2, 1000.

Each ultrafiltration experiment was initiated by transferring 270 ml of the ^{64}Cu -spiked water to an ultrafiltration cell. The water was stirred for 3 min, three 10-ml aliquots were removed for counting, the remaining 240 ml was subjected to positive N_2 pressure and constant stirring until 120 ml of filtrate was collected, and then the cell was vented. The filtrate was sampled serially to monitor the changes in ^{64}Cu concentration; a 10-ml sample was taken from each of the first, second, and third 40-ml aliquots of the filtrate. The retentate was sampled only after venting. The filters were removed and placed in vials for counting. The cell assembly was rinsed well with 50 ml of 0.1N HCl, and a 10-ml aliquot of this acid wash was counted. Recovery of the ^{64}Cu present in the initial test solution was calculated from the ^{64}Cu concentrations in the different fractions.

Bioassays

Each species was exposed to concentrations of copper that ranged from those producing no apparent effect to those producing an immediate effect. Most bioassays were conducted in flow-through systems. Mortalities were generally recorded daily for adult stages and at 4 to 6-h intervals for early-life stages. From the family of curves derived from cumulative mortality versus duration of exposure, lethal concentrations ($\text{LC}_{50}/\text{time}$) were determined. The concentration of Chelex-100 labile copper in the water was determined in each test system. Additional information about the techniques used is available from a series of publications [17-23].

Metalloprotein Isolation

Livers from bluegills were placed in nitrogen-saturated, 50mM Tris-HCl (pH 7.6) (two volumes per weight of liver) containing 10 percent sucrose, 1 percent 2-mercaptoethanol, and 200 Kallikrein Inhibitory Units of Trasylol (protease inhibitor), and then homogenized on ice with a polytron tissue homogenizer. Duplicate aliquots of each homogenate were reserved for metal analysis.

Homogenates were centrifuged at 115,000 x g for 90 min at 0°C. The final clear supernatants (S-115s) were saturated with N₂(g), frozen immediately with liquid nitrogen, and stored at -70°C until they were chromatographed. Duplicate aliquots of each S-115 and the pellets were reserved for metal analysis.

The S-115s of the livers were processed on a Waters high performance liquid chromatograph fitted with a Varian TSK 3000 SW gel-permeation column (22 x 300 mm). To separate copper metalloproteins, aliquots of the S-115s were injected and eluted with a 0.15M NaCl and 50mM Tris-HCl buffer solution (pH 7.6 at 25°C) at 4 ml/min. Molecular absorbance at 280 nm was monitored continuously and the 35 fractions collected were analyzed for metals. The column was standardized using proteins of known molecular weights.

Results and Discussion

Soluble and Particulate Copper

The ranges in the amount of copper in the soluble and particulate fractions of effluent water collected during normal operation of the power stations differed greatly (Table 3). Because soluble copper leached from the

TABLE 3--Ranges in concentration of soluble (<0.4 μ m), particulate (>0.4 μ m), and maximum total copper in effluent waters, and maximum difference in copper (Δ Cu) between influent and effluent waters at power stations.

Power station	Copper, μ g/litre			
	Soluble	Particulate	Maximum total	Maximum Δ Cu
<u>Marine ecosystems</u>				
Diablo Canyon	0.5 to 1.9	<0.1 to 1.3	3.2	...
Diablo Canyon ^a	26	1.9	28	27
San Onofre	0.2 to 1.8	<0.1 to 1.5	3.3	~2
<u>Estuarine ecosystems</u>				
Salem	2.3 to 5.0	2.9 to 5.6	11	2.1
Salem ^a	0.6	>2500	>2500	>2500
Surry	2.0 to 3.7	4.9 to 21	25	4.2
<u>Freshwater ecosystems</u>				
Fort St. Vrain	3.3 to 5.2	7.8 to 26	31	20
H.B. Robinson	18 to 61	13 to 19	79	22
Kewaunee	3.1 to 4.1	0.7 to 15	18	10
Vermont Yankee	3.2 to 4.0	0.8 to 5.0	8.4	5.1
Vermont Yankee ^b	24	25	49	41

^aConcentration following start-up of water circulating through the condenser system after a shutdown.

^bConcentration following changeover from an open- to a closed-cycle operation.

tubing may not remain in solution but may be sorbed onto particles present in the water, an increase in copper in the particulate fraction may not indicate a release of particulate copper, but only an increase in the amount of soluble copper sorbed to the particles during their transit through the cooling system. In this study, the number of sample collections were limited. Consequently, the data are meant to index the extent of variation that can be expected between different types of ecosystems and, for a given type of ecosystem, that can be expected between different sampling times.

The copper concentrations measured in water collected from the two marine ecosystems were lower than in those from the other sites. Because both stations are located in areas off the California coast receiving little runoff from land, low concentrations are not unexpected. The highest concentrations were measured at the H.B. Robinson Station. This station circulates water from an impoundment fed from a tributary of the Pee Dee River, South Carolina, a typical blackwater stream exhibiting low pH and darkly colored water. Increased quantities of copper appear to have been leached from the copper-zinc tubing of the cooling system as a result of the low pH and high organic content of the water.

Pulses of copper were released at Diablo Canyon Calif. and Salem, N.J. following start-up of water circulating through the condenser after a shutdown (Table 3). At Diablo Canyon, this pulse was primarily in the soluble form and the peak concentration was only 28 $\mu\text{g/litre}$ compared to the 7700 $\mu\text{g/litre}$ reported following a start-up that occurred before the copper-nickel tubing was replaced with titanium [24]. At Salem, the pulse released following start-up was primarily in the particulate form, which appears to have resulted from pretreatment of the condensers with the chemical CI-50. At Vermont Yankee, pulses of soluble copper were also released following changeover in the spring

from an open- to a closed-cycle operation. Here the wood in the mechanical draft-type towers was treated with Cupranol, a commercially available copper-containing preservative that appears to leach copper.

The maximum difference in copper between influent and effluent waters during normal operations was low for San Onofre, Salem, Surry, and Vermont Yankee (Table 3). The large difference at Fort St. Vrain was probably because of recirculation of water through the cooling towers before it was released as blow down. At H.B. Robinson, the difference may be because of the leaching of the tubing by the low pH water. No reason for the difference at Kewaunee was identified. For most stations, we found the differences between influent and effluent waters were not greater than those expected due to normal seasonal changes in the ecosystem.

Particles suspended in the water column differed in copper content, weight, organic carbon content, and affinity for copper as measured by the distribution coefficient (Table 4). Increases in copper concentration in the particulate fraction of the water may result from increases in the number of particles at a given concentration or increases in the amount of copper associated with each particle.

An important factor controlling the copper concentration in particulate material is uptake by planktonic organisms. The process is most evident during the time of plankton blooms [25]. Knauer and Martin studied the uptake of copper and other metals by plankton in Monterey Bay, Calif., and observed seasonal changes in the metal content of the plankton [26]. They attributed these changes to accumulation of these metals during seasons when plankton production was low and availability of metal high.

TABLE 4--Maximum values of parameters measured in particles collected in effluents from power stations.

	Nonfilterable		Particulate	Distribution coefficients
Power station	Copper, mg/g	residue, mg dry wt/litre	organic carbon, mg/litre	
<u>Marine ecosystems</u>				
Diablo Canyon	0.29	11	0.5	48 000
San Onofre	0.07	54	0.6	45 000
<u>Estuarine ecosystems</u>				
Salem	0.16	120	2.8	17 000
Surry	0.47	84	2.1	16 000
<u>Freshwater ecosystems</u>				
Fort St. Vrain	0.70	51	1.9	36 000
H.B. Robinson	2.60	12	1.1	25 000
Kewaunee	3.60	8	1.1	46 000
Vermont Yankee	1.30	12	0.5	61 000

Copper Speciation

Species of copper have been classified as labile or bound [27] and as very labile, moderately labile, slowly labile, and nonlabile [28]. The groups are defined by the techniques used and the conditions under which the measurements are made. Techniques used to differentiate between copper species include solvent extraction, ion exchange, electrochemistry, ultrafiltration, gel filtration chromatography, and ion specific electrodes. For convenience, we will continue to use the term labile and bound even though they are operational and imprecise.

We shall refer to the following as labile species: ions, ion pairs, readily dissociable (labile) inorganic and organic complexes, and easily exchangeable copper adsorbed on either colloidal inorganic or organic matter. Inorganic anions to which the copper may be complexed are hydroxides, carbonates, chlorides, sulfates, phosphates, and nitrates; organic anions are amino acids, amino sugars, alcohol, urea, etc.

We shall refer to forms that are irreversible or slowly reversible as bound species, namely stable copper-organic complexes, copper bound to high molecular-weight organic material, some inorganic complexes, and copper occluded by highly dispersed colloids. Also included in this group of bound species is a large fraction of copper complexed to humic substances (refractory organic material comprising a large fraction of organic material in natural waters).

Large differences between power stations were found in the quantities of labile and bound copper in effluents (Table 5). Quantities differed with season and matrix. Concentrations of labile and bound copper were measured in 1977 during start-up at Diablo Canyon [14], in 1979 during changeover from an

TABLE 5--Ranges in concentration of labile and bound copper ($\mu\text{g/litre}$) in effluents from power stations.^a

Power station	Labile	Bound	Reference
<u>Marine ecosystems</u>			
Diablo Canyon	0.1 to 0.4	0.4 to 1.8	14
Diablo Canyon ^b	0.4 to 21.2	0.2 to 6.5	14
San Onofre	0.2 to 0.7	0.1 to 1.6	14
<u>Estuarine ecosystems</u>			
Salem	0.9 to 4.2	<0.2 to 3.2	15
Salem ^b	0.5 to 3.3	... ^c	15
Surry	0.5 to 2.9	0.2 to 2.6	15
<u>Freshwater ecosystems</u>			
Fort St. Vrain	0.4 to 0.7	2.9 to 4.5	13
H.B. Robinson	13 to 33.9	4.9 to 26.8	16
Kewaunee	1.7 to 2.3	1.4 to 1.7	13
Vermont Yankee	1 to 2.1	1.2 to 2.4	13
Vermont Yankee ^d	133	21	13

^aChelex-100 ion exchange resin was used to differentiate between labile and bound species. A minimum of three collections were made and analyzed in triplicate.

^bValues obtained in water collected after start-up of water circulation through the cooling system.

^cNo data available.

^dValues obtained in water collected after changeover from open- to closed-cycle operation.

open- to a closed-cycle operation at Vermont Yankee [13], and in 1980 during start-up of Unit II at Salem [15]. At Diablo Canyon and Vermont Yankee, the pulse of copper discharged was primarily in soluble and Chelex-100 labile forms. We can expect, then, that the labile copper released would remain as such until it is diluted with sufficient volume of water to provide the ligands required to complex the copper. The results obtained at Diablo Canyon and Vermont Yankee were in contrast to those obtained at Salem, where the pulse of copper was primarily particles, with only small amounts of labile copper released. At Salem, the condenser tubes were pretreated with a commercially available product, CI-50. Additional studies of copper speciation in effluents during start-up are required to confirm the usefulness of pre treatment with chemicals such as CI-50, which is formulated to reduce corrosion.

Discussions of soluble forms of copper in waters are included in some recent reviews of copper [4, 5, 7, 8, 29]. It is well accepted that the chemical form of copper is important in controlling geochemical and biological processes. Consequently, attempts have been made both to calculate as well as measure copper speciation.

Concentrations of copper species have been calculated from equilibrium chemical models. These models predict how specified amounts of metals and ligands are partitioned by competing reactions in the solution and solid phases. Nordstrom et al. compared the available computerized models for equilibrium calculations in aqueous systems [30]. They evaluated consistency among models by comparing the log of the molar concentrations of free ions and complexes for a hypothetical seawater and river water. They proposed that the lack of agreement for minor species reflects primarily differences in the thermodynamic data base of each chemical model, although other factors such as activity coefficient calculations, redox assumptions, temperature corrections,

alkalinity corrections, and the number of complexes used also may affect the results. Many of the models do not address interactions with organic ligands and others suffer from the omission of polynuclear and mixed-ligand complexation [1]. As more experiments are performed and the data generated are incorporated into the model, predictive capabilities will improve.

Molecular Size Fractionation

We can better understand speciation reactions if information is available on the quantities of soluble copper in different molecular weight fractions (MWFs). The distribution of copper among MWFs differed with the ecosystem (Table 6). The average percentage of soluble copper that was in the four different MWFs in untreated water from San Onofre was about the same. Photooxidation of the water resulted in an increased percentage of the ^{64}Cu in the <1000 MWF containing the labile forms, which are considered to be more toxic than bound forms. In untreated water from Diablo Canyon, the largest percentage of copper was in the >10 000, <100 000 MWF; photooxidation of the water resulted in increases of ^{64}Cu in both the <1000 and the >100 000 MWFs. The increased percentage of copper in the >100 000 MWF is probably the result of the formation of colloidal copper or of the adsorption of copper to other metal colloids.

The pattern of copper distribution among MWFs in treated and untreated water was similar for effluents from Surry, Va. and Salem, N.J. The percentage of copper in the <1000 MWF was smaller in these samples than in those from marine ecosystems and larger than in those from freshwater ecosystems. An important factor controlling speciation in marine and estuarine waters may be the formation of chloro complexes; samples with higher salinity had increased percentages of copper in the <1000 MWF.

TABLE 6--Average percentages of soluble ^{64}Cu in different molecular-weight fractions of ultrafiltered effluent waters collected at power stations.

Power station	UV	Molecular-weight fractions, percent			
	treatment, h	<1000	>1000, <10 000	>10 000, <100 000	>100 000
<u>Marine ecosystems</u>					
San Onofre	0	28	24	25	22
	4	48	17	12	23
Diablo Canyon	0	15	21	41	23
	4	37	14	12	37
<u>Estuarine ecosystems</u>					
Surry	0	10	20	55	15
	6	20	0	13	67
Salem	0	10	38	40	12
	6	25	0	0	75
<u>Freshwater ecosystems</u>					
Vermont Yankee	0	2	3	75	20
	4	3	0	2	95
Kewaunee	0	4	10	58	28
	4	14	1	22	63
Fort St. Vrain	0	5	32	55	8
	4	9	7	6	78
H.B. Robinson	0	12	22	55	11
	6	29	1	66	4

In untreated waters from Vermont Yankee, Kewaunee, and Fort St. Vrain, the lowest percentage of copper was found in the <1000 MWF and the highest in the >10 000, <100 000 MWF. The greatest changes that occurred in copper distribution upon photooxidation of the waters were a decrease in percentage of the >10 000, <100 000 MWF and an increase in that of the >100 000 MWF.

The copper distribution among the MWFs of waters from H.B. Robinson differed from those of the other freshwater sites; higher percentages of ^{64}Cu were found in the <1000 and >1000, <10 000 MWFs. Destruction of organics by UV treatment resulted in increases in the percentages in the <1000 and >10 000, <100 000 MWFs. A possible explanation for the higher percentage of ^{64}Cu in the >10 000, <100 000 MWF than in the >100 000 MWF is that smaller colloids are present in waters of lower pH.

Apparent Complexing Capacity

The impact on ecosystems from the addition of copper from point sources is related to the concentration in the water of ligands that bind copper tightly. If the concentration of aqueous ligands is considerably higher than that of copper, then ligands are available to react with the added copper and change its physicochemical form. The formation of tightly associated copper-ligand complexes results in a form of copper that is less toxic to some biota and has a lower affinity for suspended matter [31].

The measurement of ACC of waters has been reviewed recently [32]. The methods used to determine ACC include solubilization, biological response, voltammetry, ion exchange, and selective ion electrodes. The values obtained for complexing capacity range from 32 000 $\mu\text{g Cu/litre}$ for an oil field brine

TABLE 7--The apparent complexing capacity of effluent waters from
power stations.

Power station	Apparent complexing capacity, $\mu\text{g Cu/litre}$			
	Fall	Winter	Spring	Summer
<u>Marine ecosystems [11]^a</u>				
Diablo Canyon	1	... ^b	1	1
San Onofre	1	1	1	1
<u>Estuarine ecosystems [15]</u>				
Salem	5	60	20	...
Surry	8	30	9	...
<u>Freshwater ecosystems [14]</u>				
Fort St. Vrain	...	12	100	36
Kewaunee	...	3	29	36
Vermont Yankee	...	8	1	1

^aReferences are given in brackets.

^bNo collection.

[2] to 1 $\mu\text{g Cu/litre}$ for pristine waters off the coast of California [14]. Our data indicate that in general, the ACC of marine waters are lower than those for estuarine and freshwater ecosystems [13-16].

Data on ACC are available for a limited number of effluents from seven power stations (Table 7). The ACC for marine ecosystems at both Diablo and San Onofre was low, approximately 1 $\mu\text{g Cu/litre}$. Consequently, any labile copper added to effluents of these stations should remain primarily as such and exist in chemical forms considered toxic to biota. These were probably the conditions when an abalone kill occurred at Diablo Canyon upon start-up of water circulating through the cooling system [33].

The ACC of estuarine and freshwater ecosystems was generally higher and more variable. It is expected that higher concentrations of humic and fulvic acids would be present in estuarine and freshwater ecosystems than in marine ecosystems because of runoff from land and the high productivity of most estuarine and freshwater ecosystems.

Copper Toxicity

The response to copper of aquatic organisms that were tested using the same techniques differed greatly with trophic level and life-history stage (Table 8). Of the organisms tested, oyster embryos were the most sensitive and embryonic crayfish the least sensitive to copper. Because of the high sensitivity of oysters to copper, they were used to evaluate the effect of organic chelators on the response to copper [18]. The ability of a chelator to reduce copper toxicity was related to its stability constant (Table 9). These investigators also reported that destruction of the naturally occurring copper-binding organic matter in the seawater by UV photooxidation reduced embryo survival.

TABLE 8--Toxicity of copper to some representative aquatic organisms.

Copper, µg/litre	Endpoint	Organism	Life stage	Reference
<u>Marine organisms</u>				
~10	48-h LC50	Oyster ^a	Embryo	18
~30	96-h LC50	Abalone	Adult	This paper
>30	Growth inhibition	Giant kelp	Gametophyte	34
200	96-h LC50	Anchovy	Embryo	19
200	96-h LC50	Mussel	Adult	This paper
240	96-h LC50	Herring	Embryo	20
400	24-h LC50	Anchovy	Larvae	19
440	24-h LC50	Herring	Adult	This paper
460	48-h LC50	Anchovy	Adult	This paper
650	48-h LC50	Oyster ^a	Adult	17
2000	24-h LC50	Herring	Larvae	20
<u>Freshwater organisms</u>				
28	24-h LC50	Clam	Larvae (veliger)	21
180	24-h LC50	Carp	Larvae	23
240	24-h LC50	Carp	Embryo	23
540	24-h LC50	Carp	Adult	23
600	24-h LC50	Clam	Larvae (juvenile)	21
1200	48-h LC50	Crayfish	Larvae	22
>2600	96-h LC50	Clam	Adult	21
3700	480-h LC50	Crayfish	Embryo	22
9900	48-h LC50	Crayfish	Adult	22

^aCorrected for shell closure.

TABLE 9--The effect of organic chelators on copper toxicity to Crassostrea
gigas early-life stages.^a

Copper, µg/litre ^b	Larval abnormalities at 48 h, percent					
	Control	Oxalate (8.29) ^c	Glycine (8.62, 15.59)	Citrate (14.21)	Humic matter	EDTA (18.3)
0	6.8	4.3	3.1	4.3	5.4	2.4
10	42.7	13.4	4.9	4.9	2.3	3.6
20	97.6	97.2	95.6	89.9	10.8	1.4
40	99.5	... ^d	...	...	91.2	2.3
60	100	...	...	...	97.21	2.4
100	100	...	...	...	100	84.7

^aChelators at 10^{-6} M except humic matter, which was added at 2 mg/litre [18].

^bConcentrations of total copper added to the bioassay water.

^cThe stability constants of organic chelator and copper complexes given in Martell and Calvin are shown in parentheses [35].

^dExperiments not conducted at these concentrations.

It is well documented that bioavailability, and in turn, the toxicity of copper in aquatic ecosystems, is related to the concentrations of inorganic and organic ligands in the soluble phase and the affinity for copper of metal-binding sites in the particulate phase [36]. Most data available is from controlled laboratory experiments in which the test organisms were exposed to acute levels of copper and mortality monitored. However, many exposures to copper in the field are chronic and low in concentration.

Chronic exposure to low concentrations of copper may cause sublethal effects that affect an organism's potential for growth, its reproductive capacity, its ability to resist further change, or its effectiveness in competing with other species in the ecosystem [37]. Evidence for these kinds of effects due to increased availability of copper in a field situation was obtained from studies of fish in the H.B. Robinson Impoundment. Monitoring of the abundance of adult and larval fish populations showed smaller fish populations and higher incidence of structural deformities in bluegills collected from 1976 to 1978 than those found in previous years. Considerable effort was expended investigating possible causes [38] and the results eliminated included temperature, selenium, pesticides, common water-quality properties, and vitamin C storage as causes. Because total copper and labile copper concentrations were much higher in the effluent from this power station than in those from other stations (Tables 4 and 5), studies were initiated to determine if copper was responsible for the adverse effects on fish populations.

Bluegills collected from the intake and discharge zones of the impoundment and from a control pond were examined for tissue copper concentrations and for the metals associated with metalloproteins in the livers. Separation of liver metalloproteins was performed because research on mammalian livers indicates that more information about potential adverse effects of metals can be

obtained from analysis of the kinds and quantities of metals associated with the different metalloprotein pools than of the total quantities of metals in the tissues [39]. Large differences were found in the quantities of copper associated with the three categories of metalloproteins resolved in the soluble fraction of liver homogenates (Table 10).

The low molecular weight (LMW) fraction (6000 to 40 000 daltons) contains the metallothionein-like proteins considered to be important in the homeostasis and detoxification of copper; the intermediate molecular weight (IMW) fraction (40 000 to 126 000 daltons) and the high molecular weight (HMW) fraction (126 000 to >670 000 daltons) contain metalloenzymes required for metabolic processes.

In all samples of fish, the LMW fraction contained by far the largest amount of copper. Comparison of the quantities of copper in the LMW, IMW, and HMW fractions shows that copper concentrations were much higher in fish from the intake and discharge sites than from the control site. The mean of the copper concentrations in the LMW fraction from the ten fish from the discharge site was twenty times greater than that in those from the control site; the mean from the intake site was six times greater. The quantities of copper bound to proteins in the IMW and HMW fractions were small except in the homogenates from four of the five pairs of fish sampled from the discharge site. Also in these same four pairs of fish, copper appeared to be displacing zinc from metalloenzymes in the HMW and LMW fractions. High copper concentrations in the HMW fraction and displacement of zinc is evidence that the metallothionein-like protein detoxification system was becoming overloaded [40,41].

TABLE 10--Copper in liver tissue and liver tissue compartments from bluegills collected at the H.B. Robinson Steam Electric Plant, November 1981.

Copper, 1×10^{-4} μ moles/g wet tissue					
	HMW ^a	IMW ^b	LMW ^c	Total eluted	Liver tissue
<u>Control pond</u>					
Mean	20	17	130	195	260
SEM ^d	1.5	5	30	40	50
<u>Intake site</u>					
Mean	120	120	790	1100	1400
SEM	15	30	120	170	230
<u>Discharge site</u>					
Mean	630	590	2400	3900	4900
SEM	230	210	520	950	1230

^aHigh molecular-weight fraction was eluted between 40 and 60 ml and includes proteins in the 126 000 to >670 000-dalton range.

^bIntermediate molecular-weight fraction was eluted between 61 and 72 ml and includes proteins in the 40 000 to 126 000-dalton range.

^cLow molecular-weight fraction was eluted between 73 and 92 ml and includes proteins in the 6000 to 40 000-dalton range.

^dStandard error of the mean.

These data and those from related studies [42] indicate that the labile copper released from the cooling system of the H.B. Robinson Steam Electric Plant is responsible for the increased deformities and reduced reproductive capacity found in the bluegill population in the adjacent impoundment.

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Figure Caption

FIG. 1. Ultrafiltration-procedure flow diagram. The filtrate (F) and retentate (R) were analyzed for ^{64}Cu .

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