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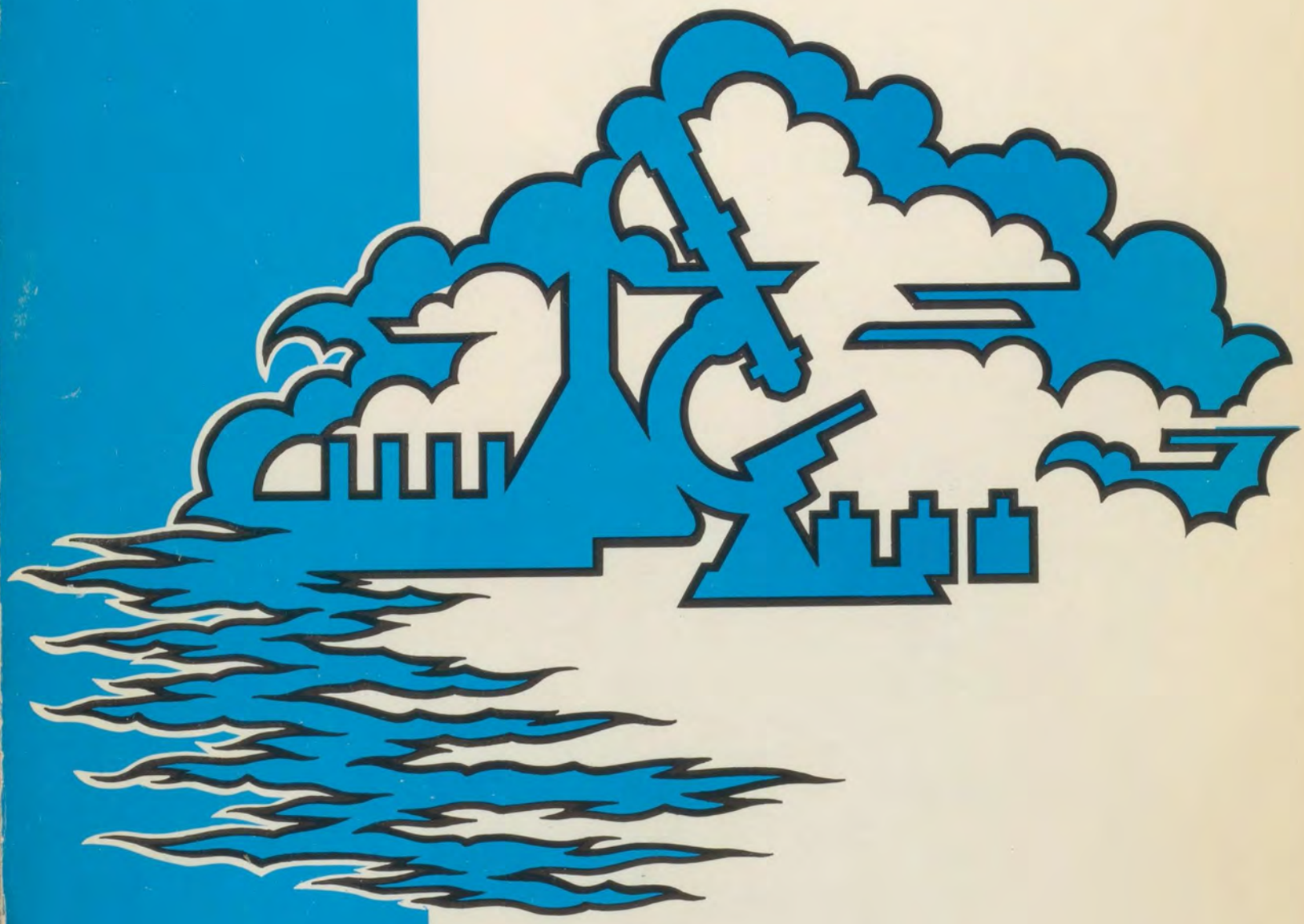
GREAT LAKES
RESEARCH ADVISORY BOARD



INTERNATIONAL JOINT COMMISSION

WORKSHOP ON TOXICITY TO BIOTA OF METAL FORMS IN NATURAL WATER

TOXICITY TO BIOTA OF METAL FORMS IN NATURAL WATER

Proceedings of a workshop held in
Duluth, Minnesota.
October 7 - 8, 1975.
sponsored by
STANDING COMMITTEE ON THE
SCIENTIFIC BASIS FOR WATER
QUALITY CRITERIA OF THE
INTERNATIONAL JOINT COMMISSION'S
RESEARCH ADVISORY BOARD.

edited by

ROBERT W. ANDREW
National Water Quality Laboratory, United States Environmental Protection Agency
Duluth, Minnesota.

PETER V. HODSON
Canada Center For Inland Waters
Burlington, Ontario.

DENNIS E. KONASEWICH
I J C Regional Office, Windsor, Ontario.

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NOTICE

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SUMMARY

This publication represents the proceedings of a workshop which discussed the toxicity to biota of metal forms in natural water. The workshop was sponsored by the Standing Committee on the Scientific Basis for Water Quality Criteria of the International Joint Commission's Research Advisory Board and was held at the Radisson Hotel, Duluth, Minnesota, on October 7-8, 1975.

The workshop consisted of formal papers on the latest research findings in the area of heavy metal forms (speciation), toxicity and cause-effect relationships. These presentations were followed by two discussion periods: one to evaluate the current "state of the art" of metal speciation determinations; and the other, to identify the immediate research needs which will enable the establishment of heavy metal objectives for the waters of the Great Lakes on the basis of metal forms rather than total metal concentrations.

INTRODUCTION

In 1970, the International Joint Commission reported that serious pollution on both sides of the boundary causing injury to health and property on the other side was due to wastes from municipal, agricultural and industrial activities discharged to these waters and their tributaries. The Governments of Canada and the United States then began a series of bilateral discussions which concluded on April 15, 1972, with the signing of the Great Lakes Water Quality Agreement by President Nixon and Prime Minister Trudeau. The Agreement sets out certain water quality objectives for the Great Lakes and outlines a wide range of remedial programs to be undertaken by the Governments to achieve them. The International Joint Commission was directed to assist in the implementation of this Agreement.

Under the terms of the Agreement, two international Boards were created to assist the Commission in the exercise of powers and responsibilities assigned to it: The Water Quality Board and the Research Advisory Board. Within the structure of the Water Quality Board is the Water Quality Objectives Subcommittee, which is charged to assess the adequacy, and propose refinement where necessary of the general and specific objectives in the Agreement, as well as to recommend specific objectives for water quality parameters named in the Agreement without such objectives. For the past two years, the Research Advisory Board's Standing Committee on the Scientific Basis for Water Quality Criteria has functioned in an advisory role to the Subcommittee dealing especially with parameters for which the data base is not well established. The Committee has established liaison with the scientific community throughout North America to obtain the most recent information to aid in the establishment of objectives.

In July 1975, the Water Quality Objectives Subcommittee submitted recommendations for water quality objectives in the Great Lakes. Notably absent were objectives for metals because appropriate scientifically defensible objectives could not be prepared at that time. Although research on heavy metal toxicity has continued for approximately 30-40 years and some general relationships to water hardness, pH, salinity and temperature, for example are known, the two groups felt that the underlying chemical and physiological basis for heavy metals toxicity in natural waters are not well understood.

Most past research and all current objectives are based on total metal concentrations. However, the toxicity of metals in the natural environment may be affected by oxidation state, solubility, complexation, ionic strength and the presence of organic matter. With recent advances in solid-state electronics and ion-sensing electrodes, there has been a resurgence of interest in developing analytical methods to enable differentiation of the various chemical states with the intention of relating the concentrations of the different forms to their toxicity to biota.

To determine whether the heavy metal objectives for the waters of the Great Lakes could be based on species of metals, the Research Advisory Board's Standing Committee on the Scientific Basis for Water Quality Criteria sponsored a workshop, in which the latest research findings in the area of heavy metal forms (speciation), toxicity and cause-effect relationships were presented. If unsolved problems did remain, the workshop was structured to define research needs to enable future concerted efforts of both aquatic biologists and analytical chemists. High priority research needs are to be transmitted and recommended through the IJC to the participating Governments for action.

This publication consists of the formal papers which were presented at the workshop as well as edited transcriptions of two discussion periods. The assistance of the authors, who submitted manuscripts of their presentations for use in these proceedings, is gratefully recognized. Editing of these manuscripts was minimal to ensure compatibility with the oral presentations.

The editors have made free use of editorial privilege to clarify the general discussions, especially those which show actual development of new ideas and illustrate the concerns of various audiences. The participants each cooperated actively in the preparation of amended remarks.

Following the workshop, most participants also aided in the clarification of research needs identified during the workshop and as well provided additional research needs. These efforts are summarized in the "Research Needs" section of this document.

Robert W. Andrew
Peter V. Hodson
Dennis E. Konasewich

RESEARCH NEEDS

This chapter presents a summary of research needs which were derived from: the formal papers within these proceedings; the discussion sessions at the workshop; and, suggestions from participants following the workshop. The summary was reviewed by many of the workshop participants and subsequently utilized by the Research Advisory Board to prepare its 1976 Great Lakes Water Quality Research Needs Report.

OVERALL

(a) There is a need for understanding of the changes that can take place in the chemical forms of heavy metals when these are introduced into the Great Lakes and of the ultimate fate of these forms.

(b) The biological impact of the various chemical forms of each heavy metal must be understood in order to adequately assess permissible concentrations in Great Lakes waters.

A more detailed itemization may be made as follows:

CHEMICAL

(a) The equilibria established between the various chemical forms of each metal in natural lake waters must be measured and the processes and rates involved understood. This requires, for the forms of each metal:

(i) measurement of equilibrium constants and steady state concentrations;
(ii) measurement of effect of change in pH, alkalinity, dissolved oxygen, dissolved solids, etc., on equilibrium constants;
(iii) measurement of solubility constants at temperatures characteristic of the Great Lakes rather than at 25 C;
(iv) measurement of the various forms of each metal in the relatively steady-state situation of open lake waters and in dynamic situations such as a mixing zone where an effluent is discharged.

(b) Methods:

(i) present methods can measure ion activity and total, particulate, and 'soluble', or non-complexed, heavy metal. Research is required on the relationship of specific forms of each metal to the above terms;
(ii) better methodology and instrumentation are required for the measurement of the concentrations of different metals forms (species);
(iii) identification of organometallic compounds is often difficult and research is required into suitable methods of analysis.

BIOLOGICAL

(a) Only those forms of heavy metals taken up by organisms are able to elicit a toxic reaction. Therefore, research is required on:

(i) biological availability of the various forms of each heavy metal;
(ii) rates of uptake and elimination of the various forms by organisms and identification of detoxification mechanisms specific for each form;
(iii) modelling of typical exposure-time-and-exposure-concentration relationships for various types of organisms in order to determine whether uptake is adequate in Great Lakes waters to elicit a lethal or sublethal response;
(iv) toxicity to biota of metals contained within their food organisms;
(v) relationship of metal forms to sublethal toxic effects.

(b) In the case of metal forms that precipitate or are not taken up by free-swimming organisms, research is required on:

(i) rates of precipitation of such forms;
(ii) accumulation of, and response to, metals precipitated either in an insoluble chemical form or adsorbed on particulate matter by benthic organisms, especially filter and detritus feeders;
(iii) physical, chemical or biological conditions under which precipitated heavy metals may be immobilized or remobilized from sediments or particulate matter and the rates at which these occur.

(c) Acute synergistic, antagonistic and additive effects of mixtures of heavy metals are known, but generally not identified nor understood. Research is required on the chronic effects of mixtures and on situations where several toxic chemical forms of a single heavy metal occur simultaneously.

(d) The sensitivity of organisms to heavy metals differs with the developmental stage. Tolerance developed in one life stage can influence response in the next life stage. Consequently the concentration-response relationship will not be constant and these have implications

for experimental design. In addition, developed tolerance could influence the degree of bioaccumulation of a metal and hence the amount passed on to the next trophic level. Research is required:

(i) to determine the life stages of each organism most sensitive to a metal and the conditions under which tolerance to a metal can be induced;

(ii) to determine whether developed tolerance represents a short-term acclimation to the metals or a long-term genetic adaptation;

(iii) to measure accumulation rates and equilibrium levels for heavy metals in relevant aquatic organisms; to determine transfer rates of metals from one trophic level to the next; and to identify those metals that will 'biomagnify' to the detriment of higher trophic levels;

(e) species of animals that are rare in the Great Lakes are likely to be those living at the extremes of their tolerance ranges for the environmental factors existing there. Consequently, these organisms may not be particularly resistant to added stresses caused by changes in water quality and serve as sensitive indicators of early change. Measures for the protection of these organisms are likely to be favourable for the protection of more common species. Therefore evaluation of the response of these organisms to heavy metals under Great Lakes conditions should be undertaken.

MONITORING

(a) Information is required on the sources and quantities of the various heavy metals (and if possible, their specific forms) entering the Great Lakes.

(b) Computer programs that adequately model metal speciation and variations in speciation with chemical characteristics of water are required for estimation of the proportion of the total concentration of a given metal present in a toxic form in any given situation.

(c) Methods developed for metal speciation in research programmes must be developed to the point that they can be used for routine monitoring of Great Lakes waters.

(d) Samples collected in the field returned to the laboratory for analysis at a later date raise problems. If satisfactory measurement of the concentrations of the various forms of heavy metals is to be achieved, the methods of sample collection, on-site preparation and preservation should be defined. Any changes in a sample during transportation and storage must be identified and assessed.

(e) Since objectives are based on experimental data with known error limits, and since monitoring involves known errors, error limits should be placed on the objective and on reported monitoring data.

(f) As methods become available for measurement of appropriate forms, spatial and temporal variations in the lakes should be measured.

STRUCTURE-ACTIVITY RELATIONS AND THE TOXICITY OF TRACE ELEMENTS TO AQUATIC BIOTA

CHAPTER 1

V. Zitko
Environment Canada
Biological Station
St. Andrews, New Brunswick E0G 2X0

ABSTRACT

The intrinsic toxicity of trace elements to aquatic biota and environmental factors (water hardness, binding by organic compounds, and particulate matter) are discussed. The intrinsic toxicity of cations may be correlated with glycine binding constants. Bicarbonate binding and competition for active sites are suggested as mechanisms of the decrease of toxicity of cations with increasing hardness. Analogous mechanisms may control the effects of organic compounds on the toxicity of cations.

STRUCTURE - ACTIVITY RELATIONS

General. Relations between structure of chemicals and their biological activity are used successfully in the development of pharmaceutically active organic compounds and pesticides. The relations help to predict quantitatively the activity of compounds, rationalize data, increase the understanding of the mechanism of action, etc. For similar reasons, structure-activity relations will find increasing applications in the assessment of environmental properties of organic chemicals.

The situation is somewhat different in the case of inorganic compounds. In comparison to organics, the number of inorganics is much smaller and the relation between chemical and physical properties of elements and their structure has been recognized a long time ago and expressed in the form of the Periodic Table. The general features of the relation between the toxicity of the elements and their position in the Periodic Table are known (see for example Suvorov 1968). Transition elements are generally more toxic than typical elements, particularly in mono- and divalent states. Some typical elements, resembling the biogenic ones are also rather toxic, and in the center of the Periodic Table, some typical elements are more

toxic than transition elements. Dawson (1974) estimated threshold concentrations acceptable for aquatic life (0.1 of 96hLC50) of a large number of elements and the data provide a priority ranking for further studies.

Two main factors influence the toxicity of inorganic compounds to aquatic life:

1. Intrinsic toxicity, determined by the element,
2. Availability to aquatic life, determined by occurrence, complexation and other chemical reactions, adsorption, etc., and summarily referred to as environmental factors.

It is not likely that the number of elements will increase much above the present 104 and, in contrast to organic compounds, there is not as much need for predictions, but there are some gaps in our knowledge of the toxic and environmental properties of the less common elements.

On the other hand, many factors influence the toxicity of inorganic compounds to aquatic life and the ability to predict the toxicity under various environmental conditions is very important. Research in this area deserves a high priority.

Intrinsic toxicity. In aqueous solution the majority of inorganic compounds exists in the form of ions whose biological activity is a function of their binding to ionizable groups in organic compounds. Strongly bound

ions block the ionizable groups and change or inhibit the normal biological functions of organic compounds.

Shaw (1954) suggested that the strength of this binding is related to the toxicity of cations and correlated the toxicity of cations, forming insoluble sulfides, with the respective solubility products. This correlation was elaborated further in a subsequent paper by Shaw and Grushkin (1967). Biesinger and Christensen (1972) confirmed the correlation between toxicity and sulfide solubility and found good correlations between toxicity, electronegativity, and binding constants to adenosine triphosphate.

Binding constants to many other organic compounds may lead to good correlations. The limiting factor is usually the availability of binding constants in the literature. An excellent collection of binding constants was published (Sillen and Martell 1964, 1971).

Glycine is a good compound for correlations, and binding constants of many cations are available. The correlation of Biesinger and Christensen's toxicity data with glycine binding constants is shown in Figure 1.

Regression equations (1) to (3) have been derived from the data of Baudouin and Scoppa (1974a) on the toxicity of cations to *Daphnia hyalina*, *Eudiaptomus padanus padanus*, and *Cyclops abyssorum prealpinum*, respectively.

$$\log c = -0.627 \log K_1 + 1.26 \qquad r = -0.869 \qquad (1)$$

$$\log c = -0.419 \log K_1 + 1.13 \qquad r = -0.782 \qquad (2)$$

$$\log c = -0.381 \log K_1 + 1.44 \qquad r = -0.804 \qquad (3)$$

c = 48hLC50, mmole/ℓ

K_1 = glycine binding constant

Number of cations = 10.

The correlation of the toxicity of cations to fish, based on a literature survey of Warnick and Bell (1969), with glycine binding constants

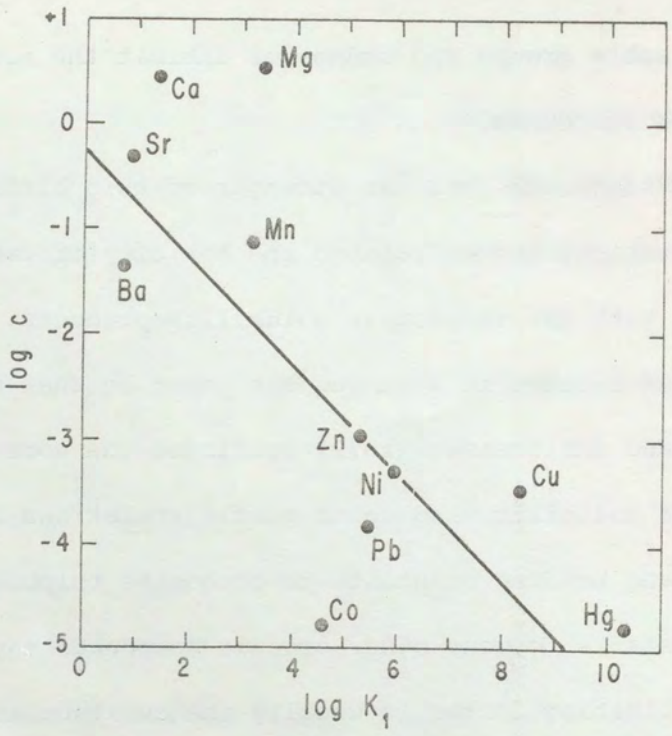

Figure 1. Toxicity to *Daphnia magna* and glycine binding constants of cations (toxicity data from Biesinger and Christensen 1972).

c = concentration causing 16% impairment of reproduction, mmole/ℓ,

K_1 = glycine binding constant

$\log c = -0.514 \log K_1 - 0.237$, $r = -0.689$

is presented in Fig. 2. The correlation, based on geometric means of the reported ranges, is quite good, but the ranges of toxicity of individual cations are very wide and this emphasizes the importance of environmental factors and probably of experimental techniques as well.

The relation between the binding of cations to organic molecules, biological and biochemical properties of the complexes is not as simple as the preceding discussion may seem to indicate. Many additional factors such

as the formation of ternary complexes (two organic compounds) play a role in these interactions (see, for example, a review by Sigel and McCormick 1970).

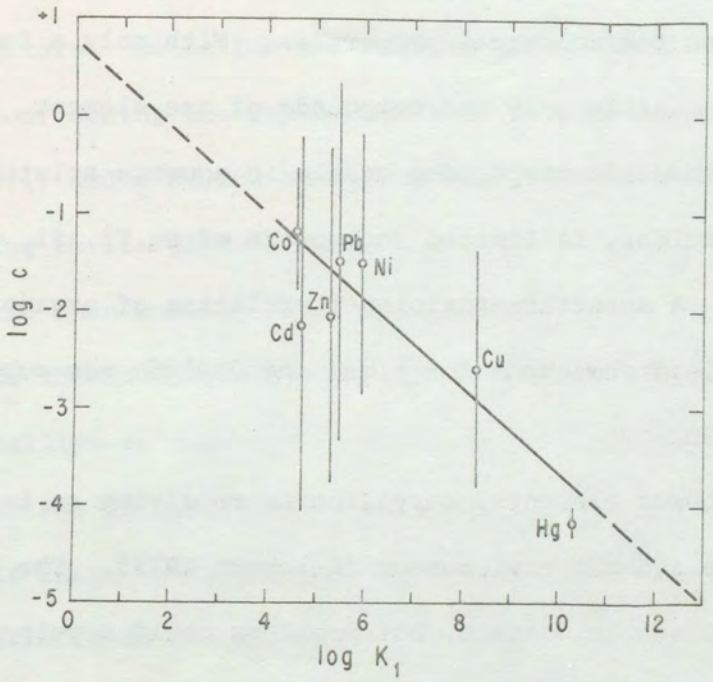

Figure 2. Toxicity to fish and glycine binding constants of cations (toxicity data from Warnick and Bell 1969).

c = toxic concentration, mmole/ℓ

K_1 = glycine binding constant,

$\log c = -0.419 \log K_1 + 0.525, \quad r = -0.892$

Correlations between the toxicity of anions to aquatic life and chemical properties have not been reported in the literature. Within the groups of the Periodic Table, the toxicity of oxygenated anions decreases with increasing electropositivity of the element (As>Sb>Bi, V>Nb>Ta, Cr>Mo>W). The situation is in some cases complicated by the existence of

several valency states. Arsenic, antimony and bismuth are probably the most environmentally important anion-forming elements and because of the large variety of forms in which they may occur in the environment, these elements have to be studied on an individual basis.

Elementorganic compounds (compounds containing covalent carbon to element bonds) do not resemble inorganic compounds of the element in physical, chemical and toxicological properties. With only a few exceptions correlations may be possible only for compounds of one element. Fortunately, the number of elementorganic compounds, stable in aqueous solutions under life-supporting conditions, is limited (compounds of Hg, Tℓ, Si, Ge, Sn, Pb, As, Sb, Se, and Te). A structure-toxicity correlation of organo-silicone, germanium, tin, and lead compounds for algae and *Daphnia* was published by Stroganov et al. (1970).

Of the less common elements, beryllium is receiving an increasing attention also in the aquatic environment (Reichert 1973). The current levels are probably not a cause for concern, but problems could develop in localized areas.

Little is known about the effects of scandium, yttrium, and lanthanides on aquatic biota. Dawson (1974) estimated the acceptable threshold of lanthanides in water as 15 µg/ℓ . The actual levels are probably much lower and problems are not likely to occur.

The group of titanium, zirconium, and hafnium is probably quite innocuous environmentally. Two reviews on zirconium have been published recently (Blumenthal 1973; Hock 1974).

Vanadium has caused some concern and two recent reports are available (Michels 1973; Committee on Biological Effects 1974). No data are available on niobium. Accumulation of tantalum was detected in some marine molluscs and crustaceans (Burton and Massie 1971).

Some data on molybdenum in seawater are available (Head and Burton 1970). The acute toxicity of molybdenum and tungsten to aquatic life is low (Tarzwell and Henderson 1960) and problems with these elements are not likely. The uranyl cation (UO_2^{2+}) may be appreciably toxic (binding to glycine log K_1 = 7.53).

Platinum metals deserve some screening in view of their use in catalytic converters. Ruthenium was studied quite thoroughly in connection with its behaviour during the reprocessing of nuclear fuels (see, for example, Beque et al. 1971) and a review on osmium was recently published (Smith et al. 1974). According to its glycine binding constant (log K_1 = 9.1), palladium may be more toxic than mercury to aquatic fauna.

Some data should be obtained on the environmental properties of gallium and indium. Thallium is appreciably toxic to fish (Zitko et al. 1975) and could cause problems in localized areas (Zitko 1975).

Little is known about the toxicity of germanium to aquatic life and no data are available on tellurium.

Toxicity and environmental properties of antimony deserve a closer look since antimony compounds are widely used as fire retardants in plastics.

The preceding discussion dealt mostly with acute toxicity. There are indications that other effects of cations may also be correlated with their binding constants. Not many comparative data on cation accumulation in aquatic fauna are available, but the logarithms of accumulation factors (F) of cadmium, copper, lead, and mercury (Majori and Petronio 1973) in mussels, *Mytilus galloprovincialis*, increase linearly with log K_1, and it is likely that correlations of this type may also be used to predict the accumulation of cations in aquatic fauna and flora.

$$\log F = 0.184 \log K_1 + 0.256 \qquad r = 0.949$$

ENVIRONMENTAL FACTORS AFFECTING TOXICITY

Hardness. It is well known that increasing hardness decreases the toxicity of cations to fish and probably to other members of aquatic fauna as well.

Available data indicate that the toxicity (incipient lethal level or 48hLC50) is a linear function of hardness and the slopes and intercepts calculated from published data are presented in Table 1.

Table 1

Toxicity of cations as a linear function of hardness

Cation	Slope x 10^8, mole/ℓ hardness	Intercept x 10^8, mole/ℓ	Hardness, mg/ℓ, $CaCO_3$	Reference
Cu^{2+}	3.18	12.4	≤53	Lloyd and Herbert
	2.45	50.7	>53	(1962)
Pb^{2+}	8.64	282	≤27	Lloyd and Herbert
	4.92	381	>27	(1962)
Cd^{2+}	10.2	-133	≤100	Brown (1968)
	17.8	-890	>100	
Zn^{2+}	29.8	445	≤46	Lloyd and Herbert
	15.6	1090	>46	(1962)
Be^{2+}	590	-14400	≤400	Slonim and Slonim (1973)
Ni^{2+}	1000	15700	≤50	Brown (1968)
	494	46000	>50	

In the majority of natural waters the most abundant anion is bicarbonate (Childs 1971; Morel et al. 1973; Baudouin and Scoppa 1974b) and the decrease of toxicity with increasing hardness may be caused by the reaction of these cations with bicarbonate. Experimental evidence confirming this assumption was published in the case of copper (Pagenkopf et al. 1974).

It can be shown that binding of a cation (M) by a ligand (NL) and the formation of a nontoxic complex (ML) would lead to a linear decrease of the cation's toxicity in terms of total concentration (M_T) with increasing hardness, if the concentration of the ligand is proportional to hardness. From the equilibrium

$$M + NL \rightleftarrows ML + N \qquad K = \frac{[ML]\ [N]}{[M]\ [NL]}$$

and mass balance $M_T = [M] + [ML]$ it follows that

$$M_T = [M] + K \frac{[M]\ [NL]}{[N]} \qquad (4)$$

Assuming that a certain constant concentration of the cation, [M] = A, is required for the measured toxic effect (LC50, incipient lethal level, etc.) and that the ligand concentration is a linear function of hardness, [NL] = B h (B = constant, h = total hardness as $CaCO_3$, mg/ℓ), equation (4) becomes

$$M_T = A + \frac{KBA}{[N]} h \qquad (5)$$

For a purely bicarbonate hardness and a calcium to magnesium ratio of 4:1 on a weight basis, the value of B is 2×10^{-5} mole/ℓ x mg $CaCO_3$/ℓ and the value of K can be calculated from equation (5) and data in Table 1, assuming that the cation-binding reaction is

$$M^{2+} + HCO_3^- \rightleftarrows MCO_3 + H^+$$

The values of K thus obtained can be compared with those calculated from published equilibrium constants (see for example Childs 1971) of reactions:

$$CO_3^{2-} + H^+ \rightleftarrows HCO_3^-$$

and

$$CO_3^{2-} + M^{2+} \rightleftarrows MCO_3$$

The results are presented in Table 2.

Table 2

Calculated and toxicity-derived equilibrium constants of the reaction.

$$M^{2+} + HCO_3^- \rightleftarrows MCO_3 + H^+$$

Cation	Equilibrium constant	
	Calculated	Toxicity-derived
Cu^{2+}	3.16×10^{-4}*	1.28×10^{-3}
Pb^{2+}	7.94×10^{-6}	1.53×10^{-4}
Zn^{2+}	5.01×10^{-6}	3.00×10^{-4}
Ni^{2+}	7.94×10^{-6}	3.20×10^{-4}

*Measured value 2.94×10^{-4} (Stiff 1971a)

It can be seen from Table 2 that with the exception of the cupric ion, the calculated constants are two orders of magnitude smaller than those derived from toxicity data and, consequently, the dependence of toxicity on hardness cannot be explained by the bicarbonate-binding mechanism alone. The equilibria are certainly more complicated in harder waters with the formation of basic carbonates also playing a role and, as indicated in Table 1, the linear dependence of toxicity on hardness for most cations has a different form above a certain hardness. Shaw and Brown (1974) suggested that both the free ion (Cu^{2+}) and cupric carbonate are toxic in hard waters.

Another possible mechanism of the dependence of toxicity on hardness is a competition for active sites in the tissues of aquatic biota between the toxic cations and those of calcium and magnesium. This competition may be described by equilibria

$$M + S \rightleftarrows MS$$

$$N + S \rightleftarrows NS$$

where M and N are cations, competing for the active site S.

The concentrations [M], [N], [MS], [NS], and [S] may be calculated from the equilibrium constants K_1 and K_2 and mass balances given below

$$K_1 = \frac{[MS]}{[M]\ [S]} \qquad K_2 = \frac{[NS]}{[N]\ [S]}$$

$$M_T = [M] + [MS]$$

$$N_T = [N] + [NS]$$

$$S_T = [S] + [MS] + [NS]$$

After obtaining [S] from equation (6), [M] and [N] are calculated from equations (7) and (8), respectively, [MS] and [NS] from the mass balances. To illustrate the behaviour of this system, four examples are given in Table 3.

$$[S]^3 + (M_T + N_T - S_T + K_1^{-1} + K_2^{-1})\ [S]^2 + (M_T K_2^{-1} + N_T K_1^{-1} - S_T K_1^{-1} - S_T K_2^{-1} + K_1^{-1} K_2^{-1})\ [S] - S_T (K_1 K_2)^{-1} = 0 \qquad (6)$$

$$[M] = \frac{M_T}{1 + K_1 [S]} \qquad (7)$$

$$[N] = \frac{N_T}{1 + K_2 [S]} \qquad (8)$$

Table 3

Competition for active sites

N_T	Other parameters	[S]	[M]	[N]	[MS]	[NS]
2×10^{-4}	I	3.0×10^{-4}	5.0×10^{-12}	1.98×10^{-4}	3.0×10^{-7}	1.5×10^{-6}
2×10^{-2}		2.0×10^{-4}	7.5×10^{-12}	1.99×10^{-2}	3.0×10^{-7}	1.0×10^{-4}
2×10^{-4}	II	2.3×10^{-4}	2.1×10^{-7}	1.27×10^{-4}	9.8×10^{-6}	7.3×10^{-5}
2×10^{-2}		6.0×10^{-6}	4.5×10^{-6}	1.97×10^{-2}	5.4×10^{-6}	2.9×10^{-4}
2×10^{-4}	III	2.9×10^{-4}	1.7×10^{-7}	1.98×10^{-4}	9.8×10^{-6}	2.0×10^{-6}
2×10^{-2}		2.0×10^{-4}	2.4×10^{-7}	1.98×10^{-2}	9.8×10^{-6}	1.0×10^{-4}
2×10^{-4}	IV	2.5×10^{-4}	8.7×10^{-7}	1.60×10^{-4}	2.1×10^{-6}	4.0×10^{-5}
2×10^{-2}		1.5×10^{-5}	2.6×10^{-6}	1.97×10^{-2}	3.8×10^{-7}	3.0×10^{-4}

	M_T	S_T	K_1	K_2
I	3.0×10^{-7}	3.0×10^{-4}	2.0×10^{8}	2.5×10
II	1.0×10^{-5}	3.0×10^{-4}	2.0×10^{5}	2.5×10^{3}
III	1.0×10^{-5}	3.0×10^{-4}	2.0×10^{5}	2.5×10
IV	3.0×10^{-6}	3.0×10^{-4}	10^{4}	10^{3}

The data in Table 3 show that for a large difference in binding constants (case I) the concentration of the bound cation, [MS], is not affected by a 100-fold increase in the total concentration of the competing species N_T. This case approximates the situation with copper. The cases II and III refer to the competition between zinc, magnesium and calcium, respectively. It can be seen that a two orders of magnitude difference between the binding constants (case II) causes a significant change of [MS] when N_T is increased. In an extreme case (IV) with only one order of magnitude difference between the binding

constants, a 100-fold increase of N_T decreases [MS] by a factor of 5.

The competition for active sites between a toxic and a nontoxic cation could, according to these samples, explain the dependence of toxicity on hardness. The closer the values of binding constants, the more pronounced this dependence would be.

The values of binding constants of cations by tissues are not known, but it is likely that the order of the constants would be similar to that observed with glycine. It can be seen that Mg^{2+} may be a much more effective competitor than Ca^{2+} (glycine binding constants 2.5×10^3 and 25, respectively).

A further indication of the bicarbonate-binding mechanism of the toxicity-hardness dependence of copper and of the competitive mechanism in the case of zinc and nickel may be obtained by comparing the data of Lloyd and Herbert (1962) and Brown (1968) with those of Tabata (1969). The former authors diluted a hard water (h = 320, alkalinity = 240 mg/ℓ as $CaCO_3$) with softened water, whereas Tabata changed hardness by adding calcium and magnesium chlorides to a water of constant alkalinity. As expected from the competitive mechanism, the toxicity of zinc and nickel decreased in both cases by a factor of 6-8 when hardness was increased 100-fold. On the other hand, under the same conditions, the toxicity of copper decreased 24 times in the diluted hard water and only 5 times in the "constant bicarbonate" water. The changes of cadmium toxicity were similar to those observed with copper, possibly due to the formation of chloride complexes in the "constant bicarbonate" water. It is interesting to note that according to Tabata, hardness has practically no effect on the toxicity of mercury, as one would expect from the competitive mechanism, due to the very high glycine binding constant of Hg^{2+}.

The discussion of toxicity and hardness was based on many simplifying assumptions and further research in this area is needed. It is surprising how

little attention was paid in the past to systematic and comparative studies of the relations between ionic equilibria, binding of ions by living matter, and toxicity.

Binding by organic matter. It has been recognized for some time that organic matter decreases the toxicity of copper to fish (see for example Wilson 1972), and probably to other aquatic fauna as well. The likely explanation is that active sites in organic molecules bind cations in the form of nontoxic complexes and an equation analogous to equation (5) could be used to describe the effect of organic matter on toxicity (h = concentration of the organic compound, B = number of active sites in its molecule). The data of Zitko et al. (1973) show that the incipient lethal level of copper (M_T, mole/ℓ) is a linear function of the concentration of humic acid (N_T, mg/ℓ)

$$M_T = 2.20 \times 10^{-7} N_T + 3.93 \times 10^{-7}$$

A competition between organic compounds and tissues to bind the toxic cations could also play a role in the toxicity-decreasing effect of organic matter. Equations (6)-(8) could be used to describe the behaviour of this system (in this case, M and N are organic ligands competing for toxic cation S).

Metal-binding constants of fulvic acid decrease in the order Cu>Ni>Co>Pb>Ca>Zn>Mn>Mg (Gamble and Schnitzer 1973) and it may be expected that the effects of organic compounds on the toxicity of cations would decrease in approximately the same order. The situation is complicated by the competition of other cations, primarily Ca^{2+} and Mg^{2+} with the toxic cations for active sites of the ligands. This may explain why humic acid ceases to decrease the toxicity of copper when hardness is higher than 60 (Wildish et al. 1971; Cook and Coté 1972). Electrostatic interactions may play a role as well.

The effects of humic acid on the toxicity of cations have not been investigated systematically, but it is known that humic acid does not decrease

the toxicity of zinc (Zitko et al. 1973), which is in agreement with the above order of binding constants.

Little is known about the effects of other organic ligands on the toxicity of cations. Wilson (1972) and Elson et al. (1973) reported that lignosulfonates decrease the toxicity of copper to juvenile Atlantic salmon, and Sprague (1968) described the effect of NTA on the toxicity of copper and zinc to the same species. On the other hand, Nishikawa and Tabata (1969) found that EDTA decreased the toxicity of Cd^{2+}, Cu^{2+}, Pb^{2+}, and Hg^{2+}, did not change the toxicity of Zn^{2+}, but increased by a factor of 10 the toxicity of Co^{2+} and Ni^{2+}. If confirmed, this observation may provide an additional insight into the cation toxicity mechanism. According to the same authors, sodium citrate decreased the toxicity of cations proportionally to their binding constants.

Binding by particulate matter. The adsorption of cations on particulate matter is also likely to decrease their toxicity to aquatic fauna, possibly with the exception of filter-feeders. A review of the properties of manganese and iron oxides is available (Morgan and Stumm 1964), and the adsorption of heavy metal ions on these has been studied (see for example Gadde and Laitinen 1974; Guy et al. 1975). Toxicity studies of cations in the presence of these materials have not been reported. There may be difficulties in the preparation of suspensions with reproducible properties.

CHEMICAL ANALYSIS OF NATURAL WATER

The conventional determination of total-metal concentrations is not sufficient for the assessment of their effects under given environmental conditions. The need for more detailed analyses of natural waters, including the determination of ionic species has been frequently emphasized (see for example Osteryoung 1972; Stumm and Bilinski 1972; West and Bustin 1972; West 1973). The methods used for this purpose are usually based on polarography,

ion-selective electrodes, and relatively selective extraction reagents (for example, Stiff 1971b; Chau and Lum-Shue-Chan 1974; Gardiner 1974). The inhibition of enzyme-catalyzed reactions may be useful to predict the toxicity of ions bound to organic ligands, but has not been used as yet for this purpose (for methods see Guilbault et al. 1967; Toren and Burger 1968; Sigel 1969; Taylor et al. 1969; Townshend and Vaughan 1970; Sheikh and Townshend 1974).

Some of the methods may not be practical for routine monitoring, but are extremely important for the determination of equilibrium constants which, once known with reasonable accuracy, make it possible to calculate the concentrations of various ionic species and complexes.

PREDICTIONS OF TOXICITY

The present state of the art is reasonably good for predictions of toxicity in certain types of waters, containing a limited number of inorganic and organic components (see for example Brown 1968; Wilson 1972; Zitko et al. 1973), and the expression of toxicity in "toxic units" is a useful approach.

The available toxicity data apply largely to fish and refer to acute toxicity. Fish are not necessarily the most sensitive members of aquatic fauna and more data on other species are needed. The additivity of toxic effects, determined at acutely toxic levels, may not be valid at chronically toxic concentrations (see for example Eaton 1973) and more data on interactions between cations should be obtained. Routine chemical analyses should be supplemented at least by a determination of heavy metal-binding capacity of the given water according to methods described by Zitko et al. 1973, Chau and Lum-Shue-Chan (1974), or Ramamoorthy and Kushner (1975).

Toxicity predictions cannot and are not intended to replace bioassays, but are a tool for better understanding of the complex processes governing the

behaviour of inorganics in natural waters.

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METAL SPECIATION IN AQUATIC ENVIRONMENTS

CHAPTER 2

Herbert E. Allen, Michael L. Crosser and Thomas D. Brisbin
Department of Environmental Engineering
Illinois Institute of Technology
Chicago, Illinois 60616

ABSTRACT

Although the majority of water quality standards specify, and environmental studies have measured only the total concentration of a trace metal, it has become apparent that the chemical form of the metal must be known to permit accurate interpretation of both its biological effects and geochemical reactions. Prediction of trace metal environmental levels, forms and transformations requires the development of mechanistic models which are based on equilibrium constants of metal complexes. Likewise, laboratory bioassays to determine acute or chronic toxicities must be conducted on the metal species which are present in the environment. Techniques used in this laboratory for these measurements are described. Applications of the ion exchange equilibrium to the study of complexation in AAP medium used for toxicity studies, complexation of metals in sewage treatment plants, and assessment of the relative significance of ligands entering the aquatic environment from various sources will be presented.

Although the majority of water quality standards specify, and environmental studies have measured only the total concentration of a trace metal, it has become apparent that the chemical form of the metal must be known to permit accurate interpretation of both its biological effects and geochemical reactions. Prediction of trace metal environmental levels, forms and transformations requires the development of mechanistic models which are based on equilibrium constants of metal complexes. Likewise, laboratory bioassays to determine acute or chronic toxicities must be conducted on the metal species which are present in the environment. Therefore, the kinetics and equilibria of the pertinent chemical reactions must be accounted for in such systems.

The objective of this paper is to explore the applicability and limitations of several analytical methods to the study of metal complexation reactions in natural and waste water systems and in controlled laboratory bioassay situations. Principal attention will be focused on electroanalytical and ion exchange techniques for the measurement of stability constants and the concentrations of ligands present. As the reactions under investigation may not be rapid, it is imperative that the investigator ascertain that the reaction has proceeded to equilibrium or to an extent equivalent to that in the system being simulated.

That the form of metals affects their biological availability has now been well documented and is acknowledged in the recent report "Water Quality Criteria 1972" (1). However, the report dismisses consideration of complexation in decreasing the toxicity of metals due to their potential transformation from less to more toxic chemical forms. In the St. Lawrence Great Lakes it is highly probable that metal complexation chemical equilibrium has been achieved due to the long residence times for the system. The ligands may originate from sources different from those for metals and both metals and ligand may be at steady-state or slowly increasing levels. We should expect that complexes will in actuality probably not transform to any appreciable extent. Therefore, the chemical states of metals in the aquatic ecosystem must be considered in establishment of water quality criteria if the criteria are to realistically reflect the toxicity observed in the natural, as opposed to the laboratory, environment.

Equilibrium models have frequently been used to predict the behavior of real systems. However, such models often do not adequately predict real world behavior which can be attributed to several factors including failure of the system to have achieved equilibrium and the application of inappropriate thermodynamic data.

In the aqueous system containing zinc and carbonate, metastable conditions may persist for periods which are very significant with respect to the manner in which aquatic bioassays are normally conducted. Patterson, et al. (2) have reported on the concentration of soluble zinc in equilibrium with zinc precipitate after a four-hour reaction period. Solubility was operationally defined by

metal which passed through a 0.45 μm membrane filter. Experiments were conducted both in solutions containing carbonate and in carbonate free media. Results of the experiments in which carbonate was present are shown in Figure 1. There is poor agreement between the experimental data points and the soluble zinc predicted by chemical equilibrium theory. The discrepancy is more pronounced in the pH region in which $ZnCO_3$ is the thermodynamically predicted stable phase than in the region in which $Zn(OH)_2$ is the phase which is predicted to be stable.

The reason for most of the discrepancy between the experimental and theoretical zinc solubility becomes apparent when one inspects Figure 2. Here data from experiments conducted both in the presence and in the absence of carbonate are compared to the theoretical solubility diagram for $Zn(OH)_2$. No difference in zinc solubility occurs because of the presence of carbonate. It can thus be assumed that even under those conditions in which $ZnCO_3$ is the stable precipitate, $Zn(OH)_2$ is the form which initially precipitates. The importance of these results to the study of toxicity of metals is obvious; one should assume neither the existance of chemical equilibrium nor the species of metal which are present in the system.

In these experiments a four-hour reaction period was utilized. After this reaction time thermodynamic equilibrium had not been achieved but this time was significantly greater than that available for chemical reaction in continuous flow bioassay systems. Most bioassay systems employ "diluters" to mix concentrated toxicant with the bioassay water. Within several minutes of mixing the water

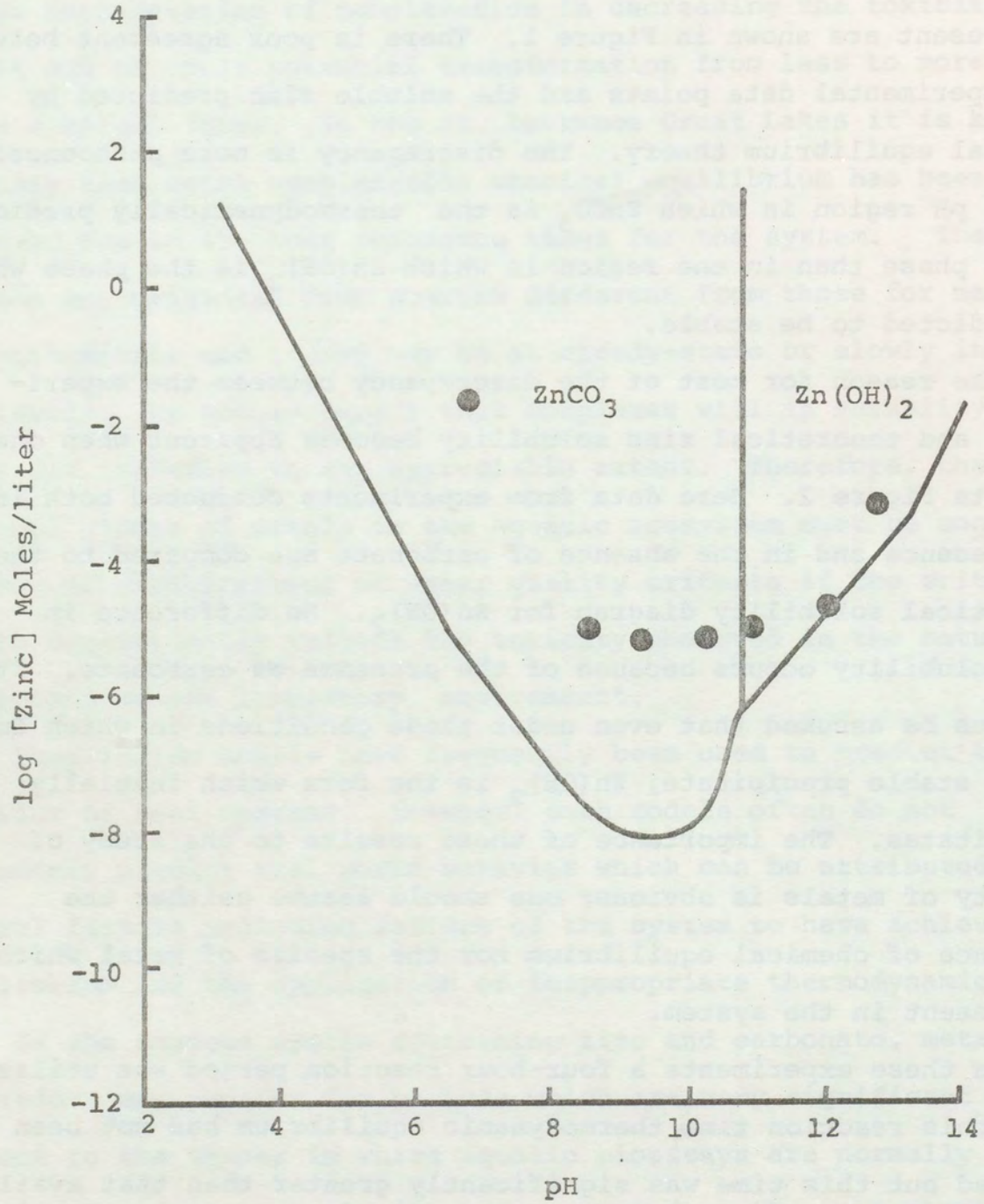


Figure 1. Comparison of Zinc Carbonate Solubility Data with Theoretical Phase Diagram. $C_T = 10^{-1.3}$ moles/liter. (From Ref. 2)

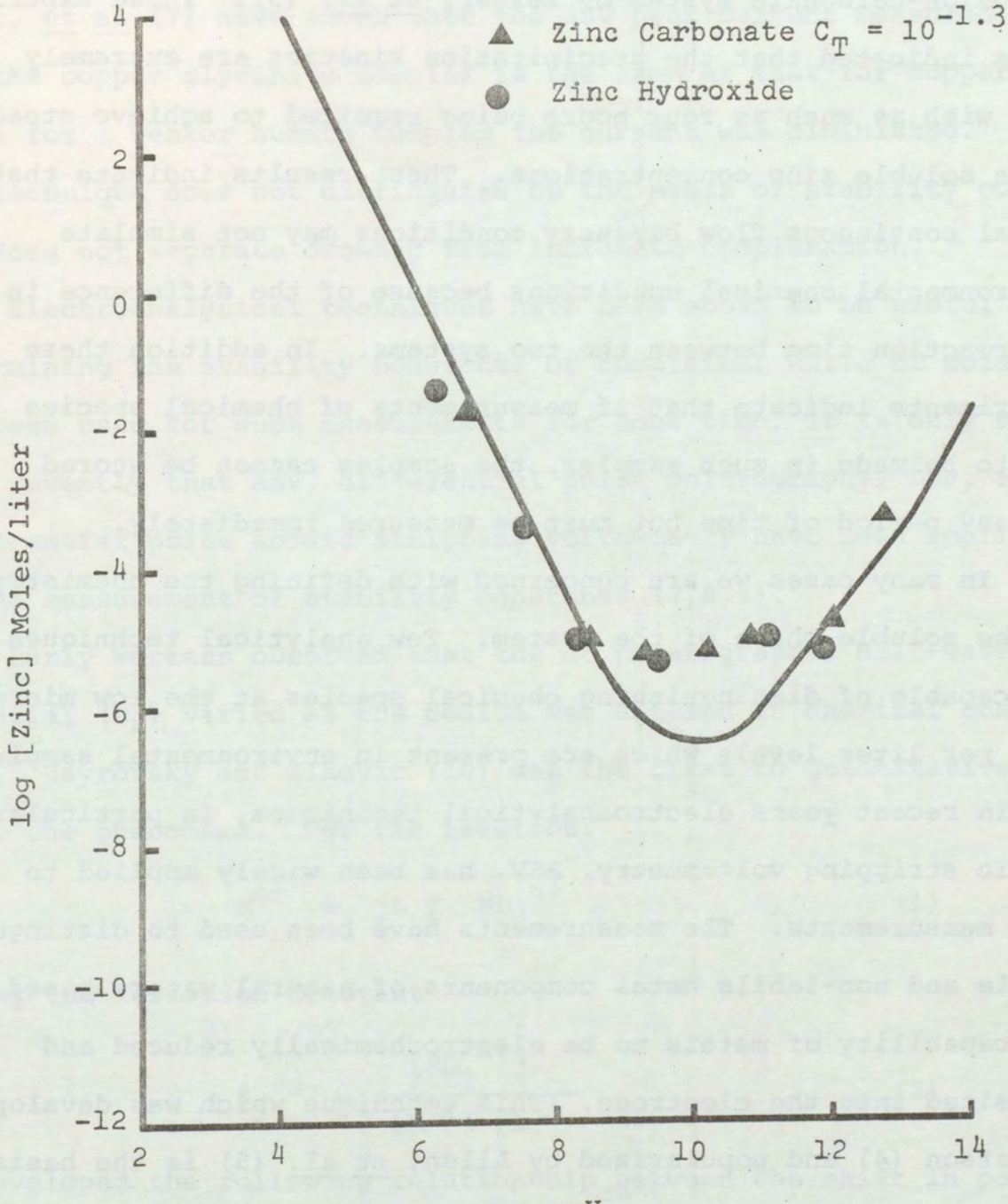


Figure 2. Comparison of Zinc Hydroxide and Zinc Carbonate Data with Theoretical Hydroxide Solubility Curve. (From Ref. 2)

is passed into the bioassay chamber where it has a residence time not exceeding several hours. Reaction kinetics in this system are most important and have been reported for the zinc-hydroxide-carbonate system by Keiser, et al. (3). These experiments indicated that the precipitation kinetics are extremely slow with as much as four hours being required to achieve steady state soluble zinc concentrations. These results indicate that normal continuous flow bioassay conditions may not simulate environmental chemical conditions because of the difference in the reaction time between the two systems. In addition these experiments indicate that if measurements of chemical species are to be made in such samples, the samples cannot be stored for any period of time but must be measured immediately.

In many cases we are concerned with defining the chemistry of the soluble phase of the system. Few analytical techniques are capable of distinguishing chemical species at the low microgram per liter levels which are present in environmental samples. Within recent years electroanalytical techniques, in particular anodic stripping voltammetry, ASV, has been widely applied to such measurements. The measurements have been used to distinguish labile and non-labile metal components of natural waters based on the capability of metals to be electrochemically reduced and deposited into the electrode. This technique which was developed by Matson (4) and popularized by Allen, et al. (5) is the basis for the electrochemical determination of complexation capacity which has been utilized by Chau, et al. (6) and others. It should be noted, however, that this technique does not provide an unambiguous

answer to the concentration of ligands present in a sample. It is accepted that metal carbonate and hydroxide complexes are electrochemically indistinguishable from free metal ions in solution. Ernst, et al.(7) have shown that the ASV peak current measured for the copper glycinate complex is the same as that for copper ion while for a weaker humate complex the current was diminished. Thus, the technique does not distinguish on the basis of stability constant and does not separate organic from inorganic complexation.

Electroanalytical techniques have been shown to be useful in determining the stability constants of complexes, while dc polarography has been used for such measurements for some time, it is only much more recently that ASV, differential pulse polarography, DDP, and differential pulse anodic stripping voltammetry have been applied to the measurement of stability constants (7,8,9).

Early workers observed that the dc polarographic half-wave potential ($E_{\frac{1}{2}}$) varied as the medium was altered in chemical composition. Heyrovsky and Ilkovic (10) was the first to quantitatively treat the phenomena. For the reaction:

$$M^{n+} + jL \rightleftarrows ML_j^{n+} \qquad (1)$$

having the formation constant

$$\beta_j = \frac{[ML_j^{n+}]}{[M^{n+}]\,[L]^j} \qquad (2)$$

he developed the following relationship between the shift in potential and the equilibrium ligand concentration:

$$\Delta E_{\frac{1}{2}} = (E_{\frac{1}{2}})_s - (E_{\frac{1}{2}})_c = \frac{2.303RT}{nF} \log \beta_j + j \frac{2.303RT}{nF} \log [L] \qquad (3)$$

where $(E_{\frac{1}{2}})_s$ is the half-wave potential of the simple, uncomplexed metal ion, M^{n+}

$(E_{\frac{1}{2}})_c$ is the half-wave potential of the metal in the presence of the ligand concentration [L]

n is the number of electrons exchanged in the reduction process

and $\frac{2.303RT}{n}$ is a constant having the value 0.0591 at 25°C.

The following assumptions have been made in the derivation of the Lingane equation.

1) The diffusion coefficient of the complex must be the same as that of the simple metal ion.

2) The complex which is formed must be electrochemically labile.

3) The ligand concentration at the electrode surface must be equal to the bulk concentration.

4) The reduction of the metal must be electrochemically reversible.

5) A single complex must predominate over a wide range of ligand concentrations.

These conditions have been discussed in detail by Crow (12) and Heyrovsky and Kuta (13).

Frequently, the condition of having a predominant complex is not fulfilled. Consequently, when the shift in half-wave potential is plotted versus the logarithm of the ligand concentration, a curve results rather than the straight line predicted by the Lingane equation. The method of DeFord and Hume (14) has been used to interpret these curves as a summation of overlapped Lingane relationships and allows the individual formation constants to be ascertained.

Many ligand-metal systems are not electrochemically reversible and therefore are not amenable to treatment by the Lingane method. However, these irreversible systems may be of considerable practical

importance and it is, therefore, important to have methods capable of studying them.

Tamamushi and Tanaka (15) developed the following modified version of the Lingane equation for an irreversible reduction process:

$$\Delta E_{\frac{1}{2}} = (E_{\frac{1}{2}})_s - (E_{\frac{1}{2}})_c = \frac{2.303RT}{\alpha nF} \log \beta_j + j \frac{2.303RT}{\alpha nF} \log [L] \qquad (4)$$

where the transfer coefficient α is the fraction of the total applied potential which favors the forward reaction. As shown by Parry and Oldham (16) the transfer coefficient can be calculated from the peak half width.

To evaluate stability constants by electrochemical methods, Ernst, et al.(7) have employed both DPP and DPASV to assure that the same value is obtained in both cathodic and anodic directions. Using this procedure one is assured that adsorption at the electrode surface has not been responsible for producing too high values for the stability constant. Typical experimental results are shown in Figures 3 and 4. The data shown in Figure 3 for the lead carbonate ion pair is for a system in which the reaction in both the anodic and cathodic direction is electrochemically reversible. The lines for both are parallel and from the slope the ligand number is determined to be 1.07 for the DPP data and 1.06 for the DPASV data. Values for the logarithm of the stability constant were 6.2 by DPP and 6.3 by DPASV. The data for the copper glycinate complex, which is shown in Figure 4 is for a system in which the reaction is reversible in neither the anodic nor the cathodic direction. By application of the electrochemical transfer coefficient, the data can be treated so that the stability constant for both processes can

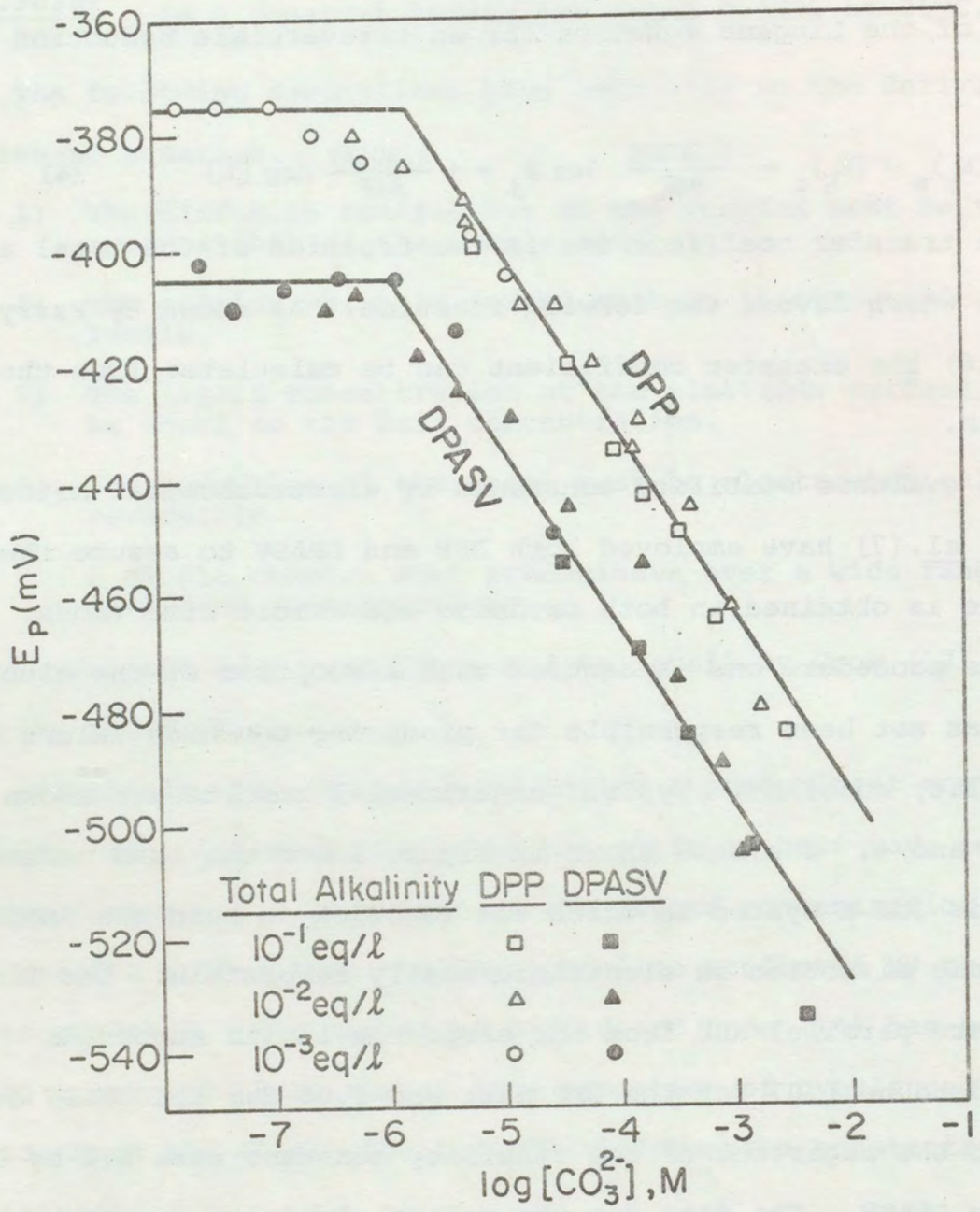


Figure 3. Variation of DPP and DPASV Peak Potentials as a Function of Carbonate Ion Concentration for 2.5 X 10^{-6}M lead. (From Ref. 7)

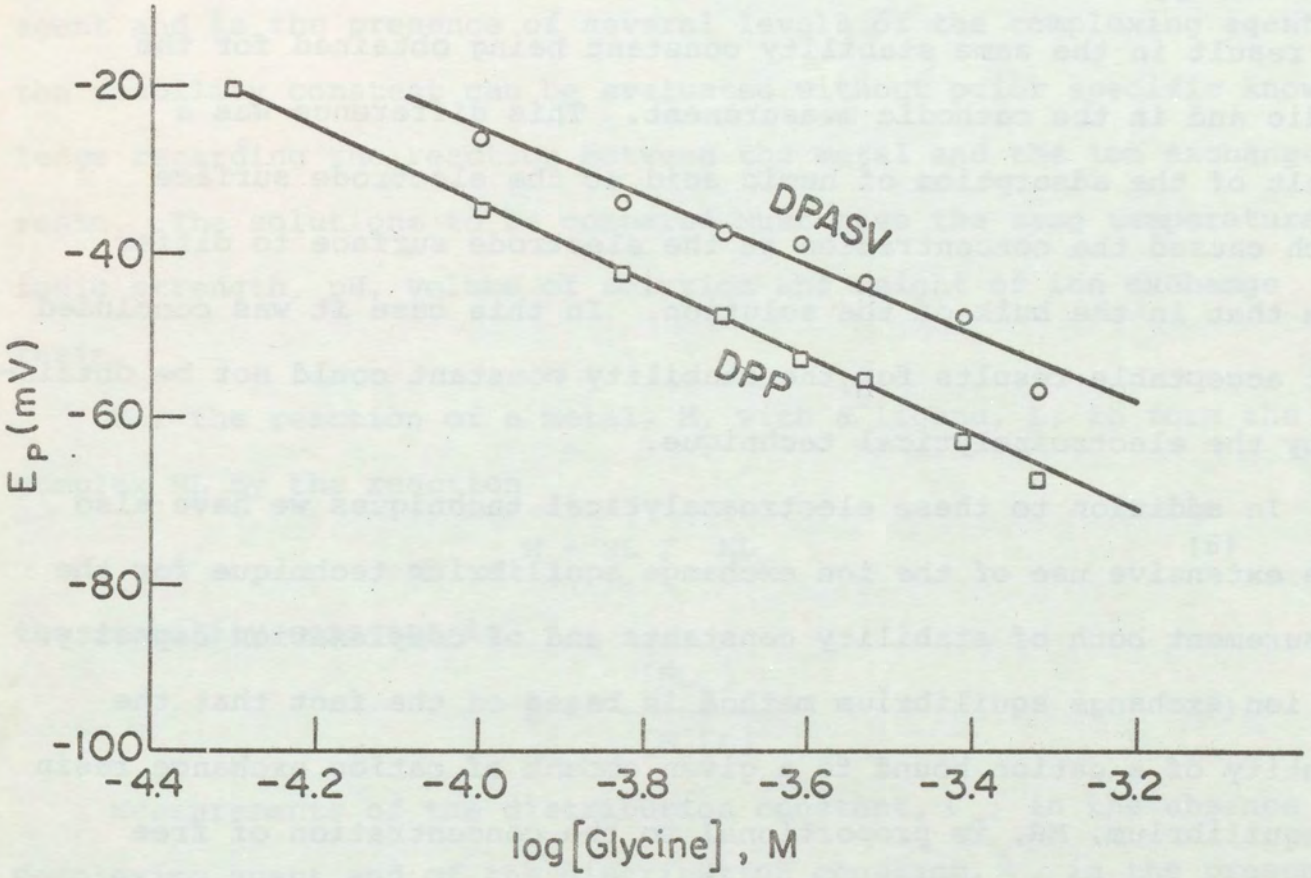


Figure 4. Variation of DPP and DPASV Peak Potentials as a Function of Glycine Concentration for 2.5 X 10^{-6}M Copper at pH 6.8. (From Ref. 7)

be treated to produce nearly the accepted value. However, ligand numbers were 1.24 and 1.28 by DPP and DPASV, respectively. Therefore, serious doubts could be placed on the significance of such determinations for measurements of ligands whose values have not previously been reported.

When metal humate complexes were investigated, it was found that the application of reversibility corrections to the data did not result in the same stability constant being obtained for the anodic and in the cathodic measurement. This difference was a result of the adsorption of humic acid at the electrode surface which caused the concentration at the electrode surface to differ from that in the bulk of the solution. In this case it was concluded that acceptable results for the stability constant could not be obtained by the electroanalytical technique.

In addition to these electroanalytical techniques we have also made extensive use of the ion exchange equilibrium technique for the measurement both of stability constants and of complexation capacity. The ion exchange equilibrium method is based on the fact that the quantity of a cation bound to a given amount of cation exchange resin at equilibrium, MR, is proportional to the concentration of free metal ions M in the solution over a wide range of concentration:

$$\frac{MR}{M} = \lambda_o = \frac{a_o V}{(100-a_o)\, g} \qquad (5)$$

where λ_o = a constant (for equilibrium between the solution and resin in the absence of complexing agent),

MR = moles of metal bound by a unit weight of cation exchange resin,

M = concentration of metal in solution at equilibrium,

a_o = percent of the total metal used which is bound to the cation exchange resin,

$100-a_o$ = percent of the total metal used which remains in solution,

V = volume of solution, and

g = weight of cation exchange resin.

By measuring the relative effects in the absence of complexing agent and in the presence of several levels of the complexing agent, the stability constant can be evaluated without prior specific knowledge regarding the reaction between the metal and the ion exchange resin. The solutions to be compared must have the same temperature, ionic strength, pH, volume of solution and weight of ion exchange resin.

For the reaction of a metal, M, with a ligand, L, to form the complex ML by the reaction

$$M + xL \rightleftarrows ML_x \tag{6}$$

the stability constant is

$$K = \frac{[ML_x]}{[M][L]^x} \tag{7}$$

Measurements of the distribution constant, λ_o, in the absence of complexing agent and of the distribution constant, λ, in the presence of complexing agent are made. Even when the complexing agent is present, the resin is in equilibrium with free metal ions as expressed by Equation 5. With the complexing agent present the concentration of free metal ions in solution is

$$[M] = \frac{MR}{\lambda_o} \tag{8}$$

In addition, the relationship between the total metal concentration in solution, $[M] + [ML_x]$, and the metal on the resin is

$$[M] + [ML_x] = \frac{MR}{\lambda} \quad (9)$$

Equations 8 and 9 may be combined:

$$[ML_x] = \frac{MR}{\lambda} - \frac{MR}{\lambda_o} \quad (10)$$

and the equilibrium constant may be written in the form

$$K = \frac{\lambda_o/\lambda - 1}{[L]^x} \quad (11)$$

of

$$\log (\lambda_o/\lambda - 1) = \log K + x \log [L] \quad (12)$$

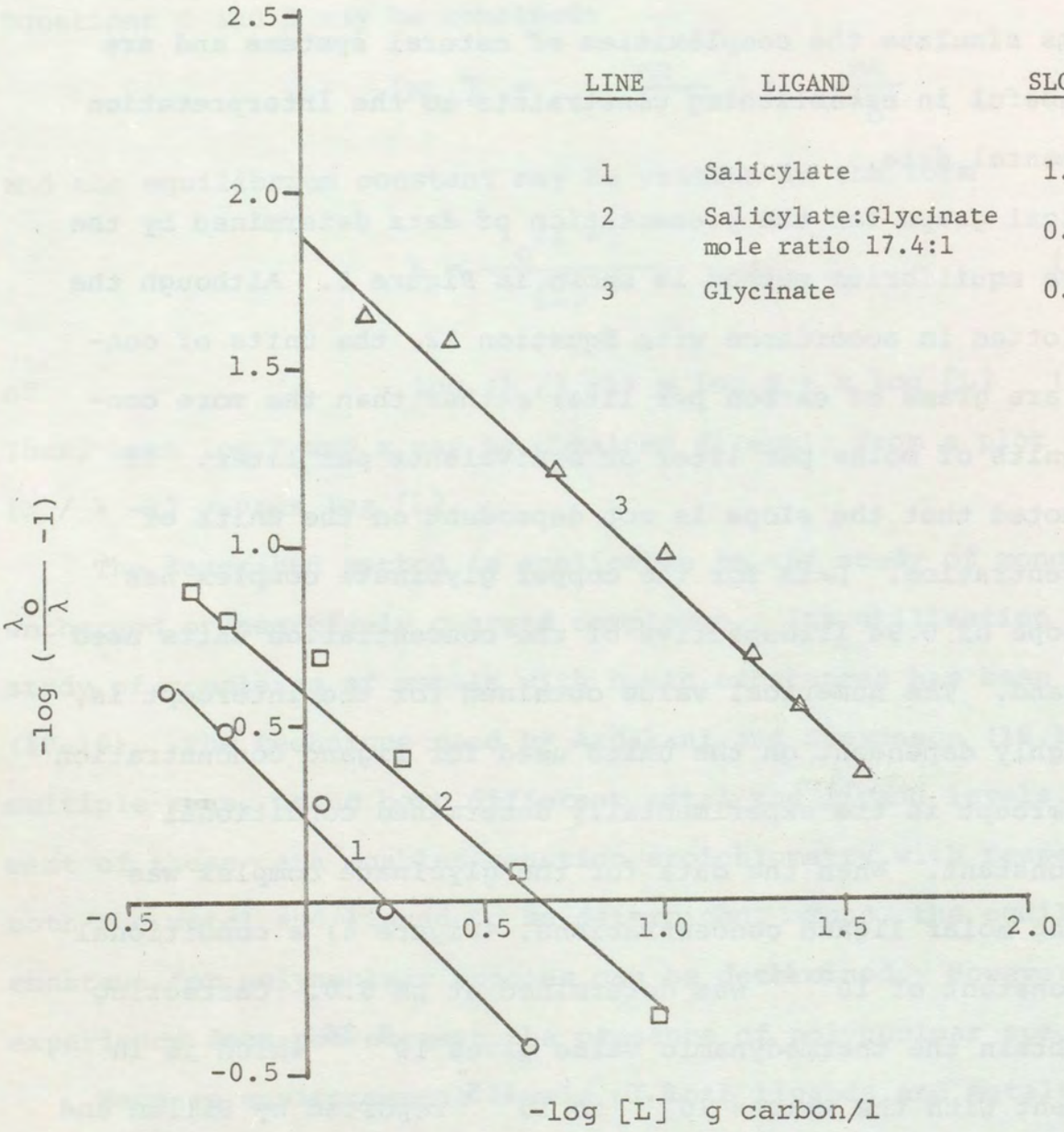
Thus, both log K and x may be obtained directly from a plot of log $(\lambda_o/\lambda - 1)$ versus log [L].

The described method is applicable to the study of monomolecular uncharged or negatively charged complexes. Its utilization in the study of complexes of metals with humic substances has been critized (17-19). The technique used by Ardakani and Stevenson (18,19) involves multiple runs, using both different metal and ligand levels. Treatment of these data enables reaction stoichiometry with respect to both the metal and ligand to be determined. Thus, the equilibrium constant for polynuclear species can be determined. However, our experience does not suggest the presence of polynuclear species.

Because environmental levels of both ligands and metals are low, and because the ion exchange equilibrium technique requires the ligand to be present in excess, sensitive analytical techniques are required for measurement of equilibrated metal levels. Such techniques include anodic stripping voltammetry or flameless atomic absorption spectrophotometry. Addition of radiotracer metal also permits a sensitive determination of the equilibrium between solution and resin.

Our present efforts have been aimed toward defining the significance of a stability constant in a multi-ligand system. Such systems simulate the complexities of natural systems and are therefore useful in establishing constraints to the interpretation of environmental data.

A typical graph for the presentation of data determined by the ion exchange equilibrium method is shown in Figure 5. Although the data are plotted in accordance with Equation 12, the units of concentration are grams of carbon per liter rather than the more conventional units of moles per liter or equivalents per liter. It should be noted that the slope is not dependent on the units of ligand concentration. Data for the copper glycinate complex has the same slope of 0.94 irrespective of the concentration units used for the ligand. The numerical value obtained for the intercept is, however, highly dependent on the units used for ligand concentration and the intercept is the experimentally determined conditional stability constant. When the data for the glycinate complex was plotted using molar ligand concentrations, (Figure 6) a conditional stability constant of $10^{3.40}$ was determined at pH 5.0. Correcting for pH to obtain the thermodynamic value gives $10^{8.26}$ which is in good agreement with the values $10^{8.0}$ to $10^{8.5}$ reported by Sillen and Martell (20). When concentrations of ligand are grams of carbon per liter the experimental conditional stability constant was determined to be $10^{1.84}$ which is the stability constant on a molar basis divided by the number of grams of carbon per mole of compound. It is possible to determine a useful stability constant for a compound irrespective of whether knowledge of the molecular weight is available; in any case the same correct value of the ligand number is obtained.



LINE	LIGAND	REGRESSION: SLOPE	INTERCEPT
1	Salicylate	1.03	.27
2	Salicylate:Glycinate mole ratio 17.4:1	0.97	.56
3	Glycinate	0.94	1.84

Figure 5. Determination of Stability Constants and Ligand Numbers of Copper Complexes of Pure Compounds and a Mixture by Ion Exchange Equilibrium Method; Ligand as Grams Carbon/ Liter. (From Ref. 21)

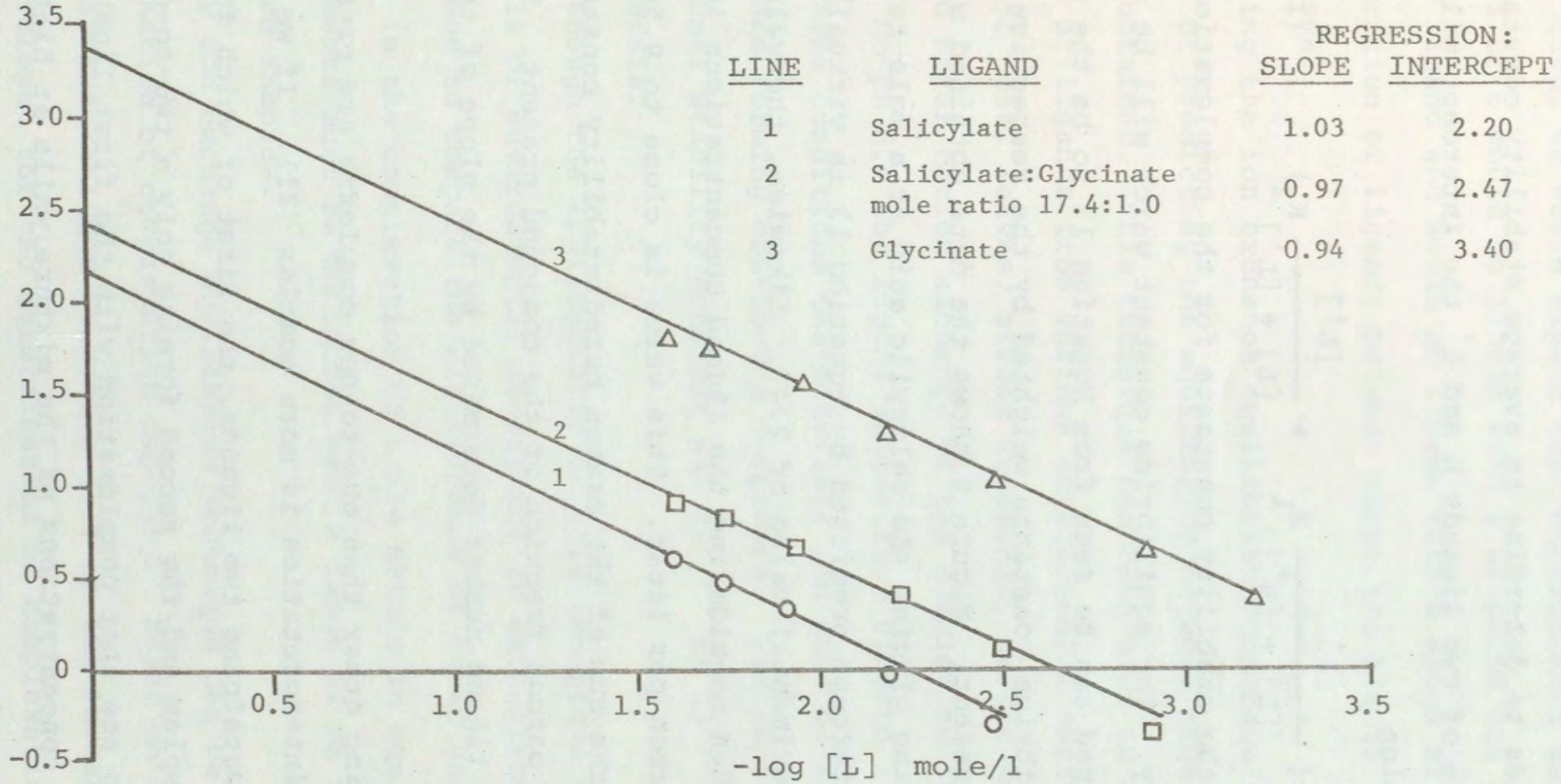


Figure 6. Determination of Stability Constants and Ligand Numbers of Copper Complexes of Pure Compounds and a Mixture by Ion Exchange Equilibrium Method. Ligand as Moles per Liter. (From Ref. 21)

The method of ion exchange equilibrium is amenable to the analysis of mixtures of ligands to determine an average stability constant. In the case of a mixture of two ligands L and L' the intercept will be given by the expression

$$\text{intercept} = \log \left(\frac{[L]}{[L] + [L']} K_1 + \frac{[L']}{[L] + [L']} K_2 \right) \qquad (13)$$

where K_1 and K_2 are the stability constants for the complexation with L and L' respectively. The equilibrium constant which will be experimentally determined can be seen from Equation 13 to be the sum of the individual equilibrium constants weighted by the respective mole fraction of ligand present. Figure 6 shows the data obtained with a mixed ligand containing glycine and salicylic acid at a mole ratio of 17.4:1. The 2.46 intercept predicted by equation 13 is virtually identical to the experimental value of 2.47. Likewise, the value of 0.56 is determined when considering the ligand concentrations in terms of grams of carbon per liter. This value is close to 0.54 which is the log of the sum of the carbon based stability constants each weighted by the carbon fraction of the compound present. It should be noted that ligand number determined by the slope of the regression is 0.97.

If ligands forming other than one-to-one complexes are present in the mixture, the interpretation is more complex (21). If we consider a mixture containing two ligands, the first of which forming only a one-to-one complex and the second forming only a two-to-one complex it is easy to see that complexation with the first ligand will dominate at low concentrations of the mixture while at high concentrations complexation with the second will be dominant. Thus,

in this case, we would expect that experimental data plotted as in Figure 5 or 6 would be linear with a slope of one at a low concentration of ligand and would be linear with a slope of two at a high concentration of ligand; between these two limits the data would be curvilinear.

Using the ion exchange equilibrium technique a stability constant capable of predicting the equilibrium between free and complexed metal can be obtained. This technique has been used in a preliminary comparison of ligands from various environmental sources. Aqueous extracts of peat, leaves, street sweepings, cattle manure and soil were analyzed after cation exchange of the samples to remove extraneous metal. At pH 5.0 the ligand ranged from 0.93 to 1.10 indicating that under these conditions the materials can each be regarded as a mixture of ligands forming one-to-one complexes. The range of values for the conditional stability constant was $10^{1.16}$ to $10^{2.05}$ expressed on a carbon basis.

Although the applicability of this technique to the study of materials of environmental importance is established, much remains to be done to compare the significance of these and other sources of ligands in the complexation of trace metals in aquatic systems. Measurements must be conducted over a range of pH values and with many such samples.

The ion exchange equilibrium technique can be extended to the determination of complexation capacity. Based on extension of the method of Ardakani and Stevenson (18) an ion exchange technique has been developed for the determination of complexation capacity (21). In this technique various metal concentrations are added to aliquots of the ligand solution and these samples are incubated with ion

exchange resin. After equilibration the concentration of metal remaining in solution ($M_f + M_c$) is determined and plotted versus the amount of metal taken up by the resin (M_R).

The ion exchange isotherm, determined in the absence of ligand gives an approximately linear relationship over a large range of metal concentration as can be seen in Figure 7. If a ligand is present in the sample, less metal is exchanged by the resin and a line of diminished slope is obtained until the amount of ligand is exceeded. After this point the slope becomes identical to that for the ion exchange isotherm. It should be noted that the slope prior to exceeding the complexation capacity is a function of the stability constant of the ligand being titrated.

Potential advantage in ion exchange equilibrium as compared to electrochemical techniques can be seen by comparison of the two techniques applied to the analysis of complexation of copper by glycine and humic acids. The stability constant for the glycinate complex could be obtained by both techniques whereas for humic material electrochemical techniques were not suitable due to adsorption effects. At pH 5.0 ion exchange equilibrium results show that the glycinate forms only a slightly stronger complex than did the peat extract when the data were compared on a carbon basis. Since the stability constants were nearly the same both ligands could be titrated by the ion exchange equilibrium method. However, in the analogous determination of complexation capacity by electrochemical methods only the humic material could be analyzed.

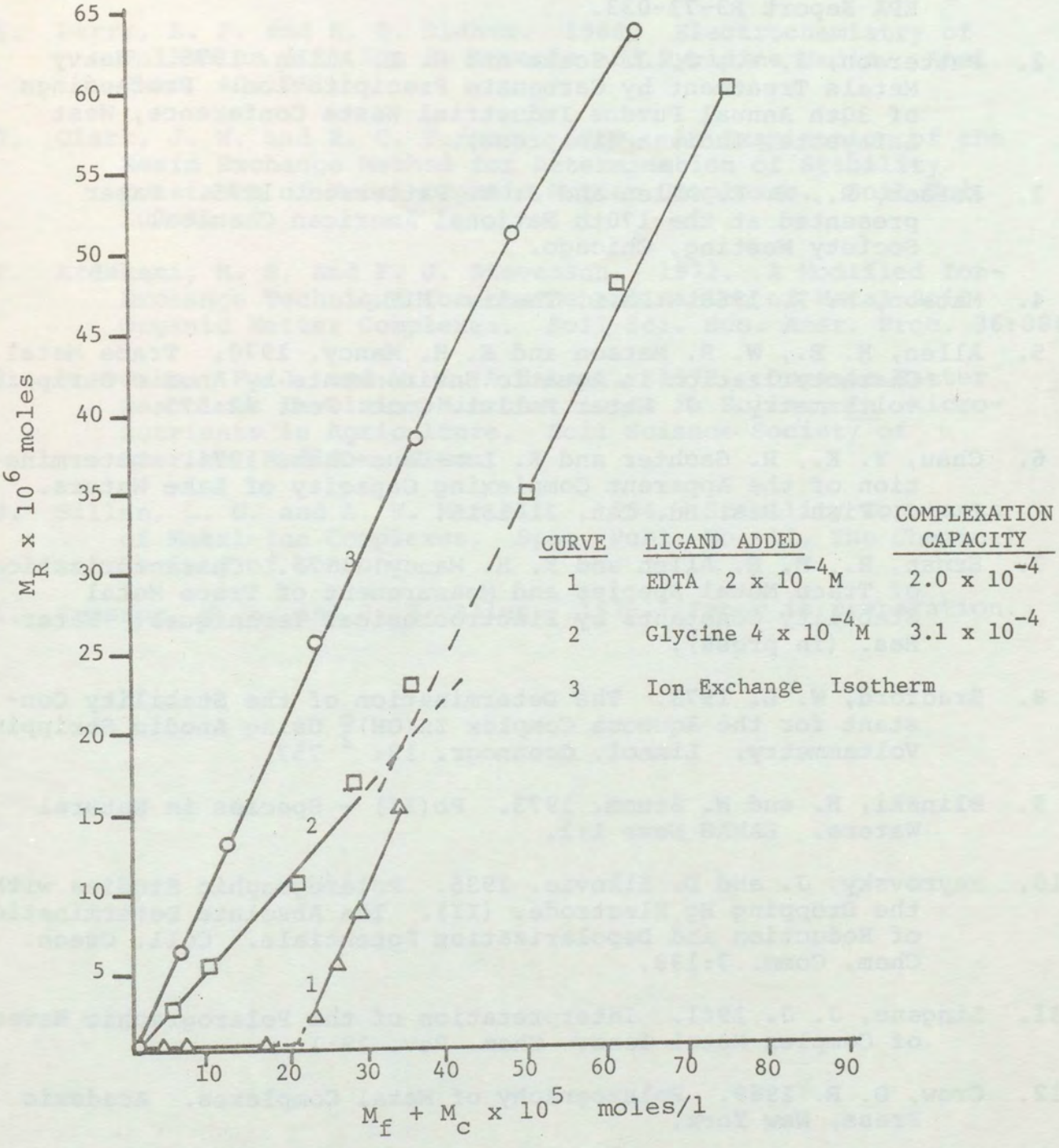


Figure 7. Determination of Copper Complexation Capacity of Glycine and EDTA by Ion Exchange Equilibrium Method. (From Ref. 21)

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ASPECTS OF HEAVY METALS TOXICITY IN FRESH WATERS*

CHAPTER 3

V. M. Brown
Water Research Center,
Stevenage Laboratory
Stevenage, England SG1 1TH

ABSTRACT

This paper will consider: (a) heavy metal contamination of waters, (b) basic aspects of toxicology of heavy metals to animals (including the more important physiological considerations), (c) the relevance of these to aquatic biota, particularly fishes, and finally, (d) describe some of our work on the chemical states of metals in natural water.

*This paper is published as a transcript to accurately reflect Dr. Brown's workshop presentation, and therefore not edited by the Editors.

Not being a chemist I shall not attempt to compete with the chemistry experts at this meeting. What I should like to do is perhaps to highlight some of the problems which the biologist sees in the heavy metals.

I came here intending to convey a little information and a lot of speculation but find that much of what I have to say was covered at our meeting yesterday - the information part in Bob Andrew's paper and the speculation part in the afternoon's discussion. Nevertheless I hope that this "essay" on metals in relation to their definition, availability, and effects on freshwater fish can still usefully contribute to our discussions.

First of all I would like to get clear what we are talking about. The marked effects which low concentrations of chemicals in fresh waters can have on aquatic organisms and ecosystems have become increasingly recognized over the last decade or so, and among substances of concern are the so-called "trace elements", "trace metals", "heavy metals" and "transition metals". Now while the chemist presumably knows what he means when he uses these terms I'm not sure that the biologist always does, and I should like therefore first of all to consider this aspect - starting with trace elements.

Trace elements in water have been defined as all of those chemical elements, both metals and non-metals, present at concentrations below about 1.0 mg/l (Rhodhe 1950). They are a diverse group of chemical substances with nothing in common but their name. However, the term has no absolute sense; what is present as a "trace element" in one component of the environment is not necessarily so in some other. Geological and biological processes both concentrate chemical elements. Thus, for example, iron, the fourth most abundant element in the rocks of the upper part of the Earth's continental crust (Fleischer 1972) is present only at trace levels in the majority of surface waters, as it is in the whole bodies of animals. But many elements can be concentrated in animals and plants to levels at which they can no longer be described as trace elements at specific localized sites. Consequently care is required in the use of the term. Schroeder (1965) recognizing this problem, speaks specifically of <u>biological</u> trace elements when discussing levels in organisms, defining such elements as those making up less than 0.01 per cent of the organism. Vallee (1959) defined trace metals as those present at from 1.10^{-6} to less than 1.10^{-12} g/g of wet organ.

Trace elements have been divided into (a) those <u>essential</u> to life and well-being of organisms and (b) those apparently <u>non-essential</u>. (Schroeder and Darrow (1973) usefully review these with regard to human health.) These are fairly well-defined categories, although Hoekstra (1972) reasonably questions the value of the distinction, and the possible significance of some elements is still in question. Sometimes distinctions are suggested (Schroeder 1965; Kneip and Lauer, 1973) between the two in terms of toxicity, but the elements of both groups, like all other substances, are toxic at sufficiently high concentrations, and this seems, therefore, an unrealistic distinction.

The assessment of upper tolerable limits of chemicals is the domain of the toxicologist, and our concern here, but it should be recognized that at lower levels of essential elements the nutritionist can detect minimal requirements. While any reduction in concentration of a nonessential element in water is probably beneficial, or at least of no consequence, reduction in concentration of essential elements below these minimal levels may lead to the onset of deficiency symptoms and to harm, although I find it difficult to imagine this happening in truly aquatic organisms, as readily as it can in terrestrial animals. (But when considering elements essential to animal life it is also important to remember that these include the major elements calcium, phosphorus, sulphur, potassium, sodium, chlorine, magnesium.

Metals, on the other hand, in contrast to "trace elements", are defined by qualitative (and not quantitative) attributes. They possess, in general, as any chemistry text-book states, properties such as lustre, malleability, good conductance of heat and electricity, form electropositive ions, and so on. And they can be divided into two classes with reference to their specific gravity, namely:

(a) the light metals - aluminium, barium, beryllium, calcium, cesium, lithium, magnesium, rubidium, scandium, sodium, strontium, potassium, and,

(b) the heavy metals, those with a specific gravity greater than 4 {International Encyclopedia of Chemical Science (1964)}. (Sometimes a limit of > 5 is defined (Passow et al 1961).

If we are to discuss metals, then, we must include the elements from both of these groups. With the exception of calcium, magnesium, sodium, and potassium all are present as trace (or minor) elements in fresh waters, and the term trace metals includes therefore all of the metals but these four.

The heavy metals occurring naturally on earth (i.e. excluding the man-made ones) number some 55 (Weast 1969) and these are listed alphabetically in Table 1. (Of the cosmic elements promethium appears to be absent from Earth.) From this table it can be seen that the greatest number of trace elements in water are in fact heavy metals so that for much of the time the two terms are often effectively synonymous. The low levels at which they are present reflect in general their low abundance (perhaps some 0.3% to 0.6%) in the Earth's crust (Mason 1966).

Table 1. Heavy metals occurring in the Earth's crust

Actinium	Gadolinium	Manganese	Protactinium*	Thorium
Antimony*	Gallium*	Mercury	Radium*	Thulium
Bismuth*	Gold	Molybdenum	Rhenium	Tin*
Cadmium	Hafnium	Neodymium	Rhodium	Titanium
Cerium	Holmium	Nickel	Ruthenium	Tungsten
Chromium	Indium*	Niobium	Samarium	Uranium
Cobalt	Iridium	Osmium	Silver	Vanadium
Copper	Iron	Palladium	Tantalum	Ytterbium
Dysprosium	Lanthanum	Platinum	Tellurium*	Yttrium
Erbium	Lead*	Polonium*	Terbium	Zinc
Europium	Lutetium	Praseodymium	Thallium*	Zirconium

*Not a transition metal

Finally, we can look at the elements termed transition metals. These are elements having an incomplete number of electrons in the next-to-outermost electron shell (the d-orbitals). All are heavy metals and, apart from antimony, bismuth, gallium, indium, lead, polonium, protactinium, radium, tellurium, thallium, and tin, the remaining heavy metals are all transition metals (Sienko and Plane 1971). Some of the transition metals play very important biological roles, having functions as vital components of enzymes, vitamins etc. However, the heavy metals, although possessing individual specificity, have universal reactivity, and are, therefore, toxic agents, being potent enzyme inhibitors (Passow et al 1961) at greater than physiological concentrations.

Now many data are available on total concentrations at which heavy metals have been found in fresh waters as well as on the toxicities which some of these metals have been found to have in the laboratory. What would appear to be lacking is reliable information on both concentrations, and states, of heavy metals in water in relation to the distribution and health of populations of aquatic animals. A small number of papers have been published describing observed or reputed distributions of various fish species in relation to particular levels of contaminants or pollutants, including some heavy metals. Total concentrations of certain heavy metals (which may not, of course, necessarily be the only ones of concern, or the main pollutional problem at any site, nor even the metals toxicologically most important at that site) have been reported, but as these must almost inevitably misrepresent the concentrations at which the metals are "available" to fishes (and the exposure involved) any correlation with fish distribution is likely to be largely an imaginary one. At very best it will be a poorly defined one. Consequently, in the present state of our knowledge, there seems to be little point in attempting to consider here heavy metal concentrations reported for fresh waters in relation to the presence of fish except to put broadly into context something of the problems they present in the field.

Because fishes evolved in waters containing trace levels of all of the heavy metals, the physiological systems developed were those able to tolerate the concentrations at which these metals were present and mechanisms for dealing with greater-than-physiological levels (particularly of the non-essential rarer ones) in water did not develop (Schroeder and Darrow, 1973). Similarly, it ought to be pointed out, that even though accumulation of the heavy metals occurred in the tissues - all organisms chemically reflecting the composition of their environment (including their food) to some degree (O'Dell and Campbell, 1970; Hoekstra 1972) - the levels reached in the tissues of fishes, before the advent of man's metallurgical activities, even in the absence of any homoeostatic control, must self-evidently have been such as to be harmless, or at least largely tolerable. {Sometimes it seems to be suggested that some substances accumulate while others don't, and that those that accumulate are harmful. However, it should be recognized that all living things are in one sense "accumulations" of chemicals and it is only when substances, such as heavy metals, are absorbed at rates faster than those at which they can be excreted, and which are more or less constantly in the environment at levels significantly above "natural" levels, that they are likely to exceed tolerable levels in the tissues and be harmful. This is just as true for essential metals as it is for non-essential ones (Schroeder, 1965).}

It is generally recognized that non-essential metals in tissues are present as contaminants without significant function, the level of which is externally controlled (Liebscher and Smith 1968). Now the normal routes of access of heavy metals to fish are (a) via food and (b) via water. Any "potential" toxic impact via the gastro-intestinal tract of the various heavy metals typically found, and often abundantly so, in food, is likely to be minimal in most cases and can be discounted because of the discrimination, both active and passive, against absorption which must occur, as is known for mammals, at that site (Davis 1972). The active (homoeostatic) component of this discrimination must in fact have arisen early in evolution in order to prevent organisms poisoning themselves during normal feeding. (Metal-poisoning via food can nevertheless still occur should metal levels be excessively high.) In contrast with the gut, however, the gill did not need to "evolve" a discriminatory function against heavy metals, which normally were, and are, present only at low concentrations in fresh waters, and it is via this route, therefore, that uptake of metals is potentially the most dangerous, particularly, as once in the bloodstream the metals initially by-pass the liver (in contrast to metals entering via the gut) and any detoxification processes which are available there. It has been amply demonstrated with radionuclides that fishes absorb heavy metals available to them in solution in a water, and the critical situation for fish here, as far as hazard is concerned, is paralleled by that for man when exposed to heavy metals in the atmosphere. In the case of fishes, the position is perhaps a little more favourable than for man, in that other metal "diluents" are available in water, and, in particular, that the rate of absorption appears to be considerably mediated by the homoeostatically-controlled element calcium, typically an abundant metal in many waters. However, because of this aspect, and other physiological phenomena involved (Hogben 1971) then I would suggest that we are not necessarily likely to resolve toxicity problems in terms of heavy metal chemistry alone, because of the over-simplification that this involves, no more than can the pharmacologist resolve drug activities in that way, a point which Barlow (1964) makes, although chemistry probably offers the most rational approach to the problem.

The importance of the calcium levels in a water to fish is emphasized by the observed toxicity of even relatively low levels (chemically) of heavy metals in soft acid waters, where conditions tend to favour maximum availability of the metals and minimal competition for gill-membrane ligand sites from calcium. Levels and rates of uptake of radionuclides of several heavy metals have been found to be inversely related to the calcium concentration in the water. (In soft waters high abundances of suspended solids, to which heavy metals become adsorbed, and organic complexing compounds, are probably the most important ameliorating factors.) Because of these considerations, therefore, should the levels of available heavy metals be increased, even by only a relatively small amount in such waters, then dangerous conditions for fish can be created.

It has been stated that toxicity of a trace metal is inversely related to the concentration at which the metal is found in sea-water (Schroeder 1973), provided that its atomic structure is such as to combine readily with organic ligands in tissues. Nevertheless, it is not consequently then the rarest heavy metals which currently cause concern in fresh waters, although

that is not to say that they might not in the future. Rather the problems arise from the somewhat more abundant heavy metals which man finds it reasonably convenient to extract and concentrate for his own purposes. In so doing, of course, he frequently inadvertently also concentrates and liberates some of the rarer metals geochemically associated with the desired metal, and Dr. Zitko has recently drawn attention to problems from thallium, arising from the processing of base ores (Zitko et al, 1975).

While it is not possible to generalize about the rates or degree of increase of heavy metals occurring in fresh waters, man is having a considerable effect here, both by direct and by indirect (i.e. atmospheric) discharges. Transfer by atmospheric routes is an important aspect which water control agencies tend to overlook. The rates at which man is mobilizing metals are much greater than those at which geochemical processes do so, and Ketchum (1972) gives some estimates of this (Table 2). Those heavy metals with the greatest utilization, and therefore with the widest distribution at relatively high levels, are the ones, particularly if they are highly soluble, most likely to give rise to problems with fresh water fishes. In reviewing contamination from all sources the US Council of Environmental Quality (1971) listed arsenic, barium, cadmium, chromium, copper, lead, manganese, mercury, selenium, vanadium, and zinc as elements of possible environmental concern. If we also added nickel then this forms a useful starting list for substances potentially likely to give rise to hazards for fish. But any heavy metal could, of course, give rise to localized problems if added to a water at sufficiently high concentrations and in environmental monitoring the need to search for abnormal abundances of rarer metals should not be overlooked. And a point which our discussions overlooked yesterday, but which it is absolutely vital to consider, is that such problems need not necessarily be associated directly with availability of the metal to fish from solution.

Table 2. Rank Order Rates of Mobilization of Materials

Element	Geological rate (G) (10^3 metric tons)	Man-induced rate (M) (10^3 metric tons)	Ratio M/G
Sn	1.5	166	110
Sb	1.3	40	31
Pb	180	2330	13
Fe	25000	319000	13
Cu	375	4460	12
Zn	370	3930	11
Mo	13	57	4.4
Mn	440	1600	3.6
Hg	3	7	2.3
Ag	5	7	1.4
Ni	300	358	1.1

The harm caused to a particular fish species of concern may well arise indirectly, originating in effects of the metal on some component of the biota other than that fish species. Hence, fundamentally in environmental management, it is ecosystems that should be studied (and not single species), concentrating on any biotic components which appear to be being affected and on changes in the input and turnover of energy in a system. In this respect, while information on the concentrations at which the various chemical species (particularly the ionic form) of any given metal are present may be of interest it seems to me that a knowledge of the total load discharged is also of very great importance and must be defined. If we are to make any proper (i.e. true) assessment of impact on a system, then we must know what these loads are, by what route and in what chemical forms a metal moves though the different components of a particular ecosystem, at what concentrations the metal is present in these different components, the rates at which it is mobilized and transferred, and the sites of greatest accumulation.

However, as is well-known, and the reason for our meeting here, it is not the total concentration at which a metal is present in a water that is of direct concern, with regard to any particular organism, but the concentrations at which forms available to that organism are present. Determining these raises great problems. Nevertheless it is essential to do so. Toxicologically it is almost as meaningless to refer to a metal by its elemental name (e.g. the toxicity of copper) as it would be simply to refer to it in general terms as "a heavy metal" - although it does perhaps focus attention on a slightly more restricted group of chemical forms for consideration. But the variation in toxicity within that group can be extremely great. Attention has been turned therefore in recent years in many laboratories towards the often very difficult problem of determining the forms in which a particular heavy metal may be present when one of its soluble salts is added to water, as well as to the abundances of these forms in waters of different qualities. Most of the studies, both toxicological and chemical, which we have made at the Water Research Centre (Stevenage) - previously the Water Pollution Research laboratory - in relation to chemical availability have been concerned with copper and, to a lesser degree cadmium and zinc - so that it is largely only studies on these metals to which I can personally refer.

One of the difficulties involved here, particularly when considering "natural" surface waters, is that of adequately sampling the water. In addition, it does not seem to be sufficiently well appreciated that when analysing for trace levels of metals then specially-designed laboratories and operational procedures are absolutely essential (Schroeder, 1973), if valid results are to be obtained. (Hamilton et al (1972) in one of a series of excellent papers on trace elements thoroughly discuss these problems.)

As we have heard, in the papers delivered so far, metals are present in natural waters both in the ionic state and as soluble and insoluble complexes, both inorganic and organic, these latter potentially varying greatly from water to water, depending on the particular ligands available and on the stability constants of the complexes. Furthermore, following the addition of soluble salts to water, adsorption of the metals onto suspended solids also takes place. These processes can effectively reduce to a variable, but often large extent, the availability of any given total concentration of metal.

This "removal" is not of course an instantaneous process, so that when fishes are placed for testing into freshly-prepared "solutions" of heavy metals, they are likely to be exposed to changing concentrations of the various forms in which a metal can be present rather than to the uniform, stable conditions which a basic test system requires. How fluctuations affect organisms is another problem altogether, but a real one. In situations where solutions are only changed batchwise after long intervals (e.g. 24h) then marked changes in chemical forms and their concentrations, as well as in total concentration of chemicals in solution are likely to occur between changes. Even in constant-flow systems similar phenomena occur but an equilibrium system will eventually be established. Of course, whether or not this reflects to any reasonable degree the equilibrium states for a particular metal in any particular surface water is open to question - and to which part of a river it might correspond in relation to distance from an effluent discharge site, a matter of real importance, must always be something of a problem. This is an important aspect which needs to be explored further. In static tests, reduction in metal concentration must inevitably occur between solution replacements, even if only from sorption of the metal by the fish, and the rate of loss from the water needs therefore to be investigated in order to define replacement rates and the limits within which test conditions can be defined as being acceptable.

At the Water Research Centre we try to make all of our studies under constant-flow conditions, but because this is not always possible, particularly in tests with industrial wastes and with suspended solids, we have looked at the rates at which metals are lost from water in order to establish at what time after preparation of a solution not being renewed continuously the system is sufficiently stable for fish to be transferred to it.

As already stated, much of the heavy metal load in natural surface fresh waters is associated with suspended solids and studies on heavy metals were therefore made initially in the presence of suspended solids. The first problem which we faced was that of setting up a stable suspended-solids system, and this involved using tanks each containing a rotating paddle beneath a perforated false base, which served to maintain the suspension. Initially kaolin was tried as the solid but this became totally precipitated with 24 h. Gravel washings of < 20 microns were then tried but precipitation again occurred after the metal was added. However, by addition of organic solids (humus-tank solids obtained from a sewage plant treating a detergent-free domestic sewage) to give a concentration of 10 per cent of the solids it was found that the precipitation problem using gravel solids was overcome and initial investigations were made in such a system (Brown et al 1974). It was then found, however, that the 48-h LC50 of zinc (Department of the Environment 1971) and that of copper, was the same at a suspended-solids concentration of 50 mg/l, no matter whether the solids alone were a mixture of organic and inorganic materials or were totally organic, and therefore in subsequent tests, for convenience of preparation, organic solids alone were used. In tests with zinc it was then shown that toxicity of a given concentration of the metal decreased as the concentration of solids increased. It was also found early in

the investigation that with zinc, copper, and cadmium, for example, the concentration of soluble metal (defined as that passing through a 0.45 µm average pore-size membrane filter) fell quickly after preparation of the mixture. Zinc, copper, nickel, and hexavalent chromium appeared to reach equilibrium within about 1 h. but the concentration of soluble cadmium continued to fall after this period, particularly over the next 5 h. (A similar decrease was found with copper, at pH 8.0 - but a much lesser one at pH 7.45 - even in the absence of suspended solids. The level of soluble zinc remained constant at pH 7.45 but at pH 7.8 continuously declined for some 16 h after preparation of the solution) (Department of the Environment 1971 b). Consequently in the tests with solids, fish were not transferred to freshly-prepared mixtures until after 1 h had elapsed. In the initial investigations in this area, then, zinc and copper, as already stated, were found to be less toxic in the presence of solids, nickel appeared equally as toxic, and cadmium appeared to be more toxic (the 48-h LC50 being four times less than when solids were absent). Because of this last finding a further series of tests were made with cadmium, in the presence and absence of organic ("humus tank") solids at a concentration of 25 mg/1. In these tests it was found that in contrast to the initial tests, no difference in toxicity was evident under the two test conditions. This draws attention to the conflicting situations which might occur in studies on natural waters. However, because of the problems associated with copper, and its general presence as a pollutant in many waters, as well as the very variable results which had been obtained with this metal at our Laboratory over the years, it was decided to concentrate our efforts on copper, rather than on cadmium.

Firstly, as described in Brown et al (1974), it was confirmed that in the presence of solids the overall toxicity of a given concentration of copper was progressively reduced as the concentration of solids increased. However, in terms of measured soluble copper toxicity apparently increased, as the solids concentration increased. This is an aspect we have not studied further.

Next we looked at the effects of some of the other classes of complexing materials commonly present in 'natural' waters, polluted and unpolluted, on the toxicity of copper. (This work went on coincidentally with studies being made by Mike Stiff of our Laboratory into the chemistry of copper in water and whose investigations served to "crystallize" many of the problems).

First we look at the effects of a good quality sewage effluent and these tests showed that in the presence of 2 mg Cu/1 survival time of rainbow trout increased dramatically as the concentration at which the sewage effluent was present increased. There is little point in making much of this observation, because a multiplicity of factors were possibly involved. Nevertheless, the effect was quite impressive.

We had, earlier on, made tests with the chelating compound NTA in the course of looking at the effects of this substance on the availability of various metals to aquatic biota and this work had confirmed John Sprague's observations with Salvelinus in soft water that a given concentration of

copper was much less toxic in the presence of NTA than in its absence (Shaw and Brown 1974). Stiff (1971) identified amino acids and "humic substances" as common classes of complexing agents in surface waters, and again, it was found that in the presence of either of these two types of compounds toxicity of a given total concentration of copper was reduced. The data for the amino-acid glycine showed that a linear relationship existed between the 72-h LC50 of copper and the concentration of glycine (at least up to 10 mg/l), and that at 10 mg/l the 72-h LC50 was about seven-times greater than it was when glycine was absent. Survival times only were compared in the presence of humic substances but again toxicity was reduced as their concentration increased.

However, the simplest product of chemical speciation is the ion, and the fact that all evidence in pharmacology and toxicology has shown that it is the metal ion which is the important form indicated this as being the most reasonable starting point for the definition of the toxicity of heavy metals. Because of the great problems which occur, as already indicated, when organic ligands are present in a water, tests were made in a hard, untreated groundwater, in which the only complexing agent was effectively carbonate. Tests were made at two pH levels which theoretically should, and in practice did, give a measurable ten-fold difference in cupric ion concentration. No difference in toxicity was observed in the two solutions and it was therefore concluded that toxicity was probably related to the total concentration at which soluble copper was present in the form both of the ion and of soluble copper carbonate, and that both forms were effectively of equal toxicity (Shaw & Brown 1974). This finding was somewhat surprising to us, and difficult to explain in view of the known effects of metal ions, but on the assumption that the findings had something to offer they were published. Nevertheless, in the original draft an alternative explanation of the findings was initially proposed but then was eventually omitted because of its speculative nature. It is, however, perhaps opportune now to quote this explanation which was as follows:- "Much of the response of living tissue to heavy metals has been shown to be related to reactions occurring at the cell membranes, rather than within the cell (Rothstein 1959). These membranes contain many ligands capable of binding metals and it may be that because of the greater competition for these ligands which would occur between cupric ions and the more abundant protons which were present in the solution at the lower pH (Sigel and McCormick 1970),, the simplest explanation of our observations (that is, that toxicity of the cupric ion varied with pH) being the right one. This fitted the observations and seemed a reasonable hypothesis because it is known that at the cell membrane exchange between ligand protons and heavy metal ions takes place readily, a simple relationship between the two having been demonstrated (Bjerrum 1950). This may not, however, be the full explanation. Not all of the binding sites at the cell membrane are of functional importance physiologically. As it was found in the test at the higher pH that a cupric ion concentration as low as about 4 µg/l was acutely lethal to 50 per cent of a test batch of fish, indicating this as being approximately the 48-h LC50 of cupric ion for rainbow trout, then ionic copper at concentrations greater than this must be in excess of that required for this effect to occur. If this is so, then at the lower test pH, where concentrations of both cupric ions and protons were greater, competition between the two would be unlikely, because of the excess of the cupric ions present, to be capable of preventing

all of the functional ligand sites eventually being complexed and "poisoned" by copper, although they might well delay onset of acute lethal effects. The data obtained (Tables 1 & 3 in Shaw & Brown (1974) indicated that such a delay did in fact occur. Thus, for example, while at a cupric ion concentrations of 50 µg/l and a pH of 7.5 the median period of survival was less than 300 min, at pH 6.5 at this same concentration the median period of survival was 1000 min. Having now heard Bob Andrew's lucid paper on copper toxicity, which unfortunately, there has been too little time to digest, it seems that while these unpublished speculations were probably correct, our published conclusions were not, and I am happy to accept that, as Bob has shown, and as Zitko et al (1973) and Pagenkopf et al (1974) have suggested, cupric ion is probably the most significant toxic form. This agrees with toxicological expectations. However, in terms of practical environmental monitoring, this information does not really resolve our problem. The data of Stiff (1971 a, 1971 b) show that there are analytical limitations and that in natural (carbonate) surface waters ionic copper is not measurable much below total copper concentrations of 100 µg/l (although in solutions where adjustment of ionic strength can be made, using potassium nitrate, then the electrode response is Nernstian down to a total copper concentration of 50 µg/l). But even in situations where adequate measurement can be made then our data (Shaw & Brown 1974) showed that the 48-h LC50 - effectively the 50 per cent asymptotic concentration - varied with pH, and that in a water with a total hardness of 100 mg/l, as $CaCO_3$, was about 4 µg/l at a pH of 7.5 but 40 µg/l at a pH of 6.5. This finding contrasts with that of Pagenkopf et al (1974) who from a theoretical approach concluded that acutely lethal concentrations were the same irrespective of pH value, even though their data, the formal units of which I do not unfortunately understand, show a trend with pH similar to that described here. Our findings indicate, therefore, that even when it is possible to measure the concentration of cupric ion, this on its own is not sufficient to describe toxicity and the concentration measured must be put into context by reference to the pH of the water in some situations. There seems to be much to commend equilibrium calculations being made as an approach here, following the model used by Pagenkopf et al (1974).

While at first sight, the conclusion arrived at here seems somewhat at variance with observations that in a hard water, where pH values tend to be high, a given total concentration of copper is always found to be less toxic than that same concentration is in soft water, where pH values tend to be low. This is not really so. Here we are looking at the toxic species, ionic copper, itself. In hard waters chemical availability of the ion tends to limit toxic concentrations whereas in soft waters it is suggested that physiological availability at the gill membrane is limiting.

These observations may serve to explain to some extent why fish can sometimes unexpectedly be found present in field situations in soft waters, where estimates of copper toxicity based on a knowledge of total copper, or of soluble copper, would make this seem unlikely. A similar situation might also exist with other heavy metals. In different natural surface waters I had further speculated that toxicity problems were likely to be complicated by differences in calcium concentrations which would give rise to different degrees of competition between calcium ions and cupric ions for binding sites,

but with copper having the greater affinity for the ligands. While I would still expect this to be so, it seems from Bob's findings that this may not in fact be a large effect.

Because of these considerations and of the non-quantifiable nature of the abundance of the different membrane ligand sites, as well as the non-specificity of both heavy metals and ligands, I feel that it is unlikely that any more precise definition of toxic relationship is possible, particularly when several heavy metals at elevated levels are simultaneously present. This is probably particularly true of field situations.

As far as cadmium is concerned, we are still looking at this metal. At levels of 1 to 2 µg/l we have observed in chronic tests (months) harmful effects on reproduction in rainbow trout and at a higher level (8 µg/l) histopathological changes. At these levels, in our hard test water, it appears that virtually all of the cadmium is present in ionic form.

In drawing to a close I would like to suggest that even in the simplest of systems, where only a single heavy metal appears to be of concern, and particularly a non-essential one, we probably still need to know the abundances with which other heavy metals are present in available forms, and perhaps also the concentrations already present in a fish. Chronic exposure of rainbow trout to cadmium, and to chromium, for example, we have found causes loss of copper from the liver and elsewhere, and some of the symptoms observed for cadmium poisoning (e.g. loss of calcium) may well be those associated with cadmium-mediated cellular copper deficiency (Mills, 1974). It is known from studies with mammals that a preliminary "loading" of the tissue with zinc can offset toxic effects of cadmium. I could foresee therefore that a non-harmful loading of copper or of zinc in the tissues of a fish might well offset at least transient exposures to cadmium and in some cases, that moderate additions of copper or zinc to a water might be beneficial with regard to cadmium contamination.

However, when it comes to practical problems of environmental management, more and more over the last two or three years I have begun to suspect that we might be in danger of out-smarting ourselves. Nationally and internationally, on the basis of tests with single substances and single species, and then often only acute lethal tests, so-called "acceptable" levels of chemicals in water are being defined, even though we know nothing of the effects of mixtures, particularly under conditions of chronic exposure, nor of fluctuating concentrations. And we totally ignore possible indirect effects of poisons even on the single species tested. So what are our limits worth? We are perhaps acting more with enthusiasm than with reason. If we do not know the answers to the real problems then, we should be prepared to admit and accept this and see where we can go from there. We might, for example, always reduce the observed acute effective level of a poison by some arbitrary factor, such as the "application factors" recommended for use in the USA, simply in order to be cautious, but is this approach good enough? As became evident in yesterday's discussion, what we are really trying to resolve in many situations, simply by chemical analysis of a water, are the effects of various and varying

mixtures of chemicals (including heavy metals in their several chemical forms). When present in the varied and variable solutions and suspensions of natural waters, on a large number of biochemically complex and very variable reacting systems - the whole thing is of such complexity that in reality it is impossible to determine a base line from which to start. Consequently, when it comes to assessing the effects of varying mixed industrial and domestic wastes we are not even in a position fully to describe such wastes chemically let alone their biological and ecological effects. But even if we could we would still not be able to predict their effects (Schroeder 1973).

If we are prepared to accept that this is so, then it seems to me that we are in exactly the same position as that which forces the pharmacologist to assess drug potency by bioassay - and that is, that he is unable to do so by chemical or physical methods of analysis. Again if this is so, then it also seems to me we are inevitably forced to do the same thing; namely, to treat each waste and each water on an individual basis, and to set standards from relevant toxicity tests - until chemical investigation of each effluent perhaps allows us to define observed toxicity in terms of a causative chemical factor or factors. But because chemical composition of an effluent can change, and in any chemical analytical programme we must always prejudge what might be present to be looked for, toxicity-testing must be a continuous on-going procedure.

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ZINC SPECIATION AND TOXICITY TO FISHES

CHAPTER 4

Gordon K. Pagenkopf
Department of Chemistry
Montana State University

ABSTRACT

A survey of the literature indicates there are at least twelve technical communications that deal with the toxicity of zinc to fishes and also contain sufficient analytical data to permit the calculation of zinc speciation. These studies deal mainly with Fathead Minnows, Bluegills, and Rainbow Trout. Several models have been developed in an attempt to ascertain which zinc species are toxic. These are equilibrium models which include homogeneous and heterogeneous phases and consider a variety of different chemical species. The discussion will focus on interpretation of species distribution and the observed toxicity.

Zinc toxicity to fishes has been the subject of a sizeable research effort during recent time. The studies have investigated effects of pH, water hardness, dissolved oxygen and temperature in attempt to elucidate what factors influence the toxicity (1), (2), (3). None of these studies has considered the speciation of zinc in the bioassay test waters.

The subject of this report is a discussion of zinc speciation in a variety of bioassay experiments and a model that considers two extremes of zinc solubility is presented. Analysis of water quality parameters and total zinc concentrations indicates that many of the test waters are supersaturated with respect to zinc carbonate. One extreme of the model is that all of the $ZnCO_3$ solid phase has formed, however, the particle size is small and thus precipitation has not occurred. The other extreme assumes that no solid phase is formed. A similar model based on chemical equilibria has been presented for the interpretation of copper toxicity to fishes (4).

EXPERIMENTAL SECTION

Bioassay Data

A survey of the literature dealing with toxicity of zinc to fishes indicates that several studies contain sufficient analytical data to permit calculation of zinc speciation. The data needed includes pH, alkalinity, hardness (calcium and magnesium), sulfate concentration and LC_{50} zinc concentration at some time, usually 96 hours. There are a sizeable number of reports that don't include all of the above data. Some of these have been included by making reasonable assumptions, however, a fairly large portion could not be used. With exception of two studies all data are for flow-through bioassays. Data is available for fathead minnows, bluegills, rainbows, goldfish, guppies, zebrafish, and Atlantic salmon.

Heterogeneous Phase Model

The bioassay studies can be divided into two groups: (1) studies unsaturated with respect to $ZnCO_3$ and (2) studies saturated in $ZnCO_3$. One study has a high pH, 8.6, and is also saturated with respect to $Zn(OH)_2(s)$. The species considered in the model are presented in TABLE 1.

TABLE 1. Chemical Species Considered in Test Waters

Zn^{2+}	Ca^{2+}	CO_3^{2-}
$Zn(OH)^{+}$	$CaHCO_3^{+}$	HCO_3^{-}
$Zn(OH)_2$ (Sol)	$CaCO_3$ (Sol)	$H_2CO_3 \cdot$ (aq)
$ZnCO_3$ (Sol)	$CaSO_4$ (Sol)	
$ZnHCO_3^{+}$	Mg^{2+}	SO_4^{2-}
$ZnSO_4$ (Sol)	$MgHCO_3^{+}$	
	$MgCO_3$ (Sol)	
	$MgSO_4$ (Sol)	
$ZnCO_3$ (Solid)		

The equilibria and equilibrium constants utilized in the calculations of species distribution are listed in TABLE 2. The constants are for 25°C and an ionic strength of 0.005 M. Both of these are representative of temperature and ionic strength of the test waters.

TABLE 2. Chemical Equilibria and Constants

Reaction	Log K	Reference
$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$	-6.34	5
$HCO_3^- \rightleftharpoons H^+ + CO_3^{2-}$	-10.17	5
$Zn^{2+} \rightleftharpoons Zn(OH)^+ + H^+$	-9.03	6
$Zn^{2+} \rightleftharpoons Zn(OH)_2(Sol) + 2H^+$	-16.77	7
$Zn^{2+} + CO_3^{2-} \rightleftharpoons ZnCO_3(Sol)$	2.74	a
$Zn^{2+} + H^+ + CO_3^{2-} \rightleftharpoons ZnHCO_3^+$	11.04	a
$Zn^{2+} + SO_4^{2-} \rightleftharpoons ZnSO_4(Sol)$	2.05	8
$Ca^{2+} + H^+ + CO_3^{2-} \rightleftharpoons CaHCO_3^+$	11.30	9
$Ca^{2+} + CO_3^{2-} \rightleftharpoons CaCO_3(Sol)$	2.94	9
$Ca^{2+} + SO_4^{2-} \rightleftharpoons CaSO_4(Sol)$	2.05	9
$Mg^{2+} + H^+ + CO_3^{2-} \rightleftharpoons MgHCO_3^+$	11.20	9
$Mg^{2+} + CO_3^{2-} \rightleftharpoons MgCO_3(Sol)$	3.14	9
$Mg^{2+} + SO_4^{2-} \rightleftharpoons MgSO_4(Sol)$	2.10	9
$ZnCO_3(s) \rightleftharpoons Zn^{2+} + CO_3^{2-}$	-10.22	10

[a]These values are predicted from comparable values for calcium and magnesium.

Calculations

Species distributions for the test waters not saturated in $ZnCO_3$ were calculated with the COMICS program (11) adapted for use with the MSU Sigma 7.

Calculations for the heterogeneous phase system are more involved and involve assumptions that will be discussed subsequently. Analytical concentrations of total zinc, total inorganic carbon and hydrogen ion in many of

the test waters are such that one predicts that $ZnCO_3$ will precipitate or at least form a solid suspension. Since the concentrations of total zinc are those observed in the test waters it is assumed that zinc carbonate suspensions are present. The particle size is not large enough to result in precipitation during the water resident time in the bioassay test tanks. Also the activity of the suspended solid phase is considered to be unity. Recent studies indicate that $Zn(OH)_2(s)$ precipitates more rapidly than $ZnCO_3$(12). These experiments were conducted with solutions of much higher total carbon and zinc and thus it isn't known how applicable they are to this consideration.

In the saturated systems the total inorganic carbon is a factor of ten larger than total zinc concentration. The majority of the zinc will be present in the solid phase and thus effectively reduces the soluble inorganic carbon concentration. The procedure for the calculation of the species distribution is summarized below:

1. Adjust total inorganic carbon downward by the amount of zinc present.

2. Calculate the concentrations of all soluble species, except zinc species, using COMICS.

3. Calculate Zn^{2+} concentration using K_{sp} and carbonate concentration from step 2.

4. Calculate other soluble zinc species using output of steps 2 and 3.

5. Sum the concentrations of all soluble zinc species.

6. Calculate zinc in solid phase by difference.

Input Parameters

Chemical speciations was calculated by using six input concentrations: (1) hydrogen ion, (2) calcium, (3) magnesium, (4) sulfate, (5) total zinc, and (6) total inorganic carbon. The LC_{50} value for 96 hours was used for total zinc. The pH of test waters was less than 8.3 (except one) and thus it is assumed that alkalinity is due to bicarbonate ion. Total inorganic carbon was calculated using the expression:

$$\text{Total Inorganic Carbon} = \text{alkalinity (eqv/l)} \quad \frac{K_{a1} + [H^+]}{K_{a1}}$$

Errors

The pH value of the test waters have standard deviations of $\pm$ 0.15 units which amount to 41 percent. The LC_{50} values for duplicate tests vary in many cases by 30 percent or more. The assumptions made in the calculations of species distribution for the heterogeneous systems have an uncertainty of about 10 percent and thus are well within the uncertainty introduced by the variation in pH.

Precipitation

The general technique that designates the presence of a precipitate is filtration through a 0.45 μ filter. The question that arises in conjunction with these studies and many others is how much solid can be formed and still pass through a 0.45 μ filter? If a molecule has a cross sectional diameter of ten angstroms (this is large for $ZnCO_3$) then an aggregation of 10^2 molecules could probably pass through the filter. How rapidly the aggregates form hasn't been well-established at this point, however, the rate may be fairly fast as evidenced by the formation of $BaSO_4(s)$ in the turbidimetric determination of sulfate. Of course the rate will be very dependent upon mechanical agitation and ionic medium. In these studies as much as 20 mg of $ZnCO_3$ is predicted to be suspended per liter of solution. With small particle size this amount of material would probably be non-detectable visually.

RESULTS AND DISCUSSION

A majority of the zinc toxicity to fishes deal with Fathead Minnows (13-17), however data is available for Bluegills (17, 18, 23), Goldfish (17), Guppies (17), Rainbows (19), Salmon (20, 21), and Zebrafish (22). The data from these studies were utilized to calculate the species distribution for the test waters. TABLE 3 lists the input concentrations and calculated soluble and solid zinc concentrations. The solid phase is expressed as a molar concentration which is not strictly correct, however, it is convenient for comparison purposes. TABLE 4 lists the zinc species concentrations and TABLE 5 lists the calcium and magnesium species concentrations. A code number has been assigned to each study for cross-referencing. The letters in the code designate fish type. The results for the Fathead Minnows will be discussed first followed by those for the other fish.

The studies with Fathead Minnows can be divided into three groups depending upon the nominal hardness of the test waters. Studies F-8, 9, 10, 11, 16 and 17 investigate the influence of pH at a nominal hardness of 50 mg/l as $CaCO_3$. Figure 1 shows a plot of zinc concentration vs. pH for these studies. Below pH 7.2 total and soluble zinc are the same. At pH values above 7.2, the system becomes saturated in $ZnCO_3$. The upper line in this region indicates zinc total and the lower line is soluble zinc.

Figure 2 is plot of zinc concentration vs. pH for a nominal hardness of 100 mg/l as $CaCO_3$, studies F-4, 5, 12, 13, 18, 19, 22, and 23. At these levels of total inorganic carbon, the system becomes saturated in $ZnCO_3$ when the pH is greater than 6. The upper line indicates total zinc whereas the lower one is the predicted soluble zinc concentrations. When comparing Figure 2 to Figure 1, it is noticed that an increase in hardness is accompanied by an increase in total zinc in the system. This is particularly evident in the lower pH studies. Also the influence of pH is more pronounced at the higher hardness level.

Figure 3 is a plot of zinc concentration vs. pH for nominal hardness of 200 mg/l as $CaCO_3$. The characteristics of this plot are very similar

TABLE 3. Input Parameters, Calculated Soluble Zinc and Predicted Solid Zinc for Zinc Toxicity Studies

Code No.	Ref.	10^4C_T	10^4Ca_T	10^4Mg_T	10^4SO_{4T}	10^5Zn_T	pH	10^4C_T adj.	10^6Zn (sol)	10^5Zn (s)
F-1	13	34.4	13.5	6.20	3.64	15.3	7.70	32.9	6.22	14.7
F-2	13	34.4	13.5	6.20	3.64	12.9	7.70	33.1	6.15	12.3
F-3	14	34.6	13.8	6.90	3.50	7.68	7.70	33.8	6.31	7.05
F-4	14	15.9	6.50	3.21	1.59	12.4	7.50	14.7	22.0	10.4
F-5	15	16.1	6.90	3.34	1.66	15.1	7.65	14.6	15.8	13.5
F-6	15	30.1	13.0	6.32	3.14	12.5	7.75	28.8	6.53	11.9
F-7	15	34.1	14.6	7.10	3.51	23.5	7.70	31.7	7.07	22.8
F-8	15	8.1	3.60	1.80	0.89	7.20	8.0	7.40	16.9	5.50
F-9	15	7.35	3.20	1.60	0.79	7.82	7.6	6.57	59.6	0.86
F-10	15	9.95	4.24	2.06	3.63	19.1	6.3	--	191.	--
F-11	15	8.53	3.63	1.76	3.54	21.1	6.1	--	211.	--
F-12	15	15.3	6.53	3.17	8.20	28.3	5.85	--	283.	--
F-13	15	16.2	6.90	3.37	7.23	38.2	6.00	--	382.	--
F-14	15	33.4	14.3	6.95	14.1	44.3	6.10	--	443.	--
F-15	15	32.8	14.0	6.81	13.9	54.3	6.10	--	543.	--
F-16	15	8.53	5.75	1.76	1.52	21.0	7.15	7.86	96.8	11.3
F-17	15	9.95	5.70	2.06	1.57	9.5	7.25	--	95.0	--
F-18	15	15.8	10.6	3.27	1.77	19.1	7.00	15.1	70.1	12.1
F-19	15	15.7	10.6	3.29	1.78	19.1	6.95	15.1	86.1	10.5
F-20	15	29.4	19.8	6.09	3.47	29.1	6.75	2.78	84.5	20.6
F-21	15	30.8	20.7	6.39	3.38	30.8	7.20	29.1	23.9	28.4
F-22	15	15.8	--	--	--	26.8	5.00	--	268.	--
F-23	15	15.8	--	--	--	9.50	8.60	--	4.48	9.35
F-24	17	3.21	1.20	0.60	0.03	1.33	7.5	--	13.3	--
F-25	16	10.2	--	--	--	1.33	7.55	--	13.3	--
B-1	18	5.76	2.82	1.62	--	4.67	7.55	--	46.7	--
B-2	17	3.21	1.20	0.60	0.30	8.23	7.50	--	82.3	--
B-3	23	61.9	24.6	12.4	6.00	17.4	7.80	60.2	2.93	17.1
R-1	19	49.6	30.0	2.00	3.64	6.12	7.80	49.0	3.48	5.77
R-2	19	7.74	4.68	0.31	0.57	2.75	7.09	--	27.5	--
GF-1	17	3.21	1.20	0.60	0.30	9.83	7.50	--	98.3	--
G-1	17	3.21	1.20	0.60	0.30	1.94	7.50	--	19.4	--
Z-1	22	3.66	0.70	0.60	--	4.59	7.00	--	45.9	--
S-1	20	2.66	1.40	--	--	5.36	7.30	--	53.6	--
S-2	21	2.66	1.50	--	--	0.84	7.30	--	8.4	--

F = fathead minnow; B = bluegill; R = rainbow; GF = goldfish; G = guppies; Z = zebrafish; S = salmonid

TABLE 4. Calculated Species Distribution for Inorganic Carbon, Sulfate and Zinc

Code No.	10^4 H_2CO_3	10^3 HCO_3^-	10^6 CO_3^{2-}	$10^4 SO_4^{2-}$	$10^6 Zn^{2+}$	$10^7 ZnOH^+$	$10^7 Zn(OH)_2$	$10^7 ZnHCO_3^+$	$10^8 ZnCO_3$	$10^5 ZnSO_4$
F-1	1.41	3.23	10.9	3.00	5.53	2.58	2.36	1.32	3.31	1.86
F-2	1.41	3.24	11.0	3.00	5.48	2.56	2.34	1.31	3.31	1.84
F-3	1.38	3.16	10.7	2.85	5.63	2.63	2.40	1.32	3.31	1.79
F-4	0.94	1.36	2.91	1.43	20.7	6.11	3.52	2.08	3.31	3.31
F-5	0.67	1.37	4.15	1.49	14.5	6.04	4.90	1.47	3.31	2.43
F-6	1.05	2.71	10.3	2.59	5.86	3.29	3.96	1.18	3.31	2.42
F-7	1.29	2.96	10.0	2.84	6.04	2.82	2.56	1.32	3.31	1.92
F-8	0.16	0.72	4.85	8.42	12.4	11.6	21.0	6.58	3.31	11.7
F-9	0.34	0.62	1.66	7.52	5.20	18.9	14.0	2.38	3.31	43.3
F-10	5.18	0.47	0.06	3.33	184.	3.41	0.12	0.64	0.64	0.68
F-11	5.40	0.31	0.03	3.27	203.	2.38	0.05	0.03	0.47	0.74
F-12	11.5	0.37	0.02	7.24	261.	1.72	0.02	7.21	0.26	2.12
F-13	11.1	0.51	0.03	6.28	355.	3.32	0.06	13.3	0.67	2.50
F-14	21.0	1.21	0.10	11.2	390.	4.58	0.11	34.9	2.20	8.48
F-15	20.6	1.19	0.10	11.0	479.	5.63	0.13	42.1	2.66	5.90
F-16	1.05	0.68	0.65	1.40	93.2	12.3	4.22	4.66	3.31	0.15
F-17	1.08	0.88	1.05	1.43	90.9	15.1	4.88	5.90	5.26	0.15
F-18	2.68	1.23	0.89	1.53	67.6	6.30	1.15	6.18	3.31	0.12
F-19	2.95	1.20	0.72	1.54	83.2	6.93	1.12	7.40	3.31	0.14
F-20	7.67	1.97	0.75	2.70	80.5	4.23	0.49	11.7	3.31	0.24
F-21	3.46	2.50	2.68	2.61	22.5	3.34	0.96	4.16	3.31	0.07
F-22	15.1	0.07	0.00	1.60	26.8	0.00	0.00	0.00	0.00	0.00
F-23	0.00	1.53	55.0	1.60	1.09	4.10	29.3	0.17	3.31	0.00
F-24	0.21	0.30	0.64	2.93	12.6	3.73	2.15	0.28	0.45	0.00
F-25	0.40	0.99	0.23	--	12.6	4.20	3.30	0.92	0.16	--
B-1	0.33	0.54	1.29	--	44.1	14.6	9.43	1.76	3.13	--
B-2	0.21	0.30	0.64	2.91	78.2	23.1	13.3	1.73	2.74	0.02
B-3	1.94	5.58	23.8	4.33	2.47	1.46	1.67	1.02	3.31	--
R-1	1.59	4.58	19.6	2.73	2.97	1.75	2.01	1.00	3.31	--
R-2	1.16	0.65	0.54	0.54	26.8	3.08	0.69	1.30	0.80	0.02
GF-1	0.21	0.30	0.64	0.29	93.4	27.6	15.9	2.07	3.28	0.03
G-1	0.21	0.30	0.64	0.29	18.4	5.44	3.13	0.41	0.65	0.01
Z-1	0.66	0.30	0.20	--	45.3	4.23	0.77	1.01	0.50	--
S-1	0.26	0.24	0.32	--	52.2	9.72	3.53	0.92	0.92	--
S-2	0.26	0.24	0.32	--	8.2	1.53	0.55	0.15	0.15	--

F = fathead minnow; B - bluegill; R = rainbow; GF = goldfish; G = guppies; Z = zebrafish; S = salmonid

TABLE 5. Calculated Calcium and Magnesium Species Distribution

Code No.	10^3Ca^{2+}	$10^5CaHCO_3^+$	10^8CaCO_3	10^5CaSO_4	10^4Mg^{2+}	$10^5MgHCO_3^+$	10^8MgCO_3	10^5MgSO_4
F-1	1.27	3.03	7.62	4.27	5.70	1.97	8.59	2.15
F-2	1.27	3.05	7.66	4.27	5.70	1.98	8.64	2.15
F-3	1.30	3.05	7.66	4.16	6.36	2.16	9.41	2.29
F-4	0.63	0.64	1.01	1.02	3.10	0.45	1.24	0.56
F-5	0.67	0.68	1.53	1.12	3.21	0.47	1.84	0.60
F-6	1.23	2.47	6.97	3.58	5.87	1.70	8.35	1.92
F-7	1.38	3.03	7.60	4.39	6.57	2.08	9.10	2.34
F-8	0.33	1.73	0.87	3.09	1.61	0.12	1.07	1.70
F-9	0.29	0.14	0.27	2.48	1.45	0.10	0.33	1.37
F-10	0.41	0.26	0.02	1.54	1.97	0.10	0.02	0.82
F-11	0.35	0.15	0.00	1.24	1.69	0.06	0.01	0.69
F-12	0.60	0.30	0.01	4.88	2.89	0.12	0.01	2.69
F-13	0.64	0.44	0.02	4.51	3.11	0.17	0.01	2.46
F-14	1.25	2.04	0.11	15.7	6.02	0.78	0.01	8.48
F-15	1.23	1.97	0.11	15.1	5.92	0.75	0.01	8.18
F-16	0.56	0.28	0.20	0.89	1.72	0.12	0.15	0.30
F-17	0.55	0.66	0.51	0.89	2.00	0.19	2.91	0.36
F-18	1.03	0.94	0.47	1.77	3.16	0.42	0.36	0.61
F-19	1.03	0.92	0.41	1.78	3.14	0.40	0.31	0.61
F-20	1.89	2.77	0.78	5.74	5.77	1.22	0.60	1.96
F-21	1.97	3.66	2.91	5.27	6.01	1.61	2.23	1.97
F-22	--	--	--	--	--	--	--	--
F-23	--	--	--	--	--	--	--	--
F-24	0.12	0.05	0.06	0.04	0.60	0.02	0.05	0.02
F-25	--	--	--	--	--	--	--	--
B-1	0.28	0.20	0.31	--	1.61	0.09	0.29	--
B-2	0.12	0.05	0.07	0.04	0.59	0.02	0.05	0.02
B-3	2.23	9.23	29.2	10.8	10.8	6.46	35.5	5.89
R-1	2.79	9.48	30.0	8.53	1.80	8.85	4.86	0.62
R-2	0.46	0.41	0.22	0.28	0.31	0.02	0.02	0.02
GF-1	0.12	0.05	0.07	0.04	0.60	0.02	0.05	0.02
G-1	0.12	0.05	0.07	0.04	0.60	0.02	0.05	0.02
Z-1	0.07	0.03	0.01	--	0.60	0.02	0.02	--
S-1	0.14	0.05	0.04	--	--	--	--	--
S-2	0.14	0.05	0.04	--	--	--	--	--

F = fathead minnow; B = bluegill; R = rainbow; GF = goldfish; G = guppies; Z = zebrafish; S = salmonid

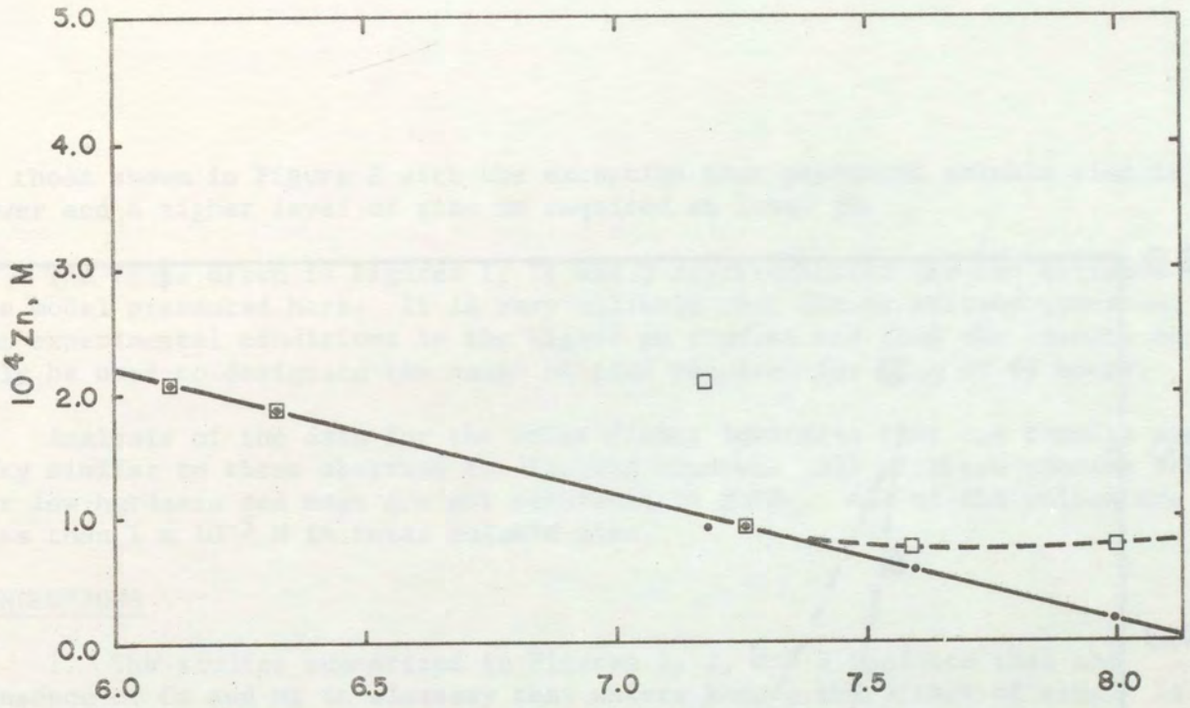

Figure 1. Toxic zinc concentrations as a function of pH for nominal hardness of 50 mg/l as $CaCO_3$, ● designates calculated soluble zinc, □ total zinc.

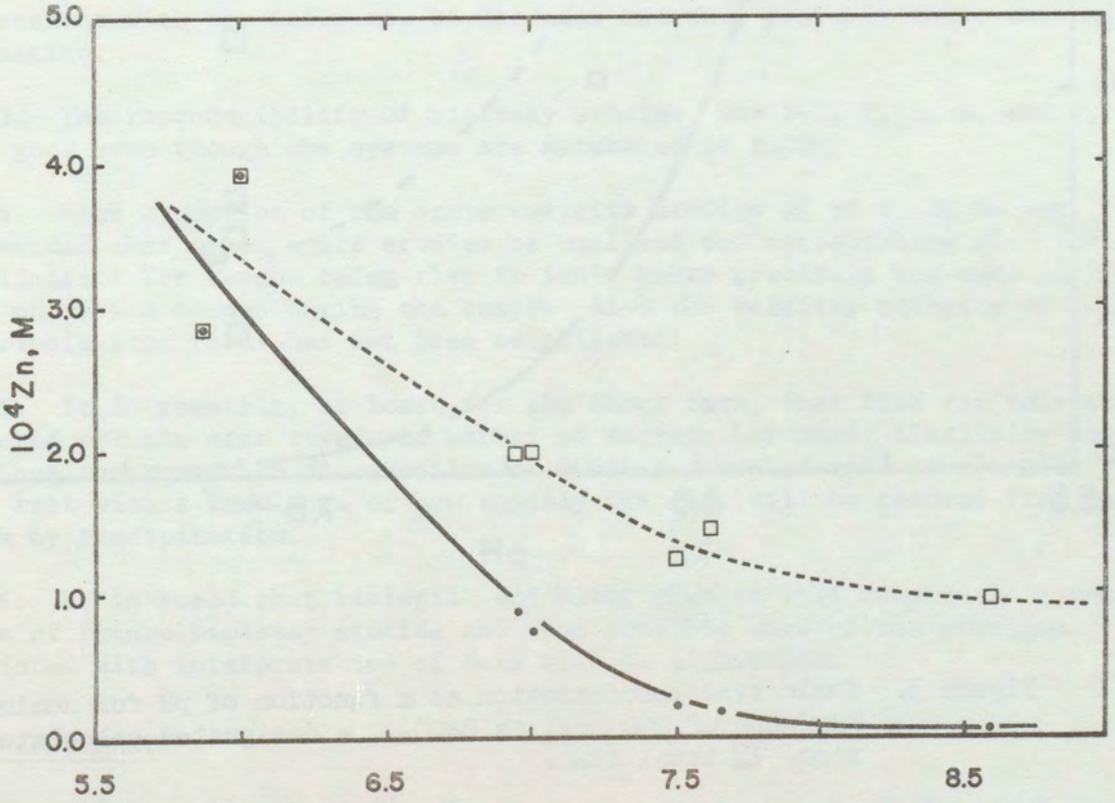

Figure 2. Toxic zinc concentrations as a function of pH for nominal hardness of 100 mg/l as $CaCO_3$, ● designates calculated soluble zinc, □ total zinc.

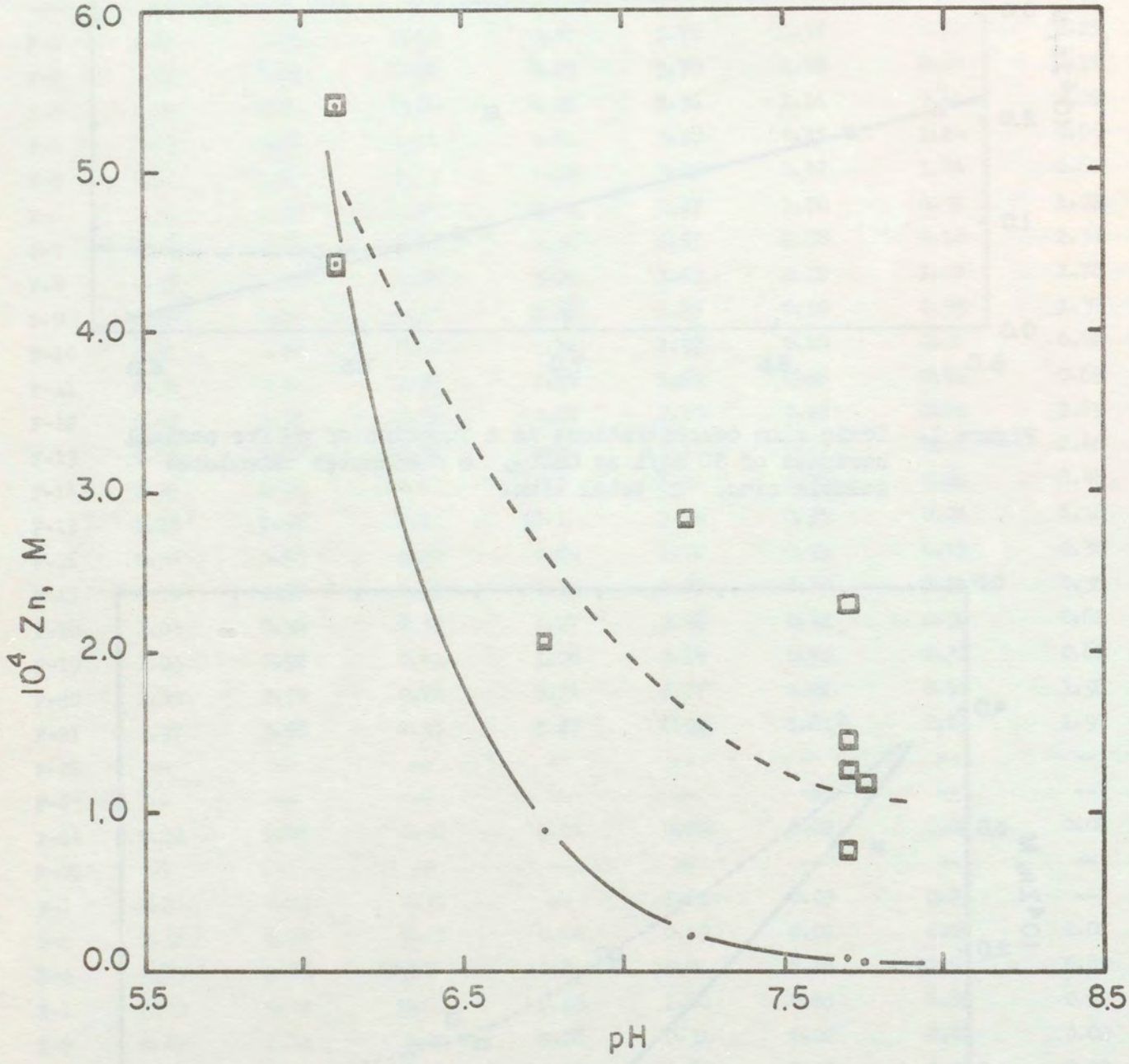

Figure 3. Toxic zinc concentration as a function of pH for nominal hardness of 200 mg/l as $CaCO_3$, • designates calculated zinc, ◻ total zinc.

to those shown in Figure 2 with the exception that predicted soluble zinc is lower and a higher level of zinc is required at lower pH.

The lines drawn in Figures 1, 2, and 3 have indicated the two extremes of the model presented here. It is very unlikely that either extreme describes the experimental conditions in the higher pH studies and thus the results can only be used to designate the range of zinc required for LC_{50} at 96 hours.

Analysis of the data for the other fishes indicates that the results are very similar to those observed for Fathead Minnows. All of these studies are for low hardness and most are not saturated in $ZnCO_3$. All of the values are less than 1×10^{-5} M in total soluble zinc.

CONCLUSIONS

1. The studies summarized in Figures 1, 2, and 3 indicate that the presence of Ca and Mg in bioassay test waters reduce the effect of zinc. It isn't known which metal is most effective in protecting the fish.

2. An increase in pH from 6-8 is accompanied by a sizable reduction in LC_{50} zinc concentrations. In most of the studies this change in pH is accompanied by an increase in the amount of solid $ZnCO_3$ potentially present in the test waters. It can be argued that the solid material is more toxic than soluble zinc and thus the decrease in total zinc is required. This is in disagreement with the influence of hardness and thus probably not a reasonable explanation.

3. The reproducibility of bioassay studies, see F-1, 2, 3, 6, and 7, is quite good even though the systems are saturated in $ZnCO_3$.

4. With exception of the acute toxicity studies at pH 6, it is not recommended that these acute studies be utilized for establishing zinc toxicity limits. The reason being that it isn't known precisely how much of the solid phase has formed during the tests. Also the relative toxicity of solid and soluble zinc forms has not been established.

5. It is possible, at least for the short term, that fish can tolerate predicted soluble zinc levels in waters of average hardness, alkalinity and pH. Thus the answer to the question of whether elevated zinc levels will be toxic rest with a knowledge of how rapidly the zinc will be removed from the system by precipitation.

6. It is hoped that biologist and water chemist will cooperate in the design of future bioassay studies and thus possibly some of the problems associated with interpretation of data will be eliminated.

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THE NEED TO ESTABLISH HEAVY METAL STANDARDS
ON THE BASIS OF DISSOLVED METALS

CHAPTER 5

P. H. Davies
Colorado Division of Wildlife,
Research Center
Fort Collins, Colorado 80521

ABSTRACT

Realistic and enforceable standards for heavy metals in natural waters can only be established if the toxic species of a particular metal is properly analyzed. This is particularly true of metals which form complexes, in varying degrees, depending on the water quality into which they are introduced. The toxicity of a particular metal can be determined irrespective of water quality when properly analyzed. This has been verified by long-term toxicity tests with lead and rainbow trout in hard and soft waters using atomic absorption (total lead) and pulse polarographic (dissolved lead) analyses. The chemistry of lead and its toxicity to trout are described. This work also demonstrates the failure of the application factor approach to predict safe heavy metal concentration in different types of water quality; when chemical analyses are based on total concentrations of metals in natural waters. When based on analyses of dissolved metals the utility of the application factor concept is verified. Its possible use may find greatest application for predicting safe concentrations where synergistic and/or antagonistic mechanisms exist in natural waters.

An evaluation is given on the use of dialysis tubing to differentiate between dissolved and complexed forms of heavy metals in natural waters. These experiments investigated the toxicity of cadmium to rainbow trout in hard and soft waters. The effects of filtration on analytical results, as a means for identifying "dissolved" metal fractions, is also discussed. A method is described for long-term testing of "so-called" insoluble compounds within limits of their solubility constants. An experiment testing the toxicity of silver iodide to rainbow trout in soft waters demonstrates the use of this method.

INTRODUCTION

The purpose of this research was to determine acute and long-term toxicity of lead to rainbow trout in hard and soft waters. Lead is a heavy metal which is readily complexed in natural waters, the degree of which largely depends upon the alkalinity (bicarbonate-carbonate concentrations) of the water into which it is released. Lead has been found to be more toxic to fish in soft water than in hard water. A partial explanation for this is that lead is partially tied up or complexed into non-toxic chemical species in natural waters. In the aquatic environment three physical states are possible and can be broadly classified as particulate, colloidal and dissolved forms of a particular heavy metal (Stiff 1971). Lead can be distributed among all three states. Yet the author believed that it is the dissolved fraction of metals that is directly toxic to fish in the aquatic environment.

The problem for the toxicologist and field men monitoring heavy metal pollutants which complex in natural waters is in identifying and analyzing that fraction of a particular heavy metal that is toxic. Realistic water quality standards can only be established when this has been accomplished. Analytical results obtained by pulse polarography were used to define the dissolved fraction of lead in hard water used in this study and were compared to those obtained by atomic absorption spectrophotometry.

MATERIALS AND METHODS

Acute Toxicity Tests

Three acute experiments are reported, two in hard water (hardness 353 mg/liter as $CaCO_3$) and one in soft water (hardness 28 mg/liter as $CaCO_3$). Lead nitrate was used as the toxic agent. Experiments were conducted with 10 rainbow trout per 35-liter aquaria, which were aerated to achieve oxygen saturation and thorough mixing. Mortalities were monitored at 24-hour intervals. LC50's from acute tests were determined by log-probit analysis (Sprague 1969). Water quality characteristics of control water for the three experiments were determined according to standard methods of the American Public Health Association (1971).

Acute toxicity tests in hard water--Two 96-hour experiments were performed under static conditions so that a narrow range of lead concentrations could be used. In the first experiment total lead concentrations were 580, 560, 540, 520, 500, and (control) 0 mg/liter. The alkalinity of this water was 267 mg/liter. Appropriate concentrations of lead nitrate were added to

each aquarium 18 hours prior to the addition of fish. This allowed formation and precipitation of lead carbonate and permitted the lead distribution to reach steady state. Lead concentrations were determined by a Perkin-Elmer Model 303 (flame) Atomic Absorption Spectrophotometer on non-acidified water samples collected at 24-hour intervals after lead nitrate was initially added to experimental aquaria. Samples stood undisturbed for several hours prior to analysis to allow settling of previously formed lead precipitates which may have been inadvertently included in the sample.

A second acute experiment in hard water was performed so that lead analyses by atomic absorption spectrophotometry (AAS) could be compared to dissolved lead concentrations obtained by a Melabs Pulse Polarographic Analyzer (Osteryoung and Osteryoung 1972). Total lead concentrations added to aquaria were 500, 490, 480, 470, 460, and (control) 0 mg/liter with a water alkalinity of 228 mg/liter. Duplicate water samples were collected in a manner similar to the first experiment except that samples were stored in polyethylene bottles placed in experimental water for analysis upon completion of the test.

Acute toxicity test in soft water--In a flow-through experiment the toxicant flow for each concentration from a proportional diluter (Mount and Brungs 1967) was divided into duplicate aquaria. Alkalinity of the experimental water was 30 mg/liter. Total lead concentrations were 2.00, 1.00, 0.50, 0.25, 0.12, and (control) 0.0 mg/liter. Daily analyses by AAS were made.

Long-term Toxicity Tests

To determine the "no-effect" concentrations for lead in hard and soft waters, two long-term experiments were used to evaluate the effects of lead on rainbow trout survival and growth. The experiments were conducted at the ambient temperature of Fort Collins city water. The well water maintained a year-around temperature of 15^{0}C and was cooled to the temperature of dechlorinated city water. The basic exposure systems consisted of proportional diluters (Mount and Brungs 1967) that delivered approximately 500 milliliters a minute to each of six 325-liter aquaria, to give a 50% replacement time of 6.3 hours (Sprague 1969). Settleable and suspended solids were removed from each aquarium by a settling trap and filter.

To determine possible growth effects induced by lead, the total weight of fish per aquarium was obtained monthly. From these data, feeding rates were adjusted daily based on monthly growth rate projections. Fish were randomly sacrificed at approximately two-month intervals to prevent overcrowding and provide length measurements. The number killed from each aquarium per sampling period varied from 10 to 40 depending on desired fish density. Analysis of variance was used to determine possible lead-induced growth differences.

Chemical and physical results for hardness, alkalinity, pH, conductance, temperature, and dissolved oxygen were obtained weekly from the experiments in accordance with standard methods of the American Public Health Association (1971). There was no significant difference in the above parameters between the test aquaria of a particular experiment.

Long-term toxicity in hard water--An experiment to measure the effect of lead on growth and survival was initiated with 70 fingerling rainbow trout averaging 83 mm in length and 6 g in weight. Added lead concentrations were 3240, 1080, 360, 120, 40, and (control) 0 µg/liter. Lead concentrations were analyzed by AAS and by pulse polarography (PP). AAS analyses were made weekly on non-acidified water samples. Water samples for PP analysis were collected five days a week and pooled in polyethylene bottles which were sealed and stored at the bottom of the test aquaria to prevent changes in the dissolved lead concentration due to absorption of atmospheric carbon dioxide. Direct PP analysis was performed on samples with concentrations above 10 µg/ liter lead, samples below 10 µg/liter lead were chelated with 1% sodium diethyldithiocarbamate and extracted in 10 ml of methylisobutyl ketone (MIBK). The experiment was terminated after 19 months of lead exposure.

Long-term toxicity test in soft water--The experiment was initiated with 280 rainbow trout fry (25 mm in length) per concentration. Total lead concentrations were 80, 40, 20, 10, 5, and (control) 0 µg/liter. Lead concentrations were analyzed weekly by AAS of extracted water samples (Fishman and Midgett 1968). The experiment was terminated after 19 months of exposure.

RESULTS

Acute Toxicity Tests

In the first experiment in hard water a 96-hour LC50 of 1.32 mg/liter analyzed lead was obtained. This compared to a LC50 of 542 mg/liter total lead. Mortality percentages at the different lead concentrations are presented in Table 1. In the second experiment in hard water 96-hour LC50's of 1.47 mg/liter dissolved lead and 471 mg/liter total lead were obtained. Comparison of AAS and PP analytical results (Table 1) demonstrates that, under conditions of the experiment, comparable lead concentrations were obtained by the two analytical methods when added lead concentrations exceeded the carbonate buffering capacity of the hard water system. From the toxicity test in soft water, a log-probit analysis yielded a 96-hour LC50 of 1.17 mg/liter lead (Table 1).

Total lead concentrations, added to aquaria in the hard water experiments, dramatically affected the water quality of the dilution water (Table 2). Alkalinity, measured as $CaCO_3$, decreased with increased lead concentration because of the precipitation of lead carbonate. The loss of carbonates also caused a decrease in pH because of the release of hydrogen ions bound in the bicarbonate buffering system as HCO_3^-. Water hardness remained unchanged with increased lead concentrations.

Long-term Toxicity Tests

In hard water the "no-effect" concentration occurred between dissolved lead concentrations (PP analysis) of 18.2 µg/liter, where no black tail effect was observed, and 31.6 µg/liter where 70% of the fish had black tails. This corresponded to total lead concentrations of 120 µg/liter and 360 µg/liter, respectively (Table 3). The "no-effect" concentration for lead in soft water was between 7.2 µg/liter (No black tails) and 14.6 µg/liter (41.3% black tails, Table 3). In soft water dissolved and total

Table 1. Acute bioassays with rainbow trout in hard and soft waters providing water quality, lead concentrations and mortality results. Lead concentrations listed as mg/liter.

Total	Analyzed AAS[a]	Analyzed PP[b]	% Mortality
Hard Water			
Experiment #1			
Days: 4 Hardness: 385 mg/liter pH: 8.15 Fish length: 86 mm Water temperature: 14 C Alkalinity: 267 mg/liter D.O.: 8.7 mg/liter			
580	6.54		100
560	5.29		100
540	0.79		30
520	0.48		0
500	0.97		0
Control	0.00		0
Experiment #2			
Days: 4 Hardness: 290 mg/liter pH: 8.78 Fish length: 130 mm Water temperature: 7 C Alkalinity: 228 mg/liter D.O.: 9.8 mg/liter			
500	7.56	7.18	100
490	5.05	4.79	100
480	2.85	2.77	100
470	1.30	1.26	30
460	0.25	0.29	0
Control	0.00	0.00	0
Soft Water			
Days: 4 Hardness: 32 mg/liter ph: 6.85 Fish length: 145 mm Water Temperature: 10 C Alkalinity: 30 mg/liter D.O.: 7.6 mg/liter			
2.00	1.60		85
1.00	0.78		5
0.50	0.43		5
0.25	0.22		0
0.12	0.13		0
Control	0.00		0

[a] Atomic absorption spectrophotometry

[b] Pulse polarography

Table 2. Effect of different lead concentrations on alkalinity and pH in hard water.

Item	Total lead (mg/liter) 500	490	480	470	460	Control
Alkalinity (mg/liter)						
phth	0	0	0	0	0	9
M.O.	7	9	10	20	21	228
pH	6.89	6.91	6.97	7.08	7.26	8.78

Table 3. Analytical, mortality and physical abnormality results from the long-term experiments in hard and soft waters.

Lead concentrations (µg/liter) Total	AAS Analysis	PP Analysis	% Mortality	% Abnormalities BT[a]	LS[b]	EC[c]
		Hard Water				
3240	2310 ± 226[d]	64.2 ± 3.9[d]	5.7	100	100	30
1080	850 ± 75	41.2 ± 2.4	0.0	90	60	20
360	380 ± 34	31.6 ± 2.6	0.0	70	10	10
120	190 ± 20	18.2 ± 2.1	0.0	0	0	0
40	100 ± 10	10.2 ± 1.1	0.0	0	0	0
Control	0 ± 0	0.5 ± 0.3	0.0	0	0	0
		Soft Water				
80	61.8 ± 3.1[d]	--	50.6	100.0	96.7	45.4
40	31.2 ± 1.7	--	15.0	84.5	43.8	38.2
20	14.6 ± 0.9	--	7.2	41.3	3.0	6.3
10	7.2 ± 0.4	--	0.6	0.0	0.0	0.0
5	3.6 ± 0.3	--	0.6	0.0	0.0	0.0
Control	0.5 ± 0.3	--	0.6	0.0	0.0	0.0

[a] Black tail

[b] Lordoscoliosis

[c] Eroded caudal

[d] 95% confidence interval

lead concentrations are the same. "No-effect" concentrations for the two long-term toxicity tests were determined from the occurrence of black tails and spinal curvatures of fish deleteriously affected by lead. The occurrence of black tails was the most sensitive criterion for measuring a long-term response to lead.

Black tails were first noted in the high concentration (64 µg/liter dissolved lead) of the experiment in hard water six months after the experiment was started. One month later fish exhibited spinal curvatures and eroded caudal fins. Spinal curvatures were of two types: lordosis (dorsal-ventral spinal flexures) and/or scoliosis (bi-lateral spinal flexures) and were generally a combination of the two, described as lordoscoliosis. The black tail effect in the soft water experiment appeared one and one-half months after initiating the experiment. Lordoscoliosis and eroded caudal fins were prevalent one month later. In severe cases of lordoscoliosis, paralysis and muscular atrophy of the flexed portion of the fish occurred. It would be impossible for fish exhibiting pronounced lordoscoliosis and paralysis to spawn, particularly in natural stream conditions. Analysis of growth data from the two experiments revealed no difference in fish growth between aquaria ($P = 0.05$).

DISCUSSION

Chemistry of Lead

Equilibrium calculations for the waters used in this study showed that the total solubility of lead was about 1.45×10^{-7} M (30 µg/liter) in hard water and 2.46×10^{-6} M (500 µg/liter) in soft water (Figs. 1 and 2). In each figure the arrow indicates the pH of the respective hard and soft waters. The curve C_{Pb} gives the solubility of lead in solution. Soluble complexes such as $PbNO_3^+$ were omitted because they represented an insignificant fraction of the total species present.

Under experimental conditions, the most important factor determining lead solubility in both of these waters was the carbonate concentration which in turn depended upon the partial pressure of CO_2 (g) and the pH. Both pH and CO_2 (g) concentrations were subject to large fluctuations due to fish respiration, metabolism, and an extremely sluggish CO_2 (g) $\rightleftarrows$ CO_2 (aq) equilibrium. In addition, the equilibria involving Pb^{++} precipitation and dissolution are very slow. Consequently, the equilibrium calculations indicate only theoretical ranges and are not necessarily an accurate description of the system, and the solubility and distribution diagrams are suggestive rather than definitive. All equilibrium constants were thermodynamic values computed at 25°C because of the lack of equilibrium constant data at the different experiment temperatures which ranged from 4 to 17°C.

Analysis and Toxicity of Lead

The analytical method employed is extremely important when determining concentrations of metals such as lead which complex and precipitate in waters of different quality. Results by AAS (and those of other methods which do not specifically analyze the dissolved fraction of a particular metal) may be very misleading when assessing toxicity or measuring heavy metal concentrations in waters of different quality.

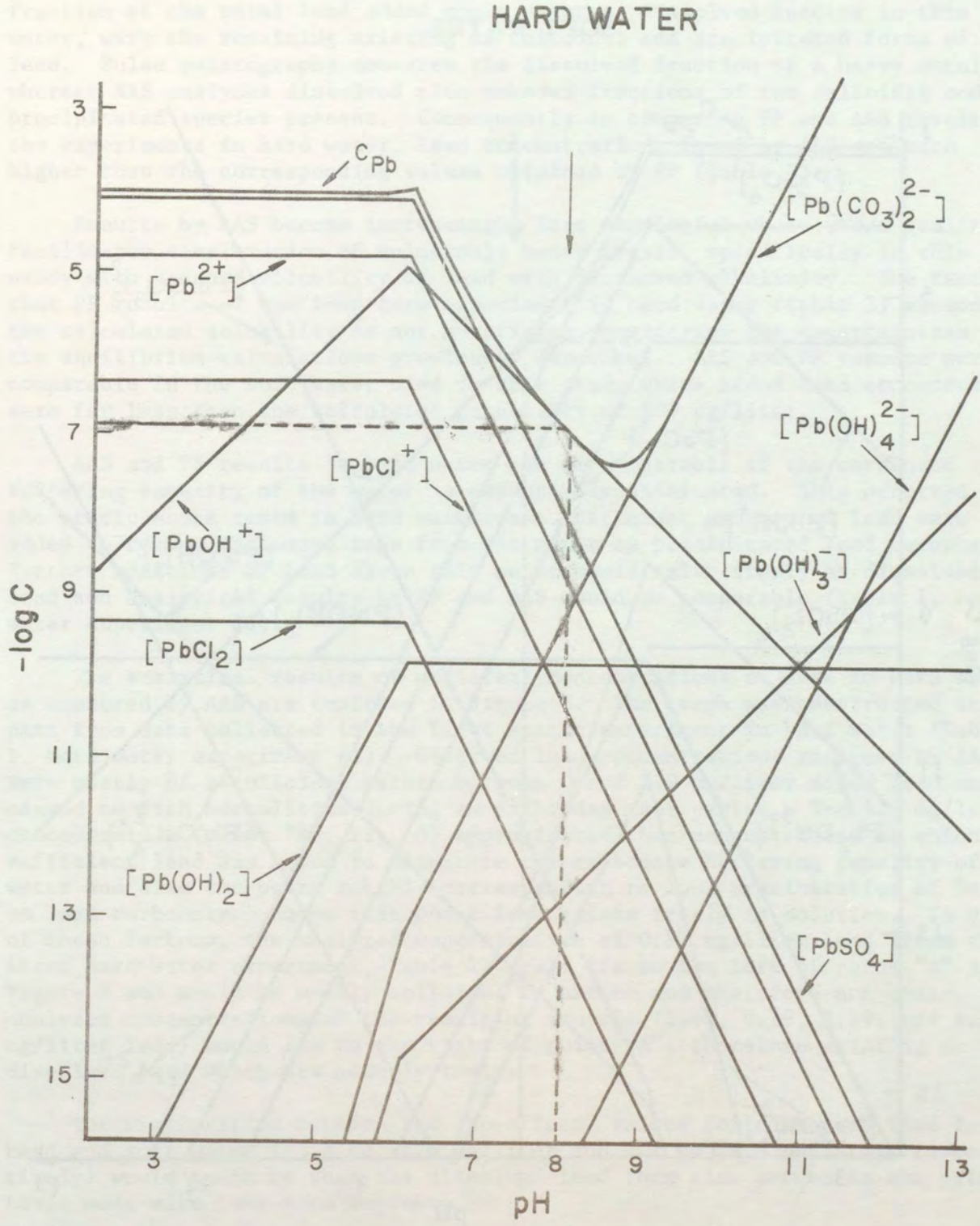

Figure 1. Solubility and species distribution of Pb++ in hard water. The curve, C_{Pb}, gives the solubility of lead at different pH values. Arrow indicates average pH in aquaria.

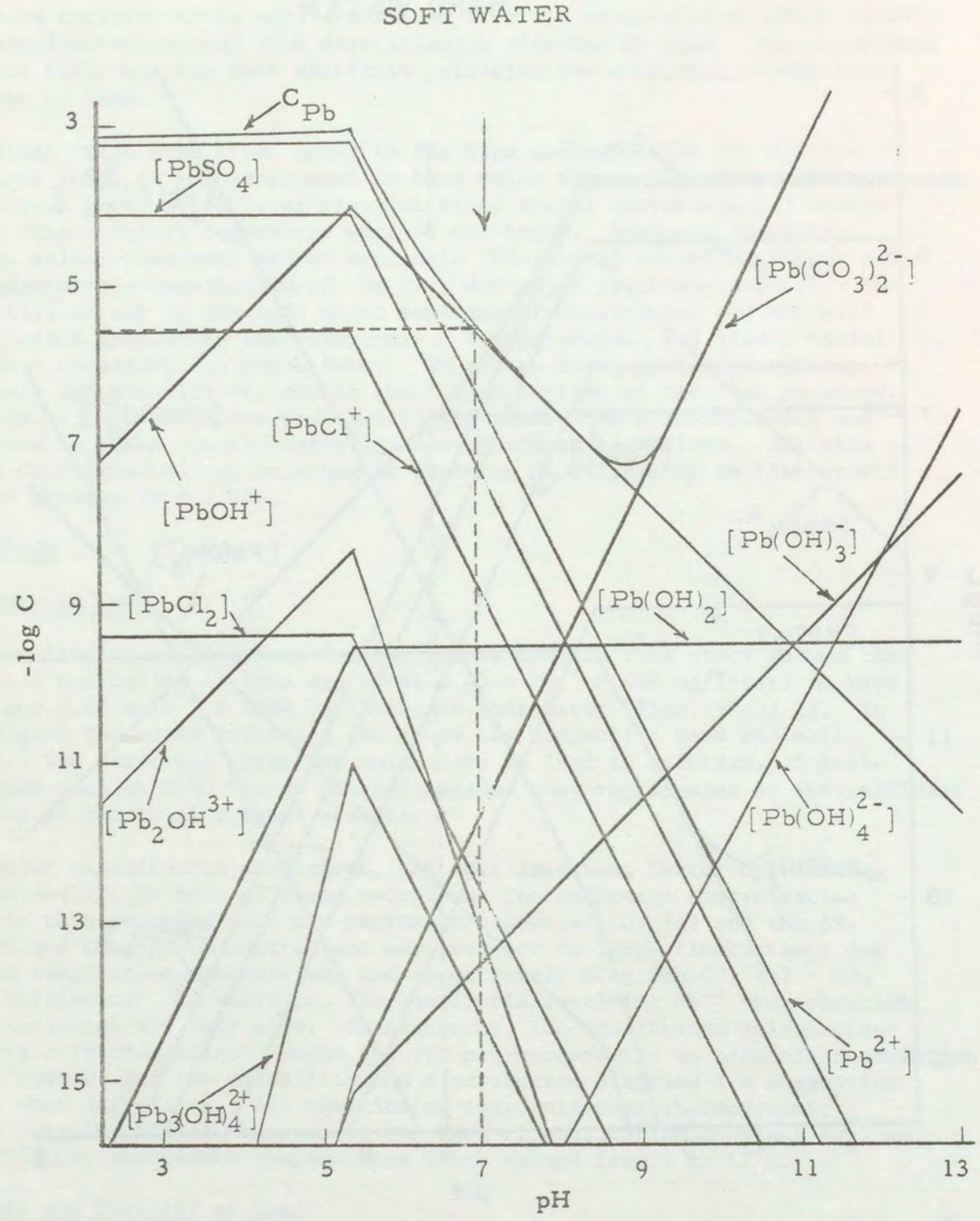

Figure 2. Solubility and species distribution for Pb^{++} in soft water. The curve, C_{Pb}, gives the solubility of lead at different pH values. Arrow indicates average pH in aquaria.

In the hard water experiment of this study, total lead concentrations greatly exceeded the calculated solubility of 30 µg/liter. Therefore, only a fraction of the total lead added would exist as dissolved species in this water, with the remaining existing as colloidal and precipitated forms of lead. Pulse polarography measures the dissolved fraction of a heavy metal, whereas AAS analyzes dissolved plus unknown fractions of the colloidal and precipitated species present. Consequently in comparing PP and AAS results of the experiments in hard water, lead concentrations found by AAS are much higher than the corresponding values obtained by PP (Table 3).

Results by AAS become increasingly less meaningful where water quality facilitates complexation of vulnerable heavy metals, specifically in this study with reduced solubility of lead with increased alkalinity. The fact that PP results of the long-term experiment in hard water (Table 3) exceeded the calculated solubility is not surprising considering the uncertainties of the equilibrium calculations previously described. AAS and PP results were comparable in the soft water used in this study where added lead concentrations were far less than the calculated solubility of 500 µg/liter.

AAS and PP results in hard water can be comparable if the carbonate buffering capacity of the water is essentially eliminated. This occurred in the static acute tests in hard water when sufficient amounts of lead were added to remove carbonate ions from the water as precipitated lead carbonate. Further additions of lead above this point would exist freely as dissolved lead and analytical results by PP and AAS would be comparable (Table 1, hard water experiment #2).

The analytical results of different concentrations of lead in hard water as measured by AAS are depicted in Figure 3. The graph was constructed in part from data collected in the first static experiment in hard water (Table 1, hard water experiment #1). Observed lead concentrations analyzed by AAS were mostly of a colloidal nature between 0 and 520 mg/liter added lead and caused no fish mortalities during an eight-day test period. The 520 mg/liter concentration (point "A", Fig. 3) approximates that concentration at which sufficient lead was added to eliminate the carbonate buffering capacity of the water and also the point roughly corresponding to 100% precipitation of lead as lead carbonate. Above this point lead exists freely in solution. In view of these factors, the analyzed concentration of 0.97 mg/liter lead (from the first hard water experiment, Table 1) would lie to the left of point "A" in Figure 3 and would be mostly colloidal in nature and therefore non-toxic. The analyzed concentrations of the remaining aquaria (0.48, 0.79, 5.29, and 6.54 mg/liter lead) would lie to the right of point "A", therefore existing as dissolved lead which was acutely toxic.

The similarities between the "no-effect" values for dissolved lead in hard and soft water (18.2 to 31.6 µg/liter and 7.2 to 14.6 µg/liter, respectively) would indicate that the dissolved lead form also serves as the primary toxic mode with long-term exposure.

ACKNOWLEDGEMENTS

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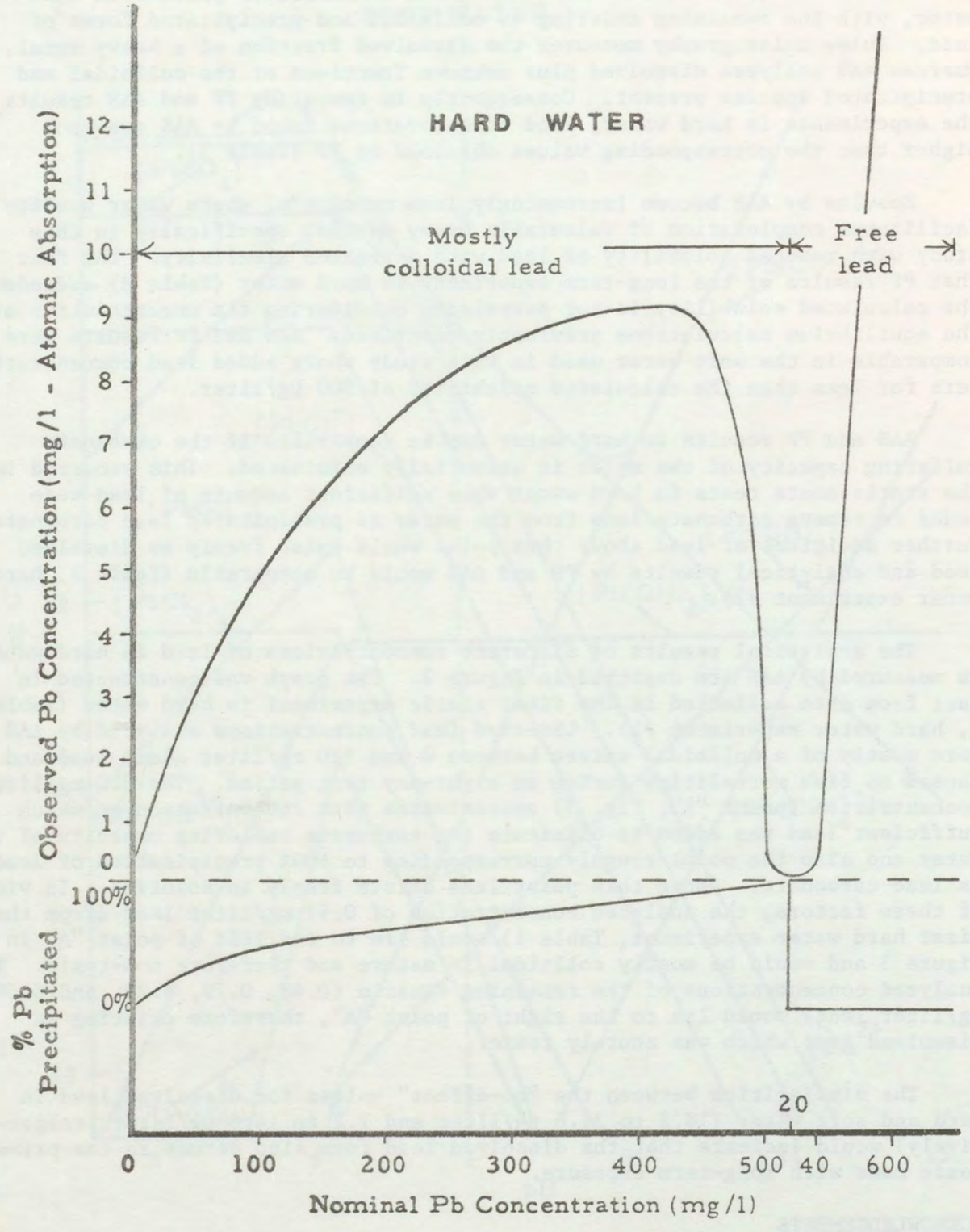

Figure 3. Graphic interpretation of the nature of lead in hard water when analyzed by atomic absorption spectrophotometry. Point "A" indicates the lead concentration at which the carbonate buffering capacity of this water is essentially eliminated.

TESTING APPLICATION FACTOR CONCEPT AS APPLIED TO RAINBOW TROUT IN DIFFERENT WATER QUALITIES

(Dr. Patrick Davies' presentation continued)

INTRODUCTION

This research was conducted to test the validity of the application factor concept as it relates to applying acute and long-term toxicity data for one quality water and predicting the safe concentration for another.

A need has existed whereby laboratory toxicity data could be used to realistically predict metal toxicities in natural waters. Henderson (1957) discussed a number of constituents involved in developing "application factors" whereby laboratory toxicity studies could be used to determine permissible concentrations of toxic substances in the aquatic environment. Mount and Stephan (1967) proposed the use of an application factor derived by dividing the maximum acceptable toxicant concentration (MATC) by the acute (48- or 96-hour) LC50 (TL_m) value for a particular toxicant. The resulting factor is used to estimate the safe concentration by multiplying it by the LC50 for a different fish species or different water quality for which long-term testing is not practical. The MATC is established on the basis of long-term exposure using the laboratory fish production index (LFPI) as a measure of effect. The LFPI reflects toxicant effects on growth, reproduction, spawning behavior, viability of eggs, and growth of fry with exposure data collected over at least one generation.

METHODS

To test the validity of the application factor concept, data is used from the experiments investigating acute and long-term toxicity of lead to rainbow trout in hard (hardness 353 mg/liter as $CaCO_3$) and soft (hardness 28 mg/liter as $CaCO_3$) waters. Figure 4 gives a diagramatic scheme for testing the application factor concept. A hard water application factor is obtained by dividing the long-term, "no-effect" concentration range by the 96-hour LC50. Theoretically, an approximation of the safe concentration of lead in soft water should be obtained by multiplying the hard water application factor by the 96-hour LC50 determined for soft water. Conversely, a "no-effect" concentration for lead in hard water is obtained by multiplying the soft water application factor by the 96-hour LC50 determined for hard water.

As explained previously, lead analyses in hard water by atomic absorption spectrophotometry (AAS) are relatively meaningless because of the inability of distinguishing the nature (dissolved, colloidal, and precipitated forms) of lead in a sample. This situation leaves two avenues by which the application factor in hard water can be approached: (1) the total amount of lead added (i.e., the nominal concentration) is known, and (2) the pulse polarographic analyses for dissolved lead is known. Therefore, application factors for both total and dissolved lead should be determined for hard water. Only

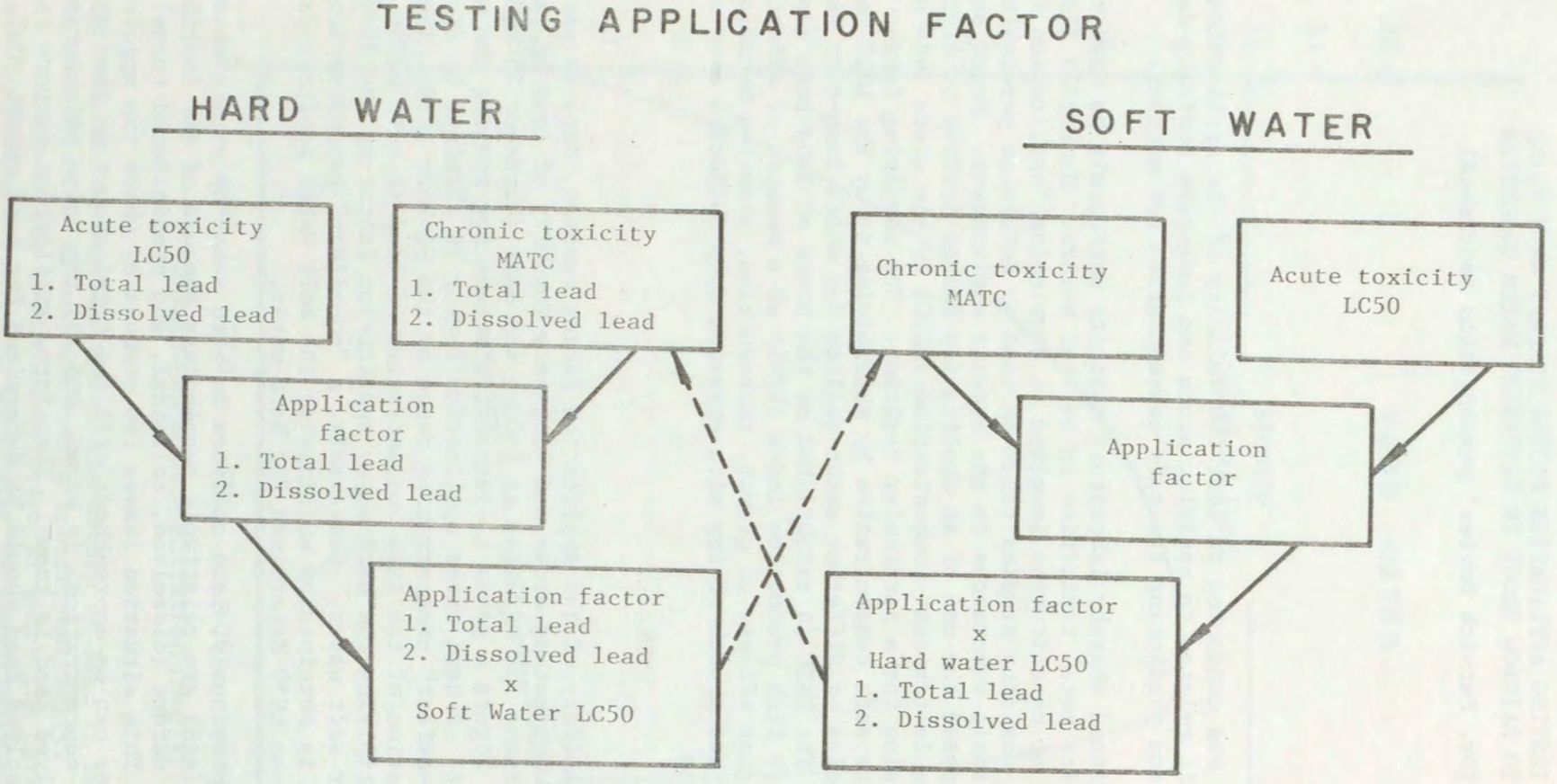

Figure 4. Schematic diagram for testing the validity of the application factor concept in regard to predicting safe concentrations of pollutants in waters of different quality.

one application factor need be determined for soft water since total and dissolved lead are essentially the same (Osteryoung and Osteryoung 1972).

RESULTS

Table 4 summarizes acute and long-term results needed to test the applicability of the application factor concept in determining safe toxicant concentrations in waters of different quality. The "no-effect" concentration application factor values appear as a range. The first of these values gives the lead concentration at which no deleterious effect was observed and the second gives the lowest lead concentration where a deleterious effect was found.

By testing the application factor data according to the scheme illustrated in Figure 4, the validity of the concept is determined. Table 5 compares experimental "no-effect" concentrations for total and dissolved lead in hard and soft waters with "no-effect" concentrations computed from application factors. The application factor concept is valid for heavy metals such as lead, which exhibit a complexing behaviour in different water qualities, only if analysis for the dissolved metal is employed. Computed "no-effect" concentrations determined on the basis of dissolved lead, are between a factor of 1 to 2 of the actual experimental values (Hard water: 9-18 µg/liter, computed versus 18 to 32 µg/liter, experimental, and soft water: 11.7-23.4 µg/liter, computed versus 7.2-14.6, experimental). Whereas on the basis of total lead, computed values greatly exceed experimental values (Table 5). This analytical limitation poses a serious and unresolved problem if the application factor concept is to find widespread use. The determination of long-term, "no-effect" and acute LC50 concentrations would require analysis of the dissolved metal. In addition samples used in monitoring pollutant concentrations in a particular water would necessitate the determination of dissolved metal concentrations if such results were to be compared to previously determined dissolved metal, "no-effect" values. Present chemical techniques analyzing specifically for the dissolved fraction of heavy metal are still somewhat experimental or research-oriented, but such techniques as pulse polarography, anodic stripping voltammetry, and use of ion selective electrodes may provide means of making such analyses. Additional research is needed to determine if dissolved analyses of other metals in one water quality adequately predict the safe concentration of the particular metal in a different water quality.

Most agencies monitoring heavy metal stream pollution problems use atomic absorption spectrophotometry (AAS) as the primary instrument for metal analysis. AAS and other techniques that provide similar results should not be expected to provide meaningful toxicological data with metals that become complexed in natural waters. The determination and implementation of meaningful water quality standards cannot be realistically made until analysis of the toxic fraction of heavy metals in natural waters can be achieved.

DISCUSSION

A question that should be raised is "Considering an ability to properly identify and analyze the toxic fractions of heavy metals in natural waters, would such techniques and manipulations as the application factor concept really be necessary?" Evidence provided by analyzing for dissolved lead would suggest not, particularly since it is highly probable that the toxicity of

Table 4. Summary of hard and soft water application factor data.

	Hard Water		Soft Water
	Total lead concentration	Dissolved lead concentration[a]	Total or dissolved lead concentration
LC50	471,000 µg/liter	1470 µg/liter	1170 µg/liter
"No-effect" concentration	120 to 360 µg/liter	18 to 32 µg/liter	7.2 to 14.6 µg/liter
Application factor	.0002-.0008	.01-.02	.006-.012

[a] Determined by pulse polarography

Table 5. Experimental and computed "no-effect" ranges in hard and soft waters.

Water	"No-effect" concentration	
	Experimental	Computed
Hard water (determined on basis):		
Total lead	120 to 360 µg/liter	2830 to 5650 µg/liter
Dissolved	18 to 32 µg/liter	9 to 18 µg/liter
Soft water (determined on basis):		
Total lead	7.2 to 14.6 µg/liter	230 to 940 µg/liter
Dissolved lead	7.2 to 14.6 µg/liter	11.7 to 23.4 µg/liter

lead would probably have been identical in both hard and soft waters, when analyzed on a dissolved basis, had the fish been of the same age when initially exposed to lead.

The application factor concept might have meaningful application where combinations of heavy metals work synergistically to increase their toxicity to the acquatic environment and would most certainly find use in predicting metal toxicities to other fish species for which long-term bioassays cannot be made. If antagonistic reactions occur in natural water to suppress metal toxicities the application factor concept again could be very useful. Numerous references have been made to the antagonistic effect of calcium to the toxicities of certain metals. Many of these effects attributed to calcium probably are the result of anion complexation of specific metals rendering a certain fraction of them non-toxic, or in a chemically inert state, thereby reducing their toxicity in certain water qualities. More investigation needs to be conducted regarding the antagonistic response of calcium or other elements to heavy metals. Analyses of metal concentrations need to be performed on a dissolved basis if a realistic evaluation of antagonistic reactions are to be made.

Chemical research is needed to develop and perfect methods of identifying and analyzing the dissolved fractions of heavy metals in natural waters. Toxicological evaluation, based on analyzing the toxic fractions of heavy metals is needed to determine if a single specific concentration of a particular heavy metal is toxic to any given species of fish, irrespective of water quality. Only when this has been accomplished can realistic water quality standards for heavy metals be implemented and meaningful evaluations of the toxicity of various metal combinations be made.

USE OF DIALYSIS TUBING IN DEFINING THE TOXIC FRACTIONS OF HEAVY METALS IN NATURAL WATERS

(Dr. Patrick Davies' presentation continued)

INTRODUCTION

Beneš and Steinnes (1974) described the method which they felt enabled the determination of "truly dissolved" forms of trace elements in natural waters. The need to define the toxic fraction of heavy metals in the aquatic environments is becoming well recognized. The method involves a dialysis cell (tubing filled with distilled water and sealed) in which only molecules or ions smaller than the diameter of pores of the dialysis membrane are able to diffuse from external water into the dialysis cell. Diffusion continues until equilibrium is attained between species inside and outside of the cell.

A distinct advantage of the method allows for analysis of the chemical makeup of water inside the cell for a total concentration of a particular element or elements with results giving only the dissolved concentration of those forms or species capable of diffusing across the membrane. This allows for the use of instrumentation, such as flame or flameless atomic absorption spectrophotometers, to analyze the dissolved fraction of trace metals where analysis is normally limited to total concentrations (dissolved and colloidal forms).

The purpose of the present research was to determine if a dialysis cell successfully differentiates between the dissolved or toxic species and other chemical forms in natural water. This work compares dialysis results from flow-through toxicity tests with lead in hard water (alkalinity - 225 mg/liter as $CaCO_3$ and hardness - 315 mg/liter as $CaCO_3$) to results obtained from long-term toxicity tests in hard water where dissolved lead concentrations were determined by pulse polarography (reported above). The dialysis method was also evaluated during preliminary toxicity experiments with cadmium using rainbow trout (*Salmo gairdneri*) in hard (hardness - 330 mg/liter as $CaCO_3$) and soft (hardness - 30 mg/liter of $CaCO_3$) waters.

METHODS

Lead Dialysis Experiment

Two different sizes (molecular weight cutoff - mwco) of dialysis tubing were used: (1) standard 12,000-14,000 mwco dialysis tubing with a mean pore diameter of 4.8 nm and, (2) an experimental grade (Spectrapor™)* with a 3,500 mwco. Dialysis cells were filled with 10 ml distilled water. Duplicate cells were sealed and submerged in each of five lead concentrations and a control.

*Spectrum Medical Industries, Inc.

Lead (as lead nitrate) was supplied to six 35-liter aquaria from a proportional diluter (Mount and Brungs 1967) set at a 50% dilution ratio. The flow rate to each aquarium was approximately 500 ml per minute. Total (nominal) lead concentrations delivered to the aquaria were 1.00, 0.50, 0.25, 0.12, 0.06, and (control) 0.00 mg/liter. Ten rainbow trout with a mean total length of 125 mm were placed in each aquaria so that metabolic and respiratory by-products would be present. Lead concentrations were determined by flame AAS and lower concentrations by flameless AAS (carbon rod atomizer). The experiment was terminated after 5 days; this was sufficient time to allow the dialysis cells to reach equilibrium. These analytical results were compared to the pulse polarographic results obtained from the long-term toxicity test of lead in hard water (described earlier).

Cadmium Dialysis Experiments

Cadmium toxicity in soft water--Experimental conditions were similar to those previously described under long-term toxicity with lead, except reagent grade cadmium sulfate was used as stock solution for the proportional diluter. Total cadmium concentrations added to the aquaria were 8, 4, 2, 1, 0.5, and (control) 0.0 µg/liter. Twenty rainbow trout, approximately 130 mm in total length, were added to each experimental concentration. Mortalities were monitored daily. A 96-hour LC50 was determined by log-probit analysis (Sprague, 1969). A duplicate set of dialysis cells was submerged in each aquarium and analyzed for cadmium at the end of the first 96 hours of the experiment. Analyses were performed with a Varian Model 1250 carbon rod atomizer. Chemical and physical results for hardness, alkalinity, pH, conductance, temperature and dissolved oxygen were obtained in accordance to standard methods of the American Public Health Association (1971).

Cadmium toxicity in hard water--Two experiments with cadmium in hard water were used to evalute the use of dialysis tubing to differentiate dissolved cadmium concentrations and the toxicity of cadmium to rainbow trout in hard water. An experiment was run under static conditions whereby cadmium as $CdSO_4$ was added to 980 ml of hard water in 1-liter linear polyethylene bottles. Added cadmium concentrations were 1.00, 0.50, 0.25, 0.12, 0.06, and (control) 0.00 mg/liter.

Duplicate dialysis bags (12,000-14,000 mwco) were filled with 10 ml distilled water, sealed and placed into each of the 1-liter bottles. The bottles were sealed and submerged in flowing hard water of the same quality to prevent changes in temperature and absorption of CO_2. Flameless AAS analysis by carbon rod atomizer was performed after a 96-hour period of equilibration. Water quality characteristics were determined in accordance with APHA (1971).

Solubility calculations were made to provide a theoretical solubility for cadmium in hard water used in these experiments. Using this calculation a second flow-through experiment was initiated using the same experimental conditions previously reported, except total cadmium concentrations delivered by the proportional diluter were 40.0, 20.0, 10.0, 5.0, 2.5, and (control) 0.0

µg/liter. Duplicate dialysis cells of 12,000-14,000 mwco and 3,500 mwco were submerged in each of the 325-liter test aquaria. Twenty rainbow trout, with a mean total length of 130 mm, were added to each experimental concentration. After reaching equilibrium cadmium concentrations in dialysis cells were analyzed using a carbon rod atomizer. Attempts were made to analyze dissolved cadmium concentrations using differential pulse anodic stripping voltommetry. Water quality parameters for hardness, alkalinity, pH, conductance, temperature, and dissolved oxygen were also determined in accordance with APHA (1971).

RESULTS

Lead Dialysis Experiment

There is no significant difference between lead concentrations of the water inside the dialysis cell and lead concentrations in the aquarium water outside of the cell (Table 6). It can also be seen that the lead concentrations inside the dialysis cell differ drastically from the dissolved lead concentrations determined by pulse polarography in the long-term experiment even where the concentration of added lead was similar. As would be expected, no mortalities occurred during this period at these lead concentrations. Water quality characteristics were: Hardness - 315 mg/liter as $CaCO_3$, alkalinity -225 mg/liter as $CaCO_3$, pH - 7.76, conductance - 1000 µmhos/cm, temperature -14°C and dissolved oxygen - 5.4 mg/liter.

Cadmium Dialysis Experiment

Cadmium toxicity in soft water--In soft water the "no-effect" cadmium concentration occurred between 0.7 µg/liter (no mortality) and 1.5 µg/liter (10% mortality). A 96-hour LC50 of 1.75 µg Cd/liter was also obtained. A comparison between aquarium and dialysis cell concentrations for cadmium is given in Table 7. As would be expected in soft water, there is no statistical difference between cadmium concentration inside and outside of the dialysis cell. Chemical and physical results for hardness, alkalinity, pH, conductance, temperature, and dissolved oxygen are given in Table 8.

Cadmium toxicity in hard water--The static experiment with duplicate dialysis cells in sealed 1-liter polyethylene bottle reveal no significant difference in cadmium concentrations inside and outside of the dialysis cells even at the relatively high cadmium concentrations present (Table 9).

From the solubility and species distribution diagrams for cadmium in the hard water used in these experiments, losses of dissolved cadmium due to precipitation as $Cd(OH)_2$ should not occur at the pH and cadmium concentration of the water involved. The solubility of cadmium in this water at a pH of 8.22 should be about 2.4×10^{-7} M (27 µg Cd^{++}/liter). Cadmium concentrations about 27 µg/liter would be subject to precipitation as $CaCO_3$ (Appendix A). Therefore, cadmium should exist freely (i.e. dissolved) in the hard water solution at and below a total (added) cadmium concentration of 27 µg/liter. Here dissolved refers to ionic species of which there are two possibilities--Cd^{++} and $Cd(OH)^{+}$. However, at a pH of 8.0 the Cd^{++} to $Cd(OH)^{+}$ ratio should be about 12.5 to 1 (Appendix A).

Table 6: Comparison of lead concentrations in hard water obtained from dialysis experiment with pulse polarographic results obtained from long-term toxicity experiment.

Dialysis experiment (mg/liter)				Long-term experiment (mg/liter)		
		Dialysis Pb				
Total Pb	Aquaria Pb	A[a]	B[b]	Total Pb	AA[c]	PP[d] Pb^{++}
1.00	0.95	0.90	0.95	3.24	2.31	0.064
0.50	0.50	0.48	0.50	1.08	0.85	0.041
0.25	0.25	0.24	0.23	0.36	0.38	0.032
0.12	0.16	0.13	0.14	0.12	0.19	0.018
0.06	0.03	0.02	0.02	0.04	0.10	0.010
0.00	0.00	0.00	0.00	0.00	0.00	0.000

[a] Dialysis tubing: 12,000-14,000 mwco
[b] Dialysis tubing: 3,500 mwco
[c] Atomic absorption spectrophotometry
[d] Pulse polarography

Table 7: Analytical results for cadmium in soft water comparing concentrations (µg/liter) inside and outside of the dialysis cells.

Total Cd	Aquaria outside dialysis	Dialysis 12,000-14,000 mwco	
		1	2
8.0	7.0	5.9	6.0
4.0	3.7	3.7	3.7
2.0	2.1	1.8	1.6
1.0	0.9	0.8	0.8
0.5	0.5	0.4	0.4
0.0	0.0	0.0	0.0

Table 8. Water quality characteristics for the dialysis experiments with cadmium in hard and soft waters.

Hardness mg/liter	M.O. Alkalinity mg/liter	pH	Conductance µmhos/cm	Temperature C	D.O. mg/liter
		Cadmium in soft water			
31	27	6.84	145	12.5	6.4
		Cadmium in hard water - static experiment			
258	208	7.54	980	14.5	--
		Cadmium in hard water - flow-through experiment			
326	234	8.22	1025	15.5	5.8

Table 9. Cadmium concentrations determined within and outside of dialysis (12,000-14,000) during the static experiment performed in 1-liter polyethylene bottles in hard water. Concentrations given in mg/liter.

	Non-acidified samples			Acidified samples		
Total Cd	Aquaria Cd	Dialysis 1	Dialysis 2	Aquaria Cd	Dialysis 1	Dialysis 2
1.00	0.81	0.77	0.78	0.93	0.89	0.89
0.50	0.33	0.31	0.31	0.39	0.40	0.40
0.25	0.18	0.17	0.17	0.24	0.24	0.23
0.12	0.10	0.08	0.08	0.11	0.11	0.11
0.06	0.05	0.04	0.04	0.06	0.05	0.05
0.00	0.00	0.00	0.00	0.00	0.00	0.00

The flow-through experiment, (high cadmium concentration of 40 µg/liter) failed to show a significant difference between cadmium concentrations within the dialysis cells to that in the water outside of the cells (Table 10). The "no-effect" concentration in the hard water of this experiment was between 13.5 µg Cd/liter (no mortality) and 21.0 µg Cd/liter (20% mortality, 4 mortalities of a total of 20 fish).

DISCUSSION

Dialysis Experiments

Dialysis results from the lead (Table 6) and cadmium experiments clearly demonstrate that dialysis membranes do not provide a suitable method for separating dissolved or toxic fractions of heavy metals from complexed or colloidal fractions in natural waters. Considering the calculated solubility (27 µg/liter) of cadmium in hard water, the dialysis results from the flow-through experiment (Table 10) in hard water were within the theoretical solubility limitation. However, total cadmium concentrations of the static experiment (Table 9) with a high of 1.0 mg Cd/liter greatly exceeded the calculated solubility; yet, there was no significant difference between cadmium concentrations within and outside of the dialysis cells.

The 12,000-14,000 mwco dialysis tubing used in these experiments was similar to that used by Beneš and Steinnes (1974). The 3,500 mwco dialysis tubing used in some of the experiments would have a pore size more than three times smaller than that used in the Beneš study. Beneš and Steinnes (1974) analyzed 20 elements from in situ dialysis experiments in Glomma River at Fetsund, Norway. They concluded that the dialysis method enables the determination of truly dissolved forms of trace elements in natural water. Chemical results obtained from dialysis cells were compared with results obtained from water samples filtered through 0.45 µm-Millipore filter and ultra filtration through Diaflo PM 10 ultra-filter. Their report makes no mention of the water quality of the Glomma River. Without a knowledge of the pH, carbonates, other complexing anions, and naturally occurring chelating agents, it is not possible to assess to what extent complexation might occur.

Filtration is not a suitable method for determining concentrations of dissolved metals in natural waters (Appendix B). Absorption losses of dissolved species on a filter can pose a serious problem. Also pore sizes of filters are not sufficiently small as to preclude the passage of colloids. This is particularly true of the 0.45 µm filter.

The conclusions reached by Beneš and Steinnes would seem to indicate one of two possibilities: (1) the elements in their experimental water were mostly dissolved, or (2) both the dialysis and filtration results failed to differentiate between the dissolved and complexed forms of the elements studied.

Toxicity of Cadmium in Hard Water

If it is true, as the author believes, that it is the dissolved fraction

of heavy metals that are toxic to fish in the aquatic environment, one would assume that the toxicity of cadmium in hard water should be similar to its toxicity in soft water, if analyzed on the basis of dissolved cadmium. This was demonstrated to be the case with the toxicity of lead, previously reported. However, this does not appear to hold true with cadmium. Cadmium in soft water had a "no-effect" concentration between 0.7 and 1.5 µg/liter, whereas in hard water the "no-effect" concentration was between 13.5 and 21.0 µg/liter.

Considering the calculated solubility of cadmium in the hard water used in these experiments (27 µg/liter) and the experimental concentrations tested, there would appear to be an abundance of dissolved cadmium in hard water to give a comparable effect as that observed in soft water. Gardiner (1974), investigating labile complexes of cadmium in synthetic and real waters, stated that humic substances constitute the major source of cadmium complexation, with inorganic carbonate second. Shapiro (1969) reported humic substances in waters between 1-20 mg/liter. Average concentrations in water have been reported to be about 4 mg/liter (Packham 1964).

The complexation of cadmium by humic substance was not considered in the solubility and species distribution diagrams of Appendix A. Yet, complexation by humic substances may contribute to the discrepancies seen in toxicity of cadmium between hard and soft water. One would expect that if humic substances caused complexation problems with cadmium in hard water that similar complexation would have also occurred in the soft water experiment.

In order to resolve this question, a water sample was collected from the aquarium with an added (nominal) concentration of 20 µg Cd/liter and analyzed by differential pulse anodic stripping voltammetry (ASV). The dissolved cadmium concentration determined by ASV was about 20 µg/liter. However, reproducibility was extremely poor, with a decrease in the cadmium concentration occurring with subsequent analysis of the same sample following stripping and replating on a new mercury drop. The decrease in cadmium was attributed to absorption of cadmium on the walls of the glass cell used in the analysis. This was further confirmed by the addition of nitric acid which caused the desorption of cadmium from the walls of the cell and the reappearance of the cadmium peak.

Considering the success of Chau and Lum-Shue-Chan (1974) in analyzing cadmium in natural waters by differential pulse anodic stripping voltammetry, the 20 µg/liter determined by ASV in this study is considered to be acceptable approximation of the dissolved cadmium concentration. The reliability of the value is further strengthened by its agreement with the added cadmium concentration and the fact that it lies within the calculated solubility of cadmium in this water. The method of analysis was essentially the same as that used by Chau and Lum-Shue-Chan (1974) except no acetate buffer was used.

It would appear that the discrepancy in the toxicity of cadmium in hard and soft water is not the result of some complexation reaction, but most probably the result of some antagonistic reaction inhibiting the toxicity of cadmium in hard water. The literature is filled with "so called" hardness

Table 10. Analytical and mortality results of the cadmium flow-through experiment in hard water comparing concentrations (ug/litre) inside and outside of Dialysis Cell A (12,000-14,000 mwco) and Dialysis Cell B (3,500 mwco).

Total Cd	Aquaria Cd	Dialysis A		Dialysis B		% Mortality
		1	2	1	2	
40.0	21.0	23.5	22.0	21.5	21.5	20
20.0	13.5	11.0	10.0	9.5	11.5	0
10.0	6.0	3.1	4.3	5.0	--	0
5.0	1.7	1.4	2.0	1.4	2.9	0
2.5	0.5	0.4	0.5	0.4	0.4	0
0.0	0.0	0.0	0.0	0.0	0.0	0

and/or calcium antagonistic reactions to the toxicity of various heavy metals, rendering them non-toxic forms. However, until dissolved concentrations of heavy metal in natural waters are properly analyzed, true antagonistic reactions will be difficult to define.

ACKNOWLEDGEMENTS

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A METHOD FOR DETERMINING THE LONG-TERM TOXICITY OF INSOLUBLE METAL COMPOUNDS DEMONSTRATED BY SILVER IODIDE IN NATURAL WATER

(Dr. Patrick Davies' presentation continued)

INTRODUCTION

Because of the extreme toxicity of dissolved silver and the current interest in silver iodide as a weather modifying agent, attempts were made to determine the toxicity of silver iodide to rainbow trout. Because of the extreme insolubility of silver iodide, sodium iodide (NaI) was used to complex or solubilize silver iodide into solution. These attempts were unsuccessful because of a toxic influence exerted by sodium iodide, which masked any possible toxicity of silver iodide. Silver iodide will disassociate to give about 0.3 µg/liter soluble silver. This value is above the toxic limits (0.09-0.17 µg/liter) found for rainbow trout exposed to silver nitrate (Goettl, et al. 1974). Consequently, silver iodide should be toxic within the limits of its solubility. The problem is that of devising a method whereby silver iodide could be added to water within the limits of its solubility for long-term testing without using a solubilizing agent.

METHODS AND MATERIALS

A preliminary experiment was set up to test the toxicity of silver iodide to rainbow trout within the limits of its normal solubility. A sealed PVC cylinder (10" inside diameter and 12" long) was constructed to permit water to flow through a dacron polyester filler material, containing 700 g of dispersed silver iodide, into a 35-liter aquarium. A one-half-inch mesh PVC support grid at the top and bottom of the cylinder held the filler material in place and prevented blockage and clogging of the inlet and outlet orifices. Flow rate to the aquarium was approximately 1 liter per minute. A control aquarium was set up in a similar manner without silver iodide in the filler material. The experiment was initiated with 100 recently hatched sac fry in each aquarium.

Following termination of the preliminary experiment, the silver iodide cylinder was rejuvinated by the addition of 250 g of silver iodide. A one-half-inch PVC tee was placed in the water supply line (on the out-flow side of an electric solenoid valve) of the proportional diluter. A valve was placed in each of the two lines from the tee with one line delivering dilution water to the W-2 cell of the proportional diluter (Mount and Brungs 1967), and the other line supplying water to the silver iodide cylinder. The effluent line from the cylinder supplied water containing dissolved silver directly to the chemical cells of the proportional diluter. The two valves were used to adjust water flows to the water and chemical cells. With a 50% diluter ratio, silver dissolved in the water flowing through the cylinder would be diluted by a factor of 50% for each of five concentrations and a control. This experiment was conducted in 265-liter fiberglass tanks.

This test was initiated with 340 eyed eggs per concentration. Experimental methods were similar to those previously described for the long-term toxicity of lead. Silver analysis was performed by flame atomic absorption of APDC-MIBK extracted water samples, and by flameless AAS carbon rod analyzer. Water quality characteristics were determined according to standard methods of APHA (1971).

RESULTS

Results from the preliminary experiment demonstrated that silver iodide, within the limits of its own solubility, was toxic to rainbow trout. This method was successful in determining the toxicities of "so called" insoluble compounds. Ninety-four percent of the exposed fish died during six weeks of exposure. No mortalities occurred in the control. Silver analysis of water through the filter was 0.88 µg/liter (range 0.5 to 1.1 µg/liter). Analysis of the control water gave 0.00 µg/liter of silver.

In addition to mortality effects, very prominent effects of retarded development and growth were observed. After a week of exposure, the fry in the control had absorbed the yolk-sac and were swim-up fry. Complete swim up did not occur with the fish exposed to silver until three weeks later. The preliminary experiment was terminated after six weeks exposure. At this time control fish were larger than exposed fish, 36 mm versus 24 mm, respectively. Effects of silver on retarded development and growth were previously reported with silver nitrate (Goettl et al. 1974). Chemical analyses for alkalinity, hardness, pH, dissolved oxygen, temperature and conductivity were performed weekly (Table 11).

A second experiment with silver iodide was initiated to more closely define the toxic limits of silver iodide. After one-year mortality, data indicates a "no-effect" concentration between 0.07 and 0.13 µg/liter silver (Table 12). This range compares quite favorably with the "no-effect" concentration (0.09-0.17 µg/liter) obtained from the long-term silver nitrate experiment (Goettl et al. 1974). Effects of retarded development and growth were not apparent. Mortalities through swim up of fry were essentially the same in all concentrations (Table 12). An error was made in placing a large number of extra eggs into the control tank. Consequently, this tank was overcrowded and the fish were considerably smaller than those in the other tanks. Therefore, data from the control is not included. Water quality data is given in Table 11. The experiment has run for one year.

Table 11. Water quality characteristics for the silver iodide experiments in soft water. Range of concentration in parentheses.

Hardness mg/liter	M.O. Alkalinity mg/liter	pH	Conductance µmhos/cm	Temperature C	D.O. mg/liter
		Preliminary silver iodide experiment			
15.2 (14-18)	12.7 (12-14)	(6.5-6.8)	99 (90-110)	15.9 (14-17)	8.5 (7.8-9.2)
		Second silver iodide experiment			
27.5 (18-34)	24.9 (14-32)	(6.64-7.37)	140 (95-160)	11.1 (6-17)	8.0 (5.6-12.0)

Table 12. Mean silver concentrations and mortality results after one-year exposure.

Item	Silver concentration[a] (µg/liter					
	0.50	0.32	0.18	0.13	0.07	0.01 (control)
Concentration	0.15-1.25	0.10-0.80	0.05-0.60	0.03-0.48	0.01-0.26	0.00-0.05
Percent mortality (through swim up)	7.9	7.4	4.7	5.9	8.5	--
Percent mortality (after swim up)	38.1	24.5	18.7	18.1	3.2	--

[a] APDC-MIBK extration analyzed by flame AA and flameless AA - carbon rod atomizer.

APPENDIX A

SOLUBILITY OF CADMIUM IN HARD WATER

Equilibrium calculations show that the solubility of cadmium used during the experiments in hard water is 2.4×10^{-7} M (27 µg/liter) at a pH 8.22. The solubility diagram for Cd^{++} and $CdCO_3$, between pH value of 6.5 to 9.5, is given in Figure 5. The arrow in the figures indicates pH of the test water. The solubility of cadmium in this water can be quite variable depending on fluctuations in pH (Table 13).

In addition to the complexation of cadmium by carbonates, complexation by hydroxides also plays an important role. The cadmium hydroxide species of interest are $Cd(OH)^{+}$ and $Cd(OH)_2$. The solubility and species distribution diagram for cadmium hydroxides in the experimental water, having a total cadmium concentration of 40 µg/liter, is given in Figure 6. As seen from Figure 6, precipitation of cadmium as $Cd(OH)_2$ should not be a problem at the experimental cadmium concentrations and pH. Figure 6 also shows the Cd^{++} and $Cd(OH)^{+}$ are the two soluble species existing in this water. The ratios for the concentrations of Cd^{++} to $Cd(OH)^{+}$ at different pH values are: 12.5 to 1 at pH 8; 4.98 to 1 at pH 8.4; and 1.25 to 1 at pH 9. At the experimental pH, Cd^{++} is by far the predominate species.

A question that might be raised is whether there is a toxicological difference between the activity of Cd^{++} and $Cd(OH)^{+}$. Analyses performed by differential pulse anodic stripping voltammetry would not differentiate between these two dissolved species, and there is probably no analytical technique capable of this at this time. The author believes that probably both species contribute to the toxicity, but probably not to the same degree.

All equilibrium constants are thermodynamic values. Consequently, the solubility and species distribution diagrams are intended to be suggestive and not definitive of the complex interrelationship of cadmium in hard water. As mentioned previously, possible effects of humic substances on the complexation of cadmium were not investigated.

Table 13. Maximum concentrations of dissolved cadmium at different pH values in the experimental hard water.

Dissolved conc. Cd	pH 7.0	7.5	8.0	8.5	9.0
Molar	4×10^{-6}	1.25×10^{-6}	4×10^{-7}	1.25×10^{-7}	4×10^{-8}
µg/liter	450	140	45	14	4.5

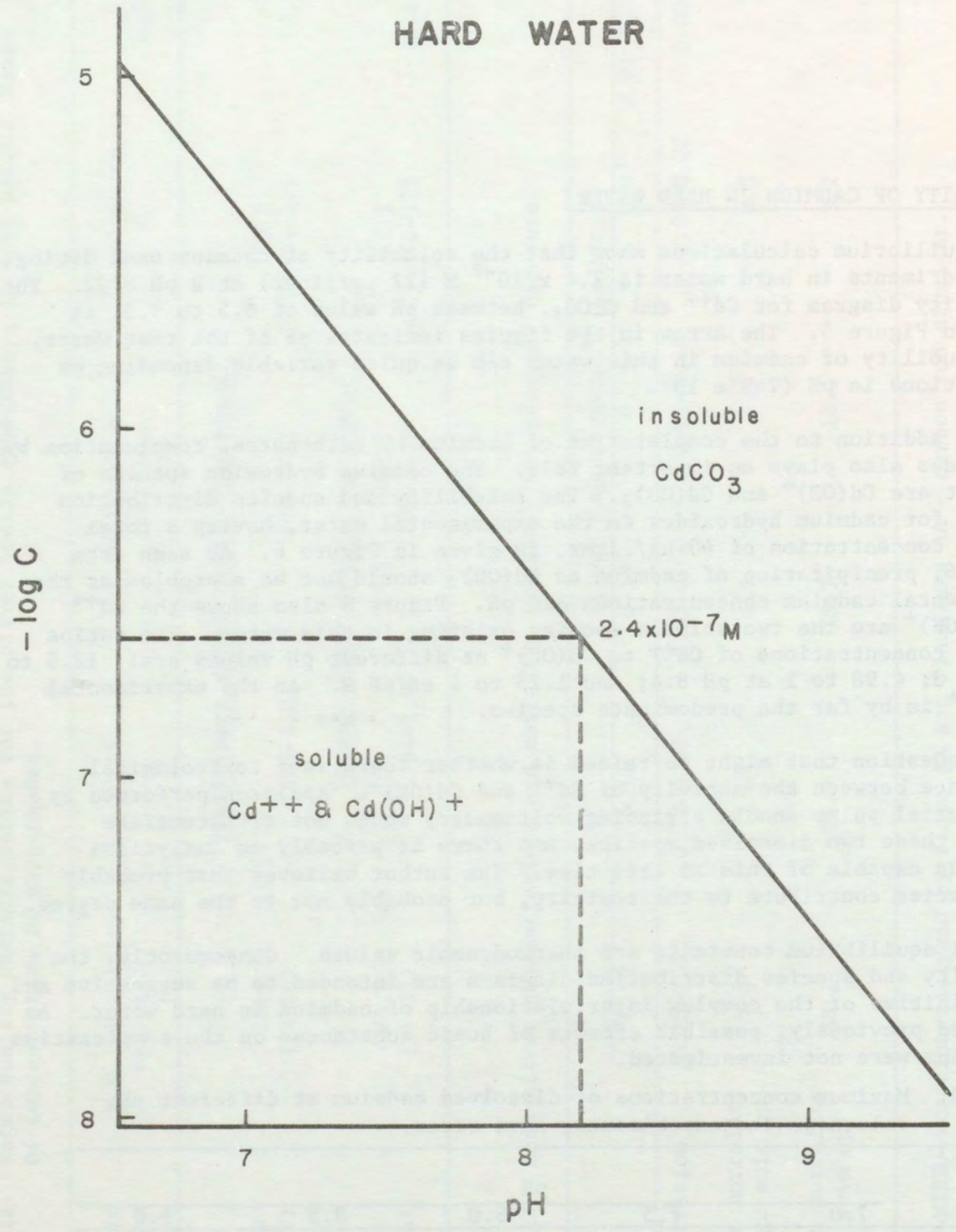

Figure 5. Solubility diagram for cadmium in hard water as determined by pH and the carbonate concentration.

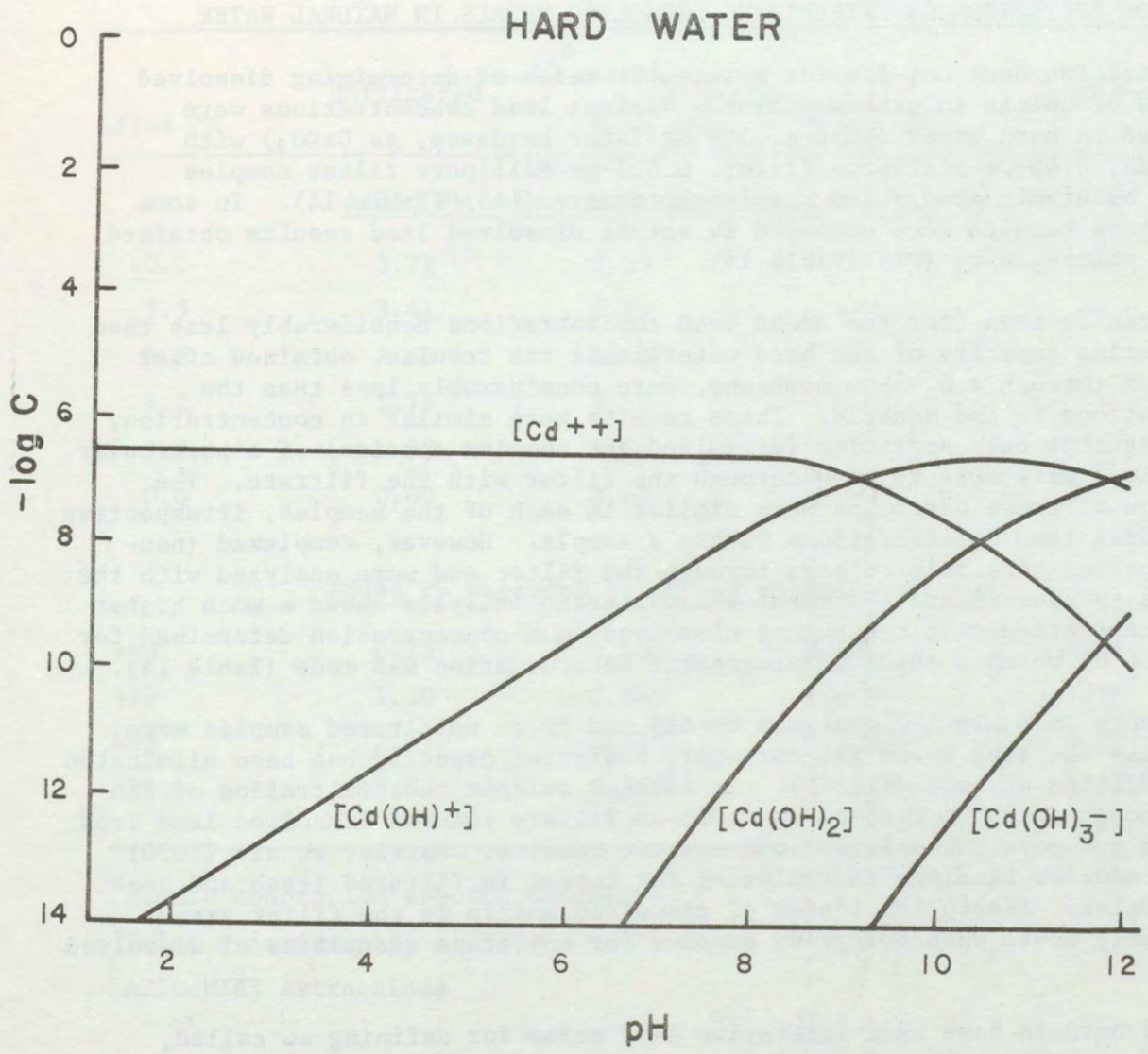

Figure 6. Solubility and species distribution diagram for cadmium hydroxide species versus pH at a total cadmium concentration of 40 µg/liter.

APPENDIX B

EFFECTS OF FILTRATION ON DETERMINING DISSOLVED METALS IN NATURAL WATER

Filtration does not provide a suitable means of determining dissolved fractions of metals in natural water. Various lead concentrations were determined in hard water (approx. 300 mg/liter hardness, as $CaCO_3$) with unfiltered, 0.45-µm Millipore filter, 0.025-µm Millipore filter samples analyzed by atomic absorption spectrophotometry (AAS) (Table 14). In some cases, these results were compared to actual dissolved lead results obtained by pulse polarography (PP) (Table 14).

It can be seen (for the added lead concentrations considerably less than the buffering capacity of the hard water) that the results, obtained after filtration through a 0.45-µm membrane, were considerably less than the concentrations in the aquaria. These results were similar in concentration, indicating that only particles (dissolved and complex species) of a particular size or less were able to pass through the filter with the filtrate. The proportion of these particles were similar in each of the samples, irrespective of the total lead concentrations within a sample. However, complexed (non-toxic) species were able to pass through the filter and were analyzed with the dissolved species in the filtrate. The filtrate analysis shows a much higher lead concentration that the actual dissolved lead concentration determined for one sample on which a pulse polarographic determination was made (Table 14).

As seen in Table 14, analyses by AAS and PP on unfiltered samples were essentially the same where the carbonate buffering capacity has been eliminated by the addition of lead (Fig. 3). It is also evident that filtration of the water through either a 0.45-µm or 0.025-µm filters removed dissolved lead from the water and gave inconsistent and erratic results. Marvin, et al. (1970) reported similar findings in analyzing for copper in filtered fresh and sea water samples. Absorption losses of dissolved metals on the filter are particularly acute when analyzing samples for any trace quantities of dissolved metals.

Many authors have used filtration as a means for defining so called, dissolved concentrations of metal in water. Stiff (1971) working with copper used filtration through a 0.45-µm membrane to define the dissolved fraction of copper in natural and polluted waters. Brown, et al. (1974) used filtration through a 0.45-µm membrane filter in their work defining the toxicity of copper to rainbow trout. As mentioned earlier, filtration was used in defining the dissolved concentrations in the dialysis experiments of Beneš and Steinnes (1974).

Filtration of water samples prior to the heavy metal analysis has been a standard procedure by many agencies, including USGS and USEPA, responsible for administrating water pollution control programs. Such practices should not be continued as a general procedure until a thorough evaluation has been made on the effects of filtration on the chemical nature, composition, and analytical results of heavy metals in natural waters.

Table 14. Effects of filtration on the analysis of lead in hard water (concentrations in mg/liter).

	AA[a]			pp[b]
	Unfiltered	Filtered		Unfiltered
Added Pb		0.45 µm	0.025 µm	
Added Pb below buffering capacity of water				
10.0	5.71	0.35	--	--
7.5	3.41	0.44	--	--
5.6	2.66	0.44	--	--
4.2	1.75	0.58	--	--
3.2	1.92	0.44	--	0.06
0.0	0.00	0.00	--	--
Added Pb exceeded buffering capacity of water				
460	0.25	0.103[c]	0.005[c]	0.29
470	1.30	0.545[c]	0.805[c]	1.26
480	2.77	0.835[c]	0.265[c]	2.85
490	5.05	0.380[c]	0.465[c]	4.79

[a]Atomic absorption spectrophotometry

[b]Pulse polarography

[c]APDC-MIBK extractions

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CHAPTER 6

TOXICITY RELATIONSHIPS TO COPPER FORMS IN NATURAL WATERS

R. W. Andrew

Environmental Research Laboratory-Duluth

Duluth, Minnesota 55804

Abstract

The acute toxicity of cupric salts to aquatic organisms, principally fathead minnows and Daphnia magna, as a function of water chemistry has been studied at this laboratory over a period of several years. Toxicity tests under a variety of experimental conditions have led to the general conclusion that copper complexes, e.g. soluble $CuCO_3$ or $Cu\ NTA^-$ are much less toxic than cupric ion (Cu^{++}). Similarly, copper precipitates are not biologically active and do not result in toxicity.

Recent work indicates that at a given pH, toxicity to both fish and Daphnia is well correlated with cupric ion activities measured using an ion specific electrode. From this, it is concluded that cupric-ion (Cu^{++}) is the major toxic form of copper. Increasing toxicity of cupric ion at high pH is hypothesized to result from interactions with sulfhydryl-containing proteins or enzymes.

Introduction

Considerable progress has been made in recent years toward determining the relationship of toxicity of the heavy metals to their aqueous chemistry. While early studies served to determine the relative sensitivity of aquatic species to the various metals and the grosser effects of such water quality parameters as hardness, pH, and dissolved oxygen concentration, recent work is directed toward determination of the precise relationships of toxicity to concentrations of ionic and molecular forms in solution, the physiological mechanisms involved, and toward application of the results to field situations. As evidenced by the number of papers at this workshop, much of this work has been conducted using copper as the toxic agent.

Numerous recent experiments have demonstrated that chelation of copper by organic reagents such as EDTA (Tabata and Nishikawa, 1969), NTA (Biesinger et al., 1974; Shaw and Brown, 1974), and amino acids (Black, 1974), effectively reduce toxicity. Reports by Japanese workers (Nishikawa and Tabata, 1969) also indicate that the reduction of toxicity is proportional to the stability constants of the chelating compounds. From these and other results it may be deduced that the chelated forms of copper are relatively nontoxic, and that reduction of toxicity is accomplished through reduction of cupric-ion activity. Although it is generally recognized that copper is similarly complexed (or chelated) by hydroxide, carbonate, phosphate, and organic compounds in natural waters (Stumm and Morgan, 1970; Stiff, 1971) a similar mechanism for the reduction of the biological activity of copper in these waters is not as readily proven experimentally.

Analytical methods have not been available until recently with sufficient accuracy, selectivity, and sensitivity for the direct determination of cupric-ion activities, especially in natural waters. Measurements of "dissolved copper" (Brungs et al., 1975; Shaw and Brown, 1974), "labile copper" (Mancy and Allen, 1974; Chau et al., 1974) and calculated ion-activities (Pagenkopf et al., 1974) have been used as a means of determining the toxic or biologically active fraction of copper. Zitko et al. (1973) and Shaw and Brown (1974) have demonstrated that it is now possible to measure cupric-ion activities in bioassay solutions at micromolar copper concentrations. These investigators also showed that reductions of toxicity by humates, NTA, and carbonate alkalinity reflected a corresponding decrease in cupric-ion activity. Shaw and Brown (1974), however, indicated as a result of experiments at two pH levels, that $CuCO_3$ (soluble) also contributed to toxicity.

The results presented herein summarize experimental toxicity studies similar to those listed above, conducted at the Environmental Research Laboratory-Duluth and its Newtown, Ohio field station over the past 8-9 years. The primary objective of these studies was to clarify the relationship between copper toxicity and water chemistry, and to apply the results toward meaningful water quality criteria for aquatic life.

Experimental Methods

The toxicity results reported here were obtained using static bioassays, except for the last experiment which utilized a flow-through design. In each of the static tests an excess of the toxic solution was provided so that effects of test animals on the dissolved oxygen concentration, pH, and CO_2 equilibria were minimized. Reagent grade chemicals (ACS) were used for all solutions and additions to the test water.

Routine procedures (American Public Health Association et al., 1971) were used to monitor Ca/Mg hardness, pH, total alkalinity, and dissolved oxygen concentrations in each of the tests. Atomic absorption spectroscopy with either flame or (more recently) graphite furnace techniques were utilized to measure total copper and/or dissolved copper (passing 0.45 μ filter) in each of the tests.

Values for LC50 using total copper, dissolved copper, or ionic copper were calculated using graphical or computer methods for fit of probit percentages vs. log copper. Mortality rates and median survival times were measured in some tests using the methods of Chen and Selleck (1969).

Stream Water Bioassays

Test waters for this first series of tests were obtained from Shayler Run, a small stream in southwestern Ohio which varied in quality due to wide fluctuations in flow and input from a sewage treatment plant upstream. The toxicity results reported were obtained using static bioassays of 5-10 juvenile fathead or bluntnose minnows in 10 l of solution, depending on the duration of the tests. Dilutions as noted in the tables, of unmodified stream water were made with demineralized water and $CaCl_2$, $MgSO_4$, and $NaHCO_3$ in the same ratios of $Ca:Mg:HCO_3$ as the stream water. Additions of phosphate in some tests were made with reagent grade $Na_2HPO_4 \cdot 7H_2O$ or $Na_4P_2O_7 \cdot 10H_2O$. Results reported in Figure 1, are for weekly tests conducted in unmodified stream water over a period of approximately 1 1/2 years. Experimental details of these tests are reported elsewhere (Brungs et al., 1975; Geckler, 1975).

Daphnia and Fry Bioassays

For tests with Daphnia magna and fathead minnow fry, 10 animals were placed in each of 300 ml. stoppered BOD bottles; pH's were adjusted with CO_2/air mixtures. Daphnia were 0 to 24 h old; fathead minnows 3.5-4.5 da old. This series of tests were conducted in Lake Superior water, and in lake water with additions of reagent grade $NaHCO_3$, $Na_2HPO_4 \cdot 7H_2O$, or $Na_4P_2O_7 \cdot 10H_2O$. All tests were replicated.

Cupric-ion activities and molecular concentrations of copper complexes in the initial tests with Daphnia magna were calculated from the dissolved copper concentrations and stability constants selected from the compilations of Sillen and Martell (1964, 1971) using a computer program. Cupric-ion activities in later experiments were measured using a cupric-ion specific electrode. The electrode was calibrated vs. activity standards prepared in 0.01 M acetate buffer using the methods of Hansen et al. (1972) and Smith and Manahan (1973).

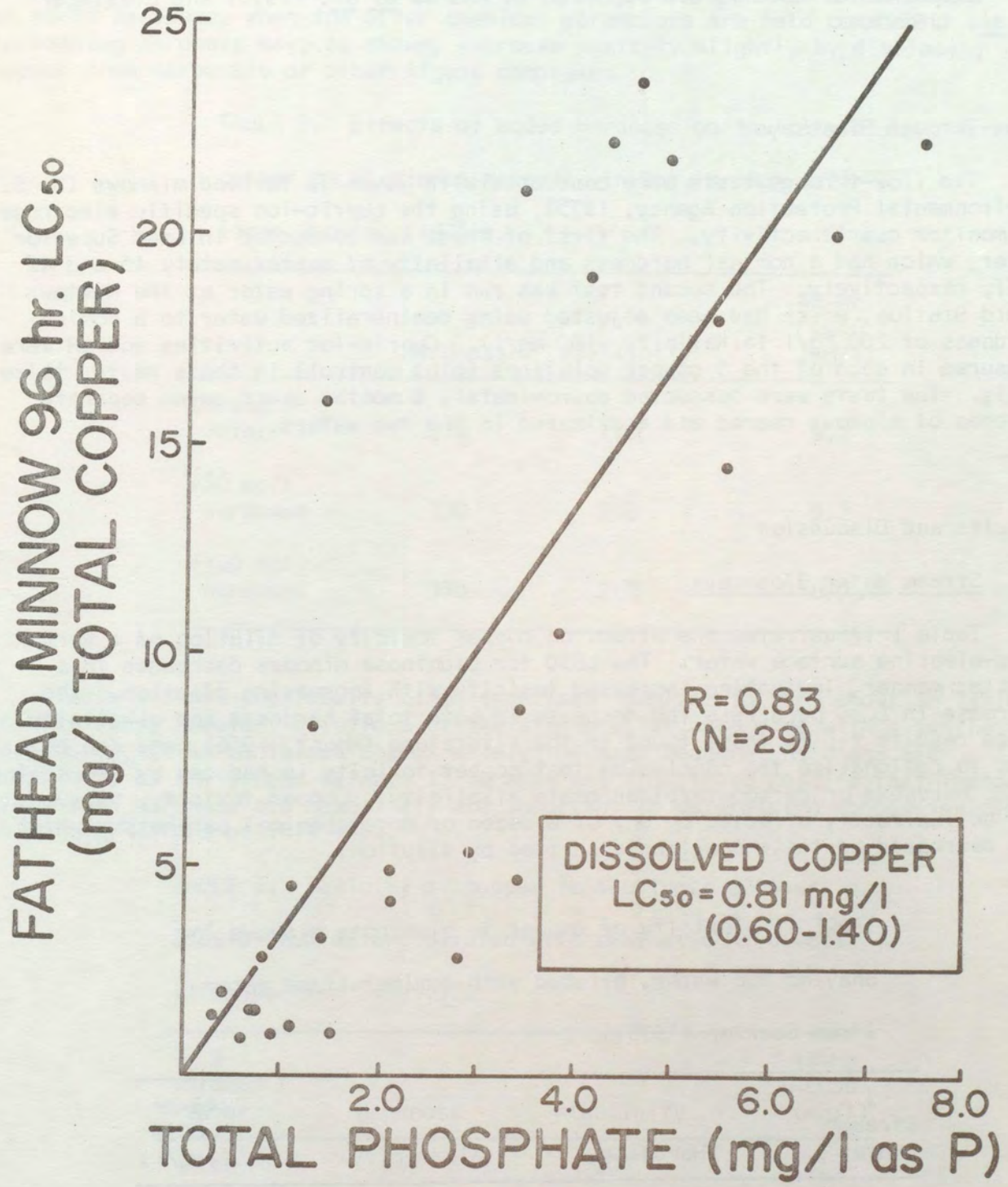

Figure 1 - Relationship of total copper 96-hr LC50 for fathead minnows and total phosphate concentration in Shayler Run water for 1 1/2 years. Inset shows dissolved copper LC50 for the same period (data from Geckler (1975)).

Experimental details are reported in Andrew et al. (1975) and Biesinger et al. (1974).

Flow-Through Bioassays

Two flow-through tests were conducted with juvenile fathead minnows (U. S. Environmental Protection Agency, 1975), using the cupric-ion specific electrode to monitor cupric activity. The first of these was conducted in Lake Superior water, which had a nominal hardness and alkalinity of approximately 45 and 42 mg/l, respectively. The second test was run in a spring water at the Newtown Field Station, which had been adjusted using demineralized water to a nominal hardness of 200 mg/l (alkalinity ~180 mg/l). Cupric-ion activities and pH were measured in each of the 5 copper solutions (plus control) in these tests, twice daily. The tests were conducted approximately 8 months apart using separate batches of minnows reared and acclimated in the two waters.

Results and Discussion

Stream Water Bioassays

Table 1 illustrates the effect on copper toxicity of dilution of a very hard-alkaline surface water. The LC50 for bluntnose minnows decreases in a regular manner, indicating increased toxicity with increasing dilution. The decrease in LC50 parallels the decrease in both total hardness and alkalinity. These results typify those found in the literature (Mount, 1966), and can be used to rationalize the conclusion that copper toxicity is reduced by increasing Ca/Mg hardness or carbonate/bicarbonate alkalinity. Copper toxicity, however, may be indirectly affected by any of a dozen or more chemical parameters which are decreased or their equilibria shifted by dilution.

TABLE 1. Toxicity of copper to bluntnose minnows in Shayler Run water, diluted with demineralized water (from Geckler (1975)).

% Stream* water	Hardness	Alkalinity	48-Hr LC50 (mg/l)
100	316	206	19
80	255	174	9.6
67	205	138	9.1
50	158	110	3.3

*Diluted with demineralized water.

In Table 2, the effects of added Ca/Mg hardness in a similar water are illustrated. In this case it can be seen that there is little or no effect of added hardness, when the other chemical parameters are held constant. Increasing hardness may, as shown, increase toxicity slightly by displacing copper from carbonate or other ligand complexes.

TABLE 2. Effects of added hardness on toxicity of copper to bluntnose minnows, in Shayler Run water (from Geckler (1975)).

	Hardness	Alkalinity	48-Hr LC50 (mg/l)
Stream water	276	216	9.2
+50 mg/l hardness	330	208	9.2
+100 mg/l hardness	370	210	8.0

Table 3 shows the results of an experiment similar to that shown in Table 1. In this case, however, dilutions of the stream water were made with a synthetic hard water, which contained approximately the same measured hardness and alkalinity as the stream water. It is apparent from these results that factors other than hardness and alkalinity are important in determining copper toxicity in natural waters of this type.

TABLE 3. Toxicity of copper to bluntnose minnows in Shayler Run water, diluted with synthetic hard water (from Geckler (1975)).

% Stream* water	Hardness	Alkalinity	48-Hr LC50 (mg/l)
100	292	204	11
75	300	204	6.7
50	302	200	3.6
25	296	202	2.9

*Dilutions with artificial hard water.

Although the solution to the problem posed in Table 3 proved to be a fairly simple one, the approach to the problem was not straightforward and led to the remainder of the investigations reported here. It was also realized at this point that a generalized analytical approach was needed for determining the toxicity of metal forms; since an investigation into the effects of all possible chemical parameters on each of the heavy metals was a nearly impossible task. Two separate approaches were eventually used, both of which led to at least partial solutions, and to additional applications.

The first of these approaches, as illustrated in Figure 1 (data from Geckler (1975)) was to correlate periodic toxicity measurements in the stream, with analytical results for each of a number of routinely measured chemical parameters. This figure shows that the total copper 96-hr LC50 for toxicity to fathead minnows is closely related to the total phosphate concentration in the stream water. Copper toxicity did not appear to be related to any of the other commonly measured parameters including hardness, alkalinity, and pH. This figure also shows that over this same period (1 1/2 years), the LC50 when based on dissolved copper measurements was relatively constant.

These facts, combined with observations of copper precipitates at the time of the tests, have led to the general conclusion that toxicity is independent of the volume or concentration of copper precipitate; and indirectly that copper precipitates are nontoxic. The results also indicate that precipitation by phosphate is a major factor, at least in some natural waters, limiting the availability and therefore the toxicity of copper.

This latter result was confirmed experimentally as shown in Table 4. In this experiment both orthophosphate and pyrophosphate significantly reduced copper toxicity, as measured by the total copper LC50. The effects of the two phosphates on copper solubility will be discussed in connection with later tests.

TABLE 4. Effect of added orthophosphate and pyrophosphate on toxicity of copper to bluntnose minnows, in Shayler Run water (from Geckler (1975)).

Addition	48-Hr LC50 (mg/l)
None	5.6
+7.7 mg/l P (Na_2HPO_4)	20
+17.4 mg/l P ($Na_4P_2O_7$)	11

Results of the previous tests also demonstrate the futility of basing the results of toxicity bioassays on total metal analyses, especially in situations where a large percentage of the metal are either precipitated or complexed. Results of such tests not only are highly variable, but are largely irreproducible even when the major chemical parameters are known. Such data are not useful to any extent, except as a general indication of the range of toxicities to be expected under certain field conditions. They may be misleading also in that they do not provide the researcher an indication of the extremes of toxicity to be encountered under extreme conditions.

Daphnia and Fry Bioassays

The second approach used, was primarily an analytical one. In each experiment, as many as possible of the various copper forms were measured, or calculated from the major ions present and the known stability constants. Initial attempts at determination of "labile" forms of copper with ASV, and of cupric-ion using the ion-selective electrode were largely unsuccessful, because of lack of sensitivity of the available instruments. However, from these first experiments we were able to calculate the ionic and molecular activities of the various copper forms and complexes. These initial experiments also demonstrated the major shifts in copper equilibria that occur with changes in carbonate/bicarbonate, phosphate, and pH equilibria; and these in turn related to changes in toxicity. Examples are shown in Table 5 and Figures 2 and 3.

TABLE 5. Effects of added bicarbonate on toxicity of 318 µg/l copper to Daphnia magna, in Lake Superior water (pH 7.4 ± 0.1) (from Andrew et al. (1975)).

Added $NaHCO_3$ (mM)	Dissolved copper (µg/l)	Cu^{++}-ion activity (µM)	$CuCO_3$ (dissolved) (µM)	Daphnia survival time (min)
None	215	0.41	2.69	~50
1	245	0.25	3.39	60
2	262	0.18	3.80	73
4	268	0.12	3.98	451
10	278	0.06	4.07	544

Table 5 shows the effect on the distribution of various copper forms and median time of survival of Daphnia with varying amounts of bicarbonate added. These results indicate that increasing carbonate alkalinity reduces copper toxicity, by increasing the concentration of soluble copper carbonate ($CuCO_3$) and decreasing the cupric-ion activity. Copper toxicity in this test was directly related to cupric-ion activities. One unexpected outcome was that toxicity was inversely proportional to the dissolved copper concentration.

The following figure (Figure 2) compares the mortality rate of Daphnia and cupric-ion activities in a series of solutions (318 µg/l total copper) with increasing pyrophosphate concentrations. As in the previous table, toxicity to Daphnia is closely related to the cupric-ion activity, and is inversely related to the formation of the soluble pyrophosphate complexes. The figure demonstrates also, the large decrease in toxicity that occurs in the presence of relatively low concentrations of a moderately strong chelating agent. Daphnia survival is extended from approximately 65 min to over 3 days, by the equivalent of only 1.5 mg total phosphorus per liter.

Figure 3 illustrates the linear relationship of toxicity (as indicated by the reciprocal of the median survival time for Daphnia) to cupric-ion activity. These results are from a large experiment where a series of copper concentrations in lake water were tested with additions of bicarbonate, orthophosphate, and pyrophosphate (Andrew et al., 1975). Although not shown in the figure, pyrophosphate had the greatest effect on toxicity, with 0.11 mM P_2O_7 reducing toxicity to negligible levels in solutions containing up to 128 µg copper/l. Cupric activities were reduced primarily through formation of soluble pyrophosphate complexes. Orthophosphate (0.21 mM), however, reduced toxicity primarily by limiting copper solubility. As shown in Table 5, bicarbonate reduces toxicity through both mechanisms, i.e., reducing solubility and through complex formation.

Tables 6 and 7 summarize the results of similar experiments with Daphnia and fathead minnows, respectively. These results are from recent experiments where cupric-ion activities have been measured using the specific-ion electrode. The results confirm earlier observations on the reduction of cupric activities by additions of bicarbonate, and the two phosphates. They also show that in spite of an approximate 50-fold greater tolerance of cupric-ion by fathead minnows, the overall response is similar.

Flow-Through Bioassays

Figure 4 shows the results of flow-through acute toxicity tests of copper to fathead minnows in Lake Superior water and Newtown spring water. Mean cupric-ion activities are plotted for each water as a function of total copper concentration. Also shown in the figure are the 96-hr LC50 and confidence limits for each of the two tests based on cupric-ion activity. These results demonstrate that (1) ion-activities in lake water are much higher at equivalent total copper concentrations and (2) LC50's are not significantly different when based on cupric-ion activity. The shape and slope of the activity curves also show graphically the complexing capacity of the two waters. These results also demonstrate that cupric-ion activities can be measured and utilized successfully in such bioassays.

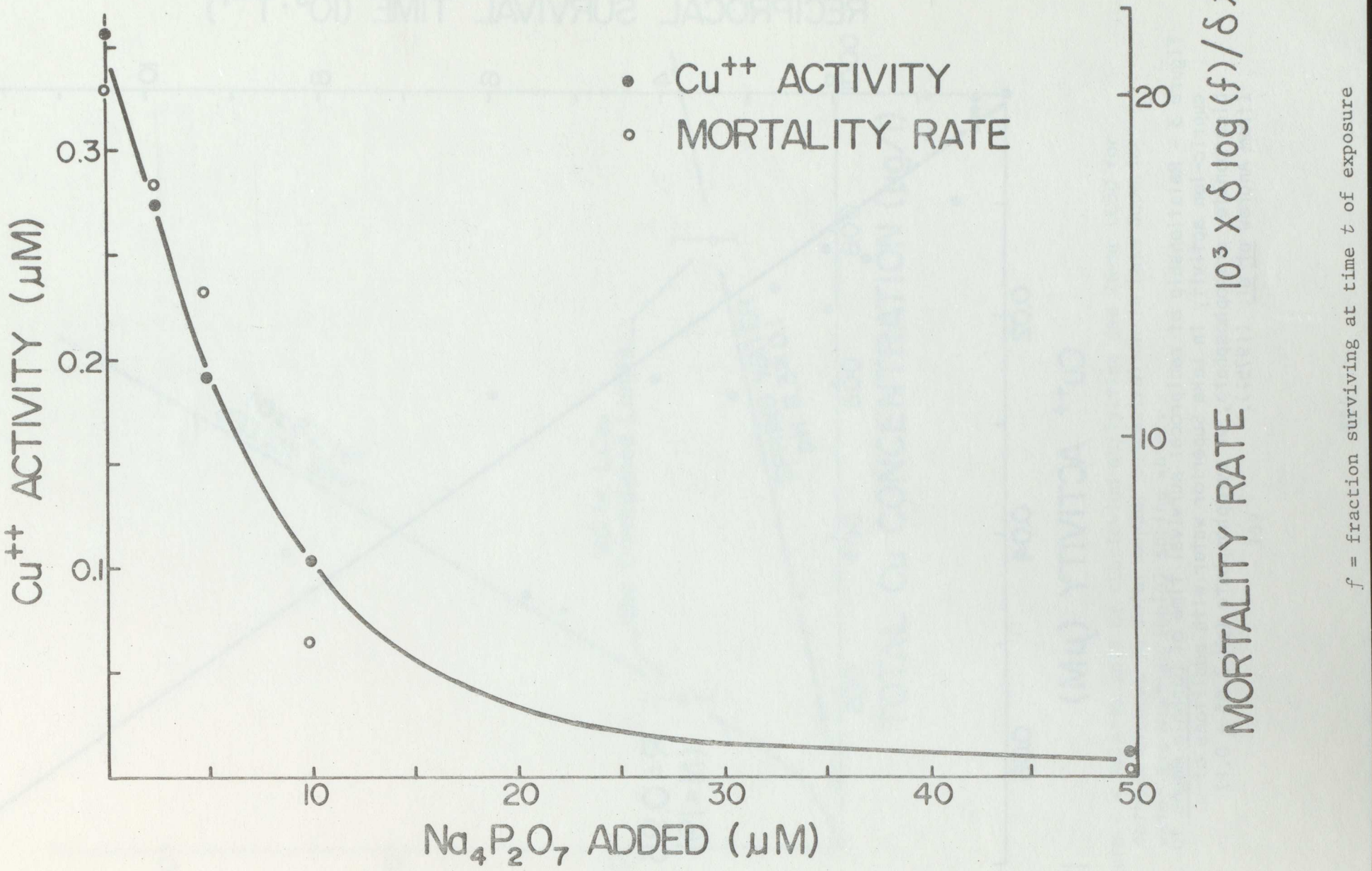

Figure 2 - Effects of added pyrophosphate on cupric-ion activity and toxicity of 318 g/l copper to Daphnia magna in Lake Superior water (pH 7.5 ± 0.1) (from Andrew et al. (1975)).

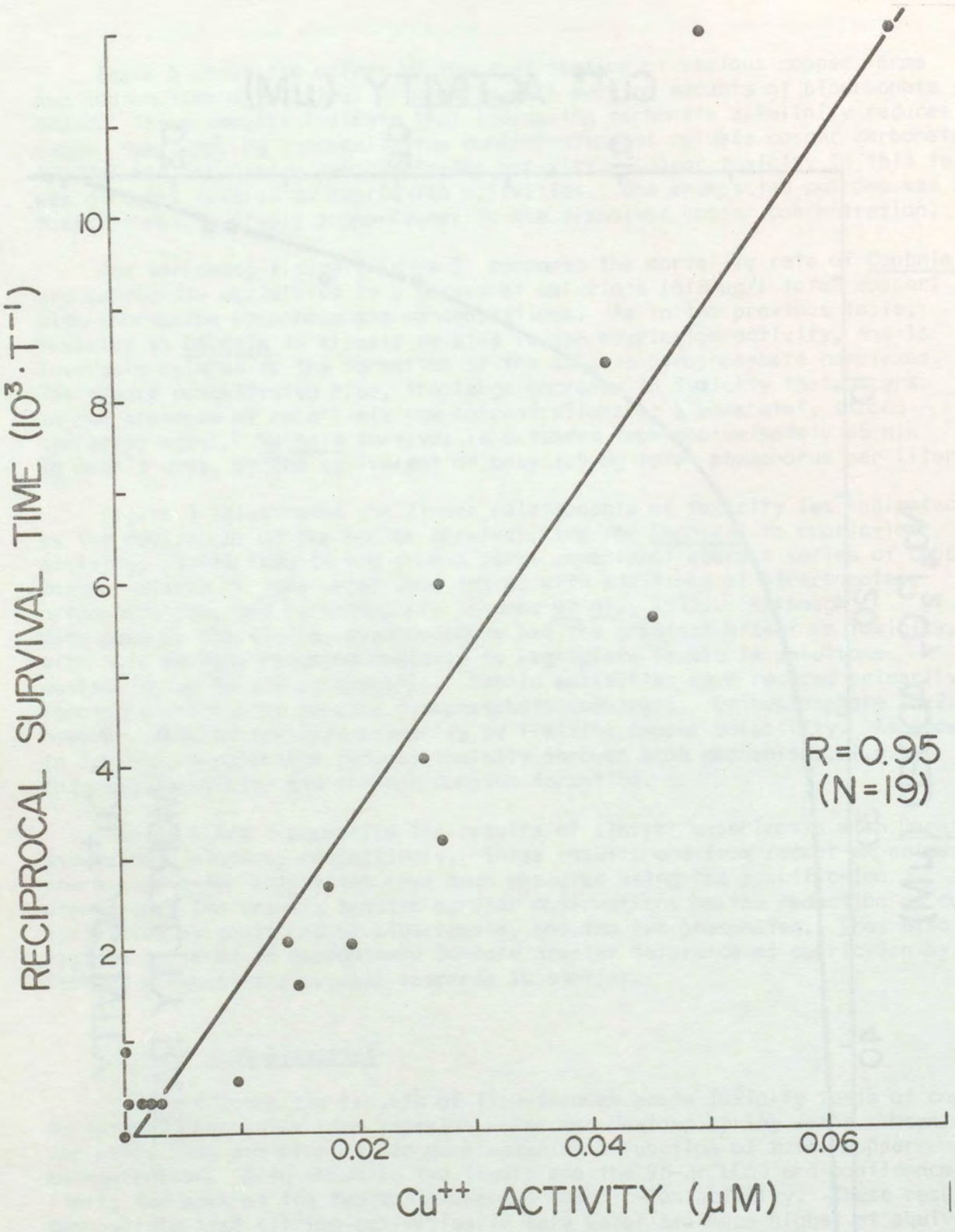

Figure 3 - Relationship of reciprocal survival time of Daphnia magna to cupric-ion activity in Lake Superior water with additions of bicarbonate, orthophosphate, or pyrophosphate (pH 7.95 ± 0.1) (from Andrew et al. (1975)).

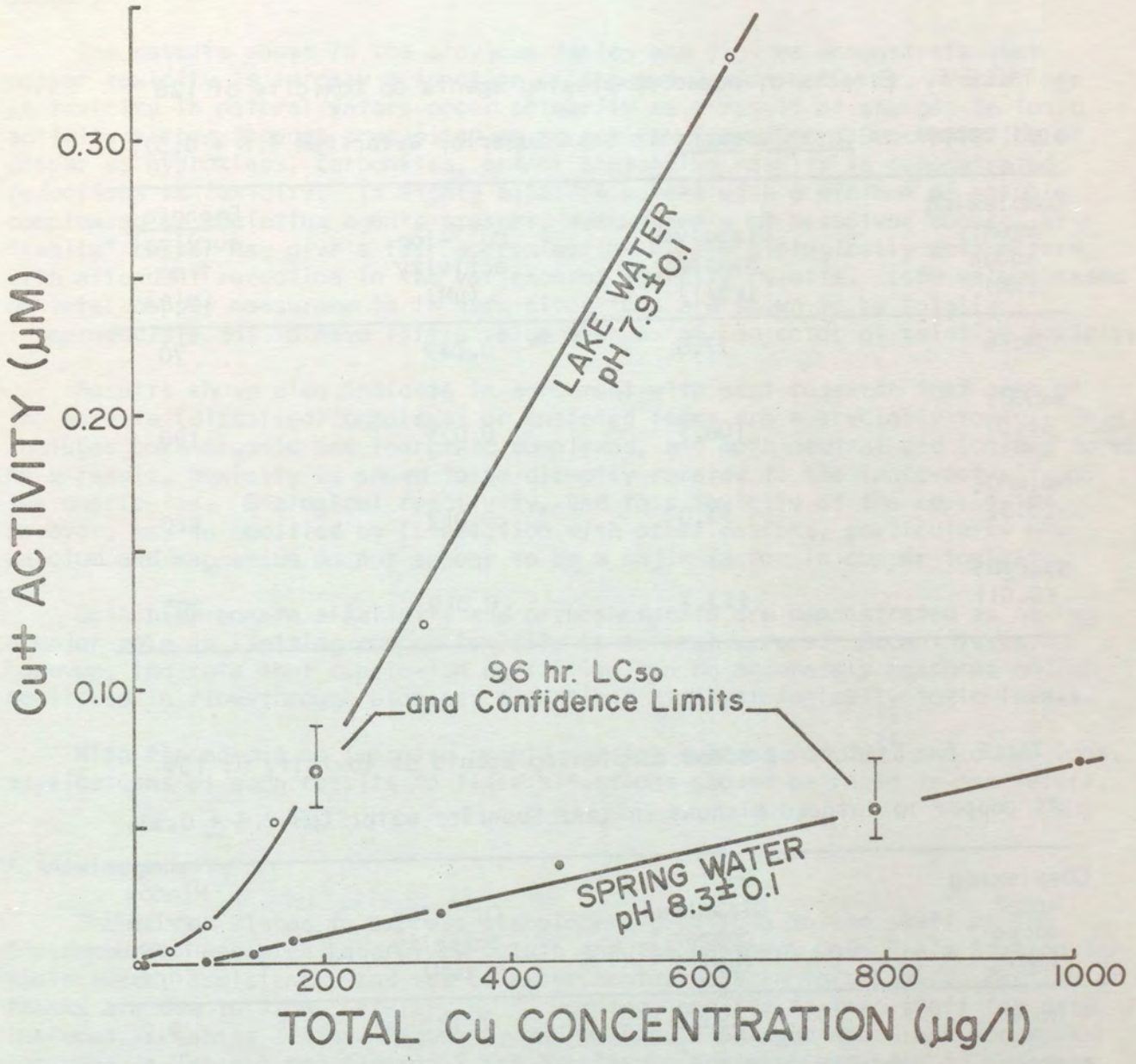

Figure 4 - Relationship of cupric-ion activities and 96-hr LC50 for fathead minnows to total copper concentration in Lake Superior water and Newtown (Ohio) spring water.

TABLE 6. Effects of added complexing agents on toxicity of 128 µg/l copper to Daphnia magna in Lake Superior water (pH 7.8 ± 0.3).

Complexing agent added (mM)	Dissolved copper (µg/l)	Cu^{++}-ion activity (µM)	Daphnia survival time (min)
None	77.5	0.049	70
$NaHCO_3$ (1.0)	108.9	0.023	190
Na_2HPO_4 (0.5)	55.8	0.017	420
$Na_4P_2O_7$ (0.01)	113.7	0.019	205

TABLE 7. Effects of added complexing agents on toxicity of 159 µg/l copper to fathead minnows in Lake Superior water (pH 7.8 ± 0.3).

Complexing agent added (mM)	Dissolved copper (µg/l)	Cu^{++}-ion activity (µM)	Minnow survival time (hr)
None	131.5	0.130	9.2
$NaHCO_3$ (1.0)	134.4	0.023	114
Na_2HPO_4 (0.5)	60.1	0.030	120
$Na_4P_2O_7$ (0.01)	155.5	0.051	67.2

Summary

The results shown in the previous tables and figures demonstrate that copper toxicity is largely a function of the cupric-ion activity. Variations in toxicity in natural waters occur primarily as a result of changes in ionic activity either through precipitation or complex formation. Precipitation of copper as hydroxides, carbonates, and/or phosphates results in demonstrated reductions in toxicity. In highly alkaline waters with a minimum of soluble complexing or chelating agents present, measurements of dissolved copper, or "labile" copper may give a fair approximation of the biologically active form, with attendant reduction in the variance of toxicity results. LC50 values based on total copper measurements in such situations are shown to be totally irreproducible and to have little value even as an indicator of relative toxicity.

Results shown also indicate in agreement with past research that none of the soluble (dissolved) complexes or chelated forms are appreciably toxic. This includes both organic and inorganic complexes, and both neutral and ionized forms. As a result, toxicity is shown to be directly related to the ionic-activity of the cupric-ion. Biological reactivity, and thus toxicity of the cupric-ion, however, may be modified by interaction with other cations, particularly H^+. Calcium and magnesium do not appear to be a major factor in copper toxicity.

Both bicarbonate alkalinity and orthophosphate are demonstrated as having a major role in limiting copper toxicity in natural waters. Recent tests, however, indicate that cupric-ion activities can be accurately measured and/or monitored in flow-through bioassay situations at physiologically toxic levels.

With the advent of improved remote sensing systems for Cu^{++} and other ions, applications of such results to field situations should be rapid in the future.

Acknowledgements

The author wishes to express his sincere gratitude to the staff of the Environmental Research Laboratory-Duluth and the Newtown, Ohio Field Station for their ideas, assistance, and use of experimental data in this paper. Particular thanks are due to J. R. Geckler, W. B. Horning, and the Newtown staff for data included in Tables 1-4 and Figure 1; and to Drs. Biesinger and Glass for work included in Table 5 and Figures 2 and 3. Thanks are also extended to numerous others who contributed to this work and the ideas embodied therein.

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EFFECTS OF ORGANIC POLLUTANTS ON COPPER TOXICITY TO FISH

CHAPTER 7

David P. Chynoweth, Jeffery A. Black* and K. H. Mancy
Department of Environmental and Industrial Health
The University of Michigan
Ann Arbor, Michigan 48105

ABSTRACT

The effect of several organic legands on copper toxicity to fish was investigated using a 96 hour flow-through bioassay. Guppies *(Lebistes reticulatus)* were exposed to copper (50 - 500 µg/liter) in the presence of various organic ligands in moderately hard water (66.8 - 98.0 mg/liter $CaCO_3$) maintained at pH 7.5. The organic compounds tested were selected on the basis of their prevalence as pollutants or their complexing properties. Glycine, cysteine, EDTA, NTA, and citric acid were added at concentrations of 36×10^{-6} M. Albumin, humic acid, and secondary sewage effluent were added to give a final concentration of 5 mg/liter (as total organic matter). Median lethal copper doses were calculated using log probit analyses of the bioassay data. The relative binding capacities of the organic ligands were determined by anodic stripping voltammetry. The bioassay toxicity results were compared to the stability of the copper-organic complexes present in the bioassay solutions. Test fish were analyzed by atomic absorption spectrophotometry to determine the amount of copper accumulation in the internal organs. An inverse relationship was observed between degree of copper binding and copper toxicity. No significant correlation was obtained between organic binding or toxicity and uptake of copper by fish. The results of this study demonstrate that bound copper is less toxic than unbound copper.

*Current Address: Department of Biology, University of Kentucky, Lexington, Kentucky.

INTRODUCTION

Copper and other heavy metals are currently the focus of investigation because of their increasing abundance as environmental pollutants and demonstrated toxicity to biota. A major goal of toxicological research on heavy metals has been to arrive at a theoretical basis for estimating "safe" levels of exposure to organisms. Most of the past research on metal toxicity to aquatic biota has been empirical in nature resulting in data describing a response (usually death) resulting from short term exposure (usually less than 96 hours) to metals administered as a dose or more often a concentration added to the surrounding medium. Water quality standards (17) have then been derived by applying an "application factor" (15) to toxicological data obtained in this manner.

Two major criticisms are apparent in applying the above types of bioassay data to prediction of safe levels of metals in the environment: (1) the use of death resulting from short term exposure as a response criterion is not useful in predicting undesired effects such as acute or chronic debilitation, teratogenic and genetic effects, and chronic deaths; and (2) the data lack reliability and validity in terms of predicting toxicity under real environmental conditions. In response to the first criticism a number of investigators of fish toxicology have studied responses such as respiration, blood oxygen levels, and ventilatory activity, coughing frequency (23), growth and reproduction rates (28), tissue and liver pathology (12), and locomotor responses (11). The second criticism may be attributed to the effect of a variety of environmental factors on the physiology of the organism and the form and distribution of the metal in the organism's environments on toxic response. The latter criticism is illustrated by reports of copper threshold limit values to fish ranging from 15 - 3000 µg/liter (13).

A variety of physical, chemical, and biological factors influence metal toxicity by altering the quality and quantity of metal species reaching the target sites of toxicity. These factors result in major variability in heavy metal toxicity data and will be the focus of discussion in this paper. Although many of the concepts discussed would be applicable to other heavy metals and organisms, the focus of this paper will be on copper toxicity to fish.

Predictive models of copper toxicity to fish are dependent upon a knowledge of (1) the concentration and distribution of copper species in the fish environment, (2) the types and concentrations of copper species eliciting a toxic response, and (3) the routes of exposure of toxic species to the target site.

DISTRIBUTION OF COPPER IN AQUATIC ENVIRONMENTS

Copper discharged in aquatic environments becomes distributed into a variety of forms which may be categorized as follows (14):

I. Soluble species
   - A. Copper ions
   - B. Copper complexes with inorganic ligands
   - C. Copper complexes with organic ligands

II. Insoluble particulates
   - A. Colloidal particulates of copper complexes or aggregates of hydrous metal oxides
   - B. Metal complexes absorbed on organic or inorganic particulates

III. Soluble or insoluble copper species contained within biomass

The distribution profile of these copper species is unique for each aquatic ecosystem and a function of a number of water quality characteristics including pH, oxidation-reduction potential, alkalinity, hardness, dissolved oxygen, cations, anions, organic matter, and suspended solids.

Numerous studies (29, 22, 20, 18, 7) have demonstrated that copper becomes concentrated in the sediment and suspended solids in the overlying water. Culp (7) and Stiff (29) reported ratios of particulate to soluble copper ranging from 47 - 88%. Copper also becomes concentrated in various components of biomass (8) which not only represent a significant component of the particulate fraction, but also a potential vector for transport of copper into fish. At pH and bicarbonate levels of most fresh waters ionic copper in excess of 0.5 mg/liter reacts with bicarbonate and hydroxide to yield malachite (29):

$$2\ Cu^{++} + HCO_3^- + 2OH^- \rightleftharpoons Cu_2(OH)_2CO_3 + H^+ \qquad \text{Eq. 1}$$

In the absence of organic ligands the aqueous chemistry in most aquatic environments of copper at low concentrations (less than 0.5 mg/liter) can be described primarily by its reaction with carbonate and to a lesser extent with hydroxide:

$$Cu^{++} + HCO_3^- \rightleftharpoons CuCO_3 + H^+ \qquad \text{Eq. 2}$$

$$Cu^{++} + 2H_2O \rightleftharpoons Cu(OH)_2 + 2H^+ \rightleftharpoons CuO + 2H^+ + H_2O \qquad \text{Eq. 3}$$

The relative distribution of these species is a function of pH and concentration of carbonate; these relationships are depicted in Table 1 taken from Mancy and Allen (14). From these calculated data it is apparent that, at low concentrations of copper (10^{-6} M) and pH ranges of 6.5 to 8.0, total copper concentration is the sum of ionic copper and copper complexed with carbonate.

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* Not included by Mancy and Allen (14)

In practice one can measure the concentration of ionic copper using an ion specific electrode (29) or anodic stripping voltammetry (1) and calculate the amount of copper carbonate if pH and carbonate concentration are known.

Table 1. Distribution of predominant species of copper as a function of pH and total carbonate. Total [Cu] = 10^{-6} M. (8)

Copper Species	$[CO_3^{=}]$ tot moles/l	-log [Cu] moles/liter at different pH values			
pH		6.5	7.0	7.5	8.0
Soluble Copper	10^{-2}	6.00	6.00	6.00	6.00
	10^{-3}	6.00	6.00	6.35	6.86
Insoluble Cu	10^{-2}	Nil	Nil	Nil	Nil
	10^{-3}	Nil	Nil	6.26*	6.06*
Cu^{++}	10^{-2}	6.83	7.41	7.96	8.50
	10^{-3}	6.21	6.55	7.35	8.35
$CuCO_3$	10^{-2}	6.07	6.02	6.02	6.04
	10^{-3}	6.45	6.16	6.41	6.89
$Cu\ [CO_3]_2^{=}$	10^{-2}	8.85	8.16	7.61	7.11
	10^{-3}	10.22	9.30	9.00	8.96

*solid species is tenorite [CuO]

In samples from aqueous environments the levels of soluble copper often exceed theoretical limits (1, 30). This has been attributed to the formation of soluble complexes with a variety of organic constituents of the water including humic acids, fulvic acids, porphyrins, amino acids, amino sugars, polysaccharides, fatty acids, and industrial pollutants such as NTA and other detergents. The dissolved organic fraction in natural waters usually ranges from 0.1 mg/liter to 10 mg/liter (7). Many of these organic ligands compete with carbonate for ionic copper thus explaining the anomolous high levels measured in natural waters. Certain organic ligands have been demonstrated to release metals from sediments (22). The binding potential of organics is frequently described in terms of stability constants examples of which are listed in Table 4. In summary, most of the soluble copper in natural waters is present as ionic copper, copper carbonate, and copper complexed with organic ligands.

TOXICITY OF VARIOUS COPPER SPECIES TO FISH

Most previous studies on copper toxicity to fish have reported exposure response data in terms of added concentration. Only a few investigators have attempted to measure the distribution of copper species in the bioassay medium (3, 4, 24, 31). In view of the fact that copper becomes distributed into a

variety of complexed forms (some of which are not toxic), it is not surprising that lethal concentrations ranging from 15 to 3000 µg/liter have been reported in the literature. Furthermore, copper toxicity studies are often conducted in media lacking soluble organic matter and particulate material typical of most surface waters. In view of demonstrated complexation of copper by this matter such studies would tend to overestimate toxic effects of copper in surface waters. In practice this source of error might be minimized by exposure of fish to copper in water obtained from the aquatic environment of interest.

A few investigators have employed electrochemical techniques in estimating soluble species of copper in fish bioassays (3, 4, 24, 31). Other studies have examined the effect of hardness (31, 19, 16) and a variety of organic compounds (3, 4, 24, 27, 31) on copper toxicity. The results of these studies will be discussed below. No literature could be found on the toxicity of particulate forms of copper to fish.

Soluble Inorganic Copper

In most natural water the predominant forms of soluble copper are Cu^{++} and $CuCO_3$. Shaw and Brown (24) examined the toxicity of these species to rainbow trout in hard water (250 mg/liter as $CaCO_3$). The relative concentrations of these two species were varied by adjusting the pH to values of 6.5 and 7.5 Identical toxicity profiles observed at both pH levels led to the conclusion that both copper species exhibit equal and additive toxicity. Although concentrations of ionic copper were measured with an ion specific electrode, the levels (3 - 140 µg/liter) were below the accurate limits of this technique (200 µg/liter).

The effect of varying carbonate alkalinity independent of pH and hardness on copper toxicity has not been examined. Roberts and Allen (21) described a method for independently controlling these variables in bioassay media by adding different concentrations of $NaHCO_3$, NaOH, and varying the concentration of CO_2 bubbled through the medium. Increase in alkalinity should result in an increase in the ratio of $CaCO_3$ to Cu^{++} which according to Shaw and Brown (24) should not effect toxicity. This relationship, however, needs further investigation under conditions where alkalinity is varied independent of pH and hardness.

Several studies have demonstrated that copper toxicity is inversely related to hardness (16, 19, 31). Since pH and alkalinity are variables associated with hardness in natural waters, the relationship of hardness and toxicity remains unclear and needs further investigation. It has been suggested that cations associated with hardness (Ca^{++} and Mg^{++}) reduce copper toxicity by reduction of gill permeability to cationic transport (9) and by competition with copper for the active sites of inhibition (5).

Effect of Soluble Organic Ligands on Copper Toxicity

A number of recent studies have examined the effects of a variety of soluble organic compounds and organic mixtures on copper toxicity to fish.

Compounds examined include nitrilotriacetic acid (NTA) (24, 27), ethylene diamine tetraacetic acid (EDTA) (27), humic substances (4, 31), glycine (4), and sewage effluent (4). These compounds or mixtures all resulted in reduction in toxicity which was attributed to formation of stabile complexes with copper. The following studies have attempted to quantitatively define the relationships of copper, toxicity, and organic ligands.

Sprague (27) exposed brook trout to various mixtures of copper and zinc in the presence of different concentrations of NTA in soft water (14 mg/liter as $CaCO_3$). Toxicity was observed to be directly related to the ratio of NTA to toxic units of metal in solution (one toxic unit is defined as the threshold concentration of metal exhibiting a lethal effect in the absence of organics). Reduction of metal toxicity by NTA adhered to the relationship that one mole of NTA complexes with one mole of metal, EDTA was also reported to reduce metal toxicity but larger concentrations are required than NTA because of the higher molecular weight of EDTA.

The effect of humic acid on reduction of copper toxicity to juvenile Atlantic salmon was studied by Zitko et al. (31). Measurements of complexation of copper versus humic acid concentration were used to predict Incipient Lethal Levels (ILL) of copper in the presence of two concentrations of humic acid. ILL values of 58 and 110 µg/liter copper were predicted at humic acid concentrations of 5 and 10 mg/liter respectively. These values were slightly lower than observed ILL values of 90 and 165 µg/liter.

Brown et al. (4) observed a direct relationship between concentrations of sewage effluent, glycine, and humic substances and reduction of copper toxicity to rainbow trout. Measurements of ionic copper with an ion specific electrode were used to determine complexation of copper at different concentrations of humic substances. A direct relationship between complexed copper and median period of survival was observed.

The effect of several organic ligands on copper toxicity to juvenile guppies was recently investigated in our laboratory using 96hour flowthrough bioassays. Fish were exposed to copper concentrations of 50 - 500 µg/liter in the presence of several organic ligands in moderately hard water (66.8 - 98.0 mg/liter $CaCO_3$) maintained at pH 7.5 and 25 C. Glycine, cysteine, EDTA, NTA, and citric acid were added at concentrations of (3-6) x 10^{-6} M. Albumin, humic acid, and secondary sewage effluent were added to give a final concentration of 5 mg/liter (as total organic matter). Median lethal copper concentrations were calculated using log probit analysis of the bioassay data. The relative binding capacities of the organic ligands were determined by anodic stripping voltammetric measurement of electroactive copper in the bioassay media. The internal copper concentrations of test fish were determined by analyzing the acid digest of fish by atomic absorption spectrophotometry. Water quality characteristics (Table 2) and copper toxicity in control chambers (Table 3) showed minimal variance during the course of this investigation.

Table 2. Summary of water quality characteristics for all bioassays.

Organic Added	Alkalinity mg/l as $CaCO_3$	pH	Hardness mg/l as $CaCO_3$	Temp °C	Resistivity ohms x 10^3
Control 1	36	7.4	87.5	25.9	3.4
Cysteine	33	7.4	96.2	24.5	3.4
EDTA	36	7.5	72.5	24.8	2.6
Glycine	39	7.5	98.0	25.0	2.9
NTA	41	7.5	92.8	25.0	3.1
Citrate	29	7.4	70.0	26.0	2.8
Egg Albumin	34	7.6	68.8	26.5	2.5
Humic Acid	28	7.5	70.0	26.0	2.6
Control 2	31	7.6	67.2	25.0	2.6
Sewage Eff.	28	7.5	66.8	25.0	2.6

Table 3. Standard Bioassay results.

BIO	Copper Dose (µg/liter)	Percent Dead After 96 Hours
I	195	90
II	175	76
III	197	89
IV	235	83
V	192	85
VI	203	100[a]
VII	232	100[b]
VIII	209	100[c]
IX	216[d]	85[d]
X	170	88

[a]Last fish died just after 48 hours.
[b]Last fish died at 96 hr.
[c]Last fish died after 72 hr.
[d]Concentration and death data taken from exposure chamber #1.

The results of this study (Table 4) show the observed values of median lethal copper concentrations (LC_{50}) and ASV copper peak measurements $(Ip)_{BS}$ for each bioassay. For comparative purposes known stability constants of copper carbonate and copper-organic complexes are included. High ASV peak currents observed for controls, cysteine, sewage effluent, and egg albumin suggest that these organics did not form stable complexes with copper. The same compounds exhibited no significant effects on the toxicity of copper to fish. The high LC_{50} concentrations observed in the presence of NTA, EDTA, humic acid, and glycine corresponded to low ASV peak current values thus relating the high binding capacities of these compounds to reduction of copper toxicity. This effect is also reflected in the relationship of toxicity to the stability constants presented. Since no significant differences were observed in the internal concentrations of test fish it was concluded that toxicity may not be related to uptake of copper.

Table 4. Relationship of toxicity, electroactive copper and stability constants

Organic Added	LC_{50} (µg/1 Cu)	$(Ip)_{BS}$[a]	Stability Constant for Cu-Org Complex
NTA	224	1.16	13.2 (32)
Humic A.	188	1.29	--
EDTA	184	1.18	18. (30)
Glycine	183	0.97	15.4 (30)
Control 2	138	2.75	---
Citrate	136	1.58	5.2 (32)
Cysteine	121	2.15	--
Control 1	112	--	--
Egg Albumin	102	3.14	3.7[b] (31)
Sewage Eff.	99	2.21	--
$CaCO_3$	--	--	3.5 (13)

a) Normalized copper peak current measured in bioassay solution containing 100 mg/1 added copper.

b) Value for bovine albumin.

Effect of Particulate-Bound Copper on Toxicity

Although most of the copper added to aqueous environments becomes complexed by particulate matter, the toxicity of this form of copper has not been investigated. Brown et al. (4) studied the effect of adding suspended organic matter (solids from an activated sludge plant) on copper toxicity to rainbow trout. Decreased copper toxicity was directly related to increased concentrations of solids. Interpretation of these data is confused by the

fact that solids of this nature could possibly change other water quality characteristics such as alkalinity and hardness which would influence copper speciation and possibly toxicity.

ROUTES OF EXPOSURE OF TOXIC COPPER SPECIES

Even if a clear understanding of copper speciation and toxicity of various copper species were known, a predictive model of copper toxicity would not be possible without a knowledge of the target sites of toxicity and routes of transport of copper to these sites. Although a number of pathological symptoms in fish have been related to exposure of copper (See Table 5), the actual mechanisms of toxicity remain unknown. Since ingestion of water by fish is minimal, the predominant route of exposure to soluble copper is probably the gills. Although the gills concentrate copper (12), exposure of fish to lethal concentrations of copper did not effect respiration of blood oxygen levels (23). The gills may concentrate copper where it may enter the bloodstream and be transported to various toxicity sites. Life (12) reported that fish exposed intraveneously or intraperitoneally concentrated copper in the liver, kidney, spleen, and intestine. Such exposure resulted in hepatocellular and renal tubular injury accompanied by anemia. Goldfish susceptibility to copper was demonstrated to be related to lack of metabolic mechanisms necessary for non-destructive transport, storage, and excretion of this heavy metal.

Table 5. Pathology of copper toxicity to fish.

Fish Type	Copper Exposure	Pathology	Ref.
Killifish	3200µg/liter	Changes in activities of several liver enzymes.	(10)
Flounder	--	Changes in gill architecture Fatty metamorphosis of liver Necrosis of kidney Destruction of hemapoietic tissue	(2)
Goldfish	18,36,90 mg/day for 70 days*	Concentration of copper in liver, kidney, spleen and intestine. Hepatocellular and renal tubular injury. Anemia Lack of mechanisms for non-destructive transport, storage, and excretion of copper	(12)

*Injected intraperitoneally

The predominant form of copper in most aquatic environments is particulate copper and copper contained in biomass. Although these forms probably represent the major vector of copper exposure to fish (via ingestion of detritus and organisms), this mechanism of exposure has not yet been studied.

Even though ingested species of copper may not be directly toxic, it is conceiveable that they could be transformed to toxic species in the milieu of chemical and biochemical reactions occurring within the fish. For example, ingested insoluble copper oxides would be solubilized by the low pH conditions of the stomach. Copper bound to soluble or particulate organic ligands might be released upon digestion of the organic matter by the metabolism of the fish or its bacterial flora.

CONCLUSION

The toxicology of copper toxicity to fish is not well delineated because of a paucity of knowledge on the mechanisms of toxicity, the toxicity of various copper species, and the route of exposure of toxic species. In view of the above discussion of these future research on copper toxicology should be directed toward the following areas:

1. The effect of alkalinity and hardness on copper toxicity.

2. Toxicity of copper complexed with inorganic and organic particulate matter.

3. Toxicity of copper contained in fish food (i.e. detritus, plankton, and invertebrates).

4. Sites and mechanisms of copper toxicity.

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COPPER TOXICITY TO PHYTOPLANKTON, AS AFFECTED BY ORGANIC LIGANDS, OTHER CATIONS AND INHERENT TOLERANCE OF ALGAE TO COPPER

CHAPTER 8

Pamela Stokes and T. C. Hutchinson
Department of Botany and
Institute for Environmental Studies
University of Toronto
Toronto, Ontario M5S 1A4

Abstract

Recently, attempts have been made to determine those factors affecting the toxicity of copper to algae in lakes influenced by the Sudbury mining and smelting activities. Since most of these lakes are typically oligotrophic, soft-water shield lakes, there is little buffering capacity and usually little organic matter present for the complexation of metals. Many of the lakes are now at a pH below 5.0, with high levels of copper (up to 1.0 ppm total) and nickel (up to 6.5 ppm total), mostly occurring in the free or labile form. Biota are poor, both in species diversity and in quantity. Isolates of green algae obtained from these lakes are nickel and copper tolerant compared with controls.

Field studies, however, have revealed that some contaminated lakes, with both high copper and nickel levels, can, nevertheless, support a much richer phytoplankton flora than one might predict from their total soluble metal contents.

A selection of lakes was made, to provide combinations of high or low metals with high and low organic matter, but with pH's selected to be above 6.0. The major variables were thus organic and heavy metal contents. There was a predictable amelioration of copper toxicity in water of high complexing capacity, but other chemical variables complicated the picture. In pure culture studies with known amounts of added acetate and EDTA, the results supported the hypothesis that bound copper is not toxic, and that strong complexing agents, such as EDTA, also prevent copper uptake by algal cells.

The ultimate toxicity of a given quantity of copper was also affected by the amounts of other cations present, for example, nickel, which is synergistic with copper, and calcium, which ameliorates copper toxicity.

While levels of total or dissolved copper (or nickel or zinc etc.) presently have a valuable place in the setting of water quality standards, a number of other variables such as the actual chemical form of the metal and its interaction with other cations and anions must, ultimately, be taken into account. In field situations single metal occurrences are non-events. Ameliorating and/or intensifying physical and chemical factors occur. Their importance is being clearly recognized but our ability to measure and to elucidate their relative importance to toxicity is still at a primitive stage. The complexing capacity of waters is one major factor in this complication.

INTRODUCTION

There appears to be general agreement that, for copper at least, the form of the metal which is directly toxic to biota is the ionic form (e.g. Andrew & Beisinger 1975). Metal in other forms - weakly or strongly complexed, absorbed on particulates, or as insoluble precipitates, may constitute a reservoir in ecosystems from which the metal can be released by chemical or biological mechanisms in ionic form. A number of pre-treatments and methods for chemical speciation of metals in water are available. (e.g. cation exchange, polarography, ion selective electrodes). Even if one measures only free or ionic copper, the actual levels of this ionic or free metal which are toxic to specific aquatic biota vary greatly. Fish, invertebrates, macrophytes and phytoplankton all show different susceptibilities to metal exposure, and even differences between populations and at different stages of the life cycle (e.g. Lisk 1972).

The present project involved field work and associated laboratory studies by which we have sought to relate the toxicity to green algae of the form and concentration of heavy metals. The presence of other cations and anions as ameliorating factors was recognized. We were particularly interested in the possible application of the concept of "labile" and "bound" metal, as defined by Chau & Lum-Shu-Chan (1974), to the relationship between chemical measurements and metal toxicity of algae.

We believe that the response of biota to waters provides useful and practical information regarding the quality of that water. Biological responses provide the ultimate test of its potential toxicity. Analytical techniques may or may not provide information on this potential toxicity, since they take but one variable at a time into account. Fish have already secured a place as accepted bioassay organisms. Their ability to respond is also governed by the ability of primary producers to survive. Indeed, the cult of the fish bioassay is synonymous in many scientific minds with the word bioassay itself. Fish, however, are not photosynthetic.

Primary producers - the planktonic algae in most aquatic systems - appear to have value as bioassay organisms. This has been more fully discussed in an earlier publication (Hutchinson & Stokes 1975). Briefly, algae can be cultured quickly in large quantities in pure culture, clones of uniform genetic composition can be perpetuated, and the methods are inexpensive. Algae are rarely troubled by disease. Since the algae constitute the base of the food chain, they are of particular importance in such operation as

reclamation of lakes and waterways. In another context, if biomagnification of metals is to occur, then we must be concerned with both the ability of algae to survive in metal-contaminated waters and with their proven ability to concentrate metal (e.g. Stokes 1975).

Differential pulse anodic stripping voltammetry and atomic absorption spectroscopy were the main analytical methods. Specific ion electrodes and ion exchange have not yet been used successfully in these studies but their successful use in a few other laboratories leads us to hope they can add further dimensions in defining critical forms.

Our field sites were centred around the Sudbury, Ontario mining and smelting region. We had already developed considerable information about the area (e.g. Myslik unpublished, Hutchinson and Whitby 1974, Whitby and Hutchinson 1974). The lakes are numerous, small, softwater oligotrophic Shield lakes. Due to smelting activities many of them have become acidified and the levels of heavy metals, especially copper and nickel, are high.

Levels of 6.4 ppm for nickel and 1.0 ppm for copper have been recorded. While most of lakes close to the Sudbury smelters had a sparse algal flora and no animal life, there were a few exceptions. Kelley Lake supported a relatively rich algal flora, and the bioassay of its water gave yields much better than predicted from determinations of total heavy metal levels alone. It had received untreated sewage for a number of years prior to the study, but was also still being subjected to the airborne and waterborne inputs of metals. Since we believed the high organic content may be a key factor in allowing a rich algal flora (by complexing the metals) we also selected from an initial extensive screening, five lakes with varying organic content, heavy metal content, and with a pH higher than 6.0. We thus eliminated the highly acidic lakes to reduce the water quality variables to manageable proportions.

Figure 1 shows the location of these lakes. Long Lake was our "control" or normal lake - some 11 Km away from the nearest smelter. It had a normal standing crop of algae for a Shield lake in this area, a scarcely elevated heavy metal level and low organic carbon. Table 1 indicates the status of the selected lakes. Background i.e. nutrient sufficient cultures, necessary for comparisons between lakes was not available. We know from previous studies that filtering and addition of nutrients have a lake specific effect on bioassay results. In view of these complicating factors, it was deemed that the best compromise was to use water filtered through a 0.45 micron filter, as soon after collection as possible, and to enrich it with modified Bold's Basal Medium (Stokes, Hutchinson, & Krauter 1973) at 0.2 strength. The controls were distilled water with the same amount of nutrients.

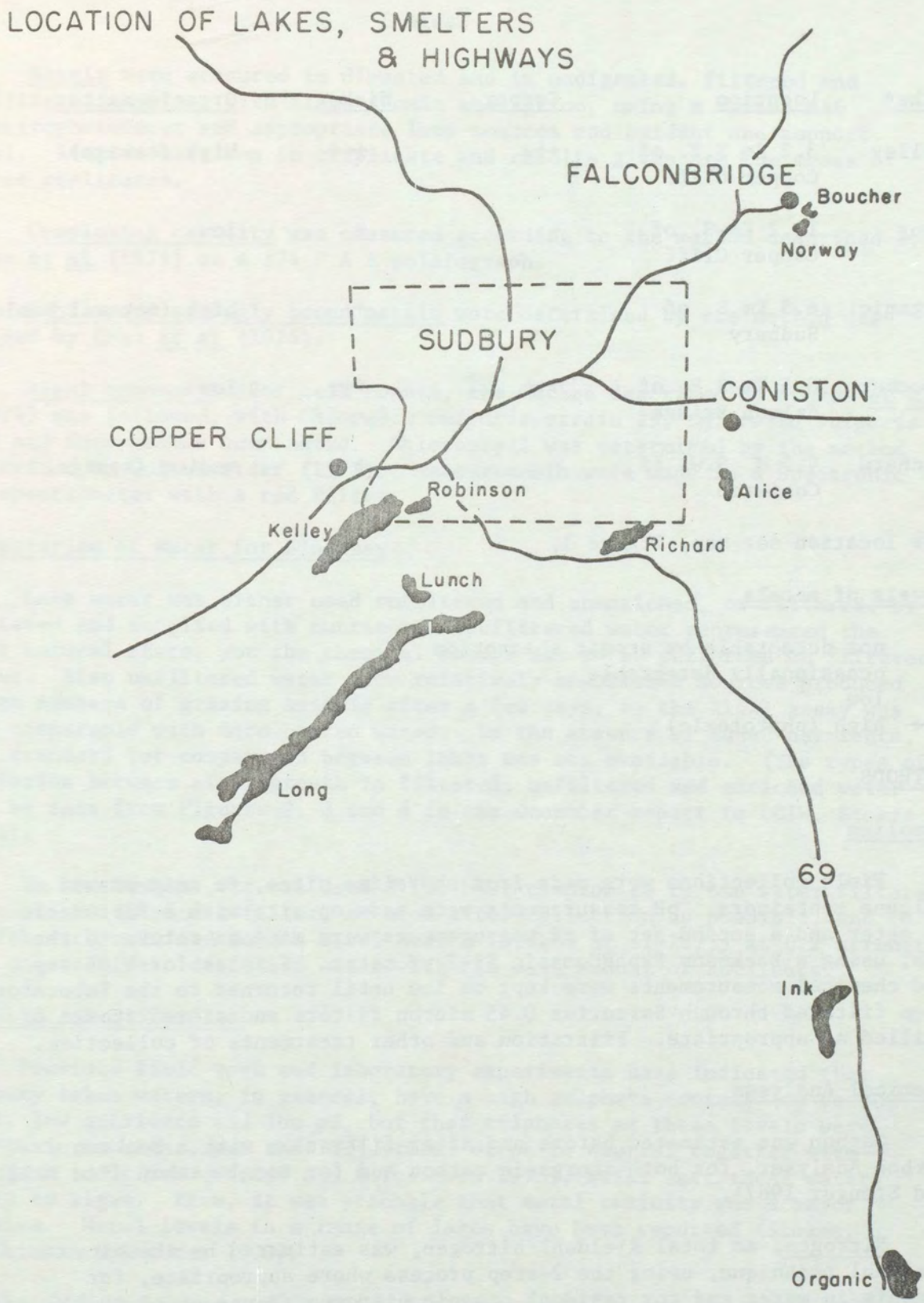

Figure 1: Map of Sudbury showing location of smelters (closed circles), highways (solid lines) and some of the lakes.

Table 1

Lake*	Location	Copper	Nickel	Organic matter
Kelley	3.2 Km S.E. of Copper Cliff	+++	+++	high (sewage)
Long	11.2 Km S. of Copper Cliff	-	+	low
Organic	6.5 Km S. of Sudbury	-	+	high (natural humics)
Boucher	0.5 Km S.E. of Falcon Bridge	+++	+++	low
Richard	4.5 M S.W. of Coniston	-	+	medium (sewage)

For location see map, Figure 1.

Levels of metals

- not detectable by atomic absorption
+ occasionally detectable
++ low
+++ high (phytotoxic)

METHODS

Sampling

Field collections were made from shoreline sites, in acid washed Nalgene containers. pH measurements were made on site with a Portamatic pH meter and a second set of pH measurements were made on return to the lab, using a Beckmann Expandomatic SS-2 pH meter. Samples for bioassay and chemical measurements were kept on ice until returned to the laboratory, then filtered through Sartorius 0.45 micron filters and stored frozen or chilled as appropriate. Filtration and other treatments of collection.

Chemical Analyses

Carbon was estimated before and after filtration with a Beckman Carbon Analyser, for both inorganic carbon and for total carbon (Van Hall and Stenger 1967).

Nitrogen, as total Kjeldahl nitrogen, was estimated by the micro Kjeldahl technique, using the 2-step process where appropriate, for ammonia in water and for residual organic nitrogen (Tavas et al 1971).

Total Phosphate was determined colorimetrically by the ANSA method, modified after Heinke (1969).

Metals were measured in digested and in undigested, filtered and unfiltered samples, with flame atomic absorption, using a Varian AA6 spectrophotometer and appropriate lamp sources and oxidant and support fuel. Samples were run in triplicate and results given are the means of three replicates.

Complexing capacity was measured according to the method described by Chau et al (1974) on a 174 P A R polarograph.

Labile and strongly bound metals were determined by the method described by Chau et al (1974).

Algal bioassay: For cell counts, the method described by Stokes et al (1974) was followed, with *Chlorella vulgaris* strain 29, *Chlorella vulgaris* 260 and *Scenedesmus acuminatus*. Chlorophyll was determined by the method described by Vollenweider (1971). Measurements were made in a Spectronic 20 spectrometer with a red filter.

Preparation of Water for Bioassay

Lake water was either used unfiltered and unenriched, or filtered, or filtered and supplied with nutrients. Unfiltered water represented the most natural state, yet the chemical assays has to be performed on filtered water. Also unfiltered water from relatively unpolluted sources produced large numbers of grazing animals after a few days, so the algal assay was not comparable with more barren water. In the absence of added nutrients, the standard for comparison between lakes was not available. (The types of variation between algal growth in filtered, unfiltered and enriched water can be seen from Figures 2, 3 and 4 in the December report to CCIW, Stokes 1974).

In conclusions, it was deemed the best compromise to use water filtered through a 0.45 micron filter, as soon after collection as possible, and to enrich with modified Bold's Basal Medium (Stokes et al 1973) at 0.2 strength. The controls were distilled water with the same amount of nutrients.

RESULTS AND DISCUSSION

Previous field work and laboratory experiments have indicated that Sudbury lakes waters, in general, have a high sulphate content (up to 300 ppm), low nutrients and low pH, but that sulphates at these levels were non-toxic to algae, and that adjustment of pH to neutral together with nutrient additions (Stokes and Hutchinson 1975), still left these waters toxic to algae. Thus, it was probable that metal toxicity was a major problem. Metal levels in a range of lakes have been reported (Stokes, Hutchinson & Krauter 1973).

Preliminary Bioassays and Chemical Analyses

For the selected lakes, levels of total soluble copper and nickel are shown in Table 2. Organic carbon and organic nitrogen and bioassay data using *Chlorella* sp. are also presented.

Table 2.

Chemical and bioassay on preliminary survey of lakes**

Filtered lake water from:	pH	Copper ppm	Nickel ppm	Organic C ppm	Organic N ppm	Assay Chlorella % control 10 days
Kelley	6.0	0.07	2.04	14	3.8	22
Long	6.0	ND	0.10	5	ND	76
Organic	6.7	ND	ND	11	0.56	122
Boucher	7.0	0.06	2.02	3	ND	4
Richard	6.7	ND	0.2	8	0.20	87
Detection limits		0.005	0.005	1.0		

** Date for Kelley, Long and Organic from June collection (original survey). Date for Boucher and Richard from July collection.

ND = Not Detectable

Further data on water chemistry are shown in Table 3. Complexing capacity for copper is also presented.

While several metals were elevated, especially in Boucher and Kelley Lakes, compared with Long Lake, copper and nickel were identified as the most serious pollutants. These two metals are known to be highly toxic to algae (Hutchinson 1973). Their presence in the Sudbury lakes, therefore, poses a serious hazard to phytoplankton. It is especially noteworthy that nickel and copper tolerance has evolved in some of these lakes (Stokes et al 1973). For example, an isolate of *Scenedesmus* from Boucher Lake was shown to be both nickel and copper-tolerant. Figures 2 and 3 demonstrate the response of the Boucher Lake *Scenedesmus* (B-4 strain) to copper compared with the response of a laboratory isolate of *Scenedesmus*. Heavy metal tolerance is often not a function of the ability of an organism to exclude the metal. Along with tolerance, there is frequently metal accumulation (see e.g. Turner 1968, Stokes 1975).

The bioassay values in Table 2 indicate a range of lake water inhibition. While Kelley and Boucher Lakes have equivalent levels of copper and nickel, Kelley Lake water is less toxic than Boucher water to *Chlorella* 29, i.e. growth of 22% compared with 4%. The major difference in algal response to these waters could be accounted for by differences in organic matter, and by their relative complexing capacity as shown in Table 3: - 2.1 for Kelley and only 0.1 for Boucher Lake. Long and Richard Lakes, with low metals and intermediate complexing capacity, permitted growth of 76 - 87%, whilst Organic Lake (complexing capacity 1.2) stimulated growth over the control.

Figure 4 shows further bioassays of the five lake waters (collected in October 1974) with two different species of algae - *Chlorella* 29 and *Scenedesmus acuminatus*. Boucher Lake water was toxic to both algal species, and both responded in similar fashion to Richard Lake. Clearly *Scenedesmus* was more sensitive to Kelley Lake water than was *Chlorella* 29. *Chlorella* 29 was subsequently shown to be a facultative heterotroph, and this ability to utilize organic carbon from organic matter *per se* was a complicating factor, which may also have caused amelioration of metal toxicity.

Copper Spiking Into Lake Waters

With some knowledge of the apparent complexing capacity of the waters, a series of experiments were designed, with copper-spiked water. Bioassays and ASV were run on parallel samples, to determine how much copper could be masked or detoxified by the water.

Figure 5 shows data for *Scenedesmus* growth with copper spikes of 0.2 ppm copper, together with the complexing capacity of the waters. The amelioration of copper toxicity matches the complexing capacity fairly well. The percentage reduction of growth by copper was rather similar for Long Lake, Richard Lake and the control. Boucher Lake, already very toxic, showed the greatest reduction of growth, when copper was added. Growth in Organic

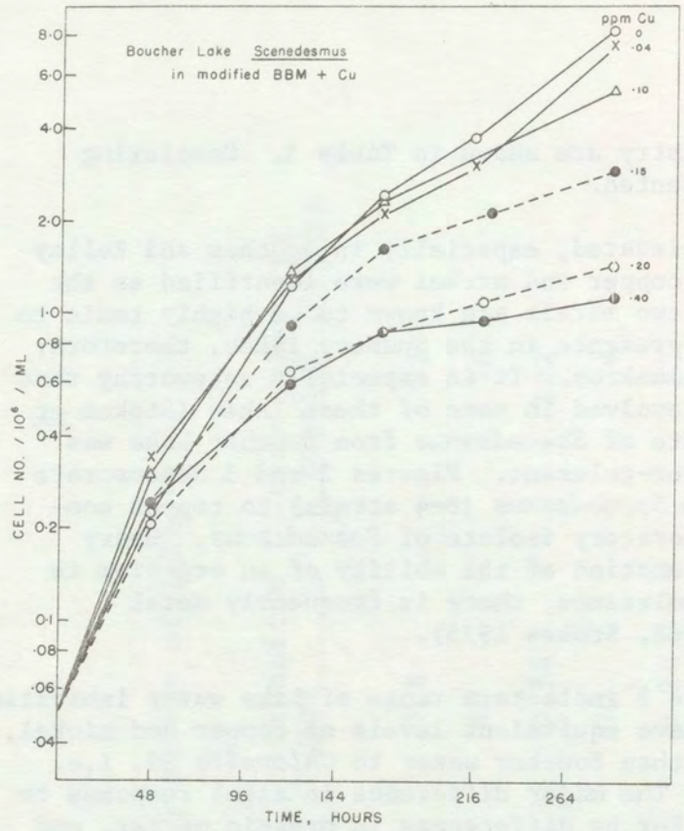

Figure 2: Growth response of Scenedesmus, from Boucher lake, in bioassay for copper.

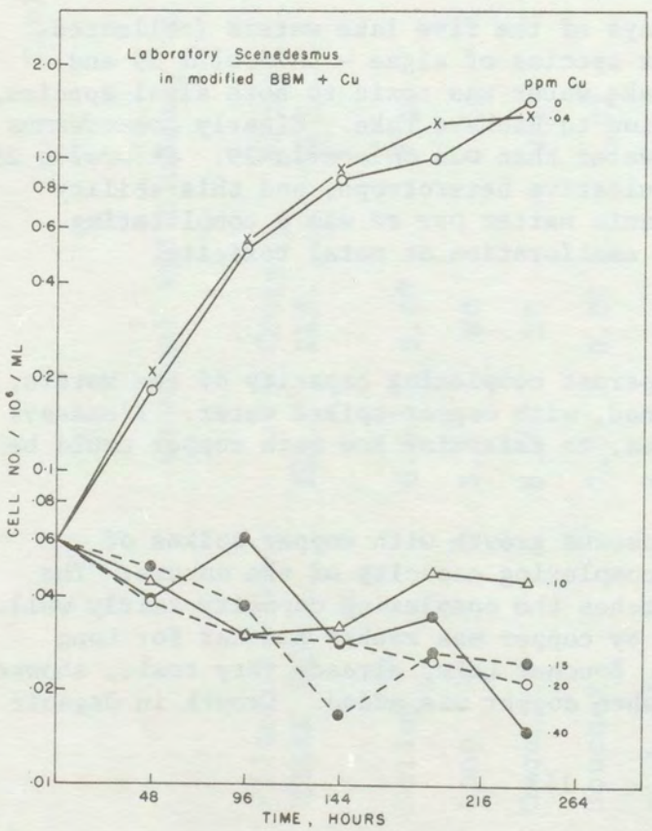

Figure 3: Growth response of Scenedesmus, laboratory strain, bioassay for copper.

Table 3

Chemical data for selected lakes (cont.)

Filtered lake water from:	Phosphate ppm	Zn ppm	Co ppm	Mn ppm	Ca ppm	Fe ppm	Complexing capacity micro-mole copper per litre
Kelley	0.1	.06	.05	.33	60.8	0.28	2.1
Long	ND	.04	ND	.03	5.0	0.02	0.6
Organic	0.1	-	-	-	-	0.10	1.2
Boucher	ND	.12	.16	.09	19.0	0.1	0.1
Richard	ND					ND	0.7
Detection limit	0.05	0.01	0.01	0.01	0.1	0.01	

ND = Not Detectable

- = No data available

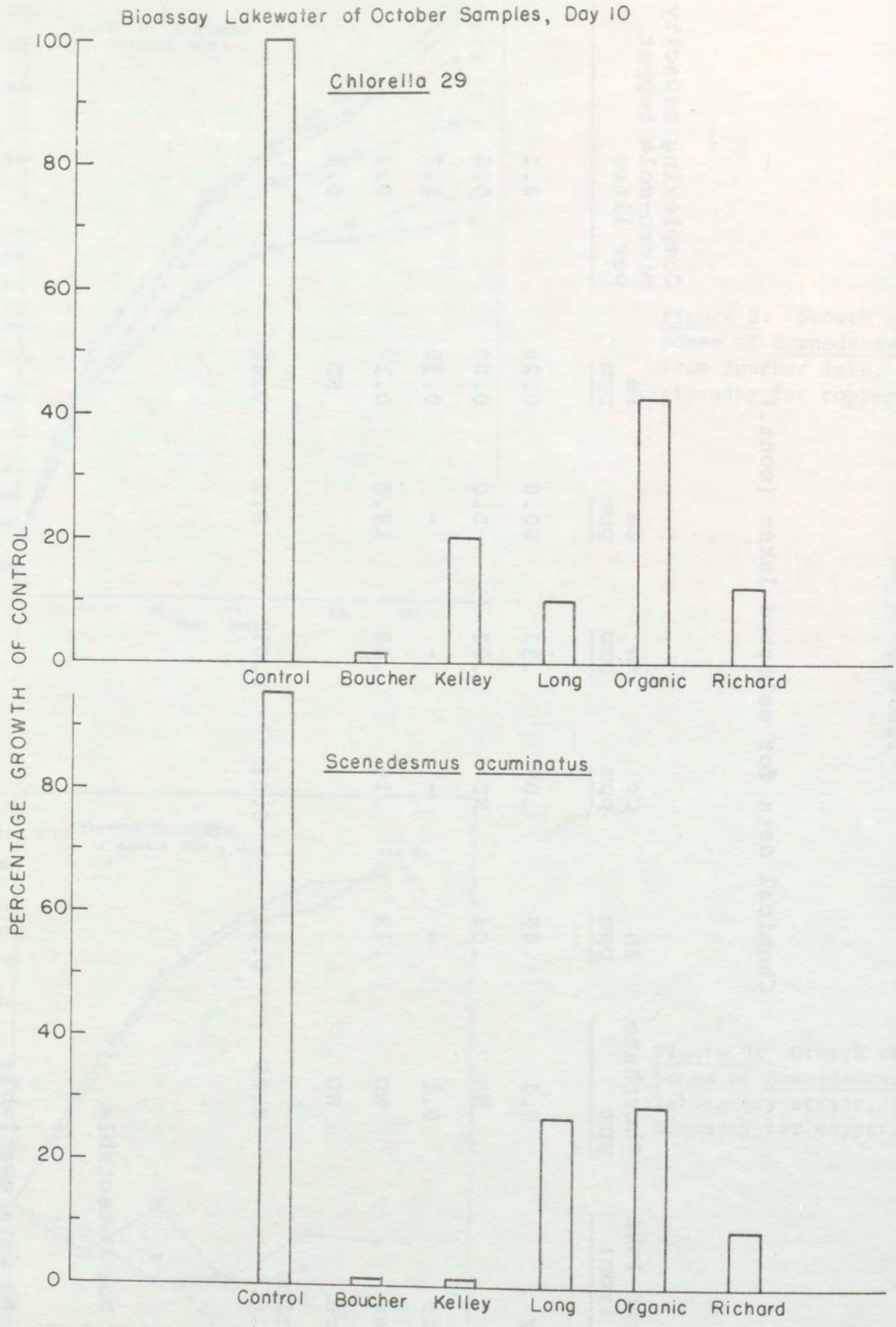

Figure 4: Response of two different species of green algae to water from five lakes, compared with distilled water control. 10-day bio-assay, with nutrients, cell numbers per ml used as criterion for growth.

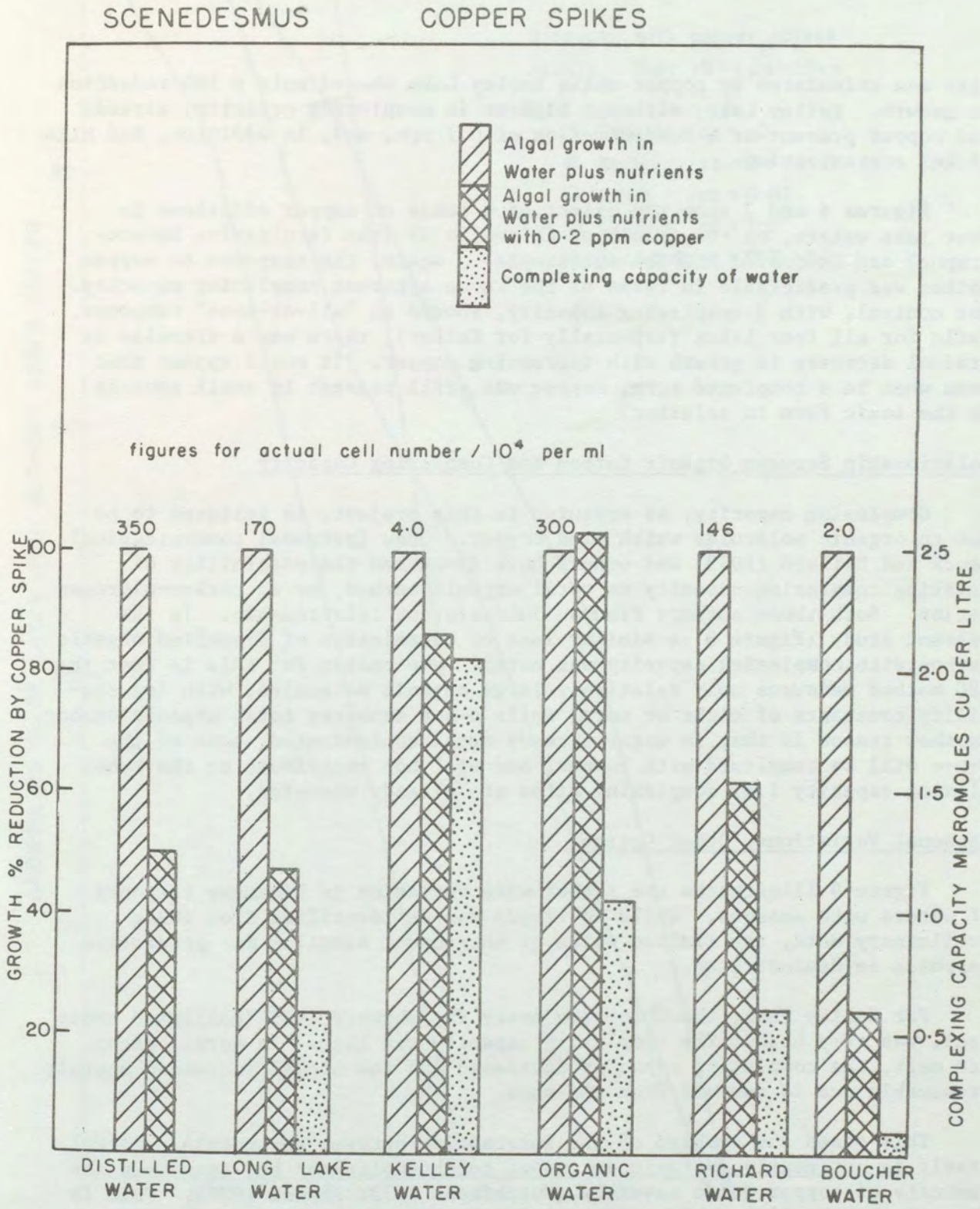

Figure 5: Response of *Scenedesmus acuminatus*, laboratory strain, to water from five different lakes and distilled water controls, with and without copper spikes.

Lake was stimulated by copper while Kelley Lake showed only a 10% reduction in growth. Kelley Lake, although highest in complexing capacity, already had copper present at a concentration of 0.1 ppm, and, in addition, had high nickel contamination.

Figures 6 and 7 show the effect of a range of copper additions to four lake waters, on the growth of *Chlorella* 29 (the facultative heterotrophe) and *Chlorella* 260 (an autotrophe). Again, the response to copper spikes was predictable in terms of the known apparent complexing capacity. The control, with 0 complexing capacity, showed an "all-or-none" response, while for all four lakes (especially for Kelley), there was a stepwise or gradual decrease in growth with increasing copper. It would appear that even when in a complexed form, copper was still present in small amounts in the ionic form in solution.

Relationship Between Organic Carbon and Complexing Capacity

Complexing capacity, as measured in this project, is believed to be due to organic molecules which bind copper. Chau (personal communication) Hanck and Dillard (1973) and others have discussed the possibility of relating complexing capacity to total organic carbon, or to carbon-nitrogen ratios. Both these authors find an inconsistent relationship. In the present study (Figure 8) a similar lack of correlation of dissolved organic carbon with complexing capacity was noted. One reason for this is that the ASC method measures only relatively large organic molecules, with log stability constants of eight or more, while d-o-c measures total organic carbon. Another reason is that in water already metal contaminated, some of the d-o-c will be complexed with copper, and will not contribute to the complexing capacity i.e. complexing sites are already occupied.

Seasonal Variations, Other Cations

Figure 9 illustrates the rather wide variation in bioassay response of waters with seasons. While no trends can be identified from these preliminary data, the limited value of infrequent sampling for predictive purposes is demonstrated.

For Kelley Lake, the *Chlorella* assay in February 1975 (collected under ice), was very high. The complexing capacity was high. In April, after ice melt, the complexing capacity decreased and the nickel increased sharply, presumably due to run-off from the snow.

This posed the problem of the interaction between two metals. Nickel itself is not nearly as toxic as copper to phytoplankton but increases the toxicity of copper quite severely (Hutchinson 1973, Stokes 1975). This is shown in Figure 10 in which high and low levels of copper (1.0 and 0.3 ppm) and high and low levels of nickel (3.0 and 1.0 ppm) were tested, alone and in combination, for a metal-tolerant *Scenedesmus*.

In Sudbury lakes and in similar situations elsewhere, copper and nickel are frequently present in combination. A cation such as nickel clearly is

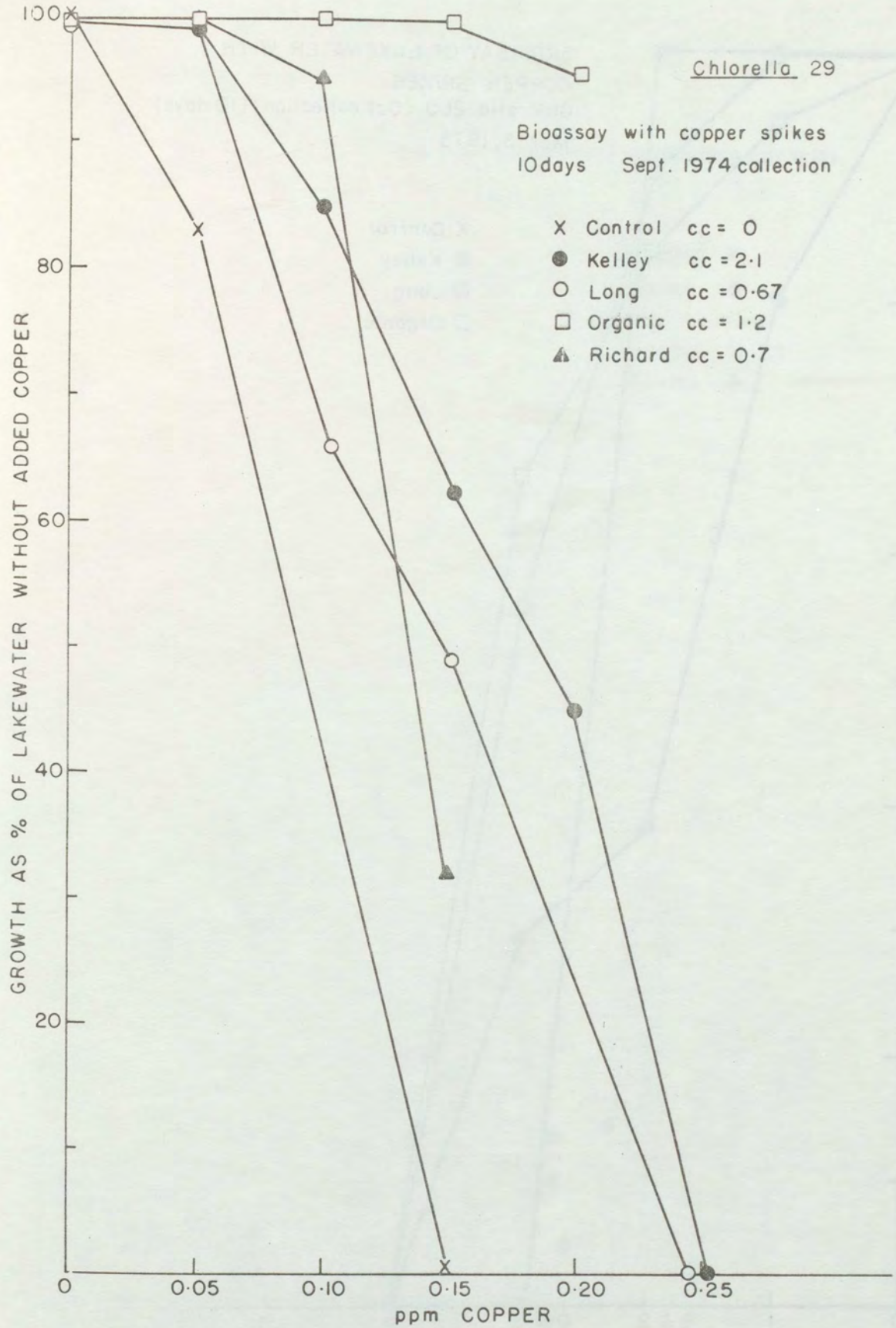

Figure 6: *Chlorella* 29 (heterotrophic) bioassay in water from four different lakes, and distilled water control, with copper spikes from 0 - 0.25 ppm. Values for complexing capacity (c.c) are given in key. Cell numbers taken at day 10.

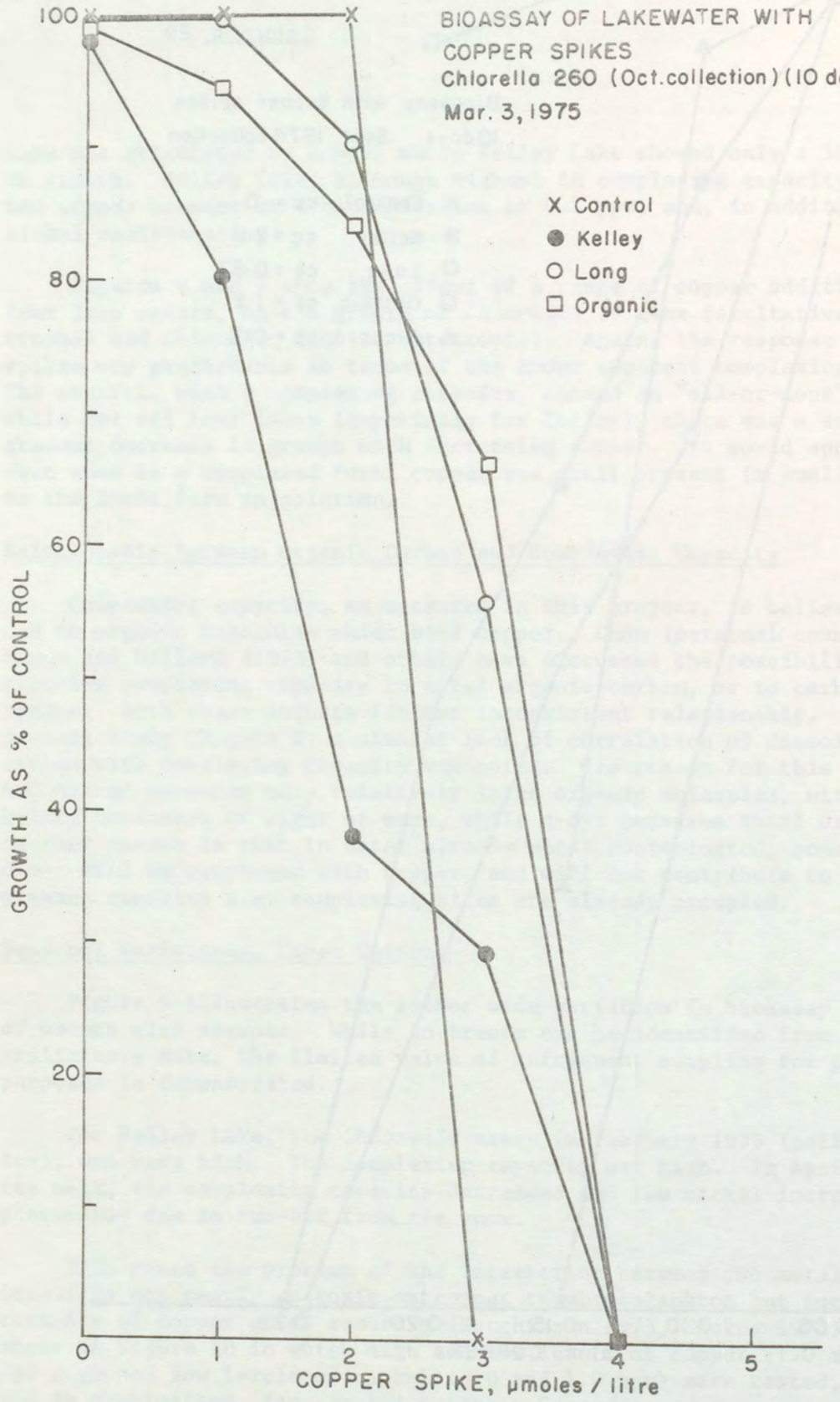

Figure 7: *Chlorella* 260 (autotrophic) bioassay in water from three different lakes, and distilled water control, with copper spikes from 0 - 4 µmoles per litre. Cell numbers taken at day 10.

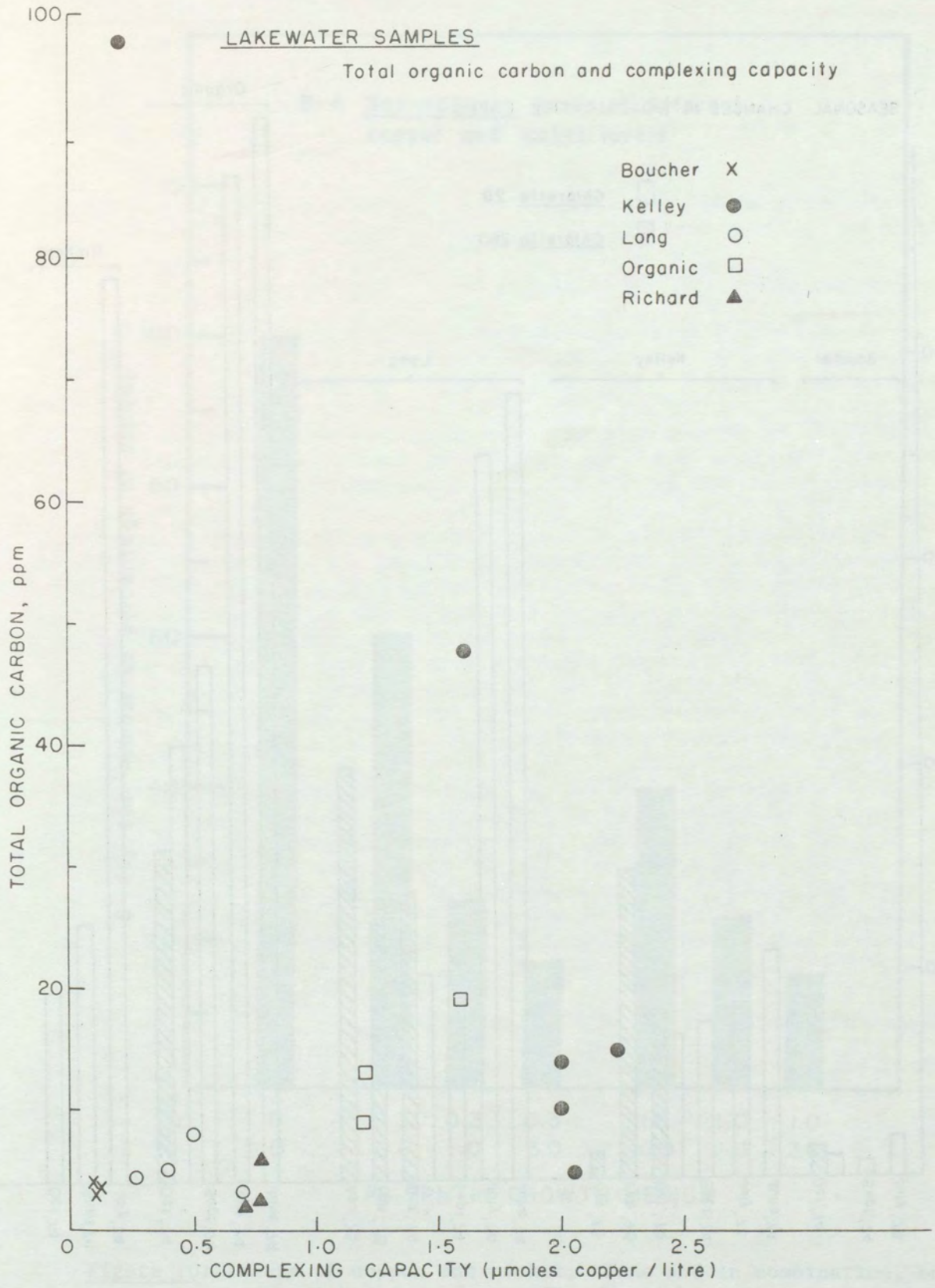

Figure 8: Total organic carbon (D-O-C) compared with complexing capacity for copper, of water from five different lakes.

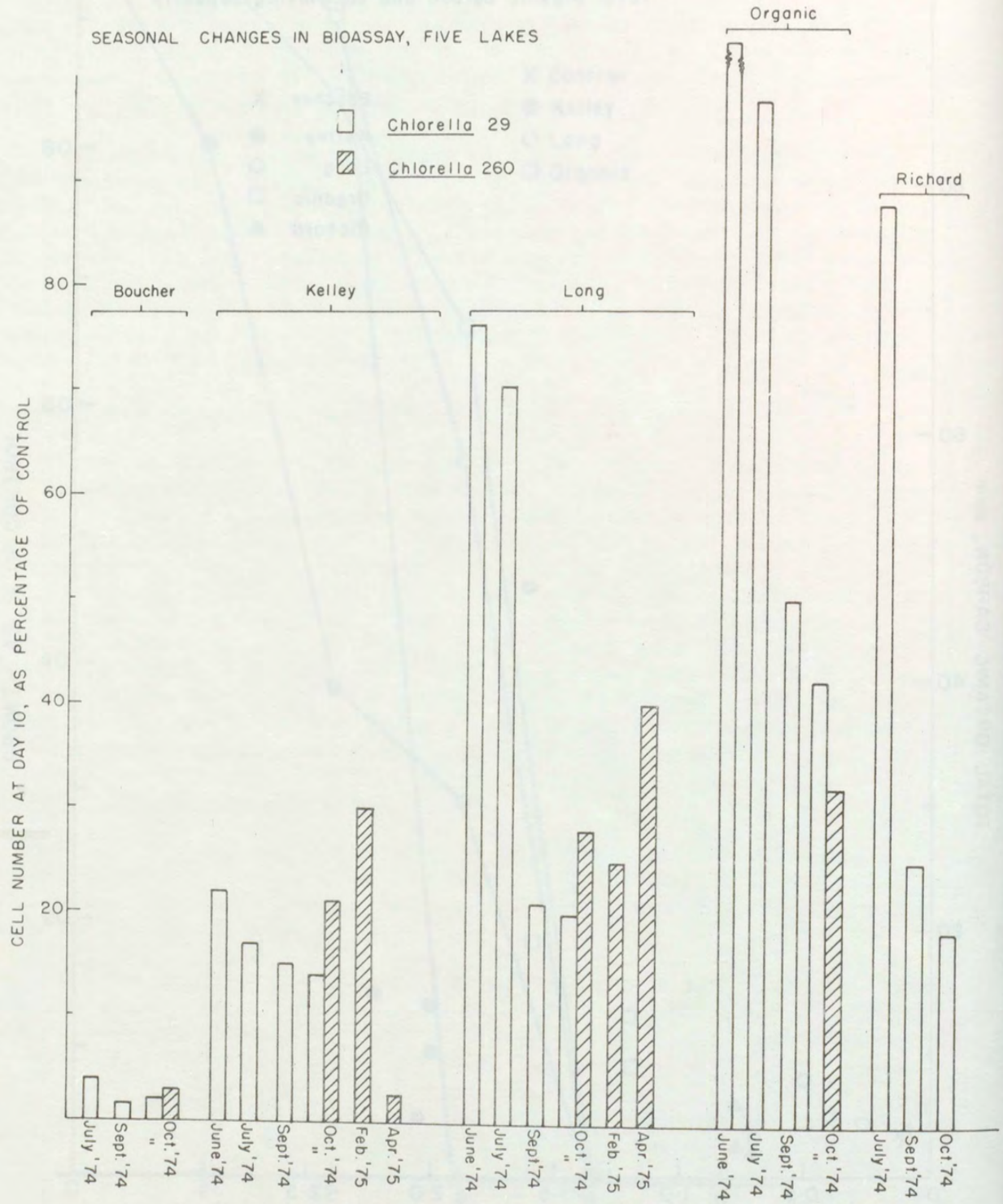

Figure 9: Seasonal changes in bioassay by *Chlorella* for five lake waters, sampled during 1974 and 1975.

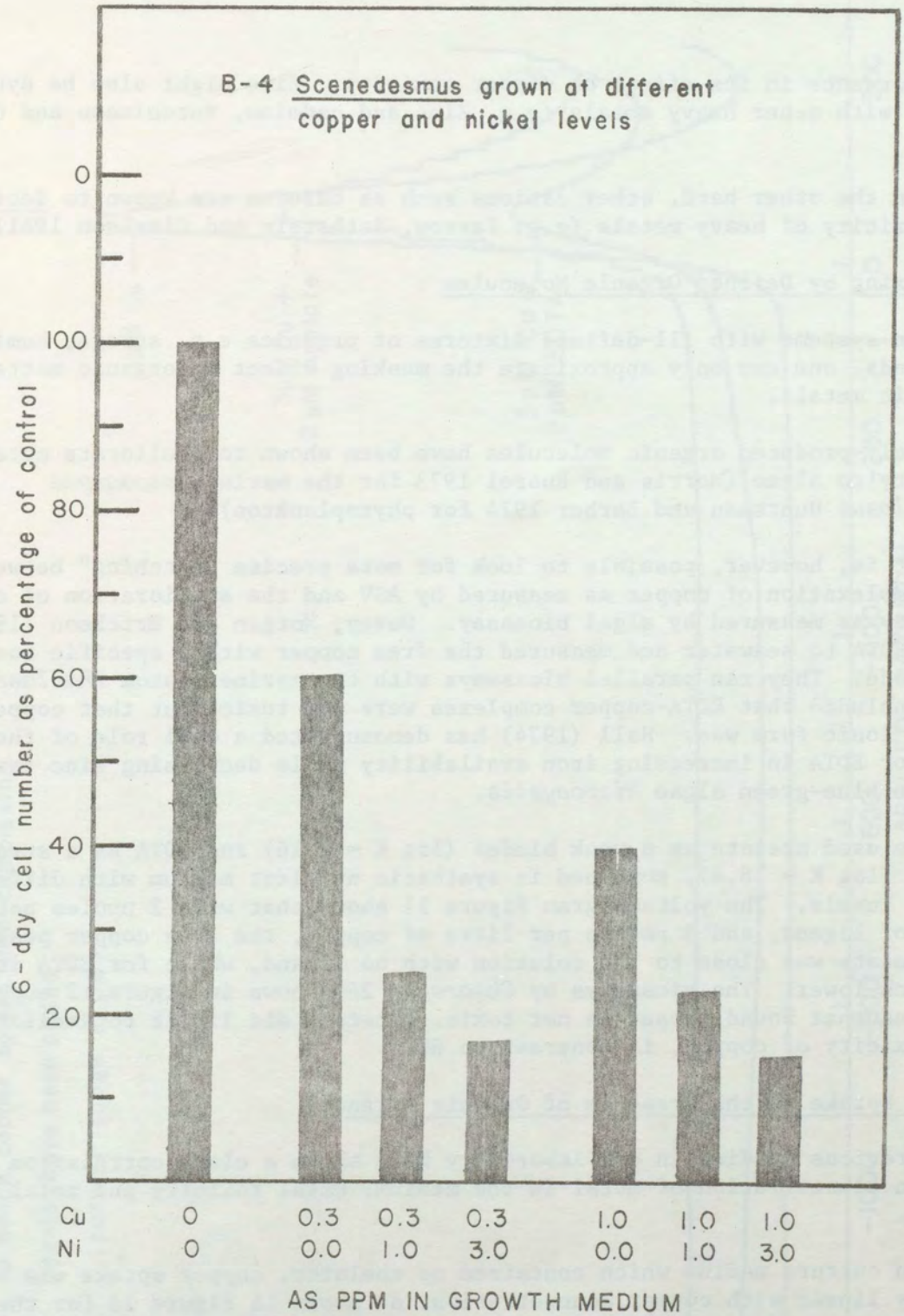

Figure 10: Effects of copper and nickel, alone and in combination, on growth of B-4 *Scenedesmus* (Boucher lake isolate). Cell numbers are taken at day 6.

of importance in its effect on copper toxicity. Zinc might also be synergistic with other heavy metals (e.g. Zinc and cadmium, Hutchinson and Czyrska 1973).

On the other hand, other cations such as calcium are known to decrease the toxicity of heavy metals (e.g. Passow, Rothstein and Clarkson 1961).

Complexing by Defined Organic Molecules

In systems with ill-defined mixtures of organics e.g. sewage, humic compounds, one can only approximate the masking effect of organic matter on toxic metals.

Self-produced organic molecules have been shown to ameliorate metal toxicity to algae (Morris and Russel 1973 for the marine *Ectocarpus siliculosus* Huntsman and Barber 1974 for phytoplankton).

It is, however, possible to look for more precise "matching" between the complexation of copper as measured by ASV and the amelioration of copper toxicity as measured by algal bioassay. Davey, Morgan and Erickson (1974) added EDTA to seawater and measured the free copper with a specific ion electrode. They ran parallel bioassays with the marine diatom *Thallassiosira* and concluded that EDTA-copper complexes were not toxic, but that copper in the ionic form was. Hall (1974) has demonstrated a dual role of the chelator EDTA in increasing iron availability while decreasing zinc toxicity for the blue-green algae *Microcystis*.

We used acetate as a weak binder (log K = 2.16) and EDTA as a strong binder (log K = 18.8), combined in synthetic nutrient medium with different copper levels. The voltammogram Figure 11 shows that with 2 µmoles per litre of ligand, and 3 µmoles per litre of copper, the free copper peak for acetate was close to the solution with no ligand, while for EDTA it was much lower. The bioassays by *Chlorella* 260 shown in Figure 12 support the idea that bound copper is not toxic. Acetate did little to ameliorate the toxicity of copper, in contrast to EDTA.

Copper Uptake in the Presence of Organic Ligands

Previous studies in our laboratory have shown a close correlation between concentration of metal in the medium, metal toxicity and metal uptake.

In culture medium which contained no chelator, copper uptake was roughly linear with copper concentration, as shown in Figure 13 for the metal-tolerant strain of *Scenedesmus*. The decreased toxicity brought about by the addition of EDTA at 50 µg/litre was paralleled by a decrease in copper uptake, as shown in Figure 14.

CONCLUSIONS

In addition to the speciation of metal in water and metal movement

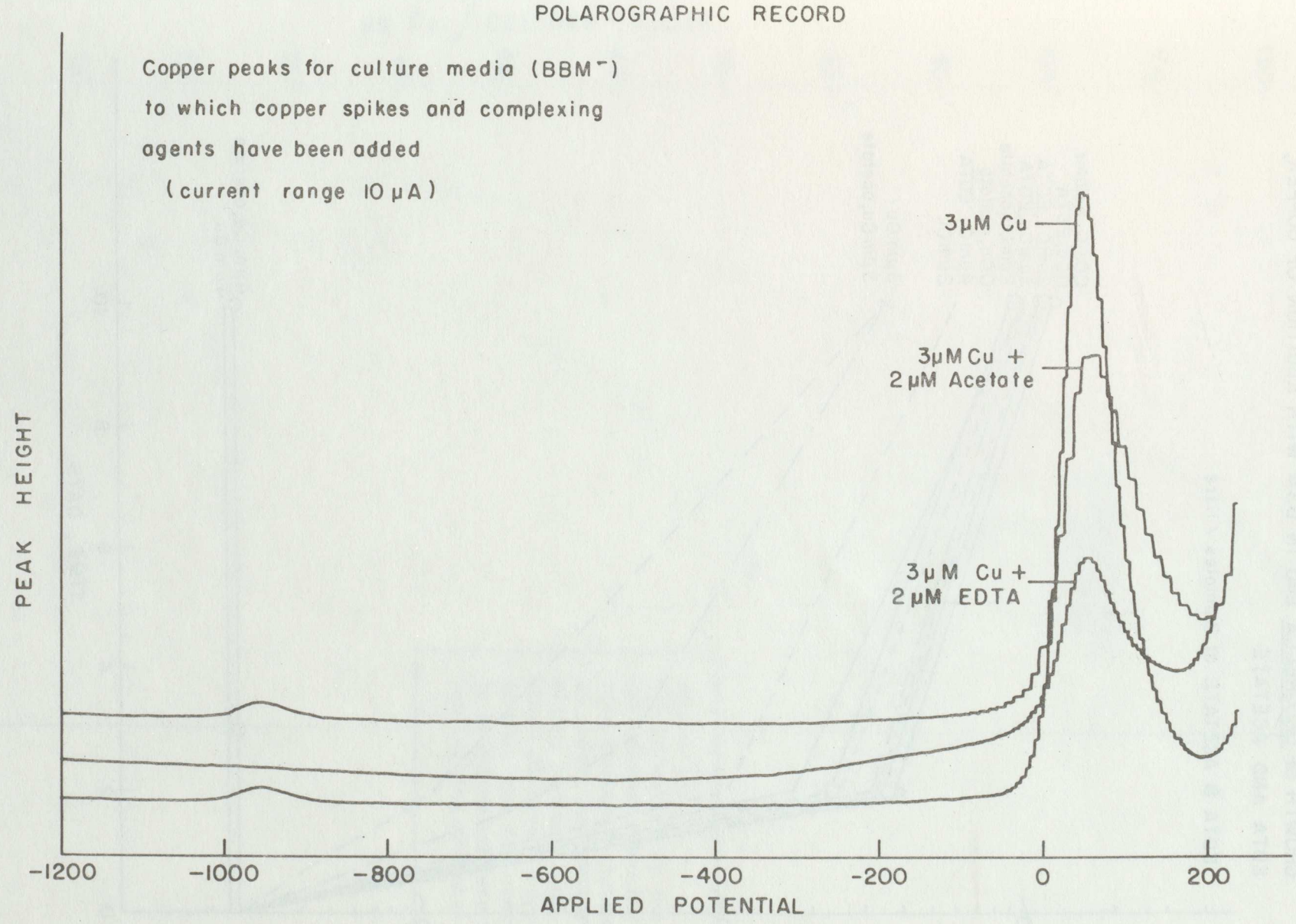

Figure 11: Voltammogram for clean culture medium with copper spikes and addition of acetate on EDTA.

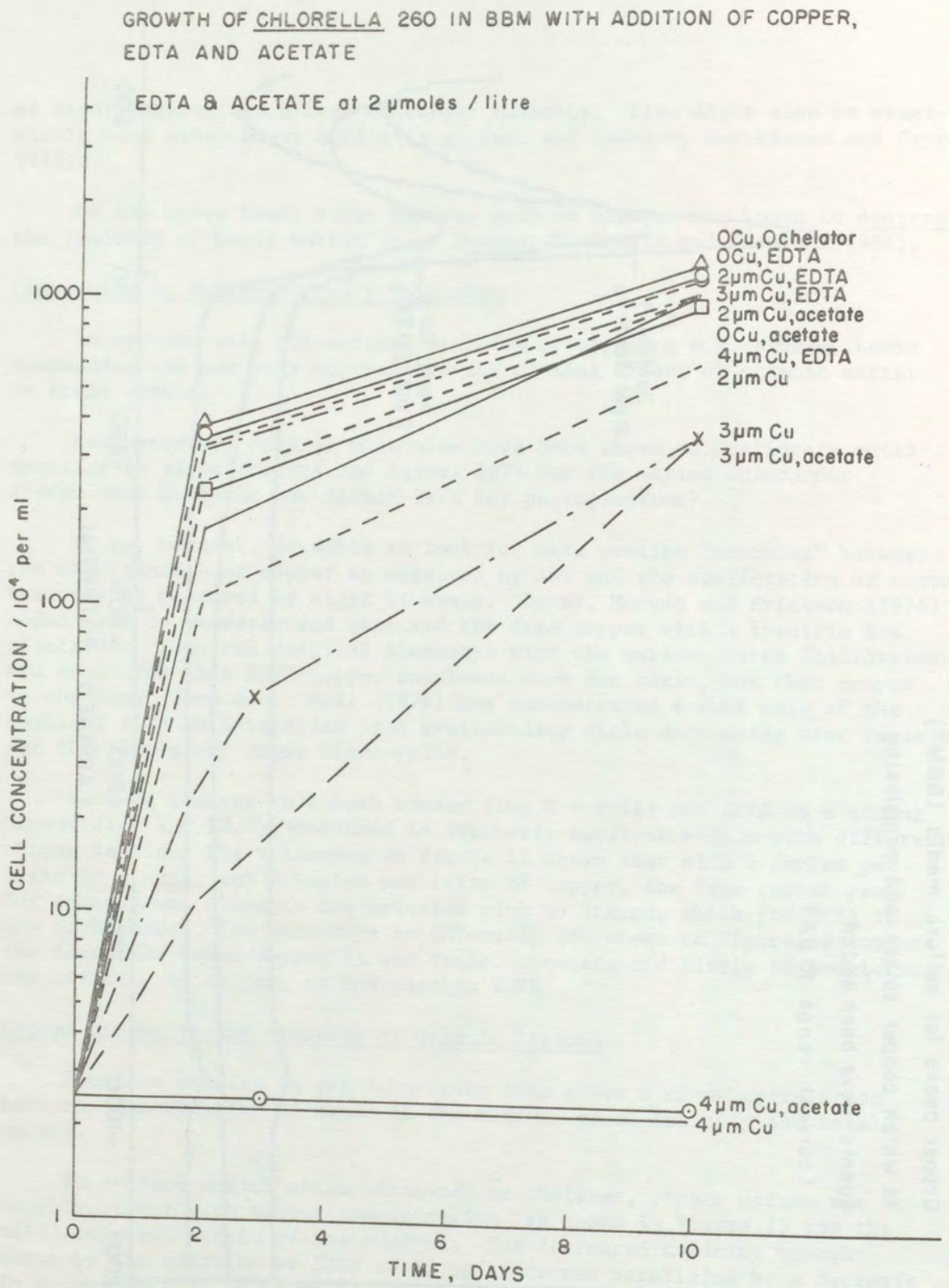

Figure 12: Bioassay, *Chlorella* 260, in culture media with addition of copper, acetate, EDTA, and combinations of these, compared with control lacking copper, acetate and EDTA.

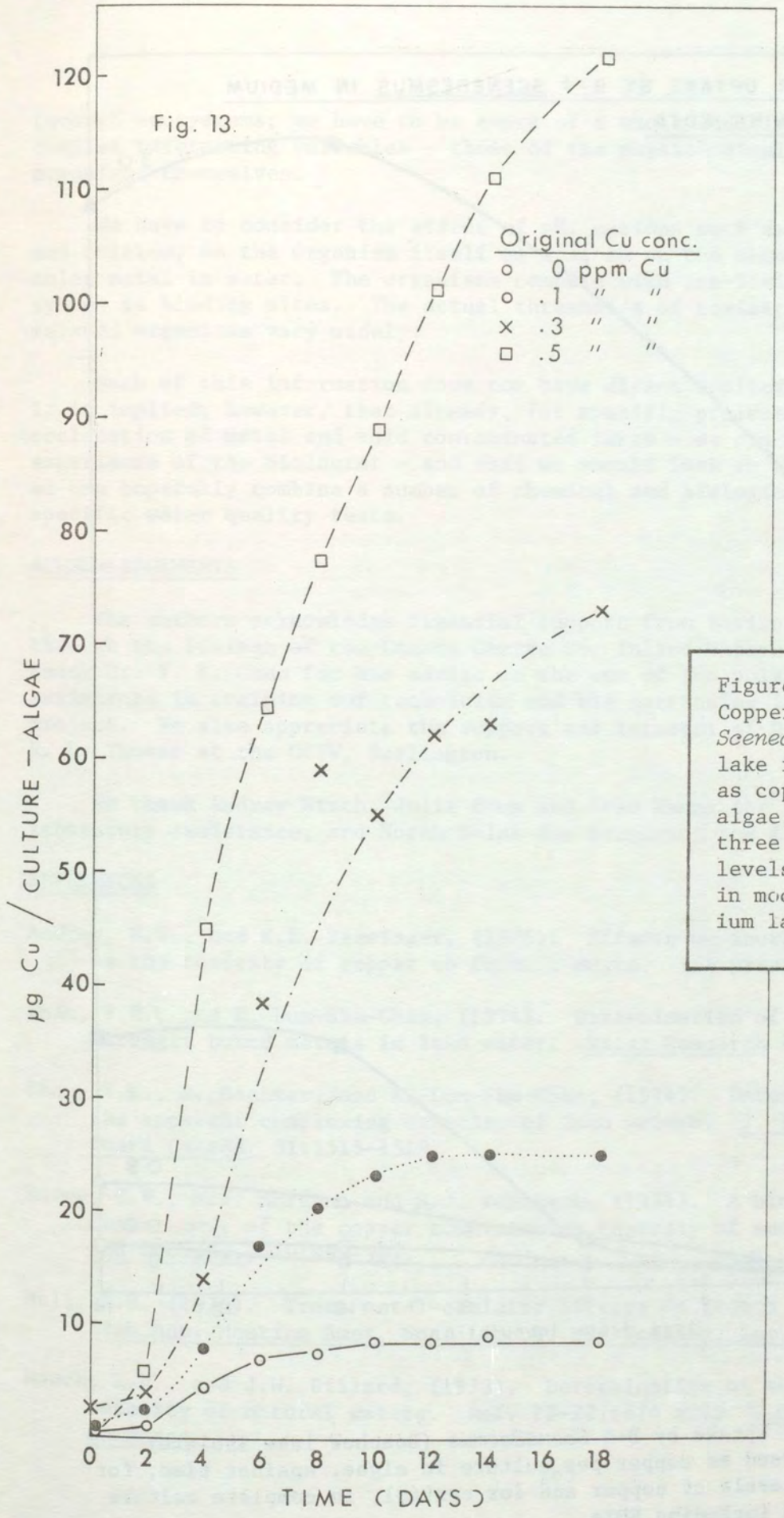

Figure 13:
Copper uptake by B-4 *Scenedesmus* (Boucher lake isolate) expressed as copper per culture in algae, against time, for three different copper levels, and for control in modified culture medium lacking chelate.

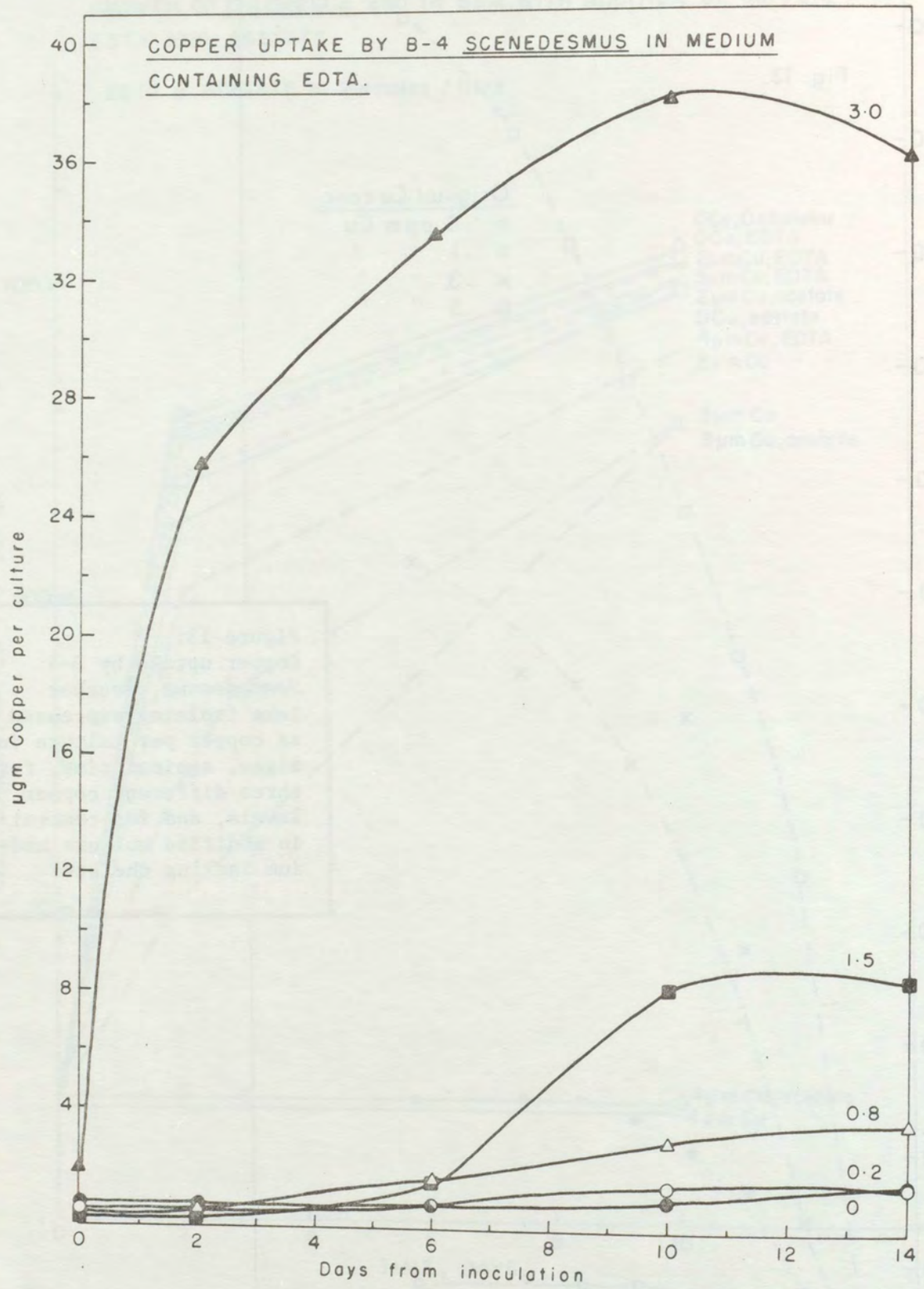

Figure 14: Copper uptake by B-4 *Scenedesmus* (Boucher lake isolate) expressed as copper per culture in algae, against time, for four levels of copper and for control, in complete culture medium including EDTA.

through ecosystems, we have to be aware of a whole additional set of more complex interacting variables - those of the physiological processes of the organisms themselves.

We have to consider the effect of pH, cations such as nickel, zinc and calcium, on the organism itself as well as on the chemistry of a particular metal in water. The organisms compete with non-living parts of the system as binding sites. The actual thresholds of toxicity even for closely related organisms vary widely.

Much of this information does not have direct application at this stage. It is implied, however, that already, for specific projects - for example, reclamation of metal and acid contaminated lakes - we can draw on the experience of the biologist - and that we should look to the future when we can hopefully combine a number of chemical and biological assays for specific water quality tests.

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COMPLEXATION OF METALS IN NATURAL WATERS

CHAPTER 9

Y. K. Chau and P. T. S. Wong
Canada Centre for Inland Waters
Burlington, Ontario

ABSTRACT

A significant fraction of the trace metals in natural waters is believed to exist in complexed forms with the miscellaneous organic ligands which regulates the availability of these metals in the system. The abundance and characteristics of the ligands determine the complexing capacity of the water, hence, its regulating capability. The paper discusses the forms of metals, toxic effects, complexing capacity of natural water and its effect in ameliorating metal toxicity.

The forms of trace metals in the aquatic ecosystem are crucially important in the studies of metal toxicity and biogeochemical pathways of trace elements. In the aqueous system, dissolved trace metals are present in the form of cations (aquo-complexes) or as complexed compounds with miscellaneous organic and inorganic ligands. The functions of chelators in natural water system with respect to metal availability have been the subject of many studies[1,2,3,4,5]. Through complexation, nutrients, notably iron, may become more soluble and remain in solution to maintain growth, whereas toxic metals such as copper, lead, etc. by the same mechanism, may become less toxic. Complexation is believed to play an important role in the regulation of the availability of metals although its exact mechanism is still not well understood.

It is assumed that most heavy metals, especially those in the transition elements group, exist in complexed forms with the various ligands in a biological system. Complexing capacity is thus a measure of the concentration of strong and active complexing agents in water.

The complexing capacity of water can be conveniently measured by the amount of ionic copper complexed by the ligands in water through direct complexation and/or displacement reactions[6]. If a known amount of ionic copper is allowed to equilibrate with the complexing agents of a water sample, the amount of copper "taken up" by a sample is a measure of the amount of the active ligands in water.

The reactions are represented as follows:

$$Cu + L = CuL \quad \text{(direct complexation)}$$
$$Cu + ML = CuL + M \quad \text{(displacement reaction)}$$

where L is the ligand, M denotes other metals, ML is a labile complex. Charges have been omitted for simplicity. Copper has been chosen as the indicator cation to react with the miscellaneous ligands in water because of its strong and nonspecific reactions with almost all ligands. Since the amount of copper added exceeds the concentration of the available ligands and the complexes originally present in water are weak and labile (e.g. Ca and Mg complexes), the complexation of copper with the ligands will be practically complete.

The procedures include the addition of increasingly known amounts of copper solution (10^{-3} M) to several aliquots of a filtered sample (100 ml aliquots). After equilibrium is reached (2 hrs. at 25°C), the labile copper in each aliquot is determined by differential pulse anodic stripping voltammetry[7]. From the plot of uncomplexed copper left in each aliquot of samples (represented by the copper peak current in μA) as y-axis vs. the concentration of copper added on the x-axis (Fig. 1), the intercept on the x-axis represents the complexing capacity of the sample expressed as μmoles/litre of copper equivalent. It can be readily computed from the equation of the regression line by putting y = 0.

By measuring the copper complexing capacity of several known complexing compounds of which the stability constants of their copper complexes are known, tartrate (log K ~ 5), citrate (log K ~ 6), glycine (log K ~ 8), NTA (log K ~ 13) and EDTA (log K ~ 19), it is found that the present technique measures only those ligands which complex copper with log stability constants greater than about 13. With CuNTA as an approximate guide, only those ligands that form complexes with copper with log conditional constants greater than about 10 will be measured.

Although it is expected that the complexing materials of a water body are related to its dissolved organics, we have found no correlation between complexing capacity with either dissolved organic carbon or dissolved organic nitrogen or both together. Since lake water is a complex biological system, the various organic compounds that have this complexing capacity may not relate to their number of carbon or nitrogen.

COMPLEXING CAPACITY AND METAL TOXICITY

The relationship of complexing capacity of the medium and its inhibition of algal photosynthesis by copper has been studied[8]. Although no absolute quantitative relationship can be derived from the numerical values of complexing capacity and its detoxification effect on phytoplankton, there was evidence that water of higher complexing capacity has a higher ability to ameliorate the toxicity of copper. The toxic effects of metals in a biological system depend greatly on several factors, complexing capacity is a significant one. Among other factors, alkalinity, hardness, nutrient concentration and pH of the medium also have certain effects. Unless it is possible to vary the complexing capacity of water samples without varying the other parameters, the sole effect of complexing capacity cannot be quantitatively assessed. It is at present not possible to vary the amount of ligands in water without changing the nutrient level, the alkalinity, etc.

COMPLEXING CAPACITY AND ALGAL GROWTH

A relationship between the complexing capacity of lake water and its ability to support algal growth was studied. A fresh water alga, *Ankistrodesmus falcatus*, was used to grow on filter-sterilized lake waters from 12 Sudbury area lakes at 20°C in a shaker under 5000 lux illumination with 18 hours light and 6 hours dark cycle. Results in Fig. 2 show that all

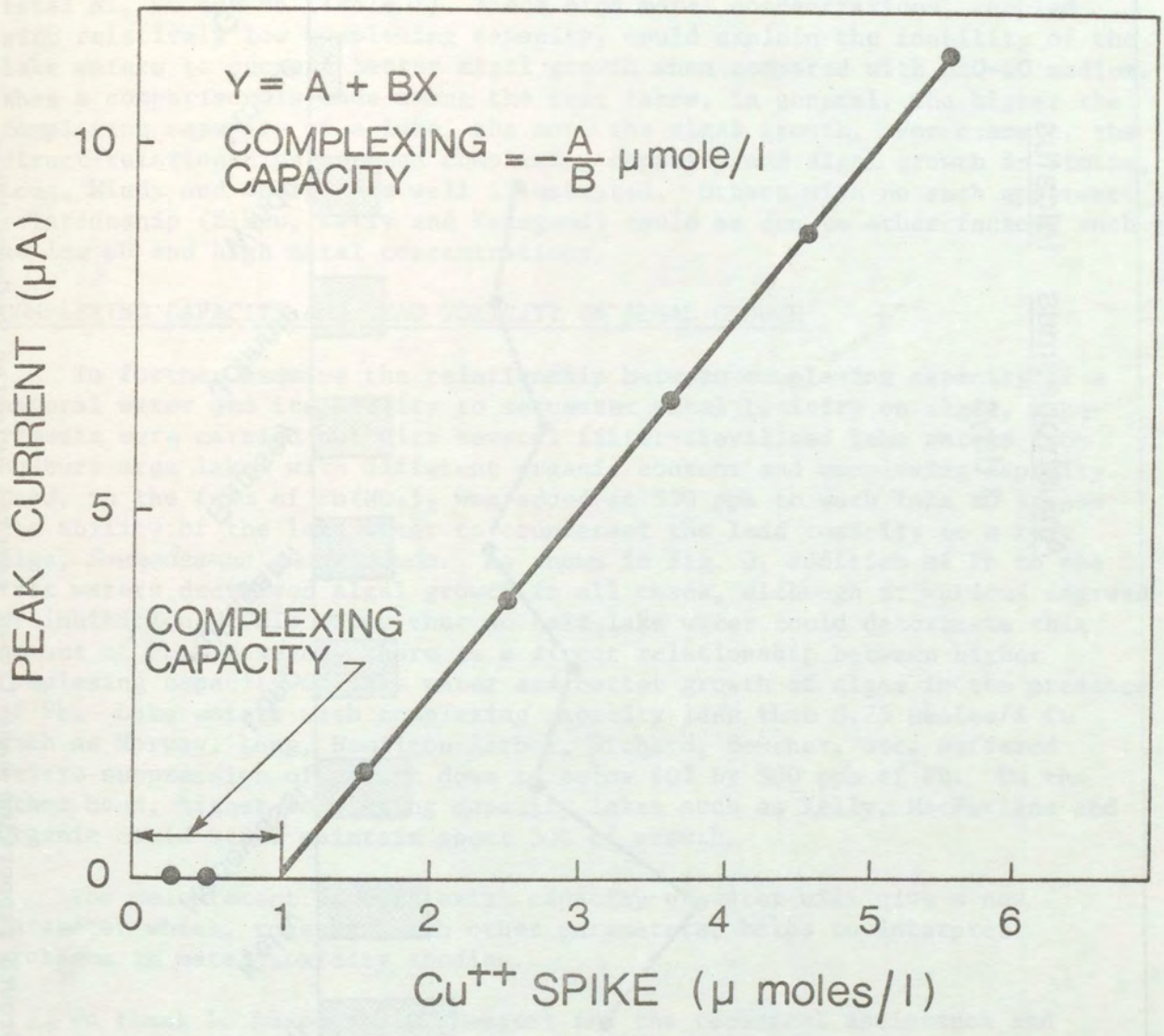

Figure 1. Schematic diagram of complexing capacity measurement.

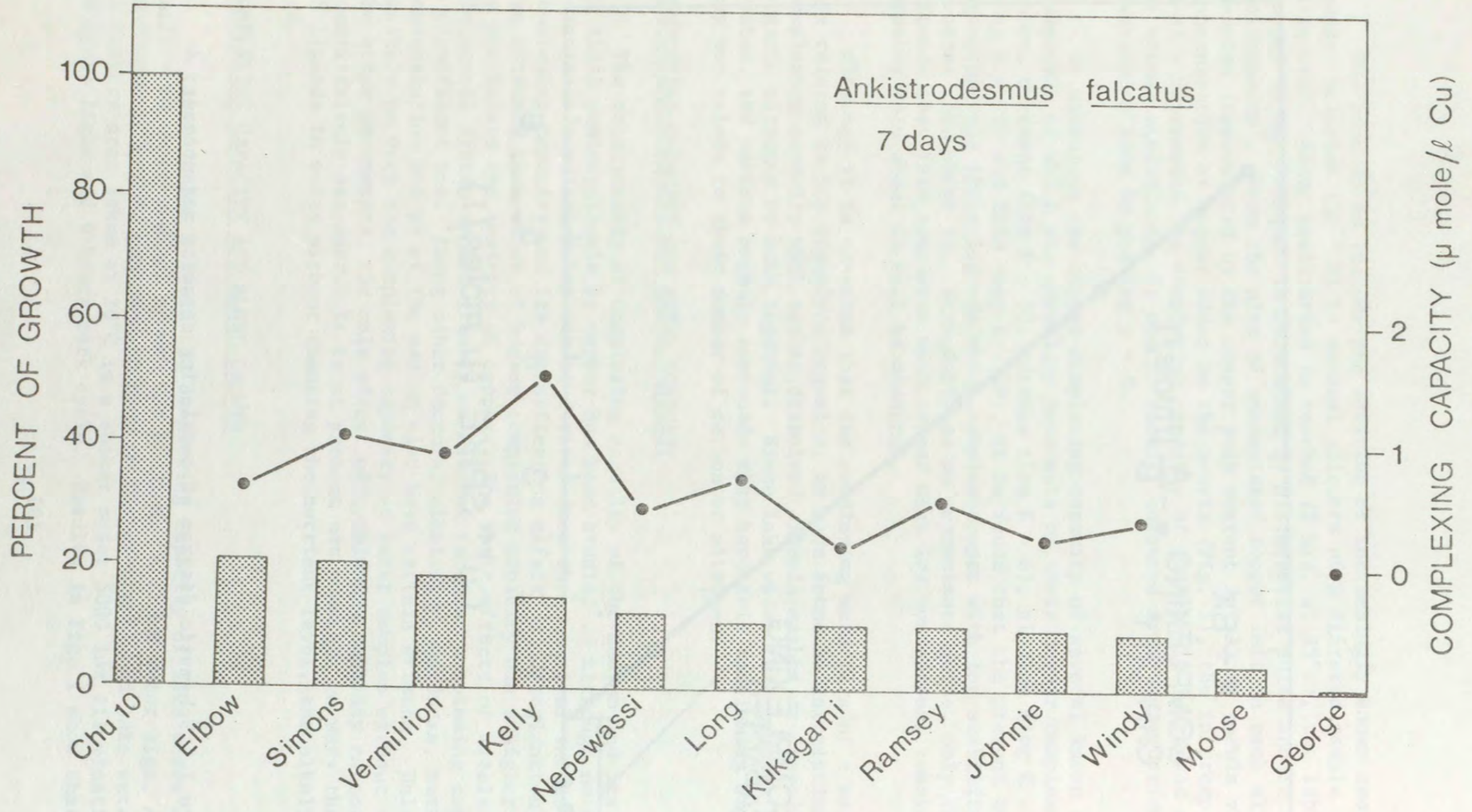

Figure 2. Growth supporting capacity of several selected Sudbury lakes (Growth determined by microscopic cell counts).

test waters exhibit various degrees of growth inhibition when compared with a laboratory medium CHU-10[9]. An examination of metal concentrations of test waters reveals high levels of Zn (both total and labile) as well as total Ni, Cu and Pb (Table 1). These high metal concentrations, coupled with relatively low complexing capacity, could explain the inability of the lake waters to support better algal growth when compared with CHU-10 medium. When a comparison is made among the test lakes, in general, the higher the complexing capacity of a lake, the more the algal growth. For example, the direct relationships between complexing capacity and algal growth in Simons, Long, Windy and George are well illustrated. Others with no such apparent relationship (Elbow, Kelly and Kukagami) could be due to other factors such as low pH and high metal concentrations.

COMPLEXING CAPACITY AND LEAD TOXICITY ON ALGAL GROWTH

To further examine the relationship between complexing capacity of a natural water and its ability to sequester metal toxicity on algae, experiments were carried out with several filter-sterilized lake waters from Sudbury area lakes with different organic content and complexing capacity. Lead, in the form of $Pb(NO_3)_2$ was added at 500 ppb to each lake to assess the ability of the lake water to counteract the lead toxicity to a test alga, *Scenedesmus quadricauda*. As shown in Fig. 3, addition of Pb to the test waters decreased algal growth in all cases, although at various degrees of inhibition. This shows that no test lake water could detoxicate this amount of Pb. However, there is a direct relationship between higher complexing capacity of lake water and better growth of algae in the presence of Pb. Lake waters with complexing capacity less than 0.75 µmoles/ℓ Cu such as Norway, Long, Hamilton Harbor, Richard, Boucher, etc. suffered severe suppression of growth down to below 40% by 500 ppb of Pb. On the other hand, higher complexing capacity lakes such as Kelly, MacFarlane and Organic could still maintain about 50% of growth.

The measurement of complexing capacity of water will give a new parameter which, together with other parameters, helps to interpret problems in metal toxicity studies.

We thank L. Luxon and G. Bengert for the technical assistance and Water Quality Laboratories for some of the chemical analyses.

Table 1. Growth of Ankistrodesmus falcatus in lake water of different complexing capacity (July, 1975)

LAKES	pH	Conductivity (µmho / cm)	Total Hardness (ppm $CaCO_3$)	Dissol. Org. C (ppm)	Dissol. Org. N (ppm)	Complexing Capacity (µmole/ℓ Cu)	Percent Growth[1]	METAL CONCENTRATION[2] (µg/ℓ) Zn	Cd	Pb	Cu	Ni[3]
Elbow	5.7	55	23.8	6.2	0.35	0.64	21.3	48.7(80.0)	0 (1.2)	0 (6.3)	16.2(16.2)	(48)
Simons	7.3	700	164.0	4.6	0.31	1.01	20.5	19.1(83.4)	0 (0.8)	0 (2.8)	0 (7.6)	(1400)
Vermillion	8.1	80	43.1	6.0	0.33	0.91	18.2	53.6(118.9)	0 (0)	0 (3.9)	0 (2.9)	(38)
Kelly	4.2	1120	479.0	4.6	7.5	1.59	16.4	0 (26.0)	0 (1.2)	0 (14.3)	0 (15.0)	(1900)
Nepewassi	7.9	43	23.9	6.5	0.26	0.56	12.6	24.9(149.6)	0 (0.5)	0 (4.9)	0 (8.4)	(17)
Long	7.8	71	32.3	3.2	0.25	0.72	11.3	7.0(64.8)	0 (0)	0 (21.0)	0 (8.0)	(92)
Kukagami	6.7	36	18.4	2.5	0.13	0.16	11.0	63.3(227.0)	0 (0)	0 (0)	0 (4.5)	(15)
Ramsey	6.4	172	55.3	3.9	0.31	0.54	10.8	23.4(69.5)	0 (0)	0 (4.3)	0 (12.0)	(320)
Johnnie	5.5	31	12.8	2.8	0.20	0.23	10.0	156.0(371.0)	0 (0)	22.5(-)	8.5(9.0)	(20)
Windy	7.4	34.8	14.5	3.7	0.17	0.40	9.7	20.7(103.8)	0 (1.0)	0 (6.5)	0 (4.9)	(12)
Moose	3.9	1100	230.0	2.3	0.25	-	4.6	272.0(333.0)	0 (4.7)	7.3(8.2)	0 (21.3)	(500)
George	5.4	29.3	11.0	2.3	0.25	0	0	103.2(327.6)	0.7(1.0)	- (4.0)	17.0(11.4)	(23)

1. 100% growth is equivalent to 3×10^7 cells/ml after 7 days incubation in the filter-steriled CHU-10 medium.
2. Metal conc. first number is labile metal; number in brackets total metal.
3. Ni determined by AAS.

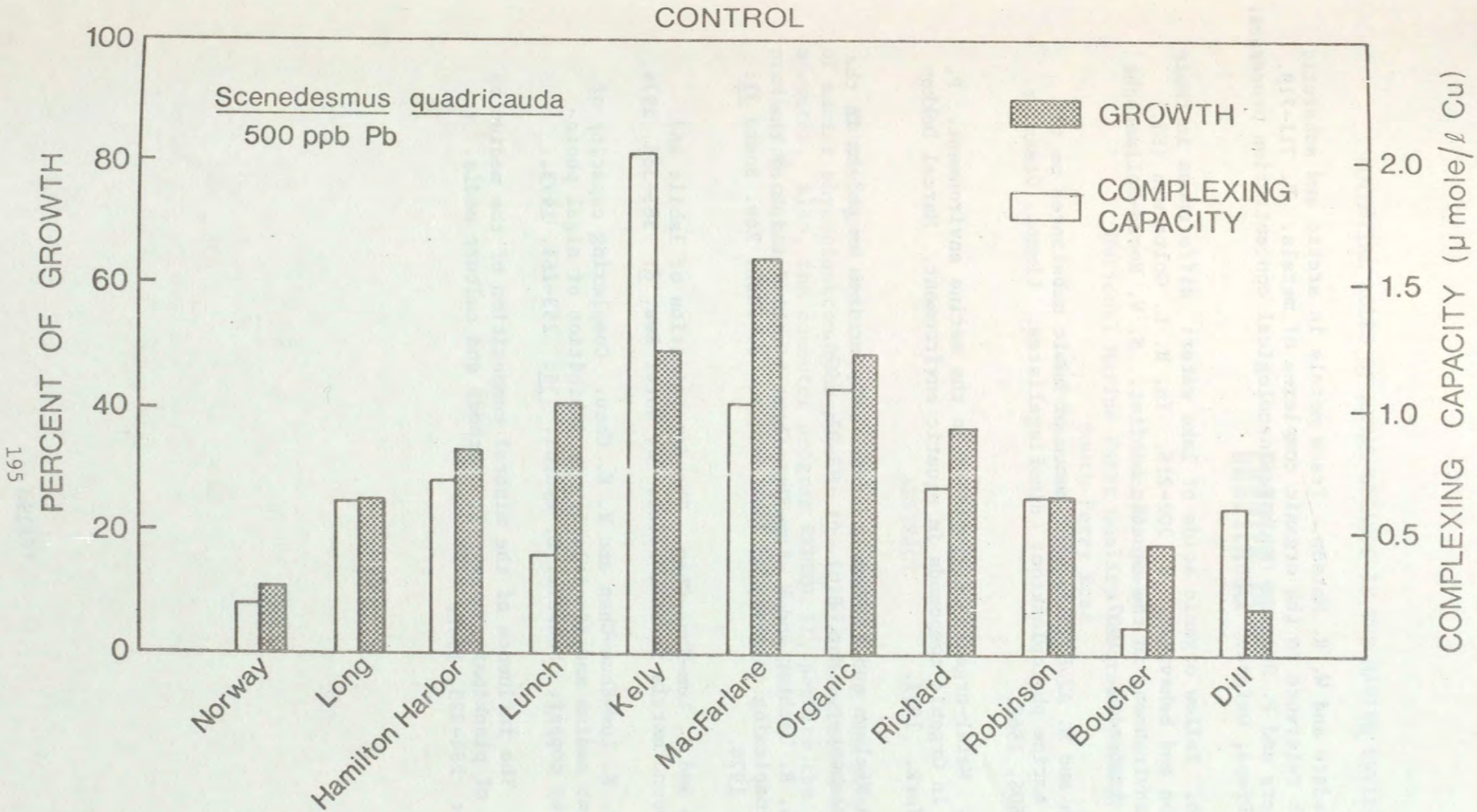

Figure 3. Effect of lead on growth of *Scenedesmus quardriacauda* in water of different complexing capacity. Lead nitrate added 500 ppb as Pb. Controls made up of respective lake waters without lead additions (Growth determined by microscopic cell counts).

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POTENTIAL ROLES OF METAL-LIGANDS IN THE MARINE ENVIRONMENT

CHAPTER 10

Earl W. Davey
National Marine Water Quality Laboratory (EPA)
South Ferry Road
Narragansett, Rhode Island

ABSTRACT

Metal-ligand interactions can be measured using the growth response of marine phytoplankton bioassays in trace metal controlled artificial seawater. Also, the computer program REDEQL II, permits the potential prediction of metal-ligand interactions in seawater.

Erickson (1972) found that the growth depression of *Thalassiosira pseudonana* (an oceanic diatom previously called *Cyclotella nana*) by copper in unenriched seawater samples from Narragansett Bay and vicinity varied with season and location of collection. These variations in copper toxicity could be due to the presence of differing concentrations of copper complexing compounds. Barber and Ryther (1969), Barber et al. (1971) and Lewis et al. (1971) have suggested the occurrence of natural chelators in seawater. The purpose of this paper is to present the development of a quantitative technique for the biological measurement of copper binding capacity of seawater.

METHODS AND MATERIALS

Artifical seawater (ASW) media were prepared according to the formulation of Kester et al. (1967). Modifications included the deletion of NaF, NaBr and $SrCl_2$; addition of 3 µmoles liter^{-1} NaH_2PO_4 and 30 µmmoles liter^{-1} of $NaNO_3$; and the reduction of the salinity to 30°/00. Possible trace metal contaminations were removed from the media by passage through Chelex - 100 chelating exchange resin, sodium form (Davey et al. 1970). The adsorption efficiency is shown in Table 1. Media were then stored or processed in silicone coated or quartz glassware to reduce the introduction of trace metal impurities. Media were exposed to 3 hours ultraviolet (UV) radiation according to the method of Armstrong et al. (1966) in order to eliminate possible organic contaminations. Particles formed by the UV treatment were removed by filtering the media through 0.22 µ acid-washed and deionized water-rinsed Millipore filters. Chelators, when required by the experiment, and vitamin B_{12} were added to the media prior to pasteurization for 3 hr at 60°C. Media were stored after pasteurization for at least 24 hr. During this period media were checked for sterility with DC synthetic seawater (Prager 1963) enriched with 500 mg DL alanine and 50 mg nutrient agar liter^{-1}.

Table 1. Adsorption efficiency of purified sodium Chelex-100 for trace metals in seawater.

Isotope	% Adsorption
^{65}Zn	>99
^{115m}Cd	>99
^{54}Mn	>99
^{64}Cu	>99
^{210}Pb	95
^{63}Ni	92
^{59}Fe	92
^{110m}Ag	33

Particle background was determined using a Model B Coulter Counter. Media contained less than 1 μg Cu liter^{-1} as measured by anodic stripping voltammetry. A mixture of trace metals (in μg liter^{-1}, 10 Fe, 2.0 Mn, 0.2 Zn, and 1.0 Mo as chlorides) was added to the culture media at the time of innoculation. The experimental organism, *T. pseudonana*, was maintained in the artificial seawater and had a consistent eight hour doubling time. The copper bioassays were run according to the method of Erickson (1972). Copper bioassays were run according to the method of Erickson (1972). Copper ion specific electrode titrations of ethylenediaminetetraacetic acid (EDTA) against copper in ASW were modified from a procedure of Orion Research Incorporated (1968).

RESULTS

Although the copper ion specific electrode was not sensitive enough for the direct measurement of copper in seawater, it could be used to quantitate, by copper titration, 10^{-3}M concentrations of EDTA (Fig. 1). The equivalence point (EP) is the point of maximum inflection of the sigmoid curve (Fig. 1). At the equivalence point, the moles of copper added are equal to the moles of EDTA present in solution.

We noted a similarity in response between the copper ion specific electrode and the growth responses of *T. pseudonana* in copper bioassays performed by Erickson (1972). This analogy suggested that the *T. pseudonana* copper bioassay could be utilized as an endpoint detector for the quantitative measurement of the copper binding capacity of seawater. In order to test this hypothesis, *T. pseudonana* growth experiments were performed in an essentially organic free (except for 1 μg liter^{-1} vitamin B_{12}) and trace metal controlled artificial seawater. The growth responses of *T. pseudonana* to increasing concentrations of copper in this media are illustrated in Fig. 2.

If copper concentrations of Fig. 2 are plotted against the cell number, represented as the percentage of control after a specific period of sustained logarithmic growth, then one obtains the no EDTA curve in Fig. 3. When a chelator is added to the copper bioassay ($1x10^{-7}$M and $5x10^{-7}$M-EDTA, Fig. 3) there is a shift from the no EDTA curve towards higher copper levels. The chelated curves are similar to the copper ion specific electrode copper titrations of EDTA in Fig. 1. Analogously, the midpoints of the sharp inflection (EP) in Fig. 3 could be used to quantitate the concentrations of added EDTA to within $\pm$5% at a sensitivity down to $1x10^{-7}$M EDTA.

The *T. pseudonana*-copper bioassay of $5x10^{-7}$M histidine (Fig. 4) indicates that natural organic chelators which might occur in seawater could be quantitated by copper-bioassay titration.

The *T. pseudonana*-copper bioassay was performed on two samples of unenriched seawater (Fig. 5). The Charleston Pond sample was taken from a relatively unpolluted area 16 km west of the entrance of Narragansett Bay. The sample had a dissolved organic content of approximately 2 mg C liter^{-1} and a copper titration equivalence point (EP) of $0.4x10^{-7}$M indicating a very low copper complexing level. The second sample was taken from Brayton Point located at the heavily populated and industrialized northern

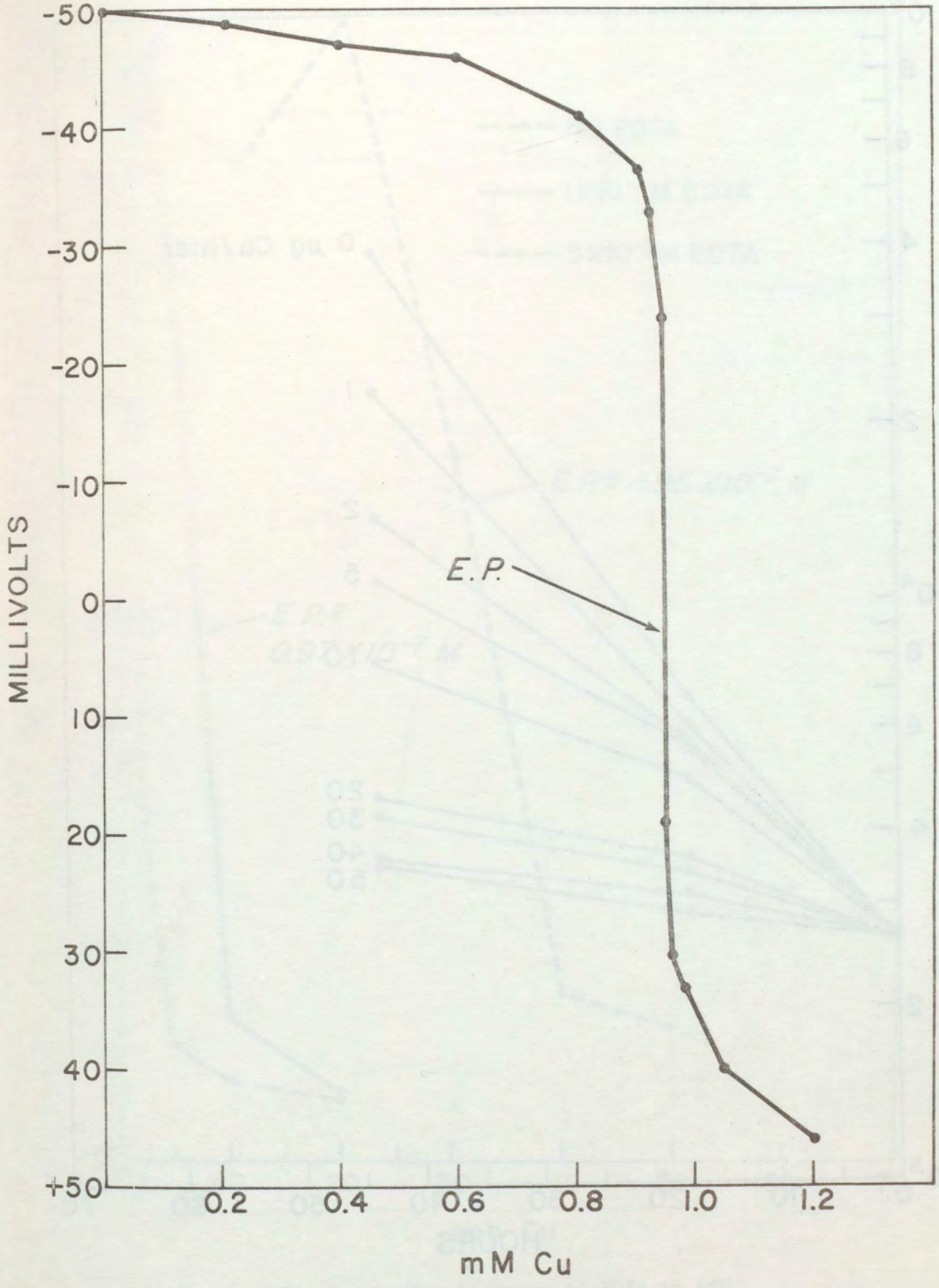

FIG. 1. Copper ion specific electrode titration of 10^{-3}M EDTA in ASW.

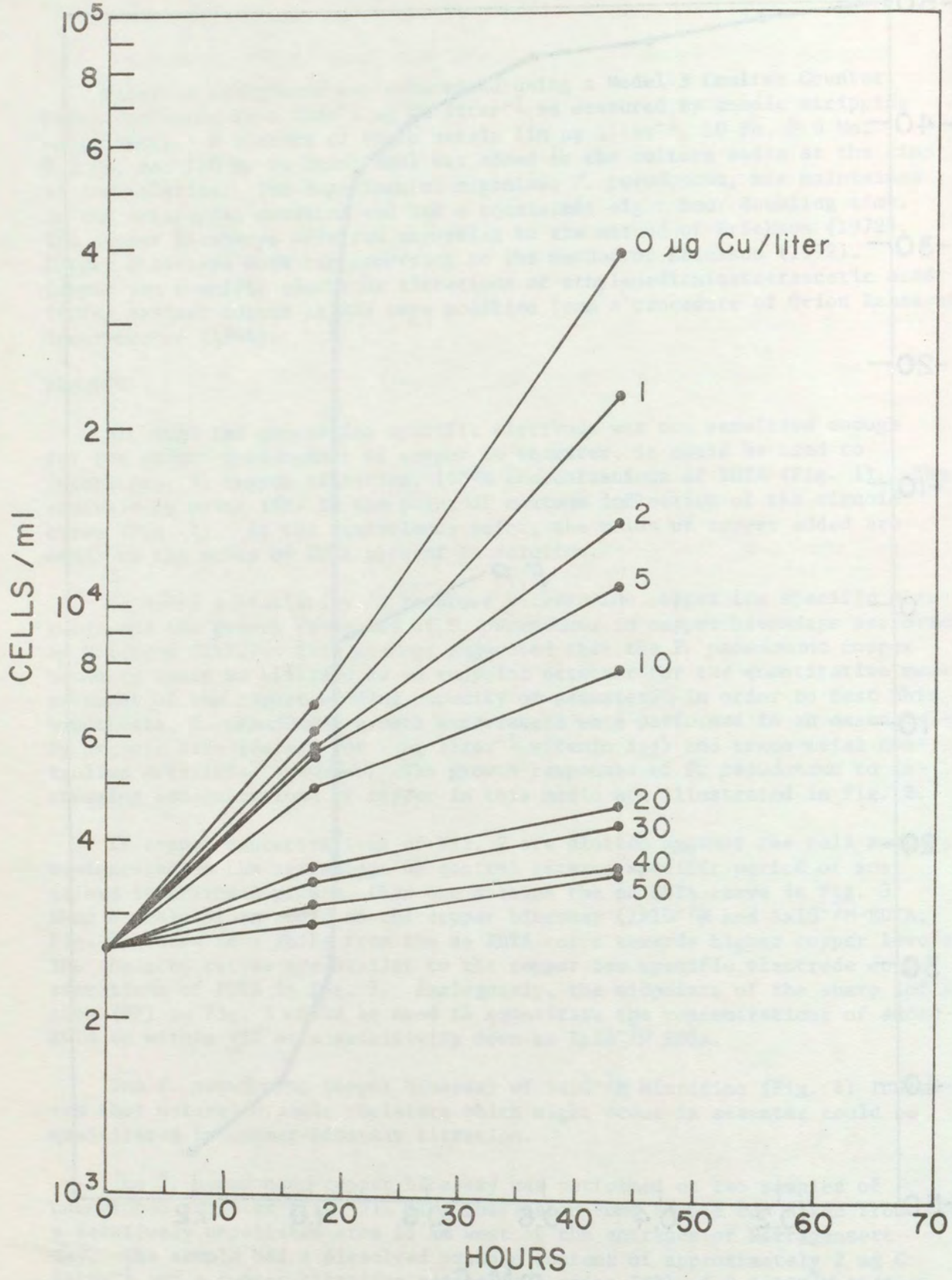

FIG. 2. Copper bioassay of *Thalassiosira pseudonana* in ASW.

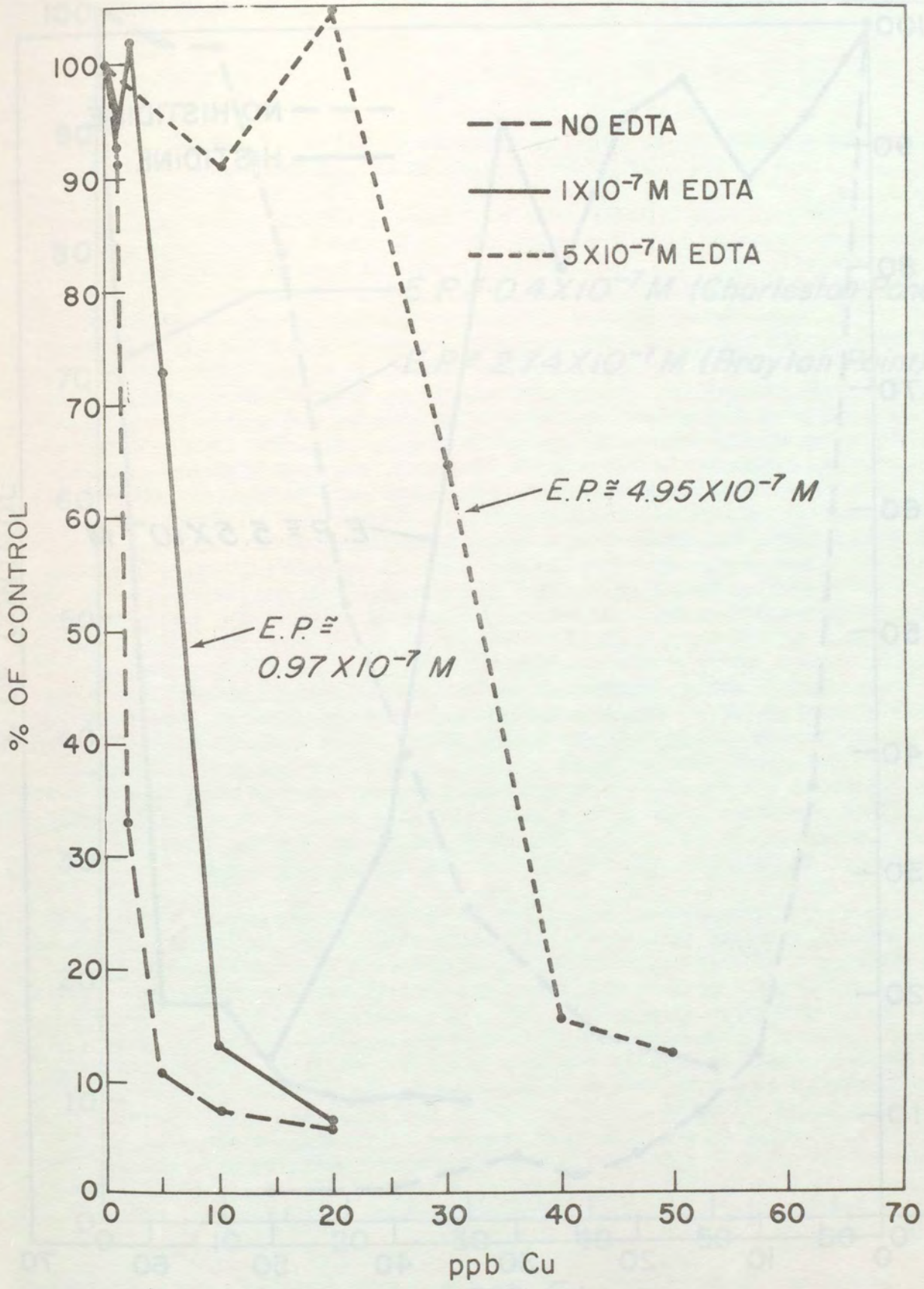

FIG. 3. Copper bioassay of EDTA in ASW.

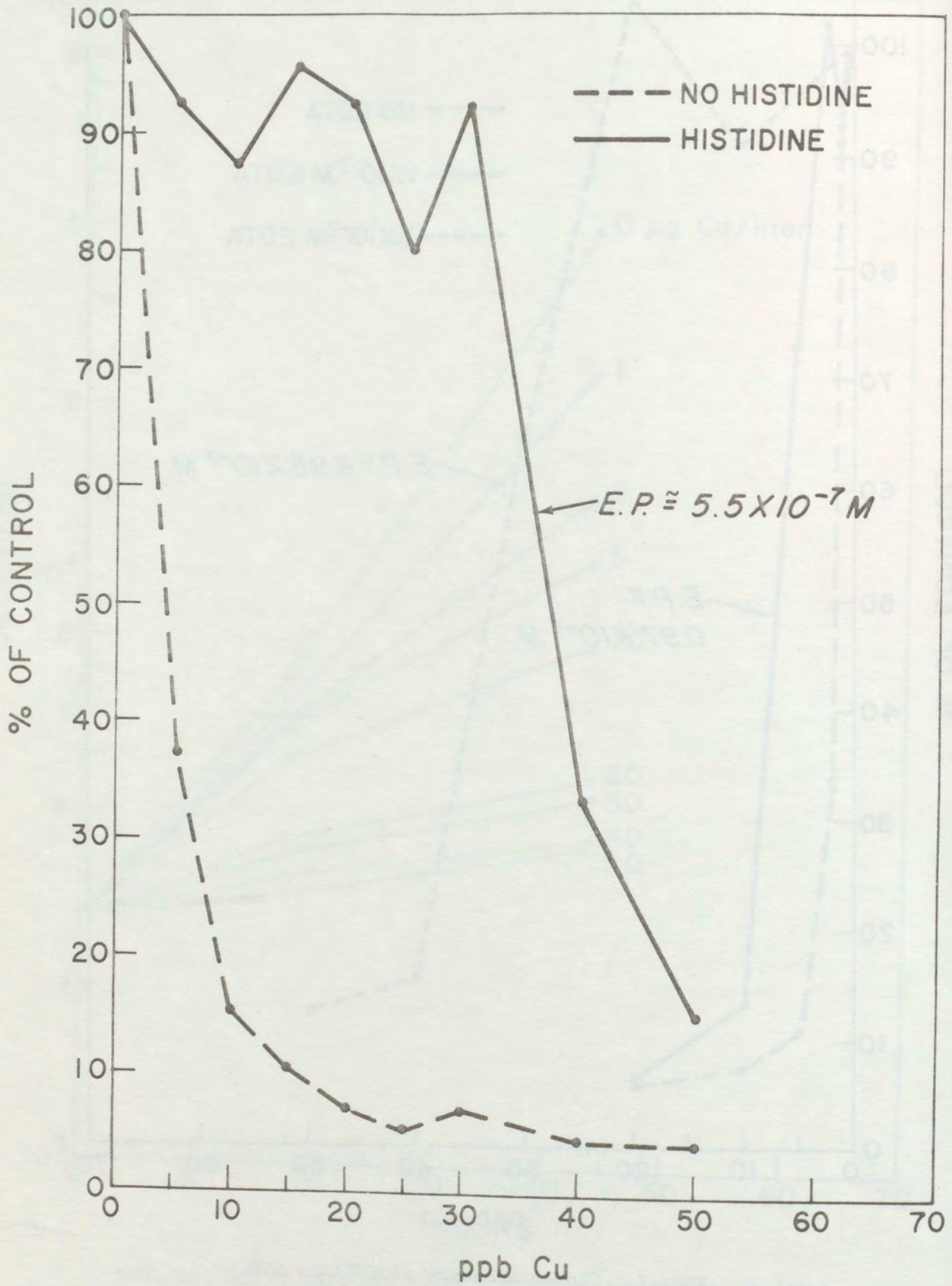

FIG. 4. Copper bioassay of $5x10^{-7}$M Histidine in ASW.

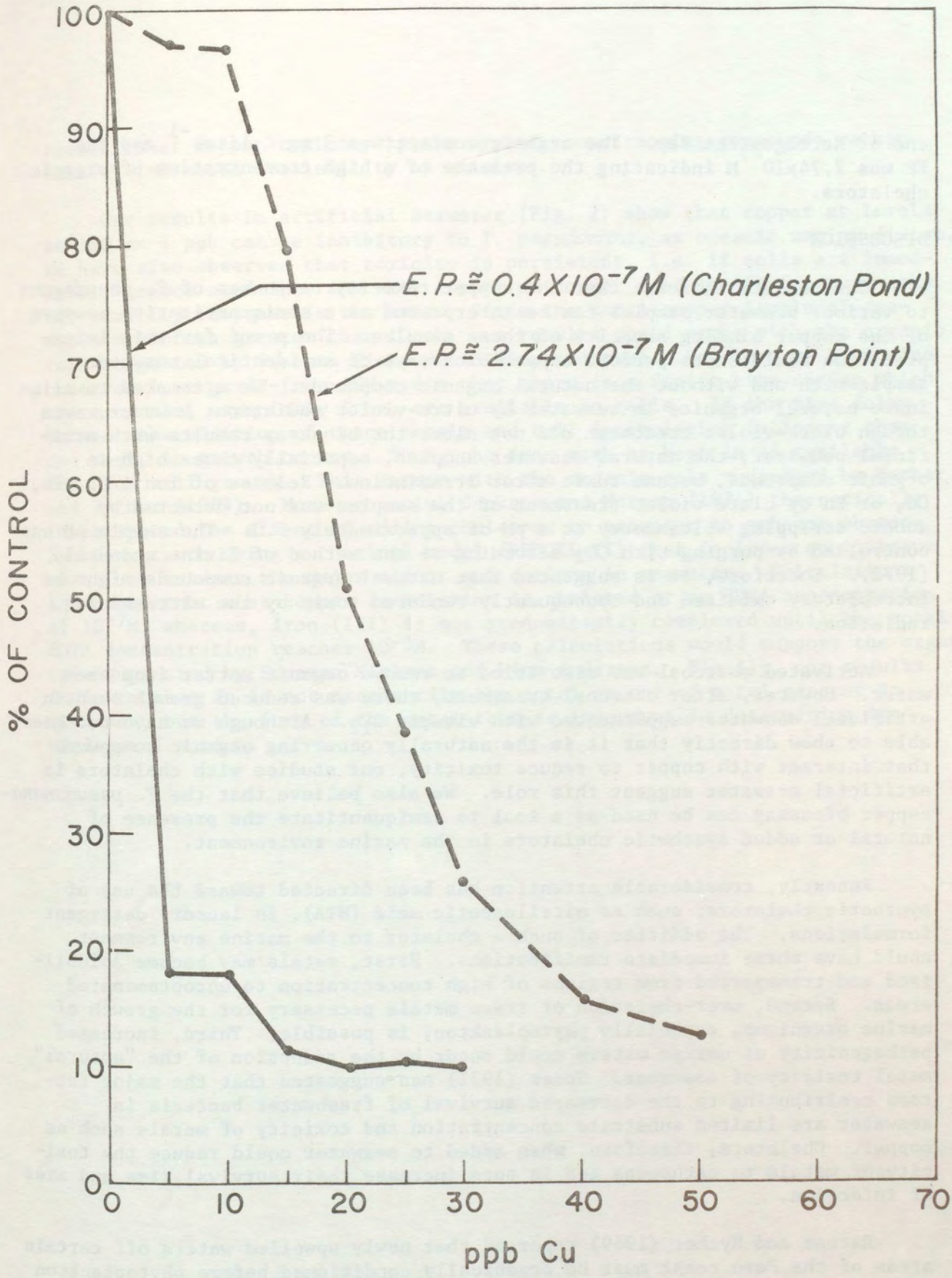

FIG. 5. Copper bioassay of seawater.

end of Narragansett Bay. The organic content was 3 mg C liter^{-1} and the EP was 2.74×10^{-7} M indicating the presence of a high concentration of organic chelators.

DISCUSSION

Our results indicate that the copper toxicity responses of *T. pseudonana* to various seawater samples can be interpreted as a semiquantitative measure of the copper binding capacity of these samples. The proof for this interpretation would be to perform copper bioassays on an identical seawater sample with and without the natural organic compounds. We attempted to eliminate natural organics in seawater by ultra-violet radiation; however, even though ultra-violet treatment did not alter the bioassay results with artificial seawater, the natural seawater samples, especially those high in organic compounds, became toxic after irradiation. Release of ionic Cu, Pb, Cd, or Zn by ultra-violet treatment of the samples was not detected by anodic stripping voltammetry at a pH of approximately 5.0. The sample pH was controlled by purging with CO_2 according to the method of Zirino and Healy (1972). Therefore, it is suggested that natural organic compounds might be incompletely oxidized and consequently rendered toxic by the ultra-violet radiation.

Activated charcoal was also tried to remove organic matter from seawater. However, after charcoal treatment, there was reduced growth even in artificial seawater supplemented with vitamin B_{12}. Although we have not been able to show directly that it is the naturally occurring organic compounds that interact with copper to reduce toxicity, our studies with chelators in artificial seawater suggest this role. We also believe that the *T. pseudonana*-copper bioassay can be used as a tool to semiquantitate the presence of natural or added synthetic chelators in the marine environment.

Recently, considerable attention has been directed toward the use of synthetic chelators, such as nitriloacetic acid (NTA), in laundry detergent formulations. The addition of such a chelator to the marine environment could have three immediate ramifications. First, metals may become solubilized and transported from regions of high concentration to uncontaminated areas. Second, over-chelation of trace metals necessary for the growth of marine organisms, especially phytoplankton, is possible. Third, increased pathogenicity of marine waters could occur by the reduction of the "natural" metal toxicity of seawater. Jones (1971) has suggested that the major factors contributing to the decreased survival of freshwater bacteria in seawater are limited substrate concentration and toxicity of metals such as copper. Chelators, therefore, when added to seawater could reduce the toxicity of metals to pathogens and in turn increase their survival time and area of infection.

Barber and Ryther (1969) reported that newly upwelled waters off certain areas of the Peru coast must be organically conditioned before phytoplankton blooms can occur. They suggest that organic compounds are necessary to make trace metal available for growth. However, Steeman Nielsen and Wium-Andersen (1970), interpreted the results of Barber and Ryther (1969) as evidence of the

reduction of copper toxicity by the organic conditioning compounds rather than of increased availability of metals.

Our results in artificial seawater (Fig. 2) show that copper at levels as low as 1 ppb can be inhibitory to *T. pseudonana*, an oceanic marine diatom. We have also observed that toxicity is persistent, i.e. if cells are immediately innoculated into ASW with copper or copper aged in ASW for two weeks, the toxicity response is the same. Since the total copper levels of seawater are often greater than 1 ppb, our results would agree with the organic role suggested by Steeman Nielsen and Wium-Andersen. However, we have also observed in organic-free ASW media that there is a finite time during which trace metals, especially iron, are available to cells. If the time delay between the addition of trace metals and cell innoculation is longer than one week, no growth occurs. Thus, the iron must be kept in an available form for cell growth. This agrees with the organic role suggested by Barber and Ryther (1969). More recently, Jackson and Morgan (1974) theoretically investigated the effect of adding chelators to seawater to enhance phytoplankton growth using a chemical model (REDQEL II) that includes equilibrium reactions between the major anions and cations in seawater. Calculations indicated that most copper in solution is chelated at an EDTA concentration of 10^{-7}M; whereas, iron (III) is not predominantly complexed until the total EDTA concentration reaches 10^{-5}M. These calculations would support the organic role suggested by Steeman Nielsen and Wium-Andersen. Finally, our results indicate that it is not necessarily the total amount of metal present in seawater, but the form of the metal that is important in determining its biological action.

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DISTRIBUTION AND BIOACCUMULATION OF MERCURY IN BIOTIC AND ABIOTIC COMPARTMENTS OF A CONTAMINATED RIVER - RESERVOIR SYSTEM[1,2]

CHAPTER 11

S. G. Hildebrand, A. W. Andren,[3] and J. W. Huckabee
Environmental Sciences Division
Oak Ridge National Laboratory[4]
Oak Ridge, Tennessee 37830

ABSTRACT

The distribution and bioaccumulation of mercury in the North Fork of the Holston River - Cherokee Reservoir which receives mercury inputs from an abandoned chlor-alkali plant were studied to determine the behavior and ultimate fate of mercury in this system. Mercury in the dissolved form appears to be leaching from waste disposal ponds at the abandoned plant. Dissolved mercury is rapidly adsorbed onto suspended particulates and the main downstream transport of mercury appears to take place in the particulate phase. Total mercury and methylmercurials in fish and benthic invertebrate taxa are highest immediately below the mercury source then decrease downstream. This pattern is similar to the downstream distribution of mercury in the water and sediments. Total mercury in rockbass *(Ambloplites rupestris)* 83 miles downstream from mercury inputs exceeds 1.0 ppm. Approximately 80% of mercury in fish species in the river is methylmercury and on the order of 50% of mercury in benthic invertebrates is methylmercury. We have not detected methylmercury in bed sediments in this system. Dietary uptake of methylmercury by fish species feeding on benthic invertebrates may be a significant route of entry of methylmercury into food webs in this river. Future research necessary to more completely understand the biogeochemical cycling of mercury in aquatic ecosystems is suggested.

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[3]Present address: Water Chemistry Laboratory, University of Wisconsin, Madison, Wisconsin 53706

[4]Operated by the Energy Research and Development Administration under contract with Union Carbide Corporation.

INTRODUCTION

The Minamata and Niigata incidents in the early 1950's set in motion a considerable research effort in several countries concerned with the toxicity, distribution, environmental transformation, and bioaccumulation of mercury in aquatic ecosystems. An excellent recent review[1] is available and the reader is referred to this paper for a concise summary of environmental mercury research to date. Mercury research in the last decade has answered many significant questions but to date the biogeochemical cycling and accumulation of inorganic and organic mercury by aquatic organisms is not completely understood. In this paper we present information on the distribution and bioaccumulation of mercury in a contaminated river system and define research necessary to more completely understand the dynamics of mercury cycling in aquatic ecosystems.

The North Fork Holston River originates in southwest Virginia and joins the South Fork Holston River to form the Holston River in eastern Tennessee. The Holston River was impounded (Cherokee Dam) in 1942 to form Cherokee Reservoir. The North Fork (Fig. 1) is a swift mountain river, with a drop in elevation of 1300 ft. from the source to its confluence with the South Fork.[2] Substrate in the North Fork ranges from silt and clay in backwaters to large boulders and bedrock in the main channel.

A chlor-alkali plant is located on the North Fork at Saltville, Virginia approximately 83 miles upstream from the mouth. In the 1950's the plant began using a mercury cathode in the electrolytic process to produce chlorine and sodium hydroxide from sodium chloride. In addition to metallic mercury lost to the North Fork through plant operation, waste from the majority of plant operations was placed in two disposal

areas adjacent to the river. Presently the two waste disposal ponds encompass approximately 120 acres and are 30-100 feet deep. The plant was closed down and finally abandoned in 1972 due to the high cost of complying with water quality standards for total dissolved solids, chlorides, and mercury. Samples of fish flesh from the North Fork analyzed for total mercury in 1970 indicated that the majority of fish species exceeded the 0.5 ppm FDA guideline.[2] The North Fork was subsequently closed to fishing in Virginia.[3] In 1974 we initiated our study investigating the distribution and transport of mercury in the North Fork and Cherokee Reservoir. The investigation is continuing and we report here our initial results.

MATERIALS AND METHODS

Water, suspended sediment, and bottom sediment were sampled on the North Fork-Cherokee Reservoir System at two stations above and 13 stations (Fig. 1) below the chlor-alkali plant in October 1973, and May and August 1974, according to standard methods.[4] Water samples to be analyzed for total mercury were stored in clean (preheated and acid washed) 250-ml glass volumetric flasks. Water samples to be analyzed for dissolved and suspended mercury were stored on ice and filtered within 48 hours through 0.4 μ Nucleopore filters. Nitric acid was added to the filtered aqueous samples to prevent adsorption losses. Sediment samples were frozen immediately upon collection. Prior to mercury analysis, all bottom sediment samples were sieved through a 44 μ screen to separate the smaller fraction for analysis. All sedimentary mercury concentrations in this report are thus from the

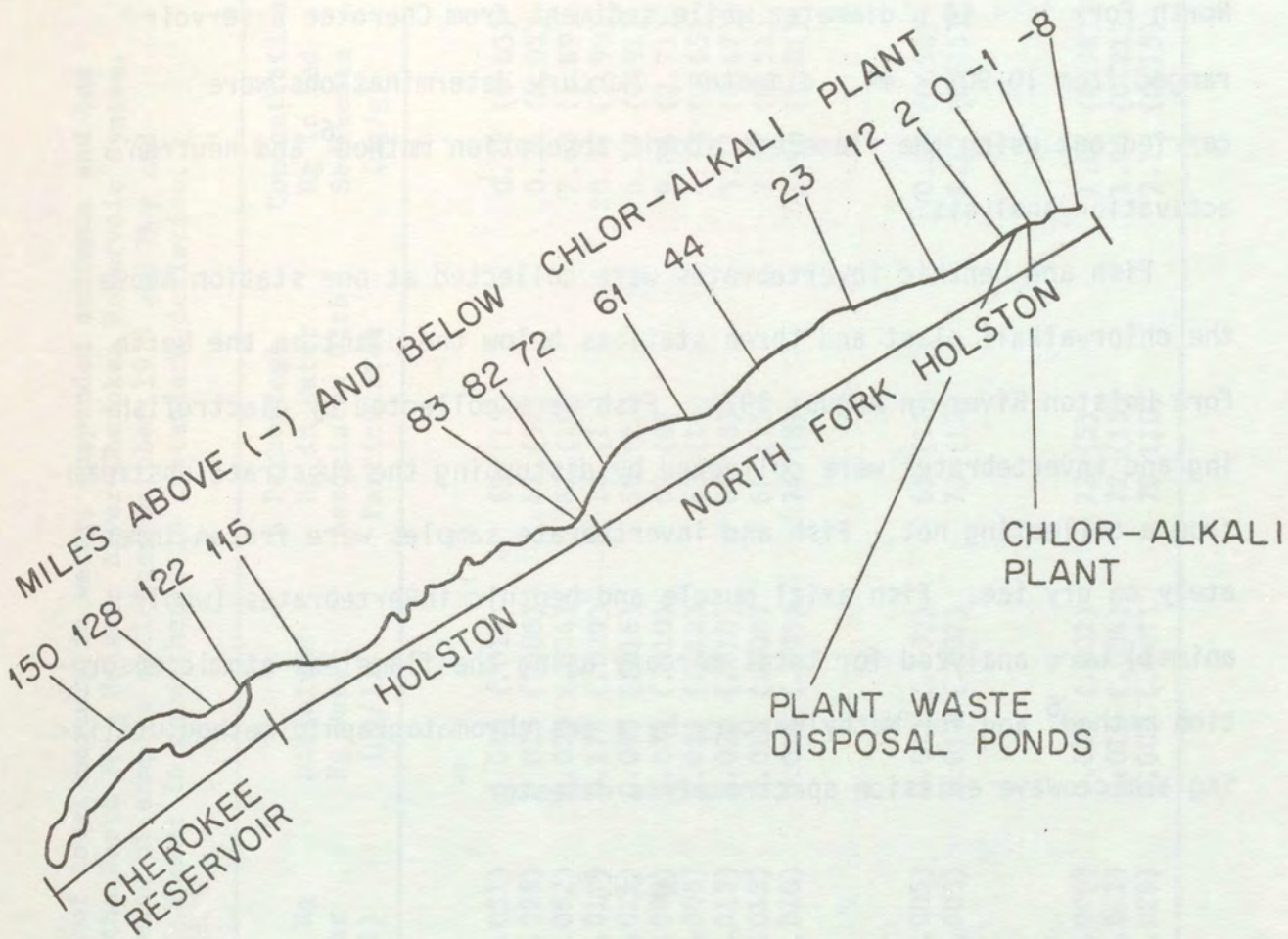

Figure 1. Schematic of the North Fork of the Holston River-Cherokee Reservoir system with location of the abandoned chlor-alkali plant, waste disposal ponds, and sampling stations.

< 46 μ fraction, and are reported as dry weight. Particle size separation is imperative for comparing mercury concentrations in river and lake samples.[5] Approximately 2% of the bed sediment sampled in the North Fork is < 44 μ diameter while sediment from Cherokee Reservoir ranged from 10-90% < 44 μ diameter. Mercury determinations were carried out using the flameless atomic absorption method[6] and neutron activation analysis.

Fish and benthic invertebrates were collected at one station above the chlor-alkali plant and three stations below the plant on the North Fork Holston River in August 1974. Fish were collected by electrofishing and invertebrates were collected by disturbing the substrate upstream from a collecting net. Fish and invertebrate samples were frozen immediately on dry ice. Fish axial muscle and benthic invertebrates (whole animal) were analyzed for total mercury using the flameless atomic absorption method[6] and for methylmercury by a gas chromatographic method utilizing a microwave emission spectrometric detector.[7]

RESULTS

Mercury in Water and Sediments

The mean concentration for three sampling dates of total mercury in water and bed sediment for stations sampled in the Holston-Cherokee system are given in Table 1. The concentration of mercury in bed sediments (< 44 μ) at two stations above the plant (miles -8 and -1) averaged 0.29 ± 0.05 (1 SD) μg/gm over the sampling interval. At mile 0 immediately below the abandoned plant effluent discharge pipe, bed sediment contained an average of 7.63 ± 1.89 μg/gm. Samples of surface sediment from

Table 1. Distribution of total mercury in water, suspended sediment and bed sediment of the North Fork Holston River-Cherokee Reservoir System. Values are means of samples collected October 1973 and May and August 1974. Number in parenthesis is 1 standard deviation.

River Miles Above (-) and Below Plant	Total Hg Water (µg/1)	Dissolved Hg Water (µg/1)	Percentage Hg in Water Associated With Particulates	Concentration Hg in Bed Sediments (µg/g)
N. Fork Holston				
-8	0.048 (.031)	0.016 (.015)	69 (13)	0.32 (0.03)
-1	0.036 (.025)	0.013 (.006)	47 (51)	0.24 (0.02)
0	0.088 (.062)	0.029 (.024)	67 (10)	7.63 (1.89)
2	0.203 (.017)	0.174 (.029)	14 (11)	20.77 (2.99)
12	0.088 (.011)	0.045 (.016)	50 (12)	6.17 (0.91)
23	0.069 (.009)	0.027 (.010)	62 (9)	9.84 (2.71)
44	0.064 (.008)	0.024 (.012)	63 (15)	4.73 (0.65)
61	0.054 (.013)	0.020 (.010)	64 (8)	3.77 (0.67)
72	0.042 (.012)	0.014 (.006)	66 (15)	3.57 (0.51)
82	0.049 (.010)	0.013 (.005)	74 (8)	1.90 (1.01)
Holston				
83	0.022 (.005)	0.011 (.002)	61 (11)	0.65 (0.50)
115	0.044 (.005)	0.012 (.007)	73 (12)	1.17 (0.25)
Cherokee Reservoir				
122	0.048 (.006)	0.013 (.002)	72 (5)	1.67 (0.38)
128	0.044 (.012)	0.011 (.004)	72 (15)	1.95 (0.21)
150	0.036 (.029)	0.009 (.007)	70 (10)	2.13 (0.15)

three different areas within the plant disposal ponds yielded an average of 151.4 µg/gm total mercury, while bed sediment in the North Fork immediately below the disposal ponds (mile 2) averaged 20.77 ± 2.99 µg/gm. Bed sediment mercury levels then generally decreased downstream in the North Fork to a mean of 1.90 ± 1.01 µg/gm 82 miles below the plant. Sediment mercury levels in Cherokee Reservoir averaged 1.91 ± 0.32 µg/gm.

Bottom sediments 2 miles, 12 miles and 23 miles below the plant were analyzed for methylmercury but no methylmercury was observed (limit of detection 0.1 µg/g).

The distribution of mercury in the water column provides insight into the mechanism of mercury transport in this system. The concentration of total mercury in water above the plant (mile -8, and -1) averaged 0.043 ± 0.026 µg/l over the sampling interval with an average of 60.2 ± 29.6% associated with suspended particulates. Below the abandoned effluent discharge pipe (mile 0) total mercury in water averaged 0.088 ± 0.062 µg/l with 67.3 ± 10.2% associated with suspended particulates. Immediately below the waste disposal ponds, however, total mercury in water averaged 0.203 ± 0.017 µg/l with only 14.0 ± 10.5% associated with suspended particulates. Total mercury in water at the remaining downstream stations averaged 0.052 ± 0.020 µg/l with 66.2 ± 11.7% associated with suspended particulates over the sampling interval.

We conclude from this observed distribution that since the chloralkali plant ceased operation in 1972 the major source of continued mercury input to the river is leaching of dissolved mercury from the plant waste disposal ponds. Mercury probably in one of its chloride or hydroxide complexes is leached from the waste disposal ponds into the

river primarily in the dissolved form. Mercury is then rapidly adsorbed onto or complexed with suspended particulates and the main transport of mercury in this river system occurs in the particulate phase.

Distribution of Mercury in Biota

Three fish species (rockbass - Ambloplites rupestris, hogsucker - Hypentelium nigricans, and shiners - Notropis spp.) were analyzed for total mercury in axial muscle at one station above and three stations below the plant on the North Fork (Table 2). The highest total mercury levels in all species were observed two miles below the plant immediately below the waste disposal ponds (rockbass = 1.59 ± 0.33 µg/g, hogsucker = 1.42 ± 0.16 µg/g, shiner = 1.70 ± 0.51 µg/g). Mercury contamination in these species is present 82 miles downstream where mean levels approaching or exceeding the 0.5 µg/g FDA guidelines were observed (rockbass = 1.26 ± 0.51 µg/g, hogsucker = 0.43 ± 0.11 µg/g, and shiner = 0.77 ± 0.15 µg/g). Mercury levels of 0.70 ± 0.23 µg/g for rockbass and 0.46 ± 0.19 µg/g for hogsucker eight miles above the plant are higher than expected for this control area (Table 2). One explanation for the high mercury levels in fish at this station may be local movement to and from the plant disposal area. We have sampled in 1975 fish species from two areas farther upstream. When mercury analyses are complete we should have a better estimate of natural levels of mercury in fish from the North Fork.

A number of each species from each station were also analyzed for methylmercury (Table 3). Estimates ranged from 78.2 ± 7.3% to 105.1 ± 5.2% methylmercury. The overall mean percentage methylmercury for 70 individual fish from the North Fork was 89.07 ± 3.24%.

Table 2. Total mercury concentration in axial muscle for three fish species at four stations on the North Fork Holston River sampled August 1974. All values µg/g ± 2 S.E. (fresh weight).

Species	River Mile Above (-) and Below Plant			
	-8	2	12	82
Rockbass (*Ambloplites rupestris*)	0.70 ± 0.23 n = 13 Size range 7.8 - 303.2 g	1.59 ± 0.33 n = 11 Size range 19.6 - 184.3 g	1.14 ± 0.19 n = 16 Size range 3.8 - 96.4 g	1.26 ± 0.51 n = 10 Size range 23.6 - 156.9 g
Hogsucker (*Hypentelium nigricans*)	0.46 ± 0.19 n = 11 Size range 11.8 - 191.0 g	1.42 ± 0.16 n = 3 Size range 41.2 - 113.2 g	1.23 ± 0.13 n = 6 Size range 45.0 - 164.1 g	0.43 ± 0.11 n = 2 Size range 53.4 - 55.6 g
Shiner (*Notropis* spp.)	0.28 ± 0.05 n = 20 Size range 7.0 - 47.1 g	1.70 ± 0.51 n = 8 Size range 1.6 - 3.7 g	1.05 ± 0.17 n = 14 Size range 1.3 - 22.2 g	0.77 ± 0.15 n = 13 Size range 1.2 - 7.9 g

Table 3. Percentage methylmercury in axial muscle of three fish species at four stations on the North Fork Holston River sampled August 1974. All values are mean % ± 2 S.E.

Species	River Mile Above (-) and Below Plant			
	-8	2	12	82
Rockbass (*Ambloplites rupestris*)	88.7 ± 8.3 n = 10	83.7 ± 7.0 n = 7	99.5 ± 4.0 n = 6	78.9 ± 16.9 n = 3
Hogsucker (*Hypentelium nigricans*)	103.8 ± 3.3 n = 9	90.9 ± 13.8 n = 3	86.3 ± 5.8 n = 6	84.6 ± 28.7 n = 2
Shiner (*Notropis* spp.)	84.5 ± 11.2 n = 10	105.1 ± 5.2 n = 3	83.8 ± 10.7 n = 5	78.2 ± 7.3 n = 6

Estimates of total mercury and percentage methylmercury in benthic invertebrate taxa collected in August 1974 at the same stations where fish were collected are given in Tables 4 and 5. Benthic invertebrates two miles below the plant contain total mercury at about the same level (mean 1.550 ± 0.271 μg/g) as fish species (Table 2). Total mercury in benthic invertebrates 8 miles above and 12 and 82 miles below the plant appears to be lower than fish species at these stations (Table 2). Mean percentage methylmercury in benthic invertebrates at all stations was on the order of 50% (Table 5) but considerable variability exists between taxa at any given station. Mercury levels reported for benthic invertebrates in the North Fork are for the whole animal thus include possible sediment contamination in the gastrointestinal tract.

The concentration of total mercury in fish and invertebrates appears to follow the concentration of total mercury in the water column and bed sediments at the four stations sampled (Figs. 2-4). When the results of the second year of investigation are complete we hope to be able to determine which factors in the abiotic environment account for the variability in total mercury levels in fish species in this system.

DISCUSSION

Our observations on the distribution of total mercury in water and sediments of the Holston-Cherokee system indicate that mercury input to the North Fork continues even though the chlor-alkali plant ceased operation in 1972. The source of mercury appears to be the large (120 acre) waste disposal ponds adjacent to the North Fork. Elimination of mercury leaching from these disposal ponds will not be a simple task due to their large surface area and depth (30-100 ft.).

ORNL-DWG 75-12871

Table 4. Total mercury concentration in benthic invertebrate taxa (whole animal) at four stations on the North Fork Holston River August 1974. All values are μg/g fresh weight.

Taxa	River Mile Above (-) and Below Plant			
	-8	2	12	82
Hydropsychidae	--	1.680	0.560	--
Corydalus	0.088	1.940	0.640	0.222
Decapoda	0.065	--	1.120	0.270
Psephenidae	0.016	--	--	--
Composite Remaining Taxa	0.035	1.030	0.840	0.110
Mean of Taxa Present ± 2 S.E.	0.051 ± 0.032	1.550 ± 0.542	0.790 ± 0.250	0.201 ± 0.094

ORNL-DWG 75-12872

Table 5. Percentage methylmercury in benthic invertebrate taxa (whole animal) at four stations on the North Fork Holston River August 1974.

Taxa	River Miles Above (-) and Below Plant			
	-8	2	12	82
Hydropsychidae	--	29.17	41.07	--
Corydalus	65.91	40.72	49.20	63.93
Decapoda	51.81	--	48.21	49.11
Psephenidae	37.5	--	--	--
Composite Remaining Taxa	51.43	83.50	40.48	--
Mean of Taxa Present ± 2 S.E.	51.66 ± 11.60	51.13 ± 33.09	44.74 ± 4.60	56.52 ± 14.82

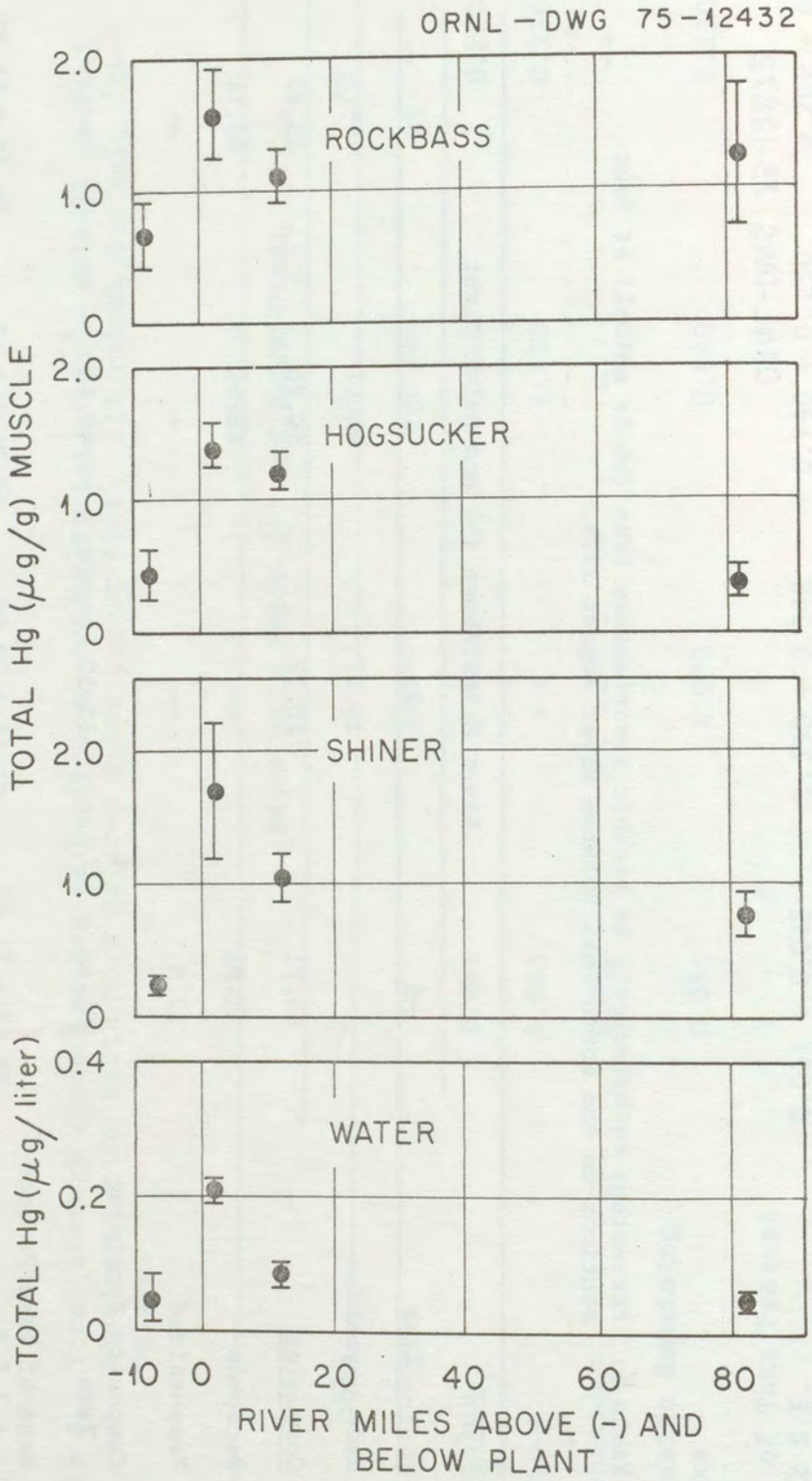

Figure 2. The distribution of total mercury in axial muscle of select fish species (August 1974) and in water (mean of October 1973, May and August 1974 - unfiltered samples) in the North Fork of the Holston River above and below the chlor-alkali plant. Error bars for fish are 2 S.E. and for water are 1 S.D.

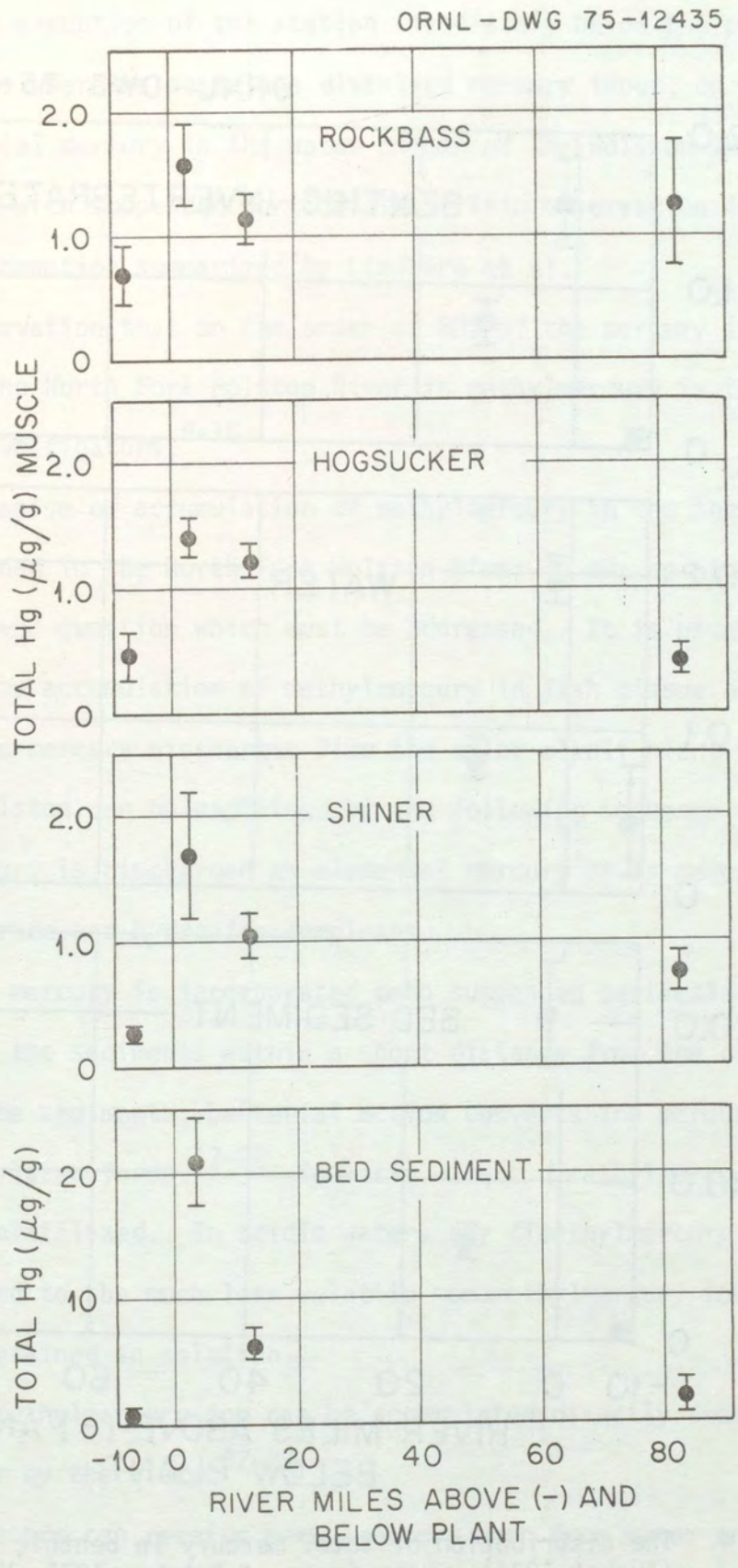

Figure 3. The distribution of total mercury in axial muscle of select fish species (August 1974) and in bed sediment (mean of October 1973 and May and August 1974) in the North Fork of the Holston River above and below the chlor-alkali plant. Error bars for fish are 2. S.E. and for bed sediment are 1 S.D.

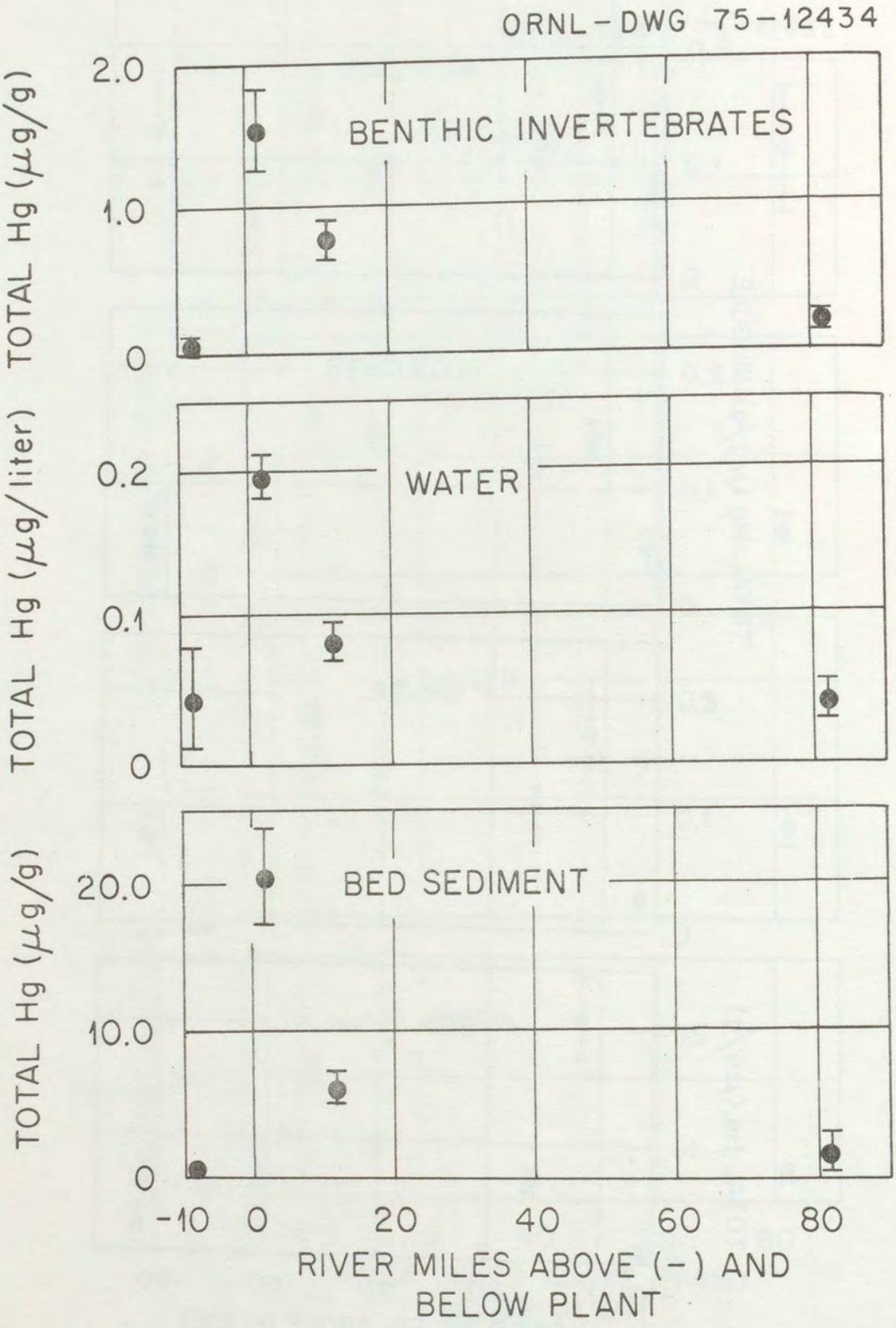

Figure 4. The distribution of total mercury in benthic invertebrates (August 1974), water (mean of October 1973, May and August 1974 - unfiltered) and bed sediment (mean of October 1973, May and August 1974) in the North Fork on the Holston River above and below the chlor-alkali plant. Error bars for benthic invertebrates are 2 S.E. and for water and sediment are 1 S.D.

With the exception of the station immediately below the plant waste disposal ponds where we postulate dissolved mercury input, on the order of 65% of the total mercury in the water column of the Holston-Cherokee system is associated with suspended particulates. This observation is in agreement with information summarized by Lindberg et al.[8]

Our observation that on the order of 80% of the mercury in fish muscle from the North Fork Holston River is methylmercury is in agreement with other investigators.[9-16]

The mechanism of accumulation of methylmercury in the three fish species examined in the North Fork Holston River in our opinion is the major unresolved question which must be addressed. It is usually accepted that the accumulation of methylmercury in fish tissue downstream from inorganic mercury discharges like the chlor-alkali plant on the North Fork Holston can be explained by the following sequence of events:

1. Mercury is discharged as elemental mercury or as mercury (II) chloride and hydroxide complexes.
2. This mercury is incorporated onto suspended particulates and into the sediments within a short distance from the outfall.
3. In the sediments, bacterial action converts the mercury to methylated forms.[17-22] In basic waters dimethylmercury can be volatilized. In acidic waters any dimethylmercury is converted to the much less volatile monomethylmercury ion which is retained in solution.
4. Monomethylmercury ion can be accumulated directly from the water by the biota.[22-23]
5. Predators can receive methylmercury both from water and from the food chain.

This sequence of events has been postulated from laboratory data and appears reasonable except that with few exceptions[25-26] methylmercury has not been detected in water or sediment in natural systems. Whether this observation can be explained by bacterial demethylation of mercury in nature[27-28], rapid uptake of available methylmercury by organisms, or insufficient analytical detection limits is not known. The accumulation of methylmercury by aquatic organisms through dietary uptake most certainly occurs but the relative importance of dietary uptake versus direct uptake from water is unresolved. The entry of methylmercury into the base of food chains may also be from the diet or water.

In the North Fork Holston River approximately 50% of the total mercury in bottom fauna is methylmercury. Dietary uptake of methylmercury by fish species in this system feeding on bottom fauna may be a very significant mode of accumulation of methylmercury since methylmercury was not found in the sediments at our detection limit. The mechanism of methylmercury accumulation by the bottom fauna in this system still however is unexplained.

Another possible mechanism of methylmercury accumulation by fish and bottom fauna in the North Fork Holston River may *in vivo* methylation. Preliminary results in our laboratory indicate that brook trout (*Salvelinus fontinalis*) do not methylate mercury in vivo.[29] Muessig[30], however, reports that channel catfish (*Ictalurus punctatus*) can accumulate methylmercury in the presence of only inorganic mercury. He suggests the synthesis of methylmercury occurs at some site associated with the fish.

In the North Fork Holston River as in other systems a large percentage of mercury in the water column is associated with suspended particulates. In addition, Lindberg et al.[8] reports that on the order of 50-80% of the total dissolved mercury in Mississippi River water is associated with dissolved organic matter of the less than 500 molecular weight fraction. The availability of mercury to aquatic organisms associated with suspended particulate matter and dissolved organic matter is not quantitatively known.

Our research and available literature information suggest the following questions are not resolved to date and warrant continued investigation to more completely understand the accumulation of both inorganic and methylmercury by aquatic biota in ecosystems like the Holston-Cherokee system receiving only inorganic mercury inputs:

1. To what extent does methylmercury occur in water and sediments? This information is critical to evaluate the significance of direct uptake from water of methylmercury by aquatic organisms.
2. Is inorganic and organic mercury associated with suspended particulates and dissolved organic matter available for accumulation by aquatic organisms?
3. What is the relative importance of dietary uptake of methylmercury versus direct uptake from water for a given trophic level?
4. Is <u>in vivo</u> methylation of inorganic mercury by fish and/or bottom dwelling invertebrates a significant route of entry of methylmercury into aquatic food webs?

The answers to these questions will obviously not come easy and will require continued refinement of analytical techniques as well as novel laboratory and field experimentation. Every attempt should be made in laboratory investigations to simulate environmental concentrations of mercury so that transfer coefficients obtained will be relevant to the natural system.

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PHARMACOKINETIC APPROACHES TO THE EVALUATION OF METHYLMERCURY IN FISH

CHAPTER 12

Rolf Hartung, Ph.D.
Toxicology Program
School of Public Health
The University of Michigan
Ann Arbor, Michigan 48104

ABSTRACT

The mathematical models developed in the study of pharmacokinetics can be modified and applied to the study of the uptake, storage, and distribution of heavy metal compounds in fish.

A series of exponential equations is used to describe biological accumulation, storage and persistence.

This approach is applied to existing experimental and monitoring data. The potential exists to improve comparisons between experimentally derived and environmentally derived data. Under certain conditions the precision of bioaccumulation experiments may be improved and their duration shortened.

The evaluation of the uptake, bioaccumulation and excretion of toxic substances is a complex task. At the surface, it appears almost impossible to compare the results of experiments which have been run for varying times at different concentrations. Since problems of this nature have been explored for some time in the field of pharmacokinetics, I decided to apply some of the simpler pharmacokinetic models to the evaluation of the uptake of methylmercury by fish, and its relationship to environmental levels of methylmercury. Some complex pharmacokinetic models, containing many compartments, have been developed to explain the organ distribution and excretion of mercury compounds (Cember, 1969). The model cited above tries to duplicate many of the naturally occurring processes, and therefore makes use of many compartments and rate constants. However, to resolve such a model experimentally requires many data points, high precision, and samples from many tissues. It is usually not possible to set up a theoretical model which seeks to account for absorption, redistribution to all tissues, transport between organs, and excretion. Even in complex models the compartments are abstract mathematical concepts which do not truly represent physiological entities. For methylmercury in fish the data simply lack the precision to test the merits of complex pharmacokinetic models. Therefore, I decided to test a number of simple pharmacokinetic models by applying them to existing data. The main purpose was to gain additional insight into the dynamics of methylmercury.

The excretion of methylmercury has already been studied by a number of authors. Järvenpää et al (1970) found that after a single dose of radiolabelled methylmercury fish lost an initial increment of methylmercury fairly rapidly, and after that lost the remainder very slowly. The initial rapid loss was discernible for about 10 days, suggesting a half-life for the initial excretion of 2-4 days. After that the remainder of the mercury was excreted with average half lives shown in Table 1. The rapid loss phase was not present after chronic uptake of methylmercury in experiments by McKim et al (1975). Therefore it may be likely that the initial rapid phase was a consequence of the method of dosing which resulted in a redistribution and greater short term availability of methylmercury for excretion.

Järvenpää's data may be described by the elimination equation:

$$C_a = Ae^{-\alpha t} + Be^{-\beta t}$$

or $$C_a = 32e^{-0.231t} + 68e^{-0.000872t}$$

Where C_a = % of initial dose

α = elimination rate constant-fast phase

β = elimination rate constant-slow phase

A,B = intercepts of the single exponential regression lines

e = base of the natural logarithm

(See Fig. 1)

If the dose has been absorbed over an extended period of time, then the α phase appears to be absent, so that the excretion can be described by a simple elimination equation of the type:

$$C_t = C_o e^{-Kt}$$

Where C_t = concentration at time t

C_o = initial concentration at t = o,

K = elimination rate constant

K is related to the elimination half life ($t_{1/2}$) by:

$$K = \frac{\ln 2}{t_{1/2}} = \frac{0.693}{t_{1/2}} \quad (2)$$

This simple elimination equation would be applicable only after absorption has ceased, when the compound tested is not metabolized to any appreciable extent, when the organism can be represented as a single compartment (a compartment being a mathematical abstraction), and when the excretion is proportional to the concentration in that compartment.

The uptake of methylmercury during continuous exposure is not as readily modeled as the elimination phase. The major reason is that the elimination begins to occur as soon as the uptake begins, so that uptake and elimination interact to determine the storage of methylmercury at any one time.

The simplest assumption would be that the rate of absorption at any particular water concentration is a constant which is not affected by the concentration that has been reached inside the organism. That is, the absorption rate constant is zero order and is proportional only to the

concentration of methylmercury in water. The concentration of methylmercury in the water is assumed to remain constant during any particular exposure regimen. A constant rate of input could be achieved only as long as there is a vast excess of available binding sites for methylmercury in the organism. As soon as a significant proportion becomes occupied, the absorption rate would shift from zero order to another form, which may be more appropriately modeled by a Langmuir or a Michaelis-Menten equation.

For the zero-order absorption model the concentration C_t in the fish at time t could be described as:

$$C_t = \frac{k_o}{V\,K}\,(1-e^{-Kt}) \qquad (3)*$$

Where K = elimination rate constant

k_o = zero order absorption constant

V = apparent volume of distribution

This model is adapted from Wagner's (1975) one compartment open model with zero order input.

The model reaches equilibrium concentration

C_∞ at $t = \infty$. At that time

$$C_t = \frac{k_o}{VK}\,(1-e^{-\infty}) \qquad (4)$$

$$= \frac{k_o}{VK} = C_\infty \qquad (5)$$

or $$C_t = C_\infty\,(1-e^{-Kt}) \qquad (6)$$

Historically, the determination of the degree of bioaccumulation has been fraught with difficulties, largely because the determinations were made on systems that had not reached equilibrium. In order to compare bioaccumulation coefficients, they should be calculated at equilibrium concentrations C_∞. Since C_∞ is extrapolated, I chose to call this a Theoretical Bioaccumulation Coefficient B_c.

Thus: $$B_c = C_\infty/W_c \qquad (7)$$

Where W_c is the concentration of methylmercury in water.

The assumption, that the uptake follows zero order kinetics, can be verified. If the quantity $(1-e^{-Kt})/K$ is plotted on cartesian coordinates against the corresponding measured concentrations, a straight

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*For the mathematical derivation of equation 3, see the appendix of this paper.

line should result (Wagner, 1975). A significant deviation from the straight line fit indicates that another model may be more appropriate.

When the experimental data from McKim et al (1975) and Reinert et al (1974) are tested in this fashion, they demonstrate linear regression coefficients r = 0.933 to r = 0.996, when choosing K = 0.000990, which corresponds to $t_{1/2}$ = 700 days. This value of K was chosen as a representative value from Table 1. The test of the uptake kinetics is presented in Figures 2 and 3. The fit appears very close to linear in all cases tested. Therefore, I felt justified to calculate $\hat{C}_\infty$, the equilibrium concentration, and B_c, the theoretical bioaccumulation coefficients, from the data, and to include data by Olson et al. (1975) and Mount (1972). These data are presented in Table 2. Examination of the data indicates the following. The B_c is approximately constant for any specific experiment. Data derived from experiments of very short duration appear to be less reliable, possibly because background concentrations of mercury were not subtracted. The fathead minnow data appear to show systematic variation from $B_c = 1.56 \times 10^5$ to 2.89×10^5. The reason for this is not apparent, but may be due to an elimination rate constant significantly different from K = 0.000990. The B_c appears to increase as the environmental temperature increases. This is illustrated by plotting the average B_c vs. temperature in Fig. 4.

TABLE I

Experimental Estimates of the Elimination Rate Constant K for Methylmercury

Species	Average t 1/2 (days)	K	Reference
Pike	750	0.000924	Järvenpää et al
"	640	0.001083	"
"	780	0.000889	"
Eel	910	0.000762	"
"	1030	0.000673	"
"	1030	0.000673	"
Pike	600	0.001155	Tillander et al

The exact reasons for the observed relationship are not clear, but the model strongly suggests that the absorption constant k_o is in part determined by metabolic rate.

In an attempt to further explore possible applications for this model, some environmental data were investigated.

It has been reported previously that the mercury concentration in fish increases with age or weight (Bache et al 1971). Their data are represented in semilogarithmic form in Fig. 5. When these data are

TABLE 2

Water Concentration W_c µg/ℓ	Duration t (days)	Tissue Concentration C_t µg/g	$\hat{C}_\infty$ µg/g	B_c x10^4	Remarks	Source of Data (W_c,t,C_t)
0.03	(14)	(0.10)	(7.26)	(2.42)	Brook trout muscle conc. (C_t)	McKim, et al
0.03	54	0.19	3.59	12.0		
0.03	131	0.38	3.14	10.5	9-15°C	
0.03	239	0.96	4.55	15.2		
0.09	(14)	(0.19)	(13.6)	(15.1)		
0.09	54	0.39	7.49	8.32		
0.09	131	0.78	6.40	7.11		
0.09	239	1.84	8.71	9.67		
0.29	(14)	(0.37)	(27.2)	(9.37)		
0.29	54	1.18	22.7	7.83		
0.29	131	2.22	18.2	6.28		
0.29	239	4.51	21.4	7.39		
0.93	(14)	(1.29)	(93.4)	(10.0)		
0.93	54	3.51	67.4	7.25		
0.93	131	7.78	63.9	6.88		
0.93	239	8.64	41.0	4.41		
2.93	(14)	(1.65)	(120.)	(4.10)		
2.93	54	9.39	180.	6.16		
2.93	131	18.7	154.	5.26		
2.93	239	29.7	141.	4.81		
0.263	(14)	(0.25)	(18.2)	(6.90)	5°C Rainbow Trout (whole fish)	Reinert et al
0.263	28	0.40	14.6	5.56		
0.263	42	0.54	13.3	5.04		
0.263	56	0.71	13.2	5.00		
0.263	70	0.94	14.0	5.34		
0.263	84	1.19	14.9	5.67		
0.258	(14)	(0.51)	(37.0)	(14.4)	10°C	Reinert et al
0.258	28	0.58	21.2	8.22		
0.258	42	0.76	18.7	7.23		
0.258	56	1.10	20.4	7.90		
0.258	70	1.34	20.0	7.76		
0.258	84	1.71	21.4	8.30		

TABLE 2

(cont'd)

W_c µg/ℓ	t (days)	C_t µg/g	$\hat{C}_\infty$ µg/g	B_c x10^4	Remarks	Source of Data (W_c,t,C_t)
0.234	(14)	(0.64)	(46.5)	(19.9)	15°C	Reinert et al
0.234	28	0.81	29.6	12.7		
0.234	42	1.09	26.8	11.4		
0.234	56	1.50	27.8	11.9		
0.234	70	1.86	27.8	11.9		
0.234	84	1.96	24.6	10.5		
0.018	336	1.47	5.19	28.9	25°C Fathead	Olson et al
0.036	336	2.50	8.83	24.5	Minnow (Whole	
0.063	336	4.48	15.8	25.1	Fish)	
0.114	336	7.06	24.9	21.9		
0.247	336	10.9	38.5	15.6		
0.05	90	0.5	5.86	11.7	Trout	Mount

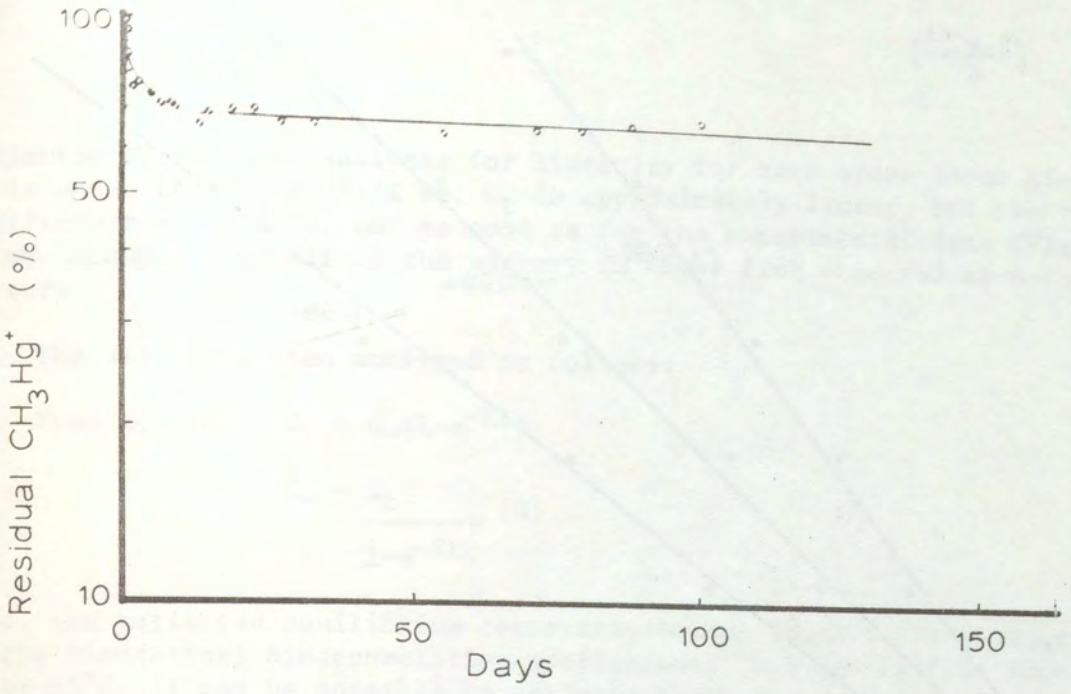

Figure 1 Excretion of methylmercury, redrawn from Järvenpää.

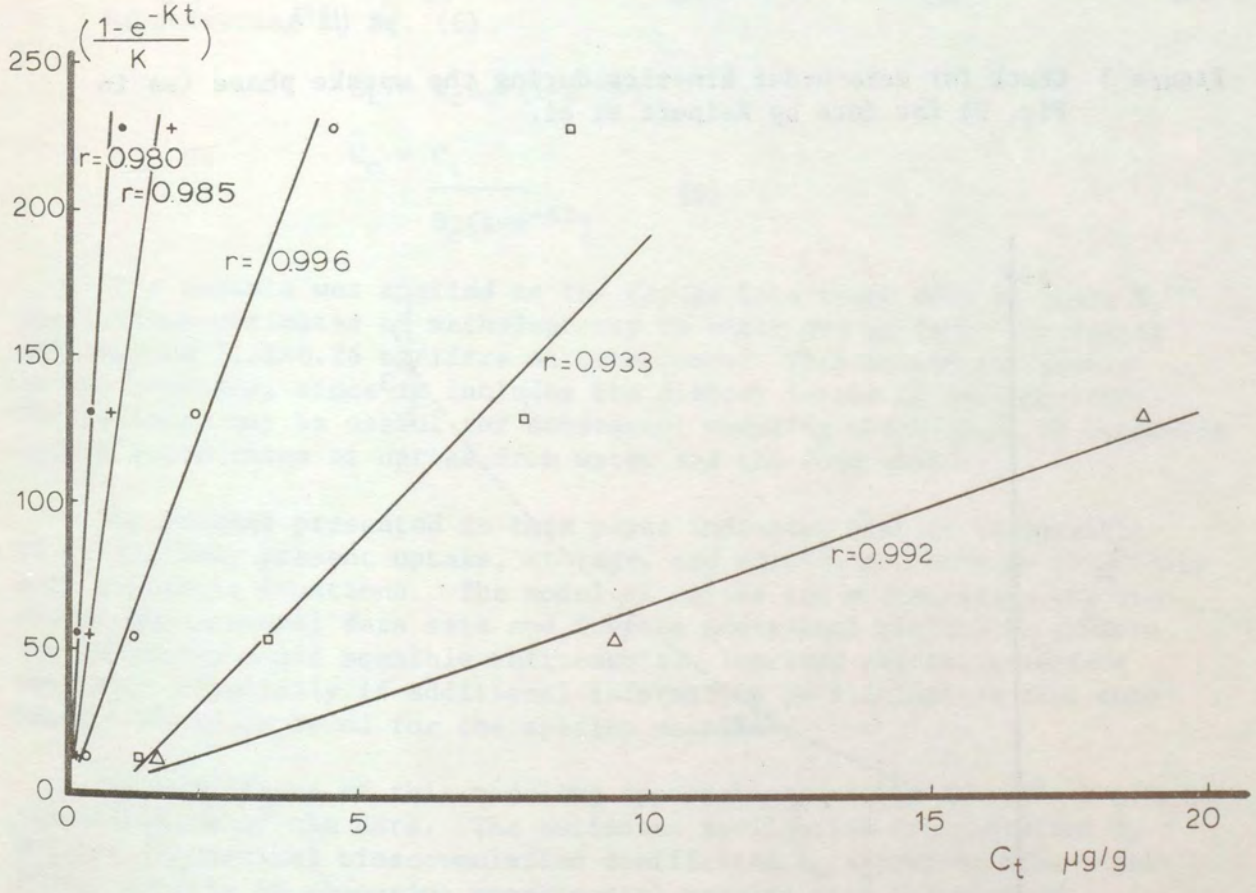

Figure 2 Check for zero-order kinetics during the uptake phase by the method of Wagner (1975) for data by McKim et al.. Linearity of the transformed regression lines implies zero-order kinetics.

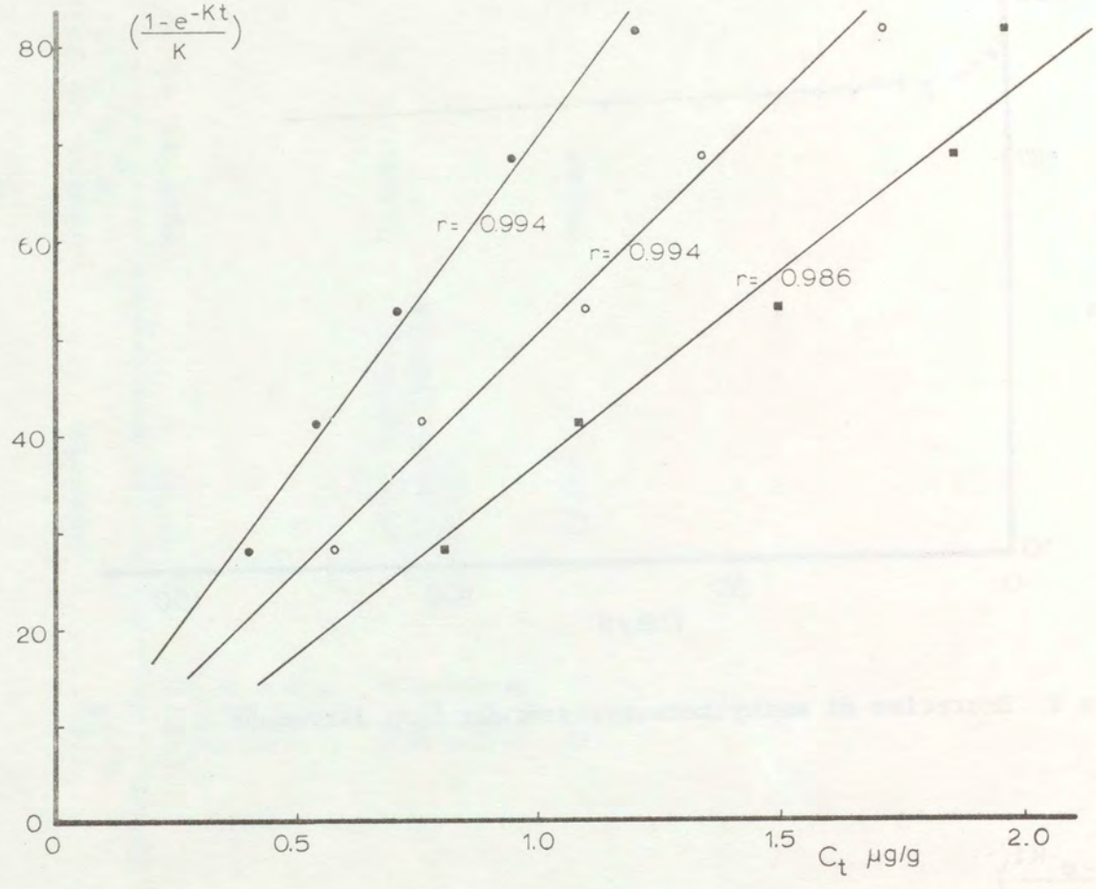

Figure 3 Check for zero-order kinetics during the uptake phase (as in Fig. 2) for data by Reinert et al.

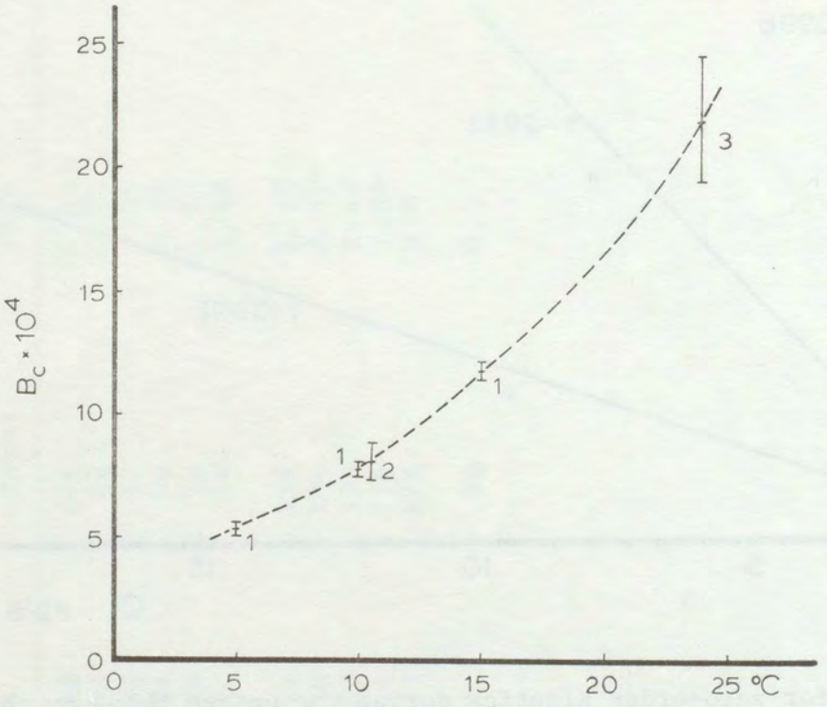

Figure 4 Effect of environmental temperature on the Theoretical Bioaccumulation Coefficient B_c (K assumed constant at 0.000990). (1) Data from Reinert et al., (2) McKim et al., (3) Olson et al.

subjected to the same analysis for linearity for zero-order input kinetics, it is shown that $(1-e^{-Kt})/K$ vs. C_t is approximately linear, but the regression coefficient r = 0.8734, not as good as for the experimental data (Fig. 6). It was assumed that all of the mercury in these fish occurred as methylmercury.

The data were then analysed as follows:

From Eq. (6) $C_t = C_\infty(1-e^{-Kt})$

$$\hat{C}_\infty = \frac{C_t}{1-e^{-Kt}} \quad (8)$$

thus, the estimated equilibrium concentration was found to be 0.58 µg/g. If the theoretical bioaccumulation coefficients (B_c) are applied from Table 2 for 10°C, it may be possible to estimate the maximum methylmercury concentration in water which could have produced this bioaccumulation.

From Eq. (7)

$$B_c = C_\infty/W_c$$

Substituting in Eq. (6)

$$C_t = W_c B_c (1-e^{-Kt})$$

or

$$\hat{W}_c = \frac{C_t}{B_c(1-e^{-Kt})} \quad (9)$$

This formula was applied to the Cayuga Lake trout data in Table 3. The maximum estimates of methylmercury in water are at least consistent and average 7.32±0.26 ng/litre methylmercury. This number is clearly an overestimate, since it includes the dietary intake of methylmercury. The estimate may be useful for subsequent modeling which seeks to determine the relative rates of uptake from water and the food chain.

The process presented in this paper indicates that it is possible to effectively present uptake, storage, and elimination data by relatively simple kinetic equations. The model of uptake and accumulation has been tested with several data sets and invites additional testing to explore its limitations and possible refinements. Improved precision appears possible, especially if additional information on elimination rate constants K could be found for the species examined.

The advantages of this modeling approach are mainly due to a better visualization of the data. The estimated equilibrium concentration $\hat{C}_\infty$ and the theoretical bioaccumulation coefficient B_c appear to have significant utility in comparing experimental results with one another.

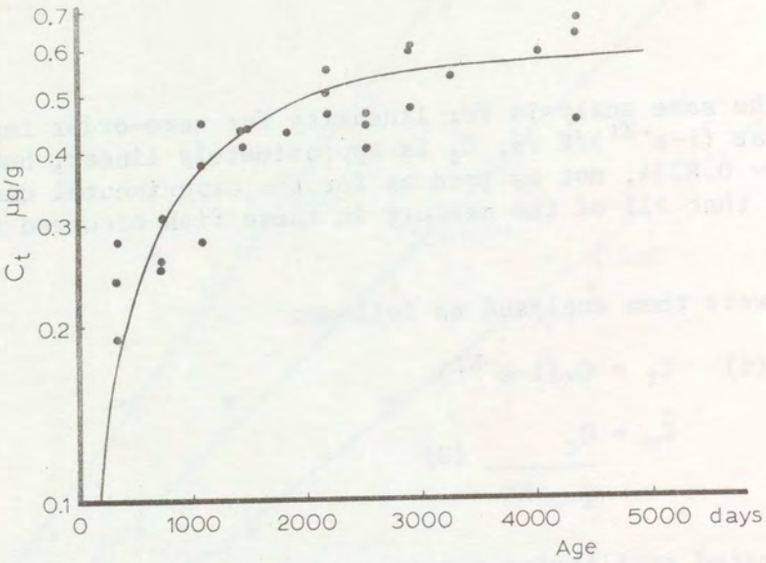

Figure 5 Concentration of mercury in lake trout from Lake Cayuga; adapted from Bache et al.

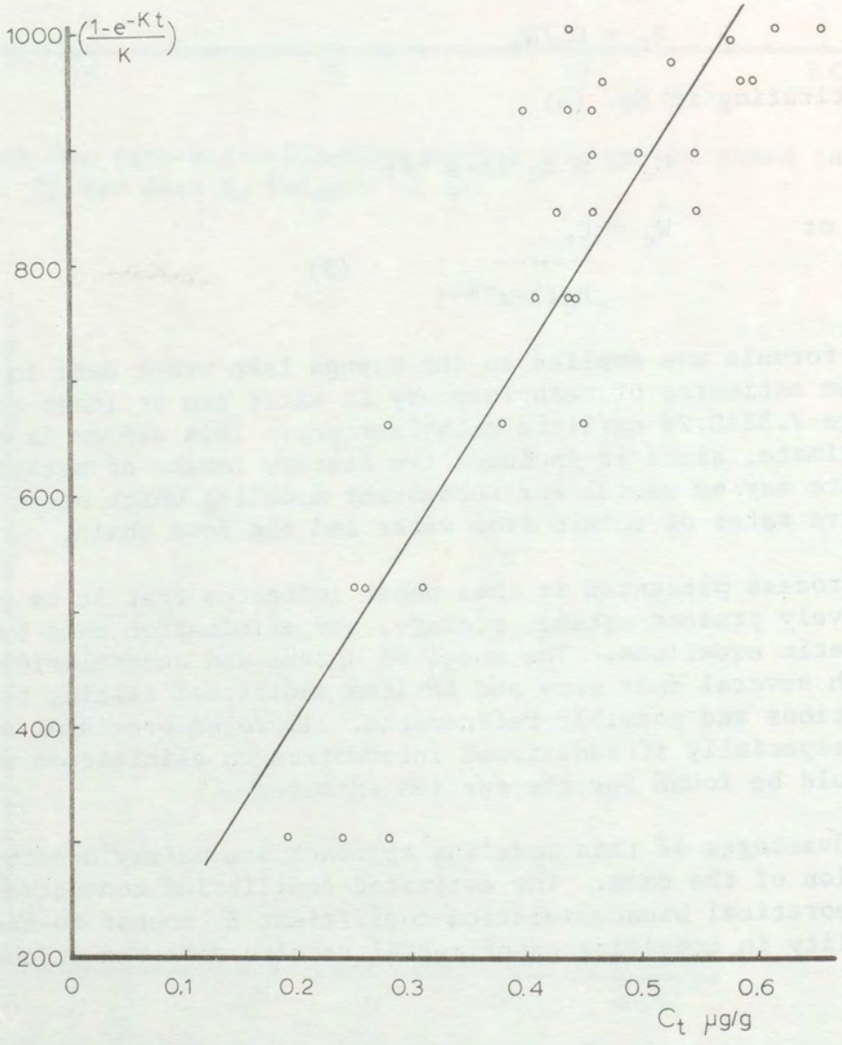

Figure 6 Check for zero-order kinetics during the uptake phase for data from Bache et al. (r = 0.8734).

TABLE 3

Application of the Proposed Bioaccumulation Model to Environmental Monitoring Data For Lake Trout From Lake Cayuga (Total mercury concentrations (C_t) from Bache et al).

Age t Days	C_t µg/g	$(\frac{1-e^{-Kt^*}}{K})$	Est. Eqil. Conc. $\hat{C}_\infty$ µg/g	Est. Max.** Water Conc. $\hat{W}_c$ ng/l
365	0.24	306	0.79	10.0
365	0.28	306	0.92	11.7
365	0.19	306	0.63	7.95
730	0.25	520	0.49	6.16
730	0.26	520	0.51	6.41
730	0.31	520	0.60	7.64
1095	0.38	668	0.57	7.29
1095	0.45	668	0.68	8.63
1095	0.28	668	0.42	5.37
1460	0.44	772	0.58	7.30
1460	0.41	772	0.54	6.81
1460	0.44	772	0.58	7.30
1825	0.43	844	0.51	6.53
2190	0.46	894	0.52	6.59
2190	0.55	894	0.62	7.88
2190	0.50	894	0.56	7.16
2555	0.40	929	0.43	5.52
2555	0.46	929	0.50	6.34
2555	0.44	929	0.48	6.07
2920	0.60	954	0.64	8.06
2920	0.59	954	0.62	7.93
2920	0.47	954	0.50	6.31
3285	0.53	971	0.55	7.00
4015	0.58	991	0.59	7.50
4380	0.62	997	0.63	7.97
4380	0.66	997	0.67	8.49
4380	0.44	997	0.45	5.66

*$K = 0.0009902$ $\hat{C}_\infty = 0.58 \pm 0.02$ $\bar{W}_c = 7.32 \pm 0.26$
$t_{1/2} = 700$ days

**based on $B_c = 7.88 \times 10^4$

Species differences with respect to uptake appear to be less significant than I would have anticipated. Temperature effects, possibly due to metabolic rate, were found to be pronounced and systematic. The kinetic analysis of data would be improved by taking more closely spaced samples during an uptake period followed by an elimination period which allows the estimation for K for each test.

The kinetic analysis is particularly useful in permitting comparisons among bioaccumulation studies and appears to have potential for establishing an approach for relating experimental data to environmental data.

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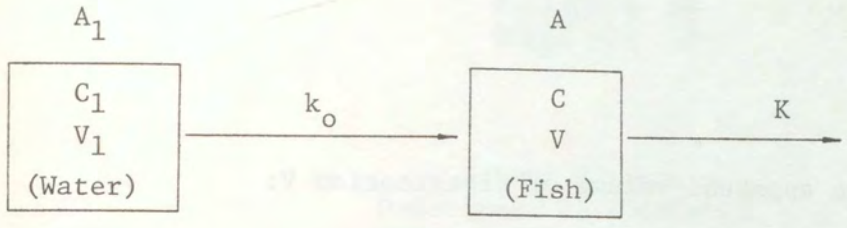
APPENDIX

Derivation of the model describing the uptake and accumulation of methylmercury in fish.



A_1, A = amounts in each compartment at any time t

C_1, C = concentrations in each compartment at t

V_1, V = apparent volumes of distribution at t

k_o = zero-order rate constant for uptake

K = first order rate constant for elimination

The differential equations describing the system are:

$$\frac{dA_1}{dt} = -k_o \qquad \frac{dA}{dt} = k_o - KA$$

Their Laplace transforms are:

$$sa_1 - D = -\frac{k_o}{s}$$ (where D is the initial total quantity of methylmercury in the system)

$$sa = \frac{k_o}{s} - Ka$$

Rearranging:

$$sa_1 = D - \frac{k_o}{s}$$

$$(s + K)a = \frac{k_o}{s}$$

Solving for a by determinants:

$$a = \frac{\begin{vmatrix} s & D-\frac{k_o}{s} \\ 0 & \frac{k_o}{s} \end{vmatrix}}{\begin{vmatrix} s & 0 \\ 0 & s+K \end{vmatrix}} = \frac{k_o}{s(s+K)}$$

Taking the antitransform:

$$A = \frac{k_o}{K} e^{0t} + \frac{k_o}{-K} e^{-Kt}$$

$$= \frac{k_o}{K} (1 - e^{-Kt})$$

Dividing by the apparent volume of distribution V:

$$\frac{A}{V} = \frac{k_o}{VK} (1 - e^{-Kt})$$

However, A/V=C

Therefore:

$$C_t = \frac{k_o}{VK} (1 - e^{-Kt}) \qquad \text{(Equation 3)}$$

MOLECULAR FORMS OF ARSENIC IN THE ENVIRONMENT

CHAPTER 13

Robert S. Braman
Department of Chemistry,
University of South Florida
Tampa, Florida 33620

ABSTRACT

A new analytical technique has recently been developed suitable for the determination of inorganic arsenic (III), arsenic (V), methylarsonic acid, dimethylarsinic acid, and trimethylarsine oxide type compounds at ambient environmental concentrations, down to approximately 0.02 ppb or 1 ng. Based upon observation of arsenic atomic emission lines in a d.c. discharge, the method also involves the separation of arsine and methyl arsines after reduction of corresponding arsenic compounds by sodium borohydride. The method has been applied to the analysis of a number of environmental samples. Methylarsenic compounds have been found in the highest concentrations in ponds high in nutrients. In seawater the methylarsenic compounds have been found associated with the pelagic Sargassum community. Soil biota rapidly biomethylate the pesticides sodium methylarsonate and dimethylarsinic acid. The inorganic arsenic (III) compound is less rapidly biomethylated to volatile forms. Transportation of methylated arsenic by air and possibly by seepage into lakes must be considered in environmental studies of this element.

ANALYTICAL METHODOLOGY

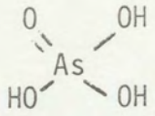
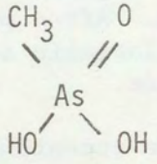
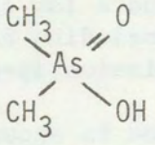
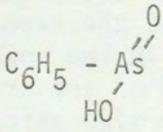
Initial analytical development work on the development of methods for arsenic involved only the determination of inorganic arsenic (III) and (V), based upon the reduction of these compounds to arsine by sodium borohydride (1). It was realized at the time that organic arsenic forms were also likely to be found and should also be studied. After preliminary work with other approaches it was found that the methylarsenic acids were reduced to corresponding arsines by sodium borohydride.

In analyzing mixtures of inorganic arsenic and methylarsenic compounds then and at expected environmental concentrations, a method was needed which included separation of the arsines and a low limit of detection (high sensitivity). All of this was achieved including easy sample handling with a cold trapping-separation U-trap and an emission spectroscopic-type detector.

Reduction chemistry of the method is shown in Table I. Acidity of the reduction conditions is important and is used to differentiate between arsenic (III) and (V). The various arsenic acids must apparently also be in the completely protonated form before they can be reduced to the corresponding arsines. The reactions do not go at higher pH values.

Aqueous solutions can be analyzed for nanogram amounts of the arsenic forms in mixtures by employing a system of the type shown in Figure 1. Samples up to 50 ml in volume are placed in the volatilization chamber and buffered to an appropriate pH. Reduction of the arsenic forms is carried out by syringe injection of $NaBH_4$ through the side port. The volatile arsines produced are scrubbed out of the sample by helium carrier gas and frozen out in the liquid nitrogen-cooled U-trap. After the reduction reaction and scrubbing is completed, typically 5-8 minutes, the liquid nitrogen is removed and the cold trap allowed to warm to room temperature. Arsines are volatilized out of the trap one at a time and carried through an electrical discharge detector of a type previously described (2) (3). The separation of arsines from each other and from water is largely due to differences in their vapor pressures. Note also that a NaOH bead trap with bypass and a provision for heating the U-trap is included. Arsine and CO_2 evolve from the U-tube at nearly the same time. The NaOH trap is used to remove the CO_2 interference. After arsine evolves, the bypass stopcock is turned. The other methylarsines are bypassed around the NaOH trap. The U-trap is heated gently to drive out the methylarsines and finally more strongly to remove absorbed water after the analysis is over. The U-trap is half-packed with glass beads, a measure found necessary to delay the volatilization of water. Figure 2 illustrates a typical

Table I Reduction Chemistry for Arsenic Acids

Arsenic acid pKa_1 = 2.25		$NaBH_4$ (5-8 ml, 2%) pH 1-1.5	AsH_3
Arsenous acid pKa = 9.23	O = As-OH	$NaBH_4$ (2 ml-2%) pH 4-5	AsH_3 b.p. -55°C
Methylarsonic acid pKa_1 = 2.60		$NaBH_4$ (2 ml-2%) pH 1-2	CH_3AsH_2 b.p. 2°C
Dimethylarsinic acid pKa = 6.19		$NaBH_4$ (2 ml-2%) pH 1-2	$(CH_3)_2AsH$ b.p. 55°C
Trimethylarsine oxide	$(CH_3)_3AsO$	$NaBH_4$ (2 ml-2%) pH 1-2	$(CH_3)As$ b.p. 70°C
Phenylarsonic acid pKa_1 = 3.59		$NaBH_4$ (2 ml-2%) pH 1-2	$C_6H_5AsH_2$ b.p. 148°C

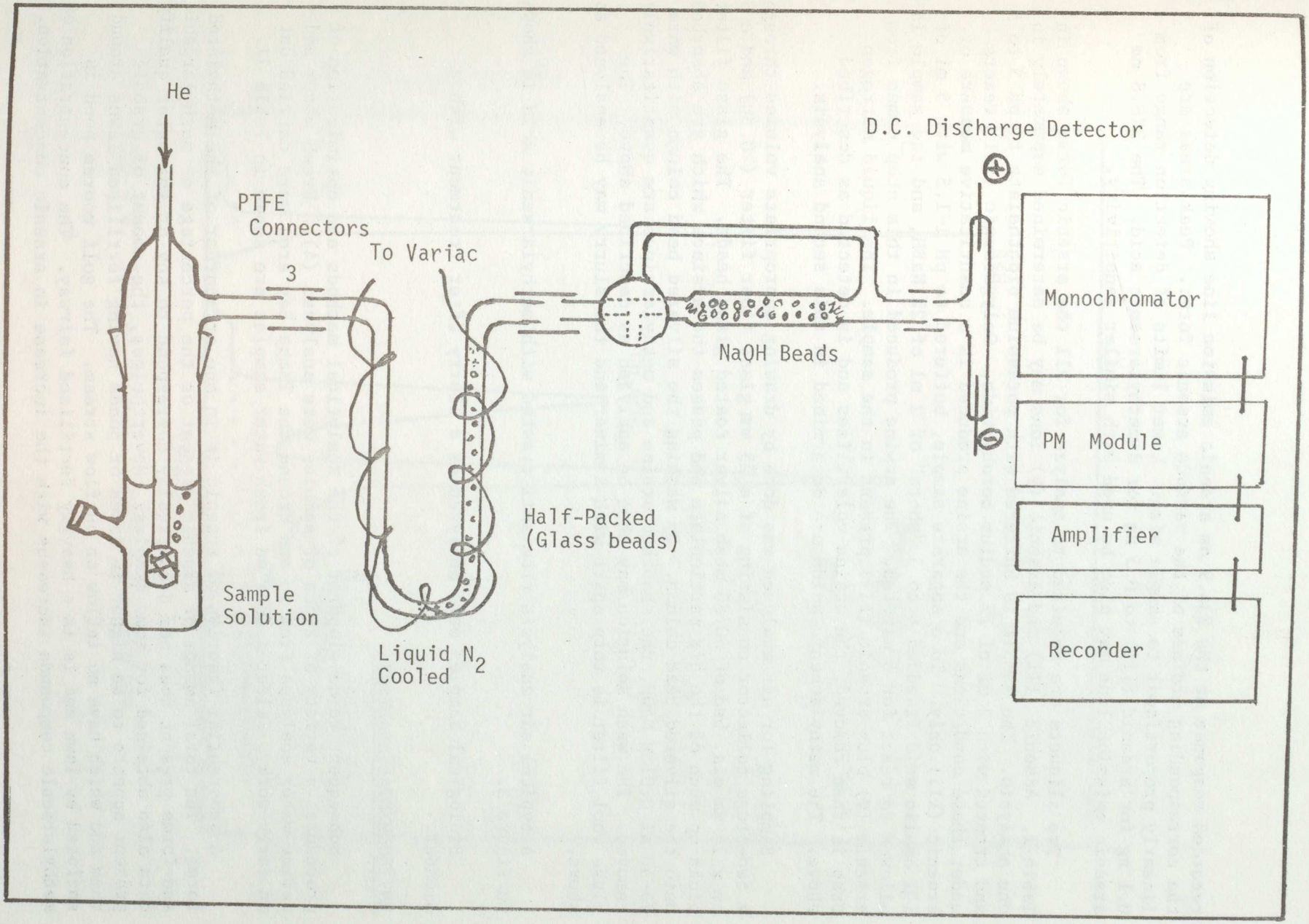


Figure 1. Apparatus Arrangement for Analyses.

recorded response at the 234.9 nm arsenic emission line showing detection of the corresponding arsines of the various arsenic forms. Peak areas are linearly proportional to sample size. Lower limits of detection range from 0.1 ng for arsenic (III) to 0.5 ng for dimethylarsenic acid. The 228.8 nm arsenic emission line may also be used with similar sensitivity.

Two aliquots are required to analyze for all the arsenic forms shown in Table I. Arsenic (III) and arsenic (V) ions may be determined separately in one analysis. The sample is buffered with potassium biphthalate to pH 3 to 5 and treated with 2 ml of 1% sodium borohydride. Only arsenic (III) reacts under these conditions and the arsine produced is a quantitative measure of arsenic (III) only. To a separate sample, buffered to pH 1-1.5 with 5 ml of 10% oxalic acid is added 4 to 5 "shots" of 2 ml of 2% $NaBH_4$ and the sample is allowed to react for 5 minutes. The arsine produced in this step comes from arsenic (V) plus arsenic (III) present in the sample. The liquid nitrogen trap is then removed, the arsine volatilizes and is detected as described above. The methylarsenic acids are determined in this second analysis.

Sampling for air analyses was done by drawing appropriate volumes through a two-stage collector consisting of a 25 mm glass fiber filter (>0.3μ) and a 3 cm x 14 mm dia. bed of 60/80 mesh silver coated glass beads. The glass filter picks up much of the air particulate and passes the arsines which are absorbed onto the silvered bead column. By washing the silvered bead column with warm 25-50 ml 0.01 M NaOH, the absorbed arsine and methylarsines are quantitatively removed. The wash solution may then be analyzed as described above. The glass wool filter is torn apart with a mixer and the slurry may be analyzed as above.

A typical air analysis from soil treated with methylarsenic acid is shown in Figure 3.

Biological samples were analyzed as a slurry after treatment with a blender.

ENVIRONMENTAL ANALYSES

Subsequent to development of the analytical methods and optimization of procedures a variety of types of samples were analyzed (4). Fresh water and saline water analyses from in and around the Tampa Bay area were carried out in early work. Selected data on fresh water samples are shown in Table II.

A substantial fraction of arsenic is in one or another of the methylated forms. The total amount of arsenic present or the percentage of methylarsenic and forms present does not dramatically correspond to any of the water quality data also obtained for some samples. Nevertheless, the amount of arsenic present appears to be higher in lakes or ponds having fertilized lawns around them and which have no inflow or outflow stream. The golf course pond is enclosed by lawn and is in a heavily fertilized fairway. The concentration of methylarsenic compounds increases with the increase in arsenic concentration.

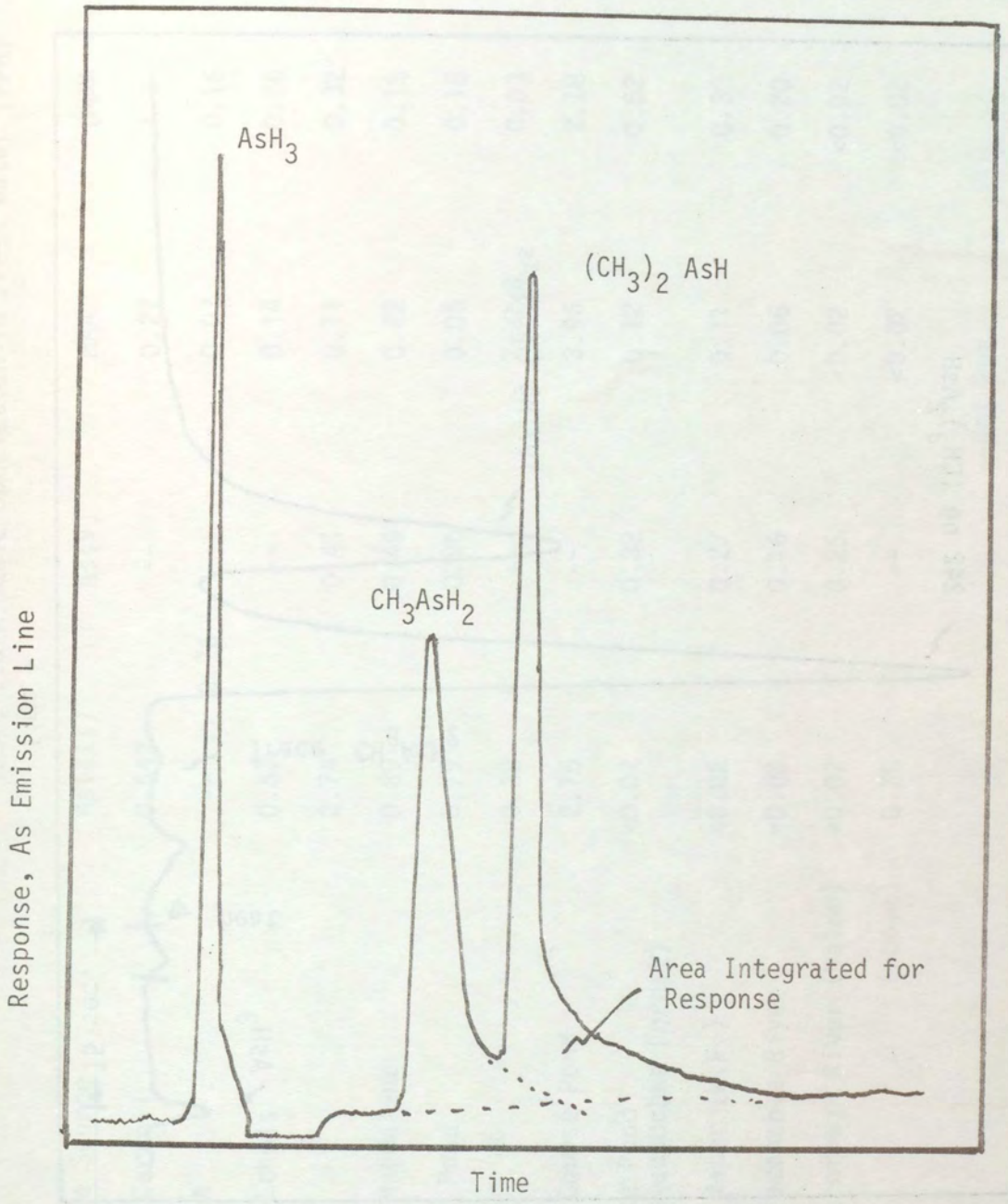


Figure 2. Typical Response Separation of Arsines

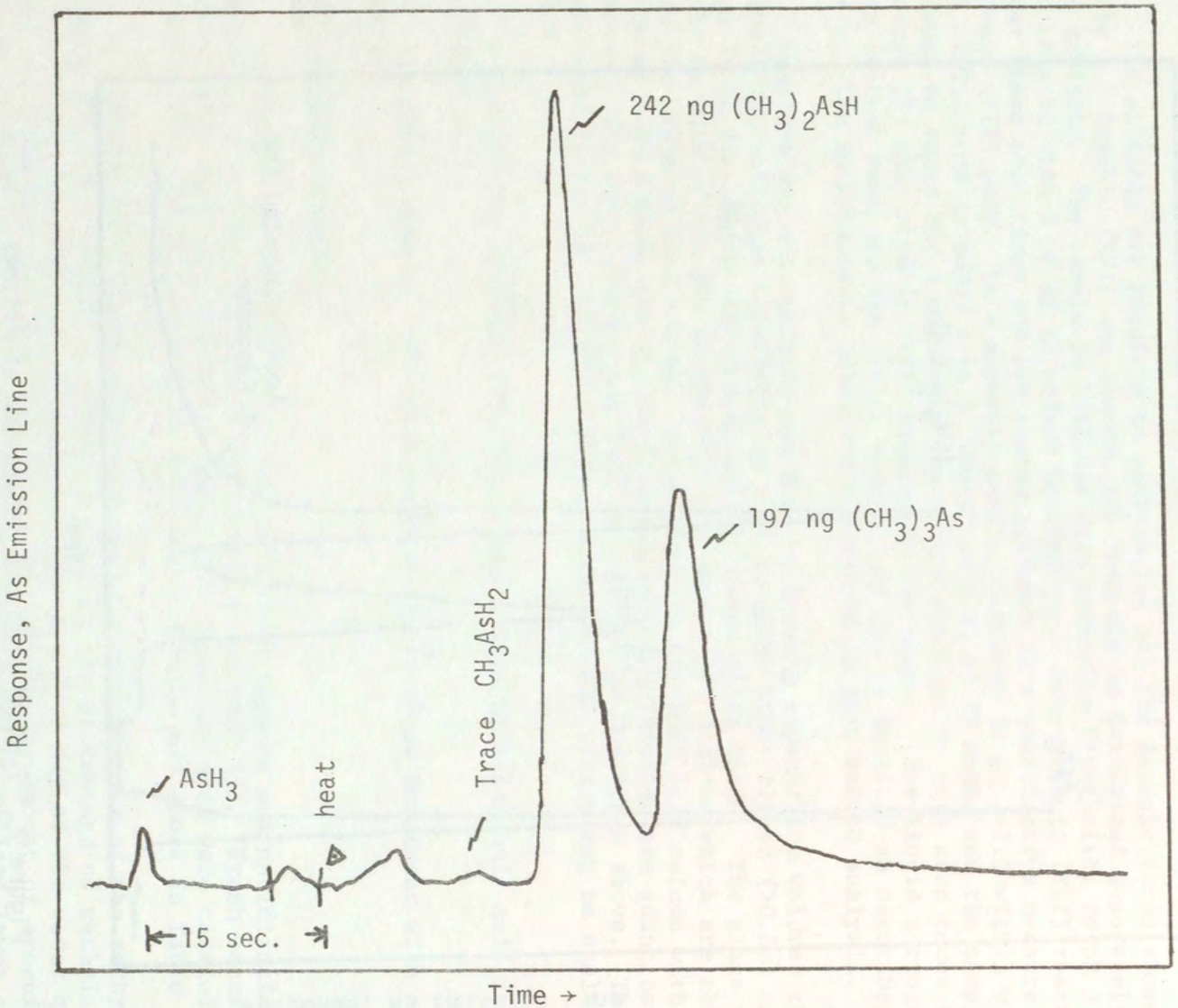


Figure 3. Response From Air Samples on Ag-Coated Glass Beads (Ref. 6)

Table IIa Arsenic Speciation in Fresh Water (PPB)

Sample Source	As(III)		As(V)	MAA	DMAA	TMA
Lake Carroll	0.542		--	0.27	1.3	<0.02
Lake A	--	0.76	--	0.07	0.16	0.14
Lake Echols	0.57		--	0.14	0.26	~0.2
	2.74		0.41	0.11	0.32	--
Lake Magdalene	0.89		0.49	0.22	0.15	--
Univ. Pond	0.79		0.96	0.05	0.15	--
	0.38		--	Trace	0.03	<0.02
Golf Course Pond	2.76		--	3.96	2.18	<0.02
Remote Pond (Withlacoochee Forest)	<0.02		0.32	0.12	0.62	--
Well Water (W.F.)	<0.02		0.27	0.11	0.30	--
Withlacoochee River	<0.02		0.16	0.06	0.20	--
Hillsborough River (below)	<0.02		0.25	<0.02	<0.02	<0.02
(above)	0.75		--	<0.02	<0.02	<0.02

--Not Run.

Table IIb Water Quality Data (Table IIa)

Sample Source	$[PO_4^{\equiv}]$	$[F^-]$	pH	COD	DO	Hardness ($CaCO_3$)
Lake Carroll	45 ppb	0.33 ppb	6.33	1.1 mg/ℓ	17.47 mg/ℓ	51 mg/ℓ
Lake A	--	--	--	--	--	--
Lake Echols	26	0	6.64	12.4	6.28	34
	--	--	--	--	--	--
Lake Magdalene	--	--	--	--	--	--
Univ. Pond	--	--	--	--	--	--
	76	0.87	7.44	3.5	3.92	90
Golf Course Pond	83	1.26	7.68	--	5.75	70
Remote Pond (Withlacoochee Forest)	--	--	--	--	--	--
Well Water (W.F.)						
Withlacoochee River						
Hillsborough River (below)[a]	1.08 ppm	4.65	7.43	39.6	4.11	87
(above)	1.59 ppb	4.91	6.64	50.0	0.84	104

[a] below discharge from water treatment plant.

Table III Arsenic in Saline Waters and Sargassum Weed (PPB)

	As(III)	As(V)	MAA	DMAA
Tampa Bay Causeway	0.12	1.45	<0.02	0.20
Tidal Flat	0.62	1.29	0.08	0.29
McKay Bay	0.06	0.35	0.07	1.00
[a]Alafia River (Brackish)	0.72	--	0.16	tr.
Seawater (Sargasso Sea)	0.24	--	<0.02	tr.
	total 1.6 (ave. of 12)			
Sargassum weed (ppm, wet)				
Burmuda Area	1.8	17.7	0.010	0.184
Gulf of Mexico	0.91	4.3	0.005	0.064
" " "	0.35	7.3	tr.	0.016

[a][2.34 ppm $PO_4^{\equiv}$, 35 ppb F^-, 6.03 pH].

In saline waters (5), Table III, the methylarsenic compounds were found in near-shore areas. Methylated arsenic was also found associated with Sargassum weed in open ocean areas. Analysis of shrimp, barnacles and fish associated with the sargassum weed also indicated the presence of small amounts of methylarsenic compounds.

Biomethylation and volatilization of arsenic as the methylarsines has been observed in soil treated with various arsenic compounds (6). Trimethylarsine and dimethylarsine were apparently the main products. Methylarsonic acid and dimethylarsinic acid are more rapidly converted to di and trimethylarsines than sodium arsenite. Some of the arsines are probably oxidized to the corresponding methylarsenic acids (or to trimethylarsine oxide). These water soluble compounds are then likely to get into ground water and possible lakes and streams.

Air transport of arsenic is a definite contamination source from polluted areas. Some evidence for this has been observed in out-of-doors ambient air analyses (7). Indoor areas with plants such as greenhouses exhibited a marked evidence of alkylarsenic compounds in air.

Further Research Needs

Environmental analyses for the several forms of arsenic have beem comparatively limited to date. Nevertheless, the mobility of the element has certainly been demonstrated. The extent of arsenic mobility in natural water systems needs further study to determine the extent of the phenomenom, especially with attention to water quality factors and total arsenic input.

Further work is also evident on the identification of arsenic compounds in biological samples.

Acknowledgment

The work reported here is part of several research projects completed or underway in the laboratories of the author. The analytical method and analyses reported were done with the assistance of C. C. Foreback, D. L. Johnson, J. M. Ammons, M. Tompkins and J. L. Bircker.

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THE MULTIPLE TOXICITY OF CERTAIN HEAVY METALS:
ADDITIVE ACTIONS AND INTERACTIONS

CHAPTER 14

Perry D. Anderson
Assistant Professor, Department of Biological Sciences
Concordia University
Montreal, Quebec
and
Lavern J. Weber
Associate Professor, Department of Pharmacology
Oregon State University
Corvallis, Oregon

ABSTRACT

Aquatic organisms inhabiting the receiving waters of manmade wastes are commonly exposed to several discrete toxicants simultaneously. Where such mixtures are concerned, the possibility of interplay between toxic constituents - either involving kinetic (i.e. uptake, accumulation, elimination) or dynamic (i.e. mode of action) - may occur. The interplay may result in a mixture being more toxic than would be predicted on the basis of an appreciation of the potency of its constituents. A laboratory study coupled with a literature survey attempts to assess the predictability of the relative toxicity of certain mixtures containing heavy metals.

Heavy metals when defined on physical parameters as those metals with a density greater than 5 compose a group of approximately 40 chemical elements. Even though some of these elements in trace amounts are essential for the proper functioning of organisms, all of the heavy metals may be assimilated at concentrations which are toxic.

Attempts to generalize about the toxicity of this diverse group may be limited to their common capacity to combine with organic molecules at ligands e.g. OH, COOH, PO_3H_2, SH_2, NH_2 and imidazole groups. Because all proteins contain some combination of these ligands, it is not surprising that heavy metals are generally potent enzyme inhibitors.

However in recognizing this common aspect of their toxicity, one must not conclude that their modes of action are the same. Each heavy metal species does not affiliate to the same degree to any given ligand. Not only may these discrete elements thereby differ in their affinities to ligands, but also their intrinsic activities may be unique. Affinity and intrinsic activity are two pharmacodynamic parameters which lend specificity to the toxicity of individual heavy metals. In addition the variation in toxicity patterns which occur due to pharmacokinetic processes, e.g. assimilation, distribution and elimination, contributes to the uniqueness of discrete heavy metal toxicity.

In view of the complexity of the toxicity of individual metallic agents, one might predict that the toxic nature of mixtures could not be simply characterized. Nevertheless there is a prevailing point of view in "water quality criteria" thinking that the toxicity of heavy metals as constituents of mixtures may be adequately described by the single concept of strict additivity. The principle of strict additivity infers that each constituent of a mixture contributes to the toxicity of the mixture in proportion to its individual relative potency.

Our investigation was aimed at evaluating the applicability of the strict addition principle or toxic unit methodology in characterizing mixtures containing heavy metals.

THEORY

Our approach to the problem of multiple toxicity of heavy metals was based on aspects of various theories originally presented by Bliss (1939), Plackett and Hewlett (1952) and Finney (1971).

The methodology devised involved the initial derivation of quantal (all or none) response curves for each toxic constituent of a mixture. The quantal response selected was death- an appropriate choice because the toxic unit principle is primarily supported by mortality studies.

Quantal response curves were converted into linear regression by plotting the dependent factor on a probit scale. Through standard probit analysis procedures, the derived linear regressions are compared for parallism (Finney, 1971).

Our first assumption in this model is that the slopes of regression lines representing constituents which have a similar mode of toxic action are parallel, i.e. the variance in the tolerance of the test organisms to each toxicant is the same. Variance is represented by the slope of the quantal response curve. Furthermore the regression coefficient of the response curve derived for a mixture in which the constituents have a similar mode of action should be the same as the common slope of the constituents' regression lines.

Theoretically the response curve for such a mixture may be predicted using the following formula,

$$Y_m = a + b \log (\pi+\rho_2\pi_2 \ldots +\rho_i\pi_i)X \quad \text{Finney (1971)} \quad \text{where} \quad \text{(I)}$$

Y_m = probit of % mortality,

X = concentration of mixture,

a = Y intercept of most potent toxicant,

b = common regression coefficient,

π = proportion of the total mixture represented by the most toxic constituent,

π_2 = proportion of the second most toxic constituent,

π_i = proportion of the least potent toxicant,

ρ_2 = relative potency between first and second toxicant,

ρ_i = relative potency between first and last constituent in potency series.

The assumption of similar action can be tested by comparing the predicted curve to a curve based on the observed results for the mixture.

This model may be a theoretical explanation for the empiracal findings supporting the principle of strict additivity. The toxic unit methodology, by which this principle has previously been investigated, uses only the LC 50 or ILL 50 parameters.

In addition to the additive principle of principle of similar action, our approach involves a second model of multiple toxicity. The second model assumes that toxicants may act differently in causing a common effect. For example, death may be the consequence of a toxicant adversely affecting neural function or death may be the consequence of another toxic agent's damage to the function of the gill epithelium. Each of these two hypothetical toxicants, as lethal agents, may act independently of each other when constituents of a mixture. Consequently no significance can be placed on the slopes of the regression lines, i.e. variance in the tolerance to toxicants as measured by slopes of the quantal response lines may or may not be different.

Theortetically, constituents of a mixture which act independently can only contribute to a common effect if their relative concentrations are above threshold.

Upon comprising a mixture using independently acting constituents whose respective concentrations are above threshold one should be able to predict the toxicity of their mixture using the following probability formula:

$$P_m = 1 - (1-P_2)\ldots(1-P_i) \quad \text{Finney (1971)} \quad \text{(II)}$$

where

P_m = proportion of individuals responding to the mixture

P_1 P_2, and P_i = respective proportions of individuals responding upon exposure to pure solutions of the first, second and last toxic constituent.

The third category of multiple toxicity is termed interaction. We presumed that, when the response to a mixture was contrary to that which was predicted for the above similar action model or independent action model, interplay either at the pharmacodynamic level of pharmacokinetic level had taken place between toxic constituents. An enhanced response to that predicted was designated synergism and a response less than predicted was termed antagonism.

PROCEDURE

Our test organism was the mature male guppy, *Poecilia reticulata*, these were grouped according to predesignated weight classes. Within each weight class test guppies were randomly divided into groups of 10.

Lethal response within each lot was recorded following 96-hour exposue to pure solutions of each toxicant in a continuous flow system which regulated the concentrations of the toxicant and modifying factors, such as pH and temperature. Hardness, alkalinity and dissolved oxygen were monitored and their values were found to be relatively constant. Some water quality characteristics are listed in Table (1).

TABLE I.

WATER QUALITY ANALYSIS

Specific conductance (microohms at 25° C) 259 pH 7.0 Temp. (°C) 25

	ppm	epm		ppm	epm
Silica (SiO_2)	32		Bicarbonate (HCO_3)	144	2.66
			Carbonate (CO_3)	0	00
Calcium (Ca)	32	1.60	Sulfate (SO_4)	1.0	.02
Magnesium (Mg)	9.9	.32	Chloride (Cl)	6.0	.17
Sodium (Na)	8.5	.37	Fluoride (F)	.1	.01
Potassium (K)	.3	.01	Nitrate (NO_3)	.3	.00
				mg/l	
			Oxygen	8.3	
Total		2.80	Total		2.86

	ppm		ppm
Aluminum (Al)		Dissolved solids: Residue on evap. at 180°C	165
Iron (Fe)	0.08	Calculated	170
Maganese (Mn)		Hardness as $CaCO_3$	124
		Noncarbonate	0
		Color	0

The test organisms were allowed to acclimate to control conditions for 1 week. They were not fed starting 24-hours prior to the experiment and throughout the experiment. Six discrete toxicants were used in our study and are listed as follows:

1) Dieldrin (HEOD)-a chlorinated hydrocarbon insecticide and suspected neural toxicant-,
2) Potassium pentachlorophenate (KPCP)-a slimicide and a known respiratory inhibitor through its uncoupling action of ATP-,
3) Potassium cyanide (KCN)-another respiratory inhibitor-,
4) The chloride salts of three heavy metals, Copper (Cu), Nickel (Ni), Zinc (Zn).

Samples of the test solutions were taken daily during the course of the experiments and analyzed in the following manner, HEOD and KPCP by gas chromatography, KCN by spectrophotometry and the heavy metals (total) by atomic absorption spectrophotometry. The mean daily concentration was used in subsequent calculations. Details on the derivation of quantal response curves have been reported (Anderson and Weber, 1975).

RESULTS

Discrete Toxicants

Figures 1-5 represent the quantal response curves obtained for the respective toxicants. Variations in tolerance through a size range of test organisms were reduced by introducing a size factor (W^h) into the abscissal quantity, where w=wet weight and h=the regression coefficient in the linear function, log LC 50=a+h log W. The weight tolerance functions were determined empirically (Anderson and Weber, 1975).

The values of h ranged from 0.3 for Zn to 0.81 for HEOD. These weight tolerance size factors were subsequently employed in studies involving mixtures.

Mixtures

I. Copper and Nickel

Statistical tests suggested an apparent parallelism between the lethal response curves for copper and nickel. In accordance with our model for similar action, i.e. strict additivity, we assumed that as constituents of a binary mixture, copper and nickel would contribute to the mixtures toxicity in proportion to their lethal potency. We submitted our test organisms to a series of binary mixtures of copper and nickel with all other conditions maintained as in the preceding experiments in which toxicants were studied individually. The linear function computed for the observed results for the mixture was compared to the predicted linear regression by a X^2 test for goodness of fit (Figure 6).

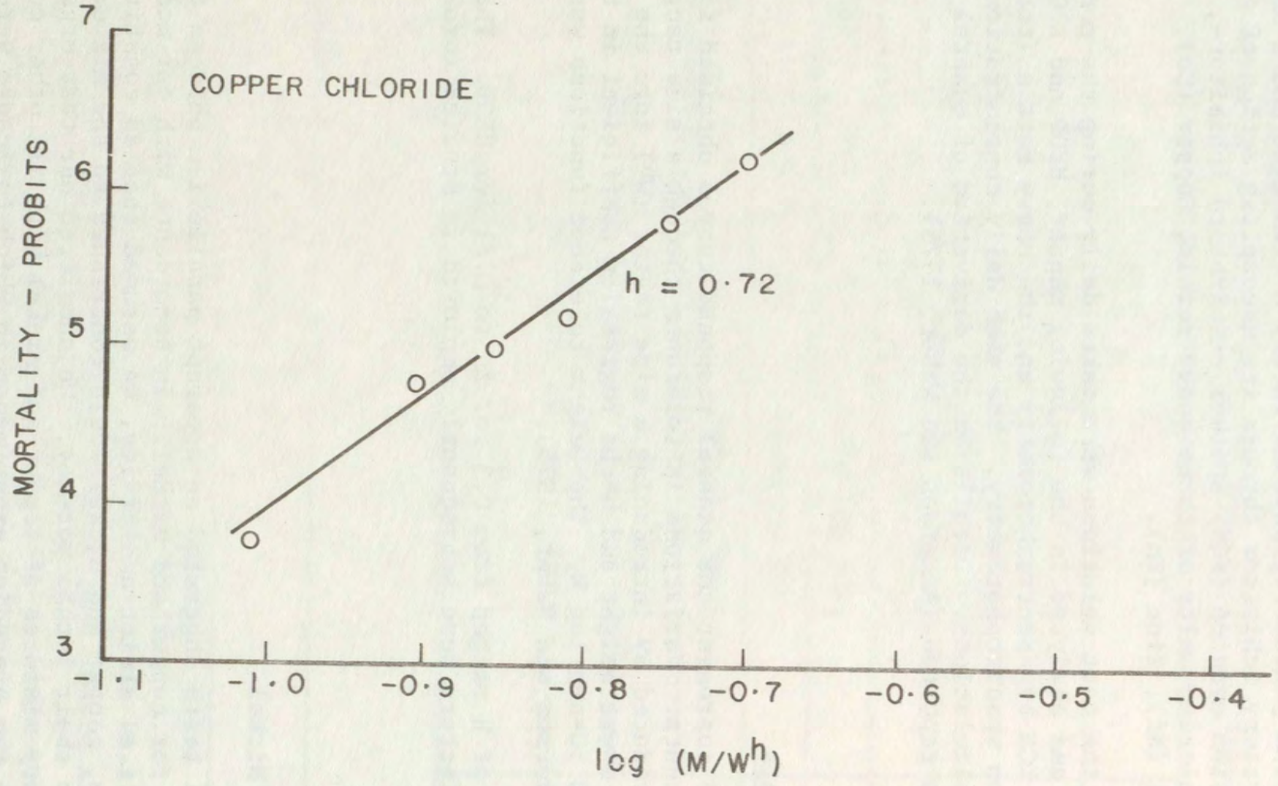

Figure 1. Lethal response curve for mature male guppies exposed for 96 hours to copper chloride.

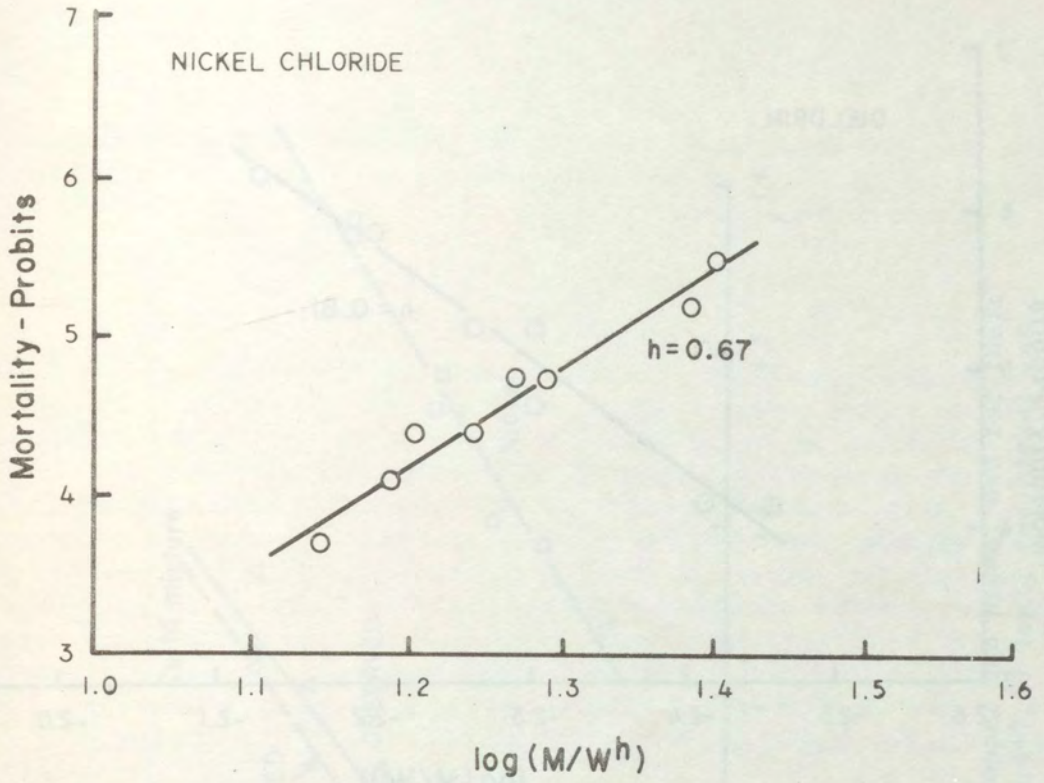

Figure 2. Lethal response curve for mature male guppies exposed for 96 hours to nickel chloride.

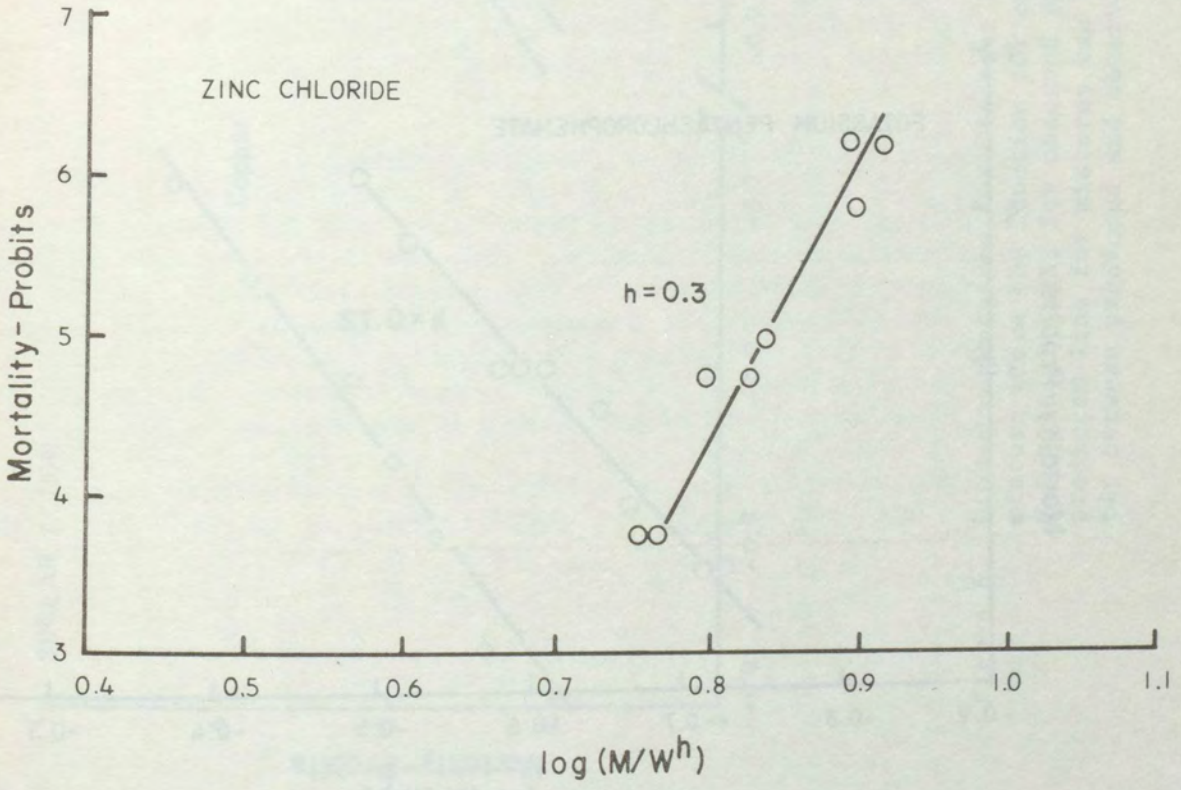

Figure 3. Lethal response curve for mature male guppies exposed for 96 hours to zinc chloride.

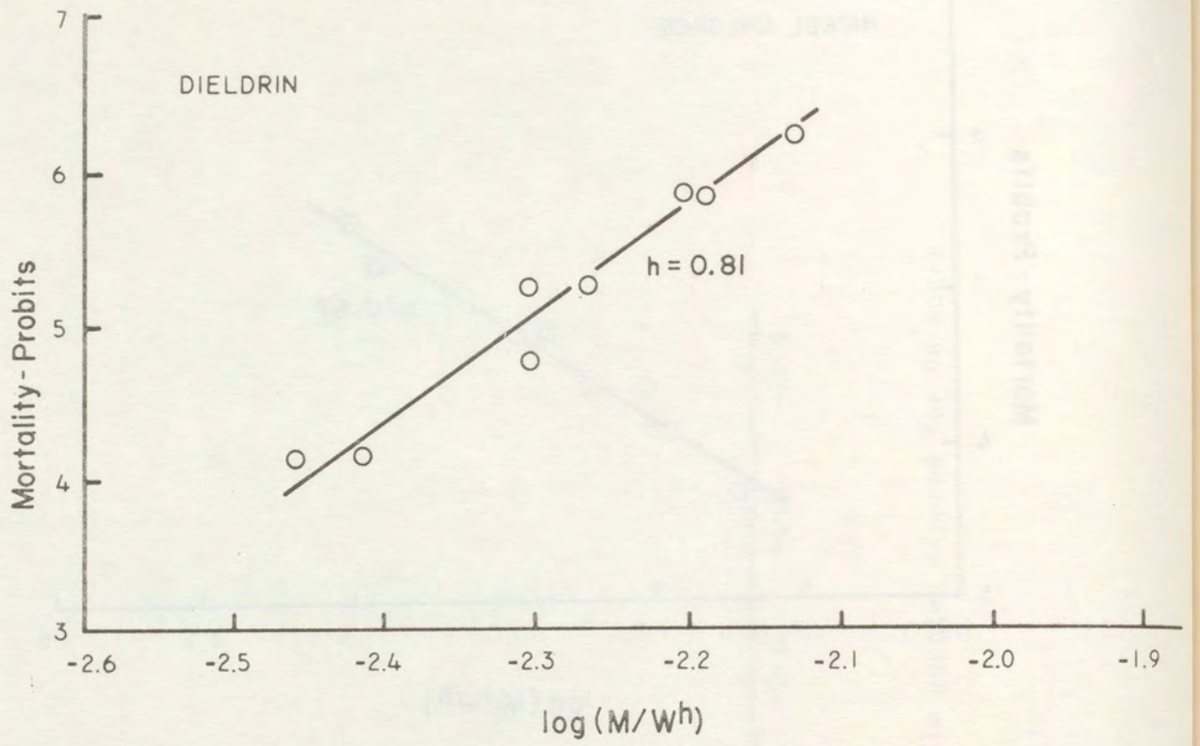

Figure 4. Lethal response curve for mature male guppies exposed for 96 hours to dieldrin.

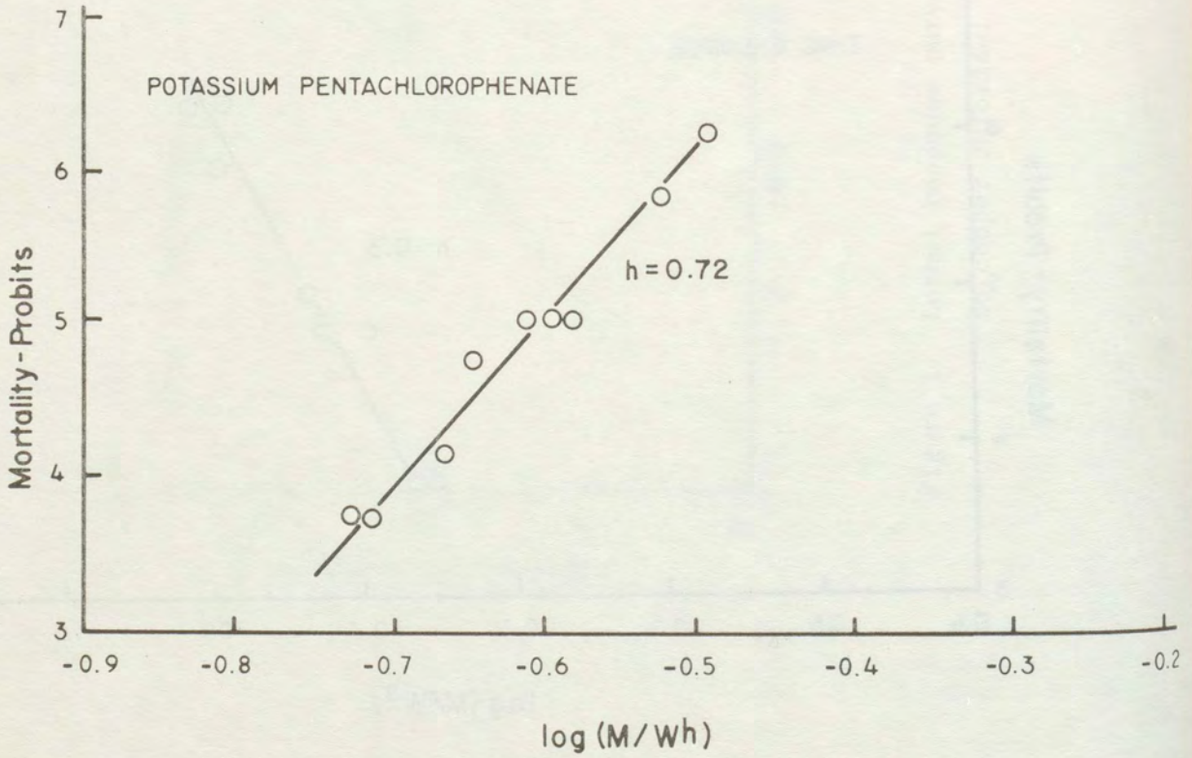

Figure 5. Lethal response curve for mature male guppies exposed for 96 hours to potassium pentachlorophenate.

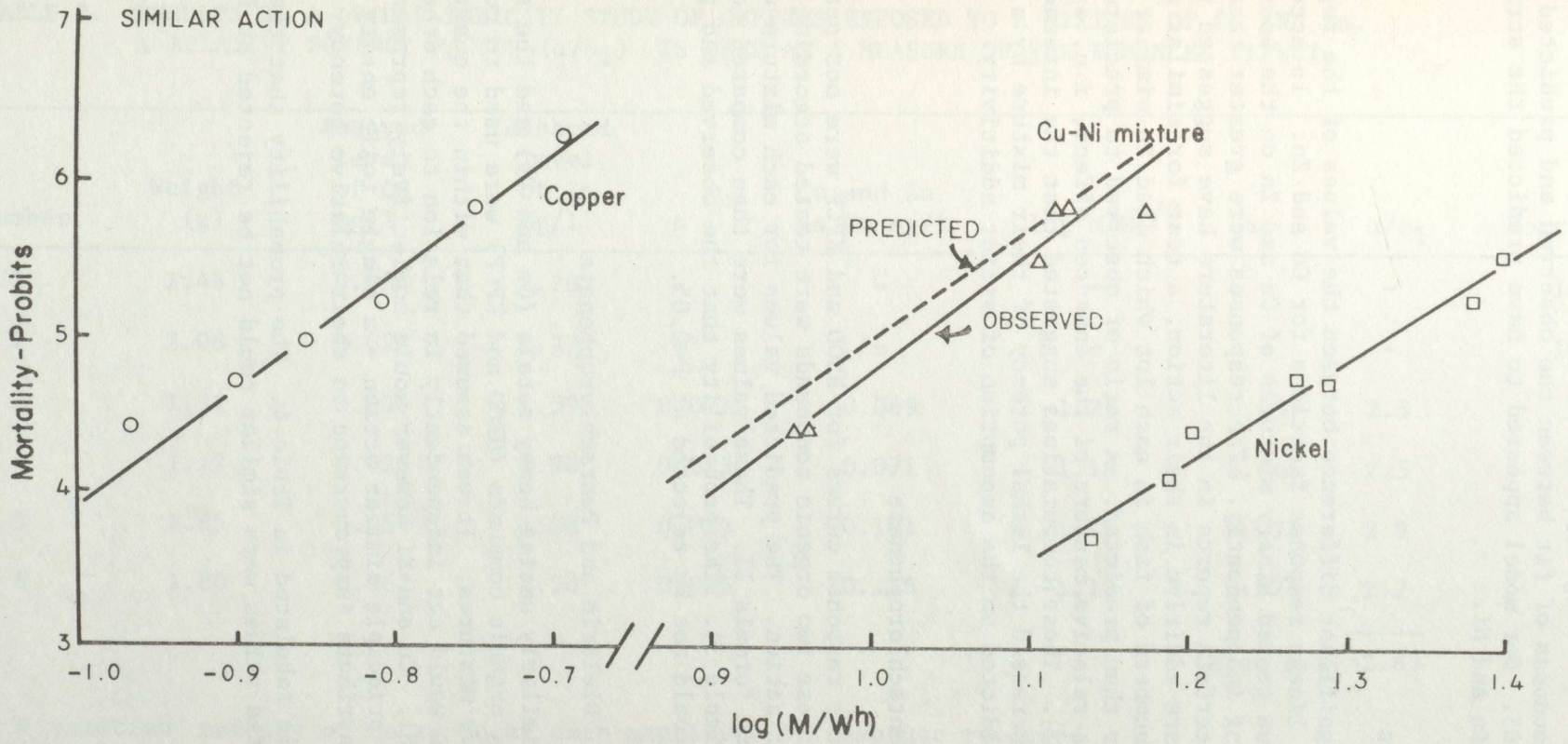

Figure 6. Linear regressions for discrete solutions of copper and nickel and for their mixture where the function for copper was Y=11.4+7.46 logX, for nickel was Y=3.32+6.32 logX, for observed points for mixtures was Y=2.52+7.37 logX and predicted line for mixtures was Y=1.99+7.05 logX. X^2 test for goodness of fit between predicted and observed=0.68 at 4 degrees of freedom(P=0.05).

The test for goodness of fit between the observed and predicted was significant at P=0.05. Our model appeared to have predicted the strictly additive action of Cu and Ni.

II. Copper and Zinc

There was a significant difference between the values of the regression coefficients of the linear response functions for Cu and Zn. In accordance with our rationale we tested binary mixtures of Cu and Zn on the assumption that they were acting independently. The responses were greater than predicted. Because certain reports in the literature have suggested that Zu and Cu in mixtures are additive in their action, a test for similar action was conducted. The numbers of fish in each lot which died in similar action studies were greater than predicted. A ratio of observed to predicted values represented a relative measure of the enhanced effect, i.e. synergistic interaction (Table 2). These computations suggested that the interactions between Zn and Cu increased the lethal potency of their mixture by a factor of 2.5 above the predicted on the assumption of strict additivity.

III. Dieldrin and Pentachlorophenate

The slopes of the response curves for HEOD and KPCP were not parallel. Binary mixtures of these two organic compounds were tested according to the model of independent action. The predicted values for each mixture were obtained by employing formula II. These values were then compared to the empirical results (Table 3). The probability that the observed and predicted values were simlar could not be rejected at P=0.05.

IV. Copper, Nickel, Dieldrin and Pentachlorophenate

The apparent similarly acting heavy metals (Cu and Ni) and the apparent independently acting organic compunds (HEOD and KPCP) were used to compose series of quarternary mixtures. It was assumed that within the quarternary mixture PCP and HEOD would act independently in relation to each other and relative to Cu and Ni. Cu and Ni however would collectively represent - in accordance with the principle similar action - a single toxic entity to which each would contribute in proportion to their relative potency.

The results were tabulated in Table 4. The probability that the observed and predicted values were similar could not be rejected at P=0.05.

TABLE 2. RESULTS OF A LETHAL TOXICITY STUDY OF GUPPIES EXPOSED TO A MIXTURE OF Cu AND Zn. A RELATIVE POTENCY FACTOR (α/α_1) IS USED AS A MEASURE OF THE ENHANCED EFFECT.

Number	Weight (g)	Assayed level of Cu mg/l	Assayed level of Zn mg/l	ρ	Cu and Zn as Cu mg/l	log $M/W^{.72}$	α_1	α/α_1	% Mortality
1	1.48	0.054	2.20	-	-	-	-	-	100
2	2.06	0.074	2.27	-	-	-	-	-	100
3	1.31	0.036	1.59	0.021	0.069	-1.25	0.057	2.5	50
4	1.12	0.026	1.96	0.023	0.071	-1.18	0.066	2.5	70
5	1.65	0.054	1.88	0.025	0.101	-1.15	0.071	2.9	90
6	1.80	0.061	2.20	0.025	0.116	-1.12	0.076	2.7	90
								Mean α/α_1=2.65	

ρ = relative potency of Zn to Cu at each magnitude of response recorded

α_1 = antilog of observed abscissal quantity log $M/W^{.72}$

α = antilog of the abscissal quantity determined from the lethal response curves of Cu (Figure I) and corresponding to the magnitude of the observed response

TABLE 3. COMPARISON OF OBSERVED AS PREDICTED MORTALITY FOR MIXTURES OF KPCP AND HEOD $X^2(4)$ FOR GOODNESS OF FIT=4.89.

Assayed level of HEOD µg/l	Assayed level of PCP mg/l	Independent variable log $M/W^{\cdot 81}$ for HEOD	% Mortality predicted or HEOD (Figure 1)	Independent variable log $M/W^{\cdot 72}$ for PCP	% Mortality predicted for PCP	% Mortality predicted for mixture $P_m=1-(1-P_1)(1-P_2)$	Observed % Mortality
5.0	0.26	-2.44	17	-0.713	11	26	10
6.45	0.40	-2.46	15	-0.639	34	44	40
6.3	0.31	-2.40	27	-0.685	17	39	50
6.4	0.40	-2.40	27	-0.58	61	72	60
4.8	0.29	-2.46	16	-0.668	24	36	70
6.9	0.41	-2.37	35	-0.594	54	70	80

TABLE 4. OBSERVED VS. PREDICTED RESULTS FOR QUARTERNARY MIXTURES OF Cu, Ni, KPCP AND HEOD.

Predicted Mortality Proportion $1-(1-P_{PCP})(1-P_{HEOD})(1-P_{Cu-Ni})=P_m$	Observed mortality proportion	Predicted numbers killed	Observed numbers killed
1-(1-.316)(1-.22)(1-.057) = 0.50	0.30	5	3
1-(1-.4)(1-.36)(1-.136) = 0.66	0.60	6.6	6
1-(1-.212)(1-.045)(1-.023) = 0.27	0.60	2.7	6
1-(1-.268)(1-.184)(1-0.045) = 0.43	0.60	4.3	6
1-(1-.758)(1-.198)(1-.084) = 0.82	0.80	8.2	8
1-(1-.655)(1-.277)(1-0.081) = 0.89	0.90	8.9	9
		$X^2 = 5.57$	
		d.f.= 4.0	

DISCUSSION

The principle of strict additivity, (similar action), as previously demonstrated for mixtures containing heavy metals (see review by Sprague, 1971) was only supported in this investigation by results of experiments on mixtures of Cu and Ni. The similar action between Cu and Ni was demonstated in mixtures where Cu and Ni were not only the sole toxic constituents but also in mixtures containing in addition to these two heavy metals the discrete organic toxicants, HEOD and KPCP.

Our assumption that strict additivity was the consequence of similar-acting chemical contaminants leads to speculation about the common mode of lethal action of Cu and Ni. At the high ambient concentrations employed in these studies, it may be postulated that Cu and Ni were acting as inhibitors of gill epitheliums' structural and enzymatic proteins. The relative lethal potency of Ni and Cu appears to be correlated with their affinity for sulphur containing ligands (Figure 7). Sulphydryl concentrations have been reported to be high in the gills of fishes (Sexton and Russell, 1955). Although these correlations are not proof of the mode of lethal action of Cu and Ni under the conditions of the experiments described herein, they offer a plausible explanation for similar action.

Why did Zn which also affiliates with ligand groups (Figure 7) not act in a similar manner to Cu and Ni? Other studies have reported a strictly additive response for Cu and Ni (Brown and Dalton, 1970). A survey of the literature reveals that besides strict additivity, Cu and Zn in mixtures may act synergistically. The discrepancy between the additive action and synergistic interaction conclusions may be due to the differences in the hardness of the water (Table 5). Calcium may interact with the assimilation of Cu and Zn or as an alternative the hardness of the water may alter the metallic forms of Cu and Zn.

Both similar-acting and synergistically interacting toxicants when present concurrently in the ambient environment present particularly hazardous forms of toxicity. Theoretically permissible levels for each constituent would not provide protection from the toxicity of their mixtures. Water quantity standards must account for the toxic potency of their mixtures. Standards based on the toxic unit principle, such as,

$$(T_c)_r = \frac{(T_{c_1} Q_1 + T_{c_2} Q_2 + T_{c_3} Q_3)}{Q_1 + Q_2 + Q_3} = 0.05 \text{ TU}$$

Tc: toxicity conc. in toxic units(TU)
Q: total effluent flow

(Seba, 1975)

may be satisfactory in establishing permissible levels for mixtures containing similar-acting toxicants. It would appear that standards for contaminants which act synergistically can only be established through an evaluation of the toxicity of their mixtures.

The approach described herein for studying multiple toxicity may provide an adequate method for (1) the identification of both similar-acting toxicants and synergistic toxicants and (2) the prediction of the mixture's toxicity of the former type of toxicants.

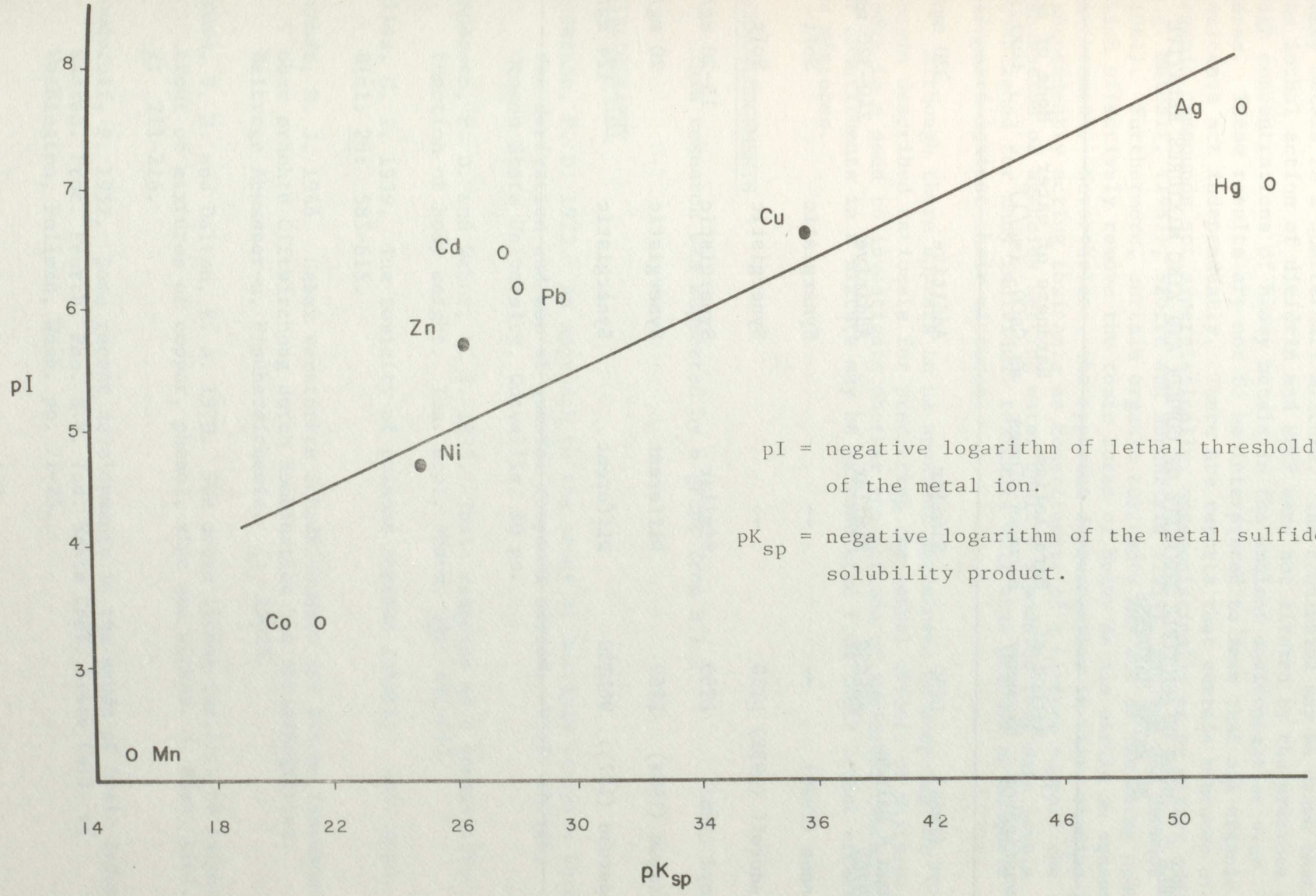

Figure 7. Correlation between sulfide insolubility, pK$_{sp}$, and a relative measure of cation toxicity to guppies, pI. (from Shaw & Grushkin, 1957)

TABLE 5. MODE OF MULTIPLE TOXICITY OF COPPER AND ZINC MIXTURES RELATIVE TO WATER HARDNESS.

Investigator	Method of bioassay	Parallelism between slopes	Hardness of water as $CaCO_3$ (mg/l)	Mode of multiple toxicity
Lloyd (1961)	LT50	Similar	Additive	320 mg/l
Brown & Dalton (1970)	48LC50	Similar	Additive	240-320 mg/l
Brandt (1946)	--	--	Synergistic	Soft
Doudoroff (1952)	LT50	--	Synergistic	Soft
Lloyd (1961)	LT50	Similar	Synergistic	15-20 mg/l
Sprague (1964)	LT50	Different	Synergistic	20 mg/l
Anderson (1973)	96LT50	Different	Synergistic	124 mg/l

In this study the organic compounds acted independently not only in relation to each other in a binary mixture but also in the presence of heavy metals in a quarternary mixture. The conclusion may be drawn that the lethal action of dieldrin and KPCP were not altered by the presence of high concentrations of heavy metals in the ambient environment or vice versa. These results are not to be interpreted to mean that all organic toxicants act independently. There are reports that certain mixtures of organic compounds act synergistically, antagonistically or by strict addition, e.g. Metcalf, (1967), Storrs and Burchfield (1954), and Sun and Johnson (1961). Furthermore, certain organic toxicants may act as chelating agents which effectively remove the toxic forms of heavy metals within an aqueous environment. Nevertheless, the apparent demonstration in these studies of independently acting toxicants as constituents of a mixture support the assumption of agencies setting water quality standards that safe levels established for many individual toxicants will also provide an adequate safeguard against their mixtures.

Although there appears to be apparent usefulness in adopting the herein described rationale for predicting the lethal effects of mixtures, there is a need to investigate whether the actions of sublethal concentrations of constituents in a mixture may be extrapolated from their lethal effects as mixtures.

ACKNOWLEDGEMENTS

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CHAPTER 15: DISCUSSIONS

A. CURRENT "STATE OF THE ART" METAL SPECIATION DETERMINATIONS

1. INTRODUCTION

HODSON: The purpose of the session is to assess the current knowledge concerning the toxicity and availability to aquatic organisms of various ionic forms and molecular complexes of metals in natural waters. The Water Quality Objectives Subcommittee of the IJC's Water Quality Board is attempting to develop water quality objectives for the Great Lakes. With respect to heavy metals, they want to specify an objective in terms of the toxic form. The chemistry of the heavy metals was reviewed, and the Subcommittee had to consider total metal, soluble metal, particulate, extractable, colloidal, chelated, complexed, bound, hydroxides, chlorides, carbonates and sulfates. At this stage, the objective is being considered in terms of total metal.

2. ANALYTICAL METHODOLOGY

In this workshop, the relationship of metal forms to toxicity is of prime interest. If we can establish that relationship, we must also ask if those metal forms can be measured on a routine surveillance basis with sufficient sensitivity, accuracy and precision? We are interested in interferences, adaptability to routine monitoring, sample preservation, transmission, and so on.

I invite each of the participants in today's session to comment on these particular topics.

ZITKO: Few of the present speciation methods could be used for routine monitoring without difficulty. Also, we must have a minimum number of parameters available to estimate toxicity of metals in any given body of water. At the moment we are limited to the use of total concentrations of metals, but we also have to know at least, hardness, alkalinity, and if possible, the concentration of organic compounds such as fulvic acid.

ALLEN: The first thing that we need to do is to define what forms of the metal are toxic. For example, the work that Bob Andrew reported, convincingly showed that the free ionic form of the metal is a toxic form, whereas, some of the other forms were nontoxic.

It is possible by at least two techniques to measure the free ionic form of the metal. The first one is the selective ion electrode. The second technique is ion exchange equilibrium which I believe can be used to measure the concentration of the ionic form of a metal in a natural water system in many cases with less difficulty than measuring even the total amount of metal.

Ion exchange equilibrium is a technique by which there is an equilibration between the metals on an ion exchange resin and the ionic form of metal in solution. In other words, the amount of metal on the resin after equilibration is directly proportional to the ion concentration that was in the solution. This technique eliminates a number of very serious current problems in trace metal analysis such as sample storage between collection and analysis.

PAGENKOPF: I would like to echo the comments made by Dr. Allen. The transport of a sample from the site to a laboratory can result in quite rapid and drastic changes in that particular sample, and I think that possibly more emphasis should be placed upon direct in-the-field analysis. The ion selective electrodes are a step in this direction but, they are subject to a variety of problems. The hydrogen ion electrode is the only one that the state of the art is sufficient such that you can get a fairly reliable field measurement at this point. However, I don't think that precludes that ion selective electrodes won't in the future become very useful tools. I also hope that additional techniques like ionic exchange equilibrium will continue to be developed. I think that each method that is ultimately developed will be somewhat site specific; in other words, what works for one person under certain water conditions may, in fact, not be too suitable for someone else. It is going to be a continuing evolutionary process but I think if all of us direct our attention to it that we can make strides quite rapidly.

CHAU: While there is no reliable and sensitive field method for measuring ppb levels of free metals in natural waters, the anodic stripping and ion exchange equilibrium techniques may be used to gain some information on the labile forms of metals in waters. To interpret the anodic stripping voltammetric results, the complexing capacity of waters should also be investigated. Although the complexing capacity of water does not provide an absolute value representing the equivalent amount of metal being masked or detoxified, it does give a relative measure of the ability of the water to mask the activity of metals. These two measurements go hand in hand as important parameters in toxicological studies.

ANDREW: I have two particular points to make. One, having used the copper ion electrode in actual flowing water bioassay systems, I am probably much more optimistic than the other members of the panel in the possibility or potential for use in field or on-site measurements in the not too distant future. There is no way of knowing, of course, how long such developments will take, but when I look back at the crudeness of our measurements in the past compared with the sophistication of some of our current measurements in other areas, I think field use of ion electrodes is most promising in the not too distant future. We are infinitely more sophisticated than we were a few years ago with regard to organic chemistry and I see no reason why we can't proceed in such a direction with inorganic chemistry.

The other point I would make is that on-site measurement of ionic activities as an estimate of toxicity or even measurement of the dissolved metal concentration is infinitely better than total metals measurements, as now practised. That is, any measurement that more closely approximates the toxic form is better than the total as far as biological interpretation

is involved. I can see some problems in trying to relate toxicity of ionic species to the total load of a metal that comes out of an effluent, where you must know the total metal concentration and the total volume of the effluent that is discharged on the given day. However, we need to know both in order to predict what will happen in a stream or lake. But I don't see this as an insurmountable problem.

HILDEBRAND: As a biologist, I am impressed by some of the sophistication that members of the panel have been devoting to attacking the problem of metal speciation in aquatic systems.

Biologists trying to absorb this information and incorporate it into experimental designs can become rapidly frustrated. I recommend that chemists work more closely with biologists and ecologists in the laboratory and in the field so that some of these speciation techniques can be incorporated into experimental designs for biologically oriented investigations.

HARTUNG: We do not have enough sensitivity to measure methyl mercury in sediments and water. I encourage analytical chemists to innovate and look at some new methodology.

I would like to reiterate that the development of toxicity data which define the chemical species that is actually producing the biological effect is of paramount importance. Subsequently, these data need to be related to monitoring data which also identify the same species of chemical compounds. We are going to encounter difficulties when we try to correlate our toxicity data, our monitoring data, and our present water quality criteria to effluent standards. We will need to develop more information on the fate of compounds after they have been released to get some idea of the dynamic processes which occur after effluents enter mixing zones and how they affect ambient water quality.

Also, there are significant effects that appear to be produced by adsorption. In other words, changes in physical state may occur even though the chemistry remains the same, so that the bioavailability of these materials may change. Therefore, we need to pay attention to bioavailability and the toxicity that is produced consequently.

HODSON: The consensus that I get from the discussion so far is that we have a number of research methods that may or may not be applicable to the field situation, and if applicable, will be useful only under certain specific situations.

3. SPECIATION IN THE ENVIRONMENT

HODSON: What are the actual forms present in the environment? Has anyone gone out and looked at the forms so that perhaps we can choose our methods from that direction?

ALLEN: In fresh water we have done very little to determine what species are actually present. The oceanographers are years ahead of us. We can go back to the classic work of Garrels and Thompson, published in 1962, on a chemical speciation model for major constituents of sea water where they showed that ion pairing in sea water was significant for many of the anions. This spurred much of the work in the 60's on the development of selective ion electrodes which confirmed most of the speciation they had proposed for sea water.

In fresh water systems, to the best of my knowledge, no one has developed a chemical equilibrium model to predict what the species are and then gone out to test if those are actually the species that occur. We certainly have the computer models, some of the equilibrium constants and concentrations of some of the constituents that are necessary. I think it's simply a question now of going ahead and really performing the necessary experimental work.

ANDREW: We have measured existing copper ion activities in two natural waters in the bioassay experiments which I presented in my paper. The waters were from Lake Superior and a surface spring water, and we measured both copper ion activity and the total copper concentration. The total concentration in both waters was around one ppb, and a maximum of two ppb. The copper ion activities in those two particular cases were on the order of 10^{-10} moles per liter, which I think agrees very closely with data that Dr. Chau presented on the calculated cupric ion activity in the presence of some of the natural chelates. We have actually measured such activities and they are extremely low. We haven't measured copper ion activity, however, in any polluted situations where there are discharges of copper. We do have some field people who are using the ammonia electrode, and possibly some of the other metal electrodes in pollution situations with the bioassay trailer on site.

CHYNOWETH: How does ionic strength, as pertaining to calcium, magnesium and alkalinity, affect copper concentration versus copper activity and, hence, how does it affect the accuracy of using the ionic copper electrode?

ANDREW: Calcium, magnesium and bicarbonate for example, affect dissolved copper measurement, principally by their effect on solubility and complexing. Carbonate, of course, increases copper solubility by ion pair formation, and, calcium and magnesium can also increase copper ion activity by displacing copper from some of the organo complexes.

Now as far as the copper ion activity is concerned, there is a very strong relationship between any of the inorganic and organic ions in solution and copper ion activity. Carbonate is a very efficient complexer of copper in natural waters and reduces copper activity on a one for one basis. Calcium and magnesium, on the other hand, influence it by increasing the ionic strength and thereby reducing the hydration of copper and thereby reduce copper ion activity. But there are any number of ionic interactions that can take place, all of which change or reduce copper ion activity, but at least in my observation all of these relationships are reflected as well by the response of the fish or the organism. These ionic relationships affect those uptake sites in the same way that they affect measurement by the copper ion electrode.

ALLEN: With your measurements of the activity of copper ion and the total copper concentration in these waters and given the pH, alkalinity, etc. you should be able to make at least a first approximation of a speciation model going through COMICS, or one of the other computer programs, to calculate what the theoretical value of the activity of copper ion in this system is. This is exactly what would be most useful, to see how much of the situation is explained by the inorganic chemistry and how far away from the measured value of the activity a theoretical calculation would be. Have you, Mr. Andrew, thought of doing this?

ANDREW: In my presentation, the two slopes of the plots of the copper ion activity versus total copper differed. There is an intercept on each one of the plots that corresponds to the strong complexing capacity in those two waters. I estimated the order of complexing at approximately 15 parts per billion copper, and I didn't get any copper activity increase in solution, until that strong chelating agent was saturated. At this point there is a linear increase. The slope is determined by the inorganic bicarbonate, as predicted. However, since the stability constants of the organic chelates are unknown, they cannot be used in the model.

CHAU: I think there are many more organics in fresh water systems than in marine water, and most of these organics are unknown. It would be difficult to use the stability constants (K values) to make up a computer program. The present computer program for speciation predictions is based mainly on compounds with known stability constants. I think, therefore, in order to have a valid speciation calculation, we should direct some of our research efforts to the identification of the major organic components in the aquatic system.

PAGENKOPF: As far as kinetics of complexation are concerned, the organic and the physical chemists are fairly well equipped to make predictions in this area with regard to how rapidly complexes will form and how rapidly they will dissociate. As we go farther into problems such as precipitation and consider the kinetics in more detail, speciation can be predicted to some reasonable degree.

4. COMPLEXATION

HODSON: There are some questions from the audience for Dr. Chau. Have you found any relationship between the complexing capacity of a lake and the type of lake involved; that is, whether it was eutrophic, mesotrophic or oligotrophic?

CHAU: I think eutrophic lakes generally have higher complexing capacity values than oligotrophic lakes because eutrophic lakes have more living organisms and organic matter in water.

HODSON: Then in a lake such as Erie where you have a very eutrophic situation, the total metal would be perhaps less biologically active than in Lake Superior, since in Lake Superior a greater proportion would be in a labile form?

CHAU: Not necessarily, the forms of metal depend on the pH of the water. At the natural pH of lake water, most of the transition metals are in bound forms. For example, very seldom can one find copper in free form in lake water.

STOKES: Is the lack of correlation between the complexation capacity of waters and the total organic carbon due in part to the complexed metals already in some heavily contaminated lakes?

CHAU: We have no idea what kind of organic compounds are involved. Low molecular weight compounds can exhibit equal complexing ability as can high molecular weight compounds. We did not find good correlation between complexing capacity with total organic carbon.

HODSON: You had suggested at one point that upon standing a water sample lost its complexation capacity. Does this suggest that in a lake there is a continuous breakdown of the complexing material releasing the metal and then a continuous uptake by other complexing agents?

CHAU: I think it may be due to a gradual breaking down of the organics by microorganisms. If the active complexing groups are broken down, the complexing capacity will be gradually weakening.

ALLEN: In the field do you actually observe that the complexation capacity is at a steady state value?

ANDREW: In our copper activity measurements, we have noted that in Lake Superior water at least, there is a seasonal change with the complexation capacity being higher in the late summer when there is a good algae boom and then decreasing after the fall overturn and during the winter. It suggests to me that there is a dynamic situation where the metal could actually be more toxic during the winter period when there is a low biological activity in the water such as low plankton biota growth. In other words, there would be higher ionic activity during the winter.

ZITKO: On the other hand, this may be compensated for by the lower metabolic rate of fish in winter. It doesn't necessarily imply that the metals would be more toxic.

ALLEN: Algae blooms will produce large concentrations of cell wall fragments, which are colloidal in size. They are broken down by enzymes, either free or bound within the organism and degraded slowly. During the period of time that those fragments exist in the environment, they have active sites which will act as a complexing site and will give a complexation capacity. This rather than a dissolved organic species may account for a good portion of the complexation capacity.

PAGENKOPF: We have also noted a transfer from the sediment to the solution phase and back to the sediments that corresponds with this yearly cycling of increased organic carbon. We may find that the activity of something like copper, or zinc remains fairly constant over the year but, in fact, the total levels may vary as the complexing capacity of the solutions increases and decreases.

HILDEBRAND: In reference to Dr. Chau's comment, I would like to ask a general quesiton of the group. When we are looking for simple parameters that we can include and monitor in both laboratory and field investigations, how relevant in the group's estimation is a dissolved organic carbon or D. O. C. measurement as an aid in determining metal speciation?

PAGENKOPF: We really don't know what an average kind of distribution of the organic material is. But in four - five years after somebody studied a natural distribution, we may well be able to take your D. O. C. level, convert it to a number of chelating sites of such and such an average strength and put that into a model and hence produce a very useful number. But right now we don't know enough about the nature of the organic material that is dissolved. There is a lot of emphasis right now on that and I feel that that's one of the measurements that in a few years will become very important.

ANDREW: Could I disagree with that just slightly? I'm basing my answer on work of Dave Hendrickson, a graduate student in our laboratory. He worked with four or five lake waters from northern Wisconsin which had a very high organic content and a very high chelating capacity. He did essentially the same sort of thing with cadmium that I did with the copper ion electrode, in that he plotted cadmium activity versus total cadmium added. He also went so far as to calculate the number of binding sites and the relative stability constants of the organic matter in the various lakes that he studied and he found that there didn't seem to be any correlation at all between the number of binding sites and the number of carbon atoms, i.e., the total carbon content.

There may be some mean reactivity with a rather large standard deviation where we can say that "on the average there are so many binding sites per carbon atom", but I'm not sure that it will have all that much predictive value.

PAGENKOPF: I would add that fulvic acid whether it comes from plant material, leeched out of the soil, or from surface degradation, is fairly comparable in its overall general characteristics. There ultimately may be some estimation possible, but by the same token, there may be quite large variations as we expand our knowledge of what types of fulvic acids are present in the environment.

WATSON: It was found about 10 to 12 years ago that the rare gasses formed hydrates. If one increases the atomic weight of the rare gasses, one finds they are more toxic. So if we were to look at it from the perspective with regard to our metals here, it might shed some light on the importance of the ligands and their complexing with regard to an atomic structure of the metals that we are referring to.

Water must be considered as a ligand, a primary ligand, with an ion possibly being displaced by those other materials that we can call in this case natural or added ligands.

ZITKO: There is a nice paper by Suvorov on the correlation of electronic structure of atoms and toxicity, which in turn could be extended to the relationship between binding to various ligands and toxicity.

PAGENKOPF: It's well established that there is a hydration sphere around metal ions, not just metal ions with a two plus charge. They are all hydrated and they have in general at least six waters of hydration that are very tightly bound to them. The loss of this water from the inner coordination spheres is one of the controlling kinetic factors involved in their rates of reaction, for complexation. During complexation with a ligand some of these waters in the inner coordination spheres are lost and replaced by donor groups of the incoming ligand, whether it's, for example, oxygen, nitrogen or sulfur. In coordination, electronic structure is significant because of the electrostatic interaction between the charge in the metal ion and the water. Some metals hold their water more tightly, their rates of hydration are larger, and so their rates of water loss are slower. So you see the different chemistry involved for the different ions that you are dealing with.

5. TRANSFORMATIONS

DEAN: You opened this discussion by a statement that the different metal species are so complicated you had to settle for total metal measurements in formulating water quality criteria. Now a great many people who are discharging metal wastes would like to settle for measurement of the active forms because then they could dump a whole lot more. I think one of the problems we have to consider is the **rate** of conversion. If the rate of conversion is rapid in both directions we are interested, of course, in total. If the rate of conversion is in one direction, as it is in the case of chromate, from a highly toxic species to an essentially nontoxic species as the chromic ion, then really we aren't concerned with total, we are concerned with only chromate. So we can't make too simple a generalization. The rate of conversion, and whether it's going to get into the system before it's tied up somewhere else, will have to be a part of the study. Here we have seen traces of copper way below background that are very toxic because they are hitting the gills. But that doesn't mean that copper has to be reduced to that level before you can discharge waste water.

HODSON: One of the things that we discussed in the Water Quality Objectives Subcommittee was that if you can get a handle on the dynamics and equilibrium involved as you just mentioned, you could perhaps write off a large proportion of the metal as being nontoxic and assume that it just drops to the sediment as an inorganic, nonsoluble, nonavailable particle. But there seems to be increasing evidence that metals in sediments can become biologically available after a period of time through changes in the chemistry of the water, or changes in the chemistry of the sediments through biological activity. So we felt that since that was another big unknown we could not simply write off a large amount of the metal as being nonavailable instantaneously to the animal. Therefore we had to consider the total metal present.

ANDREW: I think we have to keep in mind whether we are talking about a legislative standard for a discharge, or aquatic life criteria, i.e. what

they can stand or what we will permit to occur in a surface water in their environment. And a third case, really, is the laboratory research situation where we are trying to unravel the chemistry, biology and the physiology. We must keep all of these situations in mind when we are talking about whether we want to measure total, dissolved, or ionic, etc.

In the case of discharge permits, for example, I think we have to assume one of Murphy's Laws and that is, "that the worst that can possibly happen no doubt will". And so in that particular case I think the discharger should be responsible for the total metal or whatever it is that he discharges. Whenever it leaves the end of his pipe he should be held responsible for those transformations of the wastes which take place downstream. So in that particular case we should be regulating the total discharge. However, in the environment, the animal responds only to those metal forms that are present in his surroundings, and so we want to set standards or limits in that particular case on the forms to which the animal responds. Here we need a measurement of what we will allow in terms of copper ion or methylmercury or whatever may exist in his environment because these are the forms to which the animal is going to respond and potentially will kill him.

In the laboratory, where we are trying to unravel very difficult chemical situations, we need all the possible measurements, calculations, models and stability constant measurements that we can make. But then we want to use that information to interpret what is in the aquatic environment, and what we can discharge into that environment that will still allow the animal to live and function.

6. STUDIES ON TOXICITY OF METAL SPECIES

HODSON: Mr. Andrew, you speculated that the toxicity of copper increased with increasing pH. Dr. Chau states that, generally speaking, lower pH's favor the protonation of ligands, thus enhancing the dissociation of complexes. There is also the observation that decreasing pH usually enhances toxicity to the organism itself. How do you explain your speculation?

ANDREW: There are two entirely different relationships that are involved. One is that we increase copper ion activity by decreasing the pH as a result of increases in the dissociation and solubility of the carbonates, hydroxides, phosphates, etc. Actually more total copper is in solution, thereby increasing the copper ion activity.

On the other hand, lowering the pH protonates exchange groups like sulfhydryl on the surface proteins of the organism, and a lower pH actually decreases the uptake of copper at those particular sites. In other words protons (H+) interfere with the exchange of the metal at the protein site. In order to work out the toxicity relationship to pH, you must have a knowledge of both the stability constant of the external chelating agents and the stability constants of the sulfhydryl and other reactive groups in the organism. There is in my mind definitely a pH effect on the organism itself in addition to effects on all the complexation mechanisms in natural waters.

CHYNOWETH: When you observed reduction of copper toxicity at increased concentrations of bicarbonate, how do you know this effect was not due to an increase in pH or a conversion of copper to insoluble species?

ANDREW: We adjusted the pH with CO_2 after the bicarbonate was added, so that the pH was constant throughout that particular experiment. Also you may recall from the tables, copper solubility increases with increasing carbonate if the pH is held constant.

ALLEN: Mr. Andrew, is the primary site for copper toxicity the gill surface or a site inside the organism?

ANDREW: I don't know. I think in some acute tests where we have high concentrations and the animals are killed within a matter of hours, that it's caused by protein precipitation at gill membranes and the animals essentially suffocate. With the case of low concentrations of copper I don't think that is true, at least in my observations. It seems to affect a critical enzyme or a critical system in the gill surface but I don't think it's by the same mechanism.

DAVIES: In working with lead we found toxic effect levels between four and eight parts per billion when dissolved in water. We've fed lead incorporated into pelleted fish food both in the forms of a nitrate and a carbonate at levels as high as 10,000 parts per million with no toxic effect to rainbow trout. We thought possibly this was associated with an inability to uptake metals through the digestive tract. We also examined a similar situation with zinc, which is an essential element, thinking that genetically there is already a mechanism for uptake. Again at concentrations as high as 10,000 parts per million zinc in food, there was still no toxic effect. So it appears that there is no digestive uptake at least in toxic concentrations of soluble or insoluble salts of these two metals from dietary intake.

ZITKO: In general, feeding is a much less efficient way of accumulation of pollutants in fish than is uptake from water. From water pollutants are taken up directly through the gills into the bloodstream. From food they end up in the digestive tract where the absorption is much less efficient.

HARTUNG: As a generalization, what you have said holds true for metals. It does not necessarily hold true for organic compounds. Some organic compounds are very readily and very efficiently absorbed from the gastrointestinal tract. And in that case the absorption is related to the lipid-water partition coefficients. This has been studied in great detail in mammalian systems where the absorption of most divalent cations is someplace between a fraction of one percent and one or two percent, while the absorption of organic compounds, expecially of low molecular weight up to a molecular weight of 300, may be directly related to the n-octanol/water partition coefficient.

ZITKO: This is true up to a point. If you take PCB's, the uptake from water is many times more efficient that the uptake from food. If you expose fish to one part per million of Aroclor 1254 in water you go up to 50 parts per million in fish within 24 hours. If you feed them 10 parts per million in food over 200 days you may reach 10 parts per million in fish.

DAVIES: We have a need to identify what is a toxic fraction. I personally feel that it's the dissolved (or ionic) fraction. In the literature people are calling dissolved, anything that passes through a .45 micron filter, through dialysis membranes of 4.8 nanometer diameter, and ultrafiltration.

In our work, we have shown that complexed materials, which are not biologically active as toxic agents to fish, pass through these types of filtering membranes. We have also demonstrated that ionic forms of heavy metals are adsorbed onto membrane filters and fail to pass through with the filtrate. Mr. Andrew is fortunate in his selective ion work with copper in that he is dealing within a concentration range that is compatible with the use of the copper ion electrode. For lead and cadmium, the ion selective electrodes are not nearly that sensitive. When working with rainbow trout, which is the most sensitive of the trout species, selective ion electrodes do not afford much utilization. However, there are electrochemical methods, i.e., differential pulse polarography, and more specifically, differential pulse anodic stripping voltammetry, which theoretically have an unlimited detection range depending upon the plating time that you allow. I think that one of the primary things that needs to come out of a conference of this type is to clarify "what is a toxic fraction", and what are we going to be talking about when we say "dissolved".

ANDREW: Dissolved, from an analytical point of view I think has to be a rather standardized technique like passage through a .45 micron filter, even though as a chemist I don't think that's a very good definition. However, it's still a utilitarian one in the field.

ALLEN: I think we can show that dissolved metals are not synonymous with toxic metals. For instance, we can add EDTA to a solution containing copper and detoxify it even though we know that everything still is in solution. Dissolved therefore refers to soluble, but it is not synonymous with toxic.

ANDREW: One table that I presented earlier shows that the toxicity of copper actually decreases with increasing solubility of copper, in the experiment where we added carbonate. We added enough carbonate to solubilize more copper, yet the toxicity went down. So in that particular case, toxicity was actually inversely proportional to the dissolved copper fraction or dissolved copper measurement, and I think you can find examples of that in nature, where there are chelating agents present. Toxicity is not going to correlate directly with just the dissolved fraction.

DAVIES: Your dissolved form would be a copper carbonate form and I feel that probably paired ions which are electronically satisfied are toxicologically inert. Only the charged ions, like divalent cadmium or monovalent cadmium hydroxide are the toxic elements.

ANDREW: Copper EDTA has a negative charge in most of the normal pH range and yet that is also nontoxic. The same is true with NTA. It has a charge and still is nontoxic. You've raised a good question as to whether all of the toxic species are positively charged ones. I don't think we can answer it at the present time.

LOCKHART: When we move into the field and try to assess the effects of pollutants, we lack anything which would be equivalent, perhaps, to a medical history. How important is it that we know unspecific things about, for example, DDT, PCB's, in assessing the metal that may be of interest? If, for example, I'm interested in cadmium, does it really matter whether I know anything about PCB's and their exposure to the fish which I don't suspect. Can we consider these things in isolation? They don't seem to happen that way very often.

Perhaps our tendency as scientists leads us to isolate single variables so that we can relate cause to effect. Don't we saturate the multivariate experiment or the multifactorial experiment merely by stepping outside the lab door? Is it a valid approach to simplify so that we have one cause leading to one effect?

ZITKO: In order to find something about toxicity of metals we have to try to keep the other factors constant. This is the only way. If we said everything interacted with everything else, then we might as well give up.

HARTUNG: You have asked a very central kind of question and that is, what is the toxicity of mixtures and how do we predict from simple dose - response relationships for single compounds what the toxicity of mixtures might be. This is really a frontier in research even as far as human health is concerned which, as you know, is much more heavily funded than the type of research that is being discussed here. They have a couple of models which have been tested with greater or lesser effectiveness with up to three component mixtures, and in that case the studies were done in rats. At least one set of models appears to work but it is a very difficult one to apply because it requires the determination of dose response curves for each individual component with high precision and then to test at least one known mixture that incorporates all of those components. When one tries to determine the toxicity of mixtures with five, six or seven components then the number of possible combinations in this type of system is out of control, and I do not know of anyone who has been brave enough to look at that. But in some ways we are doing the same kind of tests everyday when we are eating food, which is a mixture of a great number of chemicals, any one of which when taken to excess can exhibit pronounced toxicity, including sugar, fat, salt, or spices. However, we are surviving quite nicely. I do not mean to belittle by that the central importance of looking at the toxicity of mixtures. But as the number of components of a mixture gets very large I know of no economical way to attack the problem.

DAVIES: We found with fish embryonically exposed to zinc and cadmium during the egg stage of development, that there is an acclimation effect for the hatched fry or fish. If we take fish which were not embryonically exposed to zinc or cadmium and expose them to either of these two metals, they were considerably more sensitive than the fish that had been exposed during the embryonic stage of development. So medical history can be very important.

7. TISSUE LEVELS - SIGNIFICANCE

HODSON: What utility are tissue metal levels in estimating adverse metal levels in the environment, and in a similar vein, what use might be made of uptake rates in determining metal forms or the effects thereof?

DAVIES: I think this is one advantage to analytical techniques like anodic stripping voltammetry. You are only measuring a certain type of chemical species. Presently, we cannot define by any one analytical technique what specific metal species exists in a particular water, but at least we can screen out the majority of these species. If we can find that toxicity correlates with this type of analysis we will have made significant progress, toxicologically.

HILDEBRAND: In the case of mercury, tissue levels are of great concern primarily from a human health standpoint through consumption of fish and shellfish. Actual toxic effects of mercury per se in aquatic systems haven't been generally observed. So with the specific case of mercury the tissue levels are extremely important.

HARTUNG: In the case of methylmercury, the tissue levels which have been associated with mortality in fish has been approximately 10 to 20 parts per million. There have been no effects determined in fish at tissue levels of 0.5 or one part per million. In this case when you are talking about adverse levels of metals in the environment, you are really talking about the levels in the environment which produce concentrations in fish that are unacceptable for considering the fish as source of food for man or wildlife. Therefore, in this case, we are forced to monitor fish. An analysis of total mercury in sediments or total mercury in water can only serve as an indicator. These indicators are not very good as estimators of the amount of methylmercury that is being produced in the environment and how much is available to the fish. The fish is giving you the indication as to how much methylmercury is bioavailable in the environment. What I would like to suggest is that you monitor over extended periods of time so that you can see whether you have increasing trends or decreasing trends; in other words, is the level becoming unacceptable or is it improving, or do you already have an unacceptable situation to start out with.

ANDREW: With copper and zinc, in particular, the levels in the animal tissues are high on a normal basis so that the added uptake, let's say from 20, 30, 50 parts per billion of copper or zinc in water doesn't have a great effect. It's difficult to measure uptake because of the high natural background, particularly, in liver tissues and kidney tissues where the animal normally carries a high loading of those metals. Yet these same concentrations in water are acutely toxic to the organism.

On the other hand with mercury, cadmium, and some of the other metals, there is a definite indication that when the animal accumulates levels above some certain threshold in its tissue, it begins very definitely to affect his performance, reproduction, and his survival. Dr. Hartung's presentation on mercury, I believe, gave good evidence for that.

DAVIES: We haven't finished our work with metal accumulations in fish tissues, but we have found very close correlations between the metal uptake by insects, (i.e., the invertebrate population) and heavy metal concentrations in the water. The possibility may exist for using insects as an after-the-fact indicator of a pollution situation. We have looked at this in regard to the toxicity of zinc, lead, copper, and silver on mayflies, stoneflies, and trichopterans.

ZITKO: Shellfish are very useful indicators of accumulation of heavy metals since they concentrate many heavy metals to a larger extent than fish.

8. METHYL MERCURY

ANDREW: The relative toxicity of methylmercury and mercuric ion has been compared. Also in mammalian toxicology, they have tested the dimethyl form and found that with greater methylation, the higher the partition coefficients and therefore, the higher the toxicity. What do we know about the uptake and toxicity of the ionic forms of methylmercury versus the neutral chlorides, or sulfide for example?

HARTUNG: It is my impression that probably none of the methylmercury that is found in nature occurs as the ionic form because of the very high binding energy that the material has with any sulfhydryl group, amines, carboxyl groups, and other ligands. They are usually in great excess over this particular compound. Once the methylmercury enters the organism, or as it touches the cell-walls of it, it would certainly go from any ionic state to some kind of bound state. If the bound condition was essentially a methylmercury proteinate, it would have to go then by exchange reactions from one protein molecule to the next one, which is possibly one of the reasons why the material has got such a long biological half life.

9. WATER QUALITY STANDARDS

POTOS: I would like to ask a question from a regulatory standpoint. What should we be measuring or using as a water quality standard in the next U.S. EPA revision process which takes place in about six months?

PAGENKOPF: Well I don't think we are sophisticated enough to have really adequate measure of speciation at this point. I think we are going to have to stick with the total and continue working towards more definitive speciation guidelines.

HARTUNG: I would like to disagree with that for the very simple reason that our knowledge differs from element to element. Therefore, we should stick with the best available knowledge for each individual particular element. For some of them perhaps we need to stick to total, while recognizing that this is a deficient kind of decision. For those particular materials where we have better information available we should stick with that.

ANDREW: Given a choice at this particular point in time for water quality criteria between total metal concentrations or dissolved metal concentrations, there's no question in my mind as to using the dissolved concentrations. I think measuring individual ion species is the next step in the future, but at this particular point measuring dissolved metal concentrations in both research and field situations seems infinitely better than sticking with just total metal measurement. There are enough studies in the literature now to show that the insoluble forms are not toxic.

ALLEN: If I had to make the choice I would go along with dissolved rather than total, but just let me show you where I think we can run into an extreme problem from doing this. For example, let's assume that the ionic form of zinc is toxic. Suppose we react zinc with some clay, through ion exchange with the clay, which is an insoluble form. It's going to settle out when I put it into the water. However, ion exchange reactions do equilibrate fairly easily. I would much rather include that insoluble form of zinc and not include a zinc cyanide complex, which is truely soluble, not toxic, and degrades very slowly. However, if we include that as part of the toxic zinc, and not include the portion put in on an ion exchanger or on clay, this would not be correct either.

ZITKO: We must consider that fish, cyanide, and clay are all ligands.

All these three ligands compete for zinc and those ligands which have the strongest binding constants will form non-toxic complexes. Those ligands which bind zinc more weakly will enable zinc to move from one ligand to the other. So the zinc from clay may be exchanged to the fish, so it is really a question of competition and binding constants.

DEAN: The colloidal matter of less than about a half a micron is normally filtered right through the 0.45 μ filter that we use, and is reported as soluble by convention. As a matter of fact, many precipitates such as iron hydroxide are in that range and will go right through a filter. Analytically, it is still in solution because as soon as you start adding acid to make up your analytical reagent or put it in the flame of the instrument you get all of the iron; but it's not active iron. The same can be true for a great many of the other metals. I think that some of the discrepancies are due to colloidal precipitates which increase the apparent solubility about tenfold. Dr. Allen had a slide of the solubility of zinc hydroxide. His data fitted fine except at the minimum where you would expect colloid precipitates.

Now if these colloids are in the immediate sub-micron range, their diffusion rate is too slow for them to touch the gill of a fish. So, in effect, they are out of solution even though they are analyzed as being soluble and some of your discrepancies in your data may be due to just this.

I also want to know how do you distinguish between the colloidal precipitate and a neutral ion pair?

ALLEN: You mention that the colloid is not going to get to the surface, say a gill. In the same fashion, it's not going to get to the surface of an electrode and will not be measured by electroanalytical techniques.

Secondly, when analyzed, a colloidal material will act exactly the same way as a complex. You can virtually not distinguish between a complexation reaction and adsorption reaction. For instance, if we put colloidal size clay particles into a solution we can measure an apparent complexation with these colloids in the same way as we can measure complexation with an amino acid. So from that point of view, they do become virtually indistinguishable.

We might also ask, if it is the free metal ion that is toxic, or if we know other species that are toxic. If we can develop some means either to predict their concentration or to actually measure their concentration, then does it really matter whether we include the colloidal matter as complexed material or not? Or is it simply an academic question as to whether the materials are in true solution?

ANDREW: I think use of dissolved measurements is an improvement over use of total, but I think anything beyond that is a little bit more advanced than the state of the science as we have seen here. We think we know what is happening with certain elements, but to transfer this knowledge into legally defensible water quality standards is something that is impossible to do at present, in my opinion.

DEAN: But what about chromates? Now here there is such a clear distinction between the species, the chromate and the chromic ion. The chromate anion is very toxic, irreversibly changed to nontoxic chromic cation in the field. *There is no way of making chromates in nature short of a fire, and that is hardly consistent with an aqueous system. If we treat chromates by reducing them to chromic hydroxide we have done as good a job as if we have treated sulfuric acid by neutralizing it with lime. The hydrogen ion is still around, you know, at a lower concentration but the total hydrogen is still there that was in the sulfuric acid. You don't object to neutralizing sulfuric acid, but you object to neutralizing chromates when we are reducing them?

ANDREW: I don't think any one of us would consider a total chromium measurement as adequate for measuring chromate. The most extreme example of this that I can think of is using a total chloride measurement when we know that free chlorine is the toxic form. You certainly wouldn't measure chlorine toxicity by measuring total chloride. This is the best example I think, where a separate analytical technique and separate standards have evolved for one form of the element, simply because such gross differences in toxicity were observed.

DAVIES: I think for those metal elements for which we do have enough information, we should be setting water quality standards on a dissolved (ionic) basis. However, while just about anybody in this room can run an A. A. unit; very few people in this room would be able to run pulse polarography

*Editors' Note: Recently, Schroeder, D. C. and Lee, G. F. (Water, Air and Soil Pollution, 1975) demonstrated that Cr(III) added to natural lake waters is converted very slowly to Cr(VI). Cr(III) could exist in lake water for many days. Also both valence states of chromium appear to be equally toxic on a sublethal basis and Cr(III) appears to be less toxic than Cr(VI) on an acute basis (Water Quality Criteria, 1972).

or anodic stripping voltammetry at this point in time. These aren't the simple black box techniques that A.A. is. To achieve effective monitoring and setting of standards, this is certainly going to have to be a consideration.

HODSON: So in essence what we have is a recommendation that we look at "dissolved" metal, either by a filterable technique or in the future by some electrochemical technique, and at the present moment, we ignore the bulk of the remaining metal as being biologically inactive. Is that a decent summary of what we have said?

ZITKO: It probably is a decent summary but I don't agree with it completely. Suppose you have a plant in an estuary and this plant is discharging copper, dissolved and precipitated. If we measure only the dissolved copper we don't know anything about the amount which goes out as particulate copper and may get dissolved as soon as it hits sea water. We have to know the total and the dissolved metal concentrations.

WILSON: Shellfish are filter feeders. They filter particles in the water quite indiscriminately and don't possess the ability to select metal particles from food particles. The ingestion of particulate metals of any sort by shellfish represents one route by which this form can enter the biological food chain.

DAVIES: I agree with Dr. Zitko, we should be analyzing for both total and dissolved (ionic) forms of metals. There are problems associated with changes occurring to specific metal forms, associated with a particular effluent, when released into natural waters to form various transitional species and eventually formation of particular species upon reaching equilibrium. Therefore, I think that effluent limitations, specified by the NPDS effluent standards, should be set and determined on the basis of total metal analyses. However, to ultimately assess the toxicity of a particular effluent to the biota of a stream and to realistically set water quality standards, analyses need to be based on dissolved (ionic) forms of heavy metals in natural (stream or lake) waters.

Another problem that exists is: What constitutes total metal analysis and how should it be achieved?

Total is tied up in sediments, and in conglomerative mixtures of complexed, particulate, and dissolved forms of metals moving down a stream. How do you achieve what is actually a total analysis? What constitutes biological reserves of metals, whether in lake bottom sediments, the ocean floor or suspended in a stream?

HUTCHINSON: The fact that extreme metal polluted situations already exist in nature and that nature has managed on the short term or the long term to accommodate itself to those situations is irrelevant to the general thesis that we should be concerned with. That is, that in setting water

quality standards our aim should be both to protect the environment and also, of course, as somebody has already pointed out, particularly to protect human health aspects of it. In that case, if we are interested in protection of the environment, then to err on the side of over estimating the toxicity of total elements is to err on the side of safety and the protection of the environment. We may, in fact, only find ten or five or one percent of elemental analysis of total metal to be available biologically but total is, at least, a measurable determination. And bear it in mind that we can get into problems of synergism, microbial release, and transformations of chemical form. It seems to me that we should, in fact, continue to err on the side of safety and we should, therefore, be going with the systems we can currently utilize. In setting standards we have to consider the problems of legislation and the problems of setting standards which either can't hold up in court or can't hold up in terms of variability of techniques or variability of the biological and environmental material. I suggest that if we are going to do anything at all, we should think of putting some standard errors on our total elemental analysis, rather than having standards on available forms.

DEAN: I would like to argue against that. If we err on the side of safety by a factor of a hundred fold we are, in effect, increasing the cost of treatment by approximately the same factor. Now, we can preserve the environment as long as we are here to appreciate it. If we price ourselves out of business we have overdone it.

I maintain that we have to look at real cases such as Houghton Lake, or the lakes near Sudbury mentioned by Dr. Stokes. These are the places to find out what the real toxicity is under real conditions. It doesn't mean that Bob Andrew's work isn't important. It's extremely important to understand what's happening and make it possible to extrapolate from a situation up in Sudbury to a situation down on the Gulf; we need that. But we mustn't set standards with a factor of safety of a hundred, as if dollars and energy are of no consequence. This is what you are asking us to do. I'm on the treatment side and it costs money; every time you reduce the level you pay more money. It may not be a hundred fold in the extreme, but it is a lot more expensive and I have got to get something for that money or I won't get supported. If we don't improve the environment by taking the metals out we have wasted a lot of money and somebody is going to ask embarrassing questions.

HUTCHINSON: Well I think we would both agree that the hundred fold safety factor would be quite a rare one. I think what we have to bear in mind is that it does cost money. We have got to somehow get through to the public that protection of the environment costs money and that if the government has to pass on costs, it is coming out of the taxpayer's pockets. If the companies have to pass it on it's coming out of taxpayer's pockets. It seems to me that a considerable percentage of the public when properly faced with the facts are, in fact, prepared to pay the costs of environmental protection. To allow all of the heavy metals to run up to the level at which we say, "we are near the safety limit with them", or "we are just at the edge of the whole lot of them", seems to me to be foolhardy.

DEAN: I have to agree with you. We don't want to squeeze the limits, particularly since statistics as Vince Brown pointed out for some tests are very poor. However, protecting the environment is what we are concerned with. If you say that total chromium must be removed, but, in fact, it's only the non-toxic chromic ion that is present, there may be a safety factor of 10,000. There is no biotransformation of chromic back to chromate.

That is a specific case but a factor of a hundred is very easily set and I've seen it set.

We mustn't just play safe and take a factor that's large enough so that nothing in the environment is going to be hurt with the result that the plant goes of business. This is also bad. We have got to have a balance.

HUTCHINSON: But, we are talking about setting water quality standards. We are talking about additions, future additions to the environment. We can measure total elements and we cannot, with any degree of accuracy apart from certain exceptions, measure the soluble forms. The capital investment, I put to you needed to get into the business of monitoring accurately throughout North America for soluble forms would be getting close to the sorts of expenditure that you are concerned with. We would have to train vast numbers of new people with new equipment, much of which is only currently giving us accuracy when it's in the hands of somebody like Dr. Andrew.

LOCKHART: In setting standards, whatever measure you choose, you ought to include not just the concentration but the time for which that concentration may be maintained since I think we understand that metals can be transformed back and forth in much the way that we find our pesticides degraded.

ANDREW: As I mentioned earlier, it's necessary to make a definite distinction in terms of standards as to what you use for water quality criteria for the aquatic life; and effluent standards or effluent guidelines. There has to be a distinction made between the two measurements you use in the two different cases. I think these are two different situations and two different measurements are required.

ALLEN: I would go along completely with this. What I would really question in this respect, though, is the adequacy with which we have gone from the objective water quality standards for aquatic life or other uses we are trying to protect in the environment, back to an effluent guideline. That is the most tenuous connection that has ever been made. There seems to be no rationale, really, for relating what comes out of some discharge pipe to what we find in the environment simply because we know so little about the transformations and about the hydrology of the situation. And I really think that our purpose in discussion of the forms is not as they relate to the effluent guidelines, which I agree is an extremely important question, but how they relate to the water quality objectives for the ambient water.

There is one other point that I have to make as a chemist. I agree that electroanalytical techniques are difficult. But I think we have taken this to mean that sometimes we don't get positive numbers from it; sometimes nothing

comes out on the recorder. And when we say that atomic absorption is simple what we mean is that every time that we put a sample into the atomic absorption instrument, the instrument always produces a number whether the number has any meaning or not. The concentration that I measure may have no relevancy at all to the sample concentration that I'm aspirating, yet the instrument will produce a number every time.

POTOS: If we cannot relate water quality standards to a waste load allocation or an effluent limitaiton, then the only way that we are going to protect the environment is by limiting the discharge.

ALLEN: Have we really tried to establish the links between the water quality objectives and the waste load allocations? I would question very seriously whether in all the time that we have been trying to establish water quality criteria, objectives and standards, whether we have ever really sat down, taken a good hard look on scientific grounds, and said, "we really would like to make this connection". I have asked the question fairly frequently, "How do we get from the effluent guidelines to the water quality objectives?" And the only thing that I seem to hear is "best practicable technology" and a few other terms like this, which bear no relationship at all between what's coming out of a pipe and what we need in the environment.

ANDREW: Our laboratory is working towards this. It's only been in the last two years that any of us have been forced into finding out this type of relationship. We ran into it in the Toxic Substances Hearings in Washington, in particular on discharges of cadmium and mercury mining wastes. Our witnesses, that is the EPA people, were forced into deciding whether insoluble mercury, cadmium and other compounds which were discharged, would actually go into solution and result in increased mercury and cadmium levels in the streams and lakes which were receiving these discharges.

Work on modeling of mixing zones, dilution, distribution and dispersion considering all the input sources is being carried out by the Grosse Ile Laboratory, for example. These studies are in their infancy however, and I think we are far from solving that particular question.

ALLEN: But the approach in the United States has been that we will use an effluent water quality standard that will be the same from location to location within the United States irrespective of differing receiving water chemistry, or differing receiving water volumes. That approach is not a universal one. For example, Mexico establishes their discharge water quality criteria specifically for each one of the river basins within the country and then allocates discharge loads within that river basin. That's a very direct way of relating what the load coming out of the plant is to what the water quality will be in that particular river. It may not be the best approach, and it may have some economic restraints, but it is a different way of viewing the same subject.

POTOS: I think that is exactly what we are doing. However, I don't see the need for going from an effluent limitation to a water quality objective, I see the reverse to be true. The only time that we require a best possible treatment effluent limitation is when that discharge is to an effluent quality limited stream, i.e., when you don't need anything more and you will still meet the water quality standards.

However, if a stream is fairly polluted and you do need more than best practical treatment, that's where the waste load allocation comes in. So we are not trying to relate an effluent limitation such as best practical treatment to a water quality objective, the reverse is true.

DEAN: If we said, all right, we will limit the concentration of total copper to the level of copper which is toxic considering that all of these other things can happen, you set a limit on copper that is just not practical. This is unrealistic and this is a danger if we extrapolate directly from work that Mr. Andrew has been doing. It's so low that it's unrealistic in terms of total copper. But yet you know in real wastes that so little of the total copper is really available.

ALLEN: This is true if we said that all copper was toxic and set the limit equal to that level. That is because copper ion is toxic at the hundredth part per billion level, total copper can be no greater than a hundredth of a part per billion. But I don't think that's what we're advocating. What I could envision eventually, would be a water quality objective which would state that the copper ion concentration must be below one hundredth of a part per billion, irrespective of what the total, the soluble, or any other form of copper is. In other words, if we know what the specific toxic form is we can set a water quality objective based upon that specific form of copper. When we understand the chemistry, that's the route we ought to take.

DEAN: If you set a standard at the fractional parts per billion level because it could all be active copper, that would be poisoning some species of fish, you have an impossible situation. And yet on the one hand you agree that we have to set standards based on activity; and then on the other hand we say it's so complicated we are going to have to set them on total. I don't think we are helping our regional administrators.

ALLEN: But when we do set standards six months from now we won't be using data as presented by Mr. Andrew, on copper ion activity to set the soluble copper or the total copper standards. We will be using comparable data for toxicity or protection of other uses measured in terms of soluble or measured in terms of total with the realization that these do not have the predictive capability and the rigor with which we would like to have them for future applications.

WILLINGHAM: I think there may be occasions with metals where we might want to consider both the total and dissolved form. Physical - chemical processes utilize some of the salts of iron and the salts of aluminum to produce flocs which can smother the benthic substrate if released. So I would wholeheartedly agree with Dr. Hartung in that we have to very critically

analyze and assess each metal on its own merits. There may be occasions where insoluble forms of the metal may be totally innocuous, but there are other metals where insoluble forms as well as dissolved have a dramatic effect on the aquatic ecosystem.

HODSON: Perhaps we could conclude by saying that (A) we should be looking at more than one biological response; (B) there is more than one form of metal which is toxic or has an impact on biological systems; and (C) we should not be concerned with measuring solely one form, we should be looking at the whole picture by measuring total, soluble, and at a later date one of the better means of looking at directly toxic forms of metal.

POTOS: Are you saying then that we should have a water quality standard for all the forms?

HODSON: I think since the objectives and standards are based on protecting a use, one use may be affected by one form and another use may be affected by a second form. So, consequently, we cannot take a very narrow view of things and look at only one form or one response. We have to look at the whole picture for each metal.

CHRISTIAN: The types of things that you have been bringing up are right now being formulated in terms of EPA agency policy on how to deal with the water quality criteria. The agency right now is giving very large grants to different metropolitan areas with big pollution problems and we have to have criteria for regulatory programs. To base a criteria on total we feel represents a safe approach for now. I think that Dr. Hartung has hit on the crux of the issue; you have to look at these things individually, and where you have the knowledge, modify them in the local areas. Our guidelines right now, state that you modify the criteria values, which are based on total measurements in the Section 304A water quality criteria document, given knowledge of local conditions. If you can justify a different criteria other than a total, EPA can accept that. This document is still in the draft stage. I think this type of reasonable approach is what Dr. Hartung is advocating and I don't think that the group should advocate that we should try to identify every toxic metal species in every surface water in the United States. If we do that we are going to have large problems with our regulatory program and in getting any kind of a product out of these "208" grants. A hundred and fifty million dollars has been allocated now and we need to give these people goals to shoot for. And I think that you have the basic information, and I think that Dr. Hartung's variation where you have no information right now can be factored in. We don't want to shove a total concentration standard down a state agency's throat or apply it to an individual stream if there is better information that shows that you can have a different criteria and still protect the use.

ANDREW: Mr. Potos and Mr. Christian, from an administrator's point of view; have you any difficulty in interpreting the 304A criteria for ammonia, hydrogen cyanide, and hydrogen sulfide as measurements of specifically toxic substances rather than total in those particular cases?

CHRISTIAN: Well as far as the ammonia criterion in 304A, it's dependent on pH and temperature. The un-ionized ammonia form is what the criterion calls for or restricts. I do have problems in terms of translating that into effluent limitations on a dynamic basis. Most waste treatment plants are going to have differing conditions and I'm not so sure that they can adjust their in-plant processes. So it may have to be a worst case situation where you pick the worst case and apply the ammonia criterion to the worst case unless a dynamic system can be instituted. If it can, then we will try to be reasonable and allow that type of a dynamic system to evolve.

That's where consultants from the Universities can come in. If a municipality doesn't want an arbitrary ammonia criterion that it has to meet, it can have a consultant come in and try to define different criterion for different times of the year, during winter, or during summer. We'll entertain them. Right now we have to set up the basic structure to regulate pollutants at the pipe which affect water quality. The effluent guidelines program, based on technology, doesn't do that and we have to have a water quality related program. And I'm just afraid that if there is too much bickering among scientists as to what levels to set then we may end up with a purely technology based program which may not take into account some of the metals and other things that affect aquatic ecosystems.

ANDREW: I don't think as scientists, we are trying to force anything in the way of a measurement of dissolved species or ionic species on you at the present time in any way. I think that we are just trying the best we can to review our current state of knowledge of the situation and hope to come up with some recommendations that will lead to better water quality criteria and standards two to five years from now.

I don't think we really have a consensus, but I do think the records of this particular conference and the presentations that have been made will form a basis for additional research that needs to be made furthering the relationship, in particular, as you mentioned, between water quality criteria for the surface waters and effluent guidelines. That is something that really has to be done in the next two years.

CHRISTIAN: Yes, I have high hopes myself, and this conference further adds to those hopes, but the law as it's set up calls for the revision of the water quality standards every three years. Each state will have to review its standards and any new information that's developed can be used to revise those standards and either relax or tighten up the limitations. For the time being I think that if we can provide some kind of a flexible regulatory approach; where in the specific case for a local system, the water quality criteria could be challenged to allow setting lower criteria based on more detailed knowledge of the local area, I think that we will have a good system. We are going to try to provide that flexibility to impact the programs.

B. RESEARCH NEEDS

ALLEN: The initial reasons for deciding to have this workshop came from discussions on trace metals of the International Joint Commission's Water Quality Objectives Subcommittee. Our specific objectives, I believe, are as follows: (1) to discuss and formulate the research needs relevant to the question of metals in natural waters, with specific reference to the Great Lakes; and (2) to formulate a rationale basis, for the immediate and the long term establishment of water quality objectives, and based upon these objectives, effluent guidelines to be used in their implementation.

HILDEBRAND: Direct toxicity to aquatic biota, might not be the most significant problem, but rather accumulation of these substances in aquatic biota to levels such that consumption by humans is a threat to human health. Examples might be mercury and various radionuclides. For these situations biological availability, irregardless of direct toxicity, is the central question. We have a need for continued research efforts to investigate how factors, such as organic ligands and suspended particulates, ameliorate or affect actual accumulation by aquatic organisms in addition to toxicity, at all significant trophic levels.

ZITKO: We have a definite need to study further the relationship between equilibria in natural waters and toxicity. It is relatively clear now that the equilibria or toxicity cannot be fully characterized by single numbers such as the total metal concentration or the dissolved metal concentration. We should pay attention to pH and know the concentration of other ions, calcium, magnesium and bicarbonate. We have to know the concentration of as many organic substances as possible, (in most cases, at least fulvic acid) and have some way of determining the complexing capacity of waters. We have to be able to relate all these parameters somehow to toxicity.

We have to pay more attention to other members of the aquatic fauna, not only to fish. The toxic effect may not be direct. We have an example in New Brunswick where the base metal industry cleaned up their effluent so that the concentration in the water is below levels toxic to fish. On the other hand, the levels of heavy metals in the sediments remain high. This affects the occurrence of benthic species and of aquatic insects and hence there is a very drastic difference between unpolluted and so-called cleaned up streams. The cleaned up streams have metal concentrations below the toxic levels to fish, but the heavy metals still remain in the sediments and are affecting other biota. The task for future research is to look at the ecosystem as a whole to find out what's going on.

HARTUNG: The partial consensus of this conference appears to be that the toxicologically active form is the free ionic form. I suggest that this may not be true in all cases and that indeed there may be organo-metallic forms which also may have an effect. The possibility exists that small organic ligands may actually increase the absorption of a compound rather than always protect as we have seen to date with some relatively large complexing agents.

If we start to set criteria on the toxicologically active forms, then we also have to start paying attention to the environmental dynamics which control their concentration. For example, at the present time criteria for pH are written only for the direct protection of aquatic organisms, and they have a fairly wide tolerance for pH changes. However, as we have noted, fairly small changes in pH can produce a sizeable change in the liberation of free forms of some metals, and if we consider sediments in relation to small changes in pH, we may indeed produce sizeable changes in the amount of available toxicants in the environment. Associated with that, we may also include displacement of heavy metals by common cations such as calcium, for instance, from clays. At the present time materials like calcium are not normally controlled to a great degree.

BROWN: I see as an immediate need a requirement for the development of adequate sampling techniques; firm recommendations for methods of collection; and, methods of processing and fixing samples on-site where collected. Recommendations should also be made for treatment of a sample when one gets it back to the laboratory, and how such samples should be stored?

As far as the general problem of heavy metal pollution of ecosystems is concerned, I've always felt that there is a tremendous need to define, in some sort of way, the actual distribution which occurs within the broad physical and chemical phases of any system, and if possible to detect where critical biological sites may exist. I would foresee this as involving a tremendous amount of analytical work however, even just for total metals. But this is unavoidable if the necessary information is to be obtained.

As far as effects are concerned, I think acute toxicity testing, using death as a criterion, is inadequate for defining these. While rapid death is a convenient response perhaps for quickly detecting whether a given concentration of metal is present in water in a toxic form or not, this is not really the correct thing we should look at. Relatively high concentrations of many poisons, and particularly heavy metals, produce in fact, physical destruction of the gill surface and this is not a good measure of the relative toxicity of an element in terms of its specific chemical properties. So I think we need to look at the effects in the longer term, perhaps using three-month tests on growth or something of this sort as a minimum for setting standards.

And, finally, I think it might be useful if one could look at the levels entering the blood of fish, either by chemical analysis of blood from fish which are cannulated, or by use of radionuclides, to see under what different situations one has different rates of entry of heavy metals into fishes.

DAVEY: A definite need exists to carefully inventory all natural and man-made element sources which might impact the aquatic environment. Assessing potential ocean pollutants (National Academy of Sciences, 1974) has presented an important approach for budgeting pollutants; however, the report deals only

with the metals iron, copper, and plutonium and concludes that plutonium is the only element of potential global pollution. Similar assessment should be made for all elements; however, these assessments should be focused at more localized areas, such as river, lake, or estuarine areas as well as on a global scale. These inventories would highlight elements of major environmental concern which should be carefully bioassayed in the laboratory. Also, these budgets should point out specific areas of high metal impact in the United States and Canada.

Field investigations of metal impacted areas throughout the U.S. and Canada are necessary in order to determine the extent, fate, and effects of metals on aquatic biota. Have metals per se directly or indirectly caused environmental damage and, if so, to what extent? What are the inputs, rates, routes, and reservoirs of metals within impacted areas? Special consideration should be given to areas of:

(1) Mining activities,

(2) Smelters,

(3) Industrial outfalls, especially metal plating industries,

(4) Sewage outfalls,

(5) Desalinization plants,

(6) Offshore ocean disposal areas for industrial wastes, sewage sludge, and dredge spoils.

However, it is essential that present efforts be continued and new efforts initiated to determine baseline levels of trace metals in aquatic organisms and the environmental variables that effect them. These studies should be conducted not only in contaminated environments, but also in relatively pristine or uncontaminated environments. The concentration of any trace metals can be highly variable both within and between species and influenced by a number of environmental variables. Until we understand the variability that exists in healthy ecosystems, it may be difficult to identify a contaminated ecosystem.

Because trace metals occur naturally in aquatic environments as a result of weathering and volcanic activity, the problem of determining the contribution of anthropogenic additions of trace metals to natural levels in aquatic organisms is more difficult than with halogenated hydrocarbons or refined petroleum products.

Other questions concerning potential metal pollutants which need to be answered are as follows:

1. Are certain industries, such as power plants, producing excessive metal inputs which should be controlled?

2. Can elemental transformations occur within aquatic environments to produce more lethal and/or bioaccumulated compounds such as methylmercury. If so, which elements are capable of transformation and under what circumstances?

3. Dredge spoils removed from navigational channels are often taken from areas which act as traps for sediments laden with river and estuarine bourne waste. What are the long-term effects of these dumped dredge materials upon the cleaner aquatic areas? How should dumped materials be handled to lessen environmental impact in disposal areas?

4. Liquid effluents from waste water treatment plants probably will be a major contribution of trace metals to lake, river, estuarine, and coastal waters during the next several decades. Efforts should be made to evaluate the impact that these discharges will have on concentrations of trace metals in harvestable aquatic species.

CHYNOWETH: Additional work needs to be done on toxicity of insoluble metal species (inorganic and organic particulate forms) on fish. Such forms entering fish obviously could be transformed into toxic species by microflora within the intestine of the fish or by the normal metabolism of fish.

We should study the metal concentrating potential of organisms which serve as fish food. Toxicity of metals administered to fish in food should be established.

Finally, I think it's fairly obvious that we need to study further the sites and mechanisms of metal toxicity in fish. Although we know that metals may pass through the gills, and may react with enzymes, the toxic mechanism(s) should be further elucidated.

Finally, I would also like to add that fish constitute a very minor part of the biomass in aquatic systems, and thus represent a poor test organism for assessment of metal toxicity. Metal toxicity studies should focus on micro-organisms and other types of organisms. For example, heavy metals are highly toxic to methane-producing bacteria, which are responsible for the terminal stages of decomposition in sediments. If growth of these organisms is inhibited with heavy metals, decomposition in anaerobic environments is inhibited thus interrupting the carbon cycle.

STOKES: I would like to speak in a more general sense on research needs:

1. For standard setting, we cannot use blanket policy for all metals and all situations. Whenever our knowledge is complete enough to be applied and techniques reliable, standards for a specific metal may be set with reference to the chemical forms of that metal, with some confidence. For most metals, 'total' or 'soluble' are still the most specific classification we can supply for standards.

2. There is need for greater co-operation between chemists and biologists, and attempts by each to increase knowledge of the other's fields. A biologist who prefaces his remarks by "I am not a chemist", and similarly a chemist who makes no attempt to look into the "mysterious" world of biology, are failing to make the best use of their research resources.

3. Since the ultimate test of water quality is normally its ability to support life, bioassay by standard organisms including fish, invertebrates and especially algae, should be used in conjunction with in-vitro chemical tests. The most sensitive organism (which will not be the same organism in all situations) should be used as a yardstick. Preservation of a food chain is critical: thus fish bioassay may have limited use if the primary producers in the system are more sensitive than the fish in a given system.

   Tarzwell (1975 International Conference on Heavy Metals in the Environment) has proposed a scheme for water quality control, which uses a rather limited number of organisms, but from different trophic levels. He stresses that the most sensitive organisms give us the most information about safety levels - an obvious yet surprisingly often ignored fact.

4. Research into heavy metal pollution frequently involves areas which are politically sensitive. Scientists may have to face the choice of remaining silent on an issue or making statements which implicate companies or government at the local, provincial or federal level. If his evidence is not of the '100%' variety, the scientist may be under pressure from sophisticated legal practictioners, and may eventually feel powerless to make any contribution to environmental health.

   In addition to this pressure, it is well known (though inadmissable) that granting agencies do not always look favourably on a scientist who has been outspoken on made accusations in public, concerning responsibility for some environmental problems. There is no protection for him unless he is sufficiently well established and well funded as to be immune. I do not know the solution to this problem, but I feel it has to be identified. Some of us have direct or indirect experience of being bribed to stay silent.

ANDREW: Speaking for both biologists and chemists, I think we can very easily specialize to the point that we isolate ourselves from both the perspective and the general outlook of what the real world problems are in the environment. I, personally have gained quite a bit of perspective during the past two days in seeing that steady state systems at equilibrium with a single metal or species give us simply elegant results or elegantly simple results, depending on your point of view. Yet they really don't in many ways solve the problem. The greatest challenge and the greatest need in my mind at the moment is to involve ourselves more directly with problems

in the real world environment, so that we see more realistically what is going on. I intend to do this myself in the future.

A second need is to study more closely systems that are dynamic, where effluents are actually going into streams and lakes, and where the metals in this case are both dissolving and reprecipitating and becoming parts of growing and living systems. Both chemists and biologists have shied away from dynamic systems previously, simply because they are so very difficult to study. Offering my "Quote of the Week", I think the "study of dynamic systems if like trying to nail Jello up on a wall. You nail down one part of it and the rest of it has gotten away from you". That's the way that I feel right at the moment. We have nailed down very securely a very small part of the metal toxicity problem and yet we have missed the whole bag of Jello.

BRAMAN: I think this symposium shows that the analytical chemist is not out of challenges and I think the more we interact with biologists, the more challenges they will provide.

I think in my particular instance as an example, that what we found on arsenic means that some of the standard methods for total arsenic will give low results because they don't measure the alkyl forms of arsenic. Does this mean the EPA and other monitoring operations are going to have to use my method for arsenic? I don't know; but certainly the biomethylated form is something that has to be considered. Does it mean that the method I described, is necessary for all water systems or only going to be necessary in a few water systems? That's not known either; mainly because it simply hasn't been used extensively except in Tampa, Florida and the extent of biomethylation is not known.

So we are going to have to take a look at the biomethylated arsenic forms elsewhere. We are going to have to consider biomethylated forms of other elements that probably will react in the same way, such as lead and antimony, germanium, gallium, and a few others.

DAVIES: I feel certainly that there is continued need for looking at total and dissolved, particularly the ionic fractions of heavy metals in our bioassay research work, and in the future, when possible, identifying specific forms of toxic metals in natural water systems. I think additional work is needed in evaluating what are truly synergistic and antagonistic effects, as distinct from chemical reactions in natural environments, and what are the additive effects of multiple metal interactions on aquatic biota.

Secondly, I heard reference to the COMICS computer program, I have not used it or seen it, but I think there is a need to further develop computer programs to better evaluate the complex interrelationships of metals in natural waters, both singularly and in multiple metal forms. One possibility is use of the analogue computer, as compared to the regular digital computer, which would have a greater ability to integrate and evaluate so many of these complex interrelationships.

And thirdly, I think there is also a need to investigate the uptake versus the elimiantion rates of metals in aquatic organisms particularly relating to the biochemical properties concerned with enzymatic detoxification mechanisms.

ALLEN: I note that one of the topics that's been mentioned several times during this part of the discussion, and was brought up at points yesterday but not implicitly included within the scope of the meeting was the transformation of materials once they reach the environment; transformation from one form to another or, if you wish, the biogeochemical cycling of materials. It would seem, based upon these discussions, that this is one of the large areas that we lack significant information on and perhaps it's one of the key things that we need to understand to be able to relate effluent guidelines to water quality objectives.

I would like to ask the question after almost two days of meetings at this point what the consensus might be or what the various feelings might be on what forms of metals should be included in the establishment of water quality objectives? In other words, should we include solely the toxic forms, all of the soluble forms, or total material irrespective of chemical forms, in establishment of the water quality objectives?

CHYNOWETH: We really know what the toxic forms are at this point except in the very limited laboratory situation and, therefore, I would agree with sticking with total metal concentration for the present.

ALLEN: What about for a long term objective? In other words, I think we have two questions in the establishment of any water quality objective: One is our need for immediately doing something for establishing some objectives at the present time, and the second one is in terms of a long term philosophical approach to the establishment of these objectives.

CHYNOWETH: I think we have to make some decision on what we want to protect; do we want to protect fish, algae, the entire ecosystem or what? I don't think one can define metal effects on the basis of fish bioassays. We must decide whether we wish to protect human health or the quality of the environment before a basis can be established for setting standards.

BROWN: I think it is essential to know the total amount of metal that is being put into a system - what is being transported through it as well as its fate. It would help, as a starting point if in the laboratory we could look at what chemical species are present when heavy metal salts are added to waters at high and low pH's, at high and low alkalinities, at high and low temperatures, using extremes of the environment likely to be encountered to try to give us some feel for what is going on in our environment. Also, we should consider what sort of release occurs under such conditions, from precipitated materials.

HARTUNG: I should think that, philosophically speaking, we do want to define what the toxicologically active forms are. I do not mean to imply by this that one universal form for any one particular metal is going to be equally effective in harming fish, microbes or insects. It may be different depending on the trophic level that is being affected. But to evade that issue and to consider only total metals leads us into the untenable situation which occurs, for instance, in Houghton Lake in Michigan which has been treated for a long time with copper sulfate. All of the criteria that we have written to date, based on total copper analyses, predict that this particular lake should be a biological desert. Nothing could be further from the truth, it is a beautiful fishing lake. In other words, our total copper criteria are occasionally in complete conflict with biological evidence. The basis for the conflict is that we have not identified the particular form that might be producing any effects as part of the standard setting process and we have not identified the factors that influence the release or the transformation of those particular forms of copper which could be producing an effect. We have not combined these factors into a criteria statement that makes biological sense. We have, instead, looked for simplistic answers that are easy to administer but that are not sufficiently precise to give us the real answer that would assure environmental protection.

DAVIES: In the frame of reference of talking about six months to update criteria, certainly I think at this point we are primarily limited to total metal determinations. But I think where information is starting to become available on toxic fractions, Mr. Andrew's work with copper and my work with lead, at least tentative mention should be made of these findings. Otherwise we are going to end up in another five years with a total metal analyses for which we still won't know anything about their toxicity.

One other research need is that of awareness in our studies of looking for acclimation affects as they may occur during embryonic stages of development such as we found with zinc and cadmium. In following the standard EPA method of going through all life stages starting with the egg stage, we may be producing higher concentration tolerances with embryonically exposed organisms than with organisms that have not previously been exposed to a particular toxicant. Therefore, assessments of the toxicity of heavy metals should include examining effects during both embryonic exposures and post embryonic exposures.

REEDER: I think we have to look at why we are doing this. Why are we setting water quality objectives? One of the main purposes for setting water quality objectives is, of course, to protect the environment as such.

The other thing is that we are developing for managers a tool to help them manage. And in doing this, we have to look very closely at the forms of these metals that are causing the problem. We must also very closely look at, from a manager's point of view the question! "If these are the forms that you must measure, can we measure them?" There is no use in setting an objective if we can't monitor the forms or if we can't put it in a manager's hands in

in such a way that he can make use of it. I think this is a very important phase of the problem and I keep getting the feeling that we are narrowing down too much. Water quality objectives are for whatever use the manager intends to make of that water system, and the most stringent of uses is the objective that is usually set for that purpose. We have to provide both the scientific objectives and analytical methods for the managers to be able to manage and to put these objectives to use.

HARTUNG: I agree with you. We may have many restrictions at the present time that will not let us achieve the philosophical goals that I reacted to a minute ago. However, we are also seeing the beginnings of avenues to build towards: number one, identification of what might be the toxic material; and number two, the development of dynamic models that may predict by taking into account a great number of variables, what the concentration of that active form might be. Actually, the manager might be able to get away with being able to measure total mercury or total copper, and in addition some variables which affect the solution chemistry of copper, all of which may be relatively accessible to him. But I think the idea of being able to go ahead with one single value, developed by one crude technique, is probably no longer tenable.

CHAPMAN: What is needed is a continued research effort to increase the usefulness of the newer analytical methods to field monitoring. Perhaps the one area of most pressing need is to get the researchers out into a number of specific actual field pollution situations where the findings described in the last two days can be tested to enable us to solidify the relationship between metal form and total ecological effect.

HODSON: We should actually go out into the Great Lakes, and look at what forms occur there. Until we take the electroanalytical methods on a monitoring basis and really see what is there, we may be measuring the toxicity of all the wrong forms in the lab.

DAVIES: There is still a basic lack of knowledge of the interactions even under laboratory situations, of heavy metals in waters of different quality. We are generally confined to thermodynamic values of solubility constants at 25 degrees centigrade, while in the aquatic environment and in our tests the temperature range is anywhere from three to twenty degrees centigrade. There is a general lack of solubility constant information at different temperatures. So I think there is a need on the part of the chemist to arrive at solubility constants at other than limited temperature conditions.

BROWN: Occupational and industrial health specialists have made what seem to be the sort of approaches that we ought to be making: that is determining the duration of exposure, and the maximum level which can be exceeded for what period of time before harm is done.

As far as analysis in the field is concerned, while some methods may be fine in the Great Lakes waters, when we get into our dirty rivers we have a terrible time. We will find that our electrodes don't work and the quality of

our samples changes if we carry them for more than perhaps 10 minutes or so. I don't know what the answer to this one is apart from putting an animal into a sample occasionally to see what happens biologically, and to assay total effective concentrations of toxic materials. Equilibrium calculations may be the only useful approach.

On protecting the environment, this depends basically on one's philosophy; on what one wants in the environment, or what one is prepared to allow to be discharged to a water. I think part of the philosophy of living is that there are some things which we will accept that can be put into water; for example, highly biodegradable materials at low levels. I don't, however think that human health should be the criterion. I find this awfully egocentric as a scientist and a biologist. While it is entirely attractive, it is scientifically unsound.

It strikes me that if one is going to protect the environment, then one must really look at the rare animals in that environment, not the common ones (which presumably, in view of their success, have a wide amplitude of tolerance) which is the general policy now, because those rare animals are presumably at the limits of their tolerance within the variability of that system. These are the ones that perhaps we ought to be watching to see whether they disappear or to define what will harm them. I think looking at common (i.e. abundant) animals, which can probably stand a lot of change before they go, is the wrong approach.

POE: Electroanalytical techniques and other techniques which are available to us are not really all that capable of determining what species or what form the metal exists in. You can get some idea from these techniques but there needs to be a development of the basic methods by the analytical chemist to actually determine what forms of metals actually exist in solution. And I would call for some committment to back up research and the development of basic methods for study of metal speciation.

ALLEN: If we are to advance our understanding of metal speciation in the environment, then we need to test that against a hypothesis. In other words, we need to predict, prior to the measurements, what metal species are actually present in the environment, and then go out actually to measure those concentrations in the environment. In those cases where the theoretical predictions agree with the experimental measurements, then we can infer that we do understand environmental chemistry. In those situations where they do not agree then we can infer that we require additional understanding of what is going on in the environment. Coupled with that, I would agree that what we do need is to look simultaneously at the toxic forms of materials that actually exist in real environmental segments. And for those we need to develop appropriate analytical methodologies.

REEDER: I think that there is a very great need that we have in the research area and that is for research on methods to enable a sample to be taken from the environment and transported back to the laboratory so when it gets there it hasn't deteriorated or shifted to other forms. Your analysis on that sample is only as good as the sample you receive. If you don't receive it in the same form that it is in the environment then I think a lot of our work is useless.

WORKSHOP PARTICIPANTS

ALLEN, H. E., Illinois Institute of Technology, Chicago, Illinois
ANDERL, W. H., Minnesota Pollution Control Agency, Roseville
ANDERSON, R., Environmental Research Laboratory, Duluth, Minn.
ANDERSON, P. D., Concordia University, Montreal, Quebec
ANDREW, R. W., Environmental Research Laboratory, Duluth, Minn.

BAHNICK, D., University of Wisconsin, Superior
BATCHELDER, T. L., Dow Chemical, Midland, Michigan
BEAUPRÉ, B., Commissioner, IJC, Richelieu, Quebec
BENOIT, D., Environmental Research Laboratory, Duluth, Minn.
BIERMAN, V. J., Jr., U.S. EPA, Grosse Ile, Michigan
BRAMAN, R. S., University of South Florida, Tampa
BROWN, V. M., Water Research Center, Stevenage, England
BYDALEK, T. J., University of Minnesota, Duluth
CARLISLE, D. B., Inland Waters Directorate, Ottawa, Ontario
CHAPMAN, G., U.S. EPA, Corvallis, Oregon
CHAU, Y. K., Canada Centre for Inland Waters, Burlington, Ontario
CHRISTENSEN, G., Environmental Research Laboratory, Duluth, Minn.
CHRISTIAN, J., U.S. EPA, Washington, D. C.
CHYNOWETH, D. P., University of Michigan, Ann Arbor
COOK, P., Environmental Research Laboratory, Duluth, Minn.
CRAIG, G. R., Ontario Ministry of Environment, Rexdale
CRITCHFIELD, D., U.S. EPA (OWPS), Washington, D. C.

DAVEY, E. W., National Marine Water Quality Lab., Narragansett, Rhode Island
DAVIES, P. H., Colorado Division of Wildlife, Fort Collins
DEAN, R. B., U.S. EPA, Cincinnati, Ohio

EATON, J., Environmental Research Laboratory, Duluth, Minn.
EGER, P., Minn. Dept. of Natural Resources, St. Paul, Minn.
ENGELE, R., Environmental Research Laboratory, Duluth, Minn.

FERST, C., U.S. EPA, Region IV - Atlanta, Georgia

GLASS, G., Environmental Research Laboratory, Duluth, Minn.

HALL, T., University of Toronto, Ontario
HANSON, D., Wisconsin Department of Natural Resources
HARTUNG, R., University of Michigan, Ann Arbor
HERMANUTZ, R., Environmental Research Laboratory, Duluth, Minn.

HESSE, J. L., Michigan Department of Natural Resources, Lansing
HILDEBRAND, S. G., Oak Ridge National Laboratory, Tennessee
HODSON, P. V., Canada Center for Inland Waters, Burlington, Ontario
HOLCOMBE, G. W., Environmental Research Laboratory, Duluth, Minn.
HOLMAN, W. F., Procter & Gamble, Cincinnati, Ohio
HORA, M. E., Minnesota Pollution Control Agency, Roseville
HORNING, W. B., Newtown Fish Toxicology Station, Cincinnati, Ohio
HORTON, J. W., University of Wisconsin, Superior
HUTCHINSON, T. C., University of Toronto, Ontario
HUVER, C. W., Museum of Natural History, University of Minn., Minneapolis

JOLGER, W., Wisconsin Department of Natural Resources, Madison
JONES, B., Environmental Research Laboratory, Duluth, Minn.

KLAVERKAMP, J. F., Freshwater Institute, Winnipeg, Manitoba
KONASEWICH, D. E., IJC, Windsor, Ontario

LOCKHART, W. L., Freshwater Institute, Winnipeg, Manitoba
LOESCHER, B., Ontario Ministry of the Environment, Rexdale
LONG, D., University of Minnesota, Duluth

MAGNUSON, V., University of Minnesota, Duluth
MASCHWITZ, D. E., Minn. Pollution Control Agency, Roseville, Minn.
MCCORMICK, J. H., Environmental Research Laboratory, Duluth, Minn.
MULLIN, M. D., U.S. EPA, Grosse Ile, Michigan
MURRAY, D., Ontario Ministry of Environment

NEVI, R. P., University of Minnesota Medical School, Duluth
NIIMI, A. J., Ontario Ministry of Natural Resources

OLSON, L. E., Minnesota Pollution Control Agency, Roseville

PAGENKOPF, G., Montana State University, Bozeman
PARKE, D., U.S. EPA, Kansas City, Mo.
PARKS, J., Ontario Ministry of Environment
PERRAS, A., Ontario Ministry of Environment
PICKERING, Q. H., Environmental Research Laboratory, Duluth, Minn.
POE, D., University of Minnesota, Duluth
POLDOSKI, J. E., Environmental Research Laboratory, Duluth, Minn.
PORTER, G. S., University of Minnesota Medical School, Duluth
POTOS, C. P., U.S. EPA, Chicago, Illinois
PUGLISI, F. A., Environmental Research Laboratory

RATHBUN, N. C., Arizona Game & Fish Dept., Phoenix
REEDER, S. W., Inland Waters Directorate, Ottawa, Ontario
REINKE, J., Ontario Ministry of Environment, Rexdale
ROBINSON, J., Michigan Department of Natural Resources, Lansing
RYAN, D., Wisconsin Department of Natural Resources, Spooner

SCHUETTPELZ, D. H., Wisconsin Department of Natural Resources, Madison
SHEAR, H., IJC, Windsor, Ontario
SPEHAR, R., Environmental Research Laboratory, Duluth, Minn.
STEBBINS, E., IJC Research Advisory Board Member, Cleveland
STOKES, P., University of Toronto, Ontario
STUPAVSKY, S., International Nickel Co. of Canada Ltd., Copper Cliff, Ontario

TOBIN, R. S., Canada Centre for Inland Waters, Burlington, Ontario

VANDERWAL, J., Ontario Ministry of Environment, Rexdale

WATSON, A. E. P., IJC, Windsor, Ontario
WILLFORD, W. A., U.S. Fish and Wildlife Service, Ann Arbor, Michigan
WILLINGHAM, T., U.S. EPA, Denver, Colorado
WILSON, R., University of Minnesota Medical School, Duluth, Minn
WILSON, R. C. H., Environment Canada, Halifax, Nova Scotia
WINSLOW, J. C., Minnesota Pollution Control Agency, Roseville

ZITKO, V., Fisheries Research Board of Canada, St. Andrews, New Brunswick

TERMS OF REFERENCE - RESEARCH ADVISORY BOARD

1. As used herein, "research" includes development, demonstration and research activities, but does not include regular monitoring and surveillance of water quality.

2. The functions and responsibilities of the Research Advisory Board relating to research activities in Canada and the United States concerning the quality of the waters of the Great Lakes System shall be as follows:

(a) To review at regular intervals these research activities in order to:

(i) examine the adequacy and reliability of research results, their dissemination, and the effectiveness of their application;
(ii) identify deficiencies in their scope, and inadequacies in their funding and in completiong schedules;
(iii) identify additional research projects that should be undertaken;
(iv) identify specific research programs for which international cooperation will be productive;

(b) To provide advice and consolidations of scientific opinion to the Commission and its boards on particular problems referred to the Advisory Board by the Commission or its boards;

(c) To facilitate both formal and informal international cooperation and coordination of research;

(d) To make recommendations to the Commission.

3. The Research Advisory Board on its own authority may seek analyses, assessments and recommendations from other professional, academic, governmental or intergovernmental groups about the problems of the Great Lakes water quality research and related research activities.

4. The International Joint Commission shall determine the size and composition of the Research Advisory Board. The Commission should appoint members to the Advisory Board from appropriate Federal, State and Provincial Government agencies and from other agencies, organizations and institutions involved in Great Lakes research activities. In making these appointments the Commission should consider individuals from the academic, scientific and industrial communities and the general public. Membership should be based primarily upon an individual's qualifications and potential contribution to the work of the Advisory Board.

5. The Research Advisory Board should work at all times in close cooperation with the Great Lakes Quality Board.

MEMBERSHIP LIST
RESEARCH ADVISORY BOARD

UNITED STATES SECTION

Dr. A. F. Bartsch (Chairman - resigned March/76)
Director
National Environ. Research Center
200 S. W. 35th Street
Corvallis, Oregon 97330

Dr. D. I. Mount (Chairman - effective March 1976)
Director
Environmental Research Laboratory
6201 Congdon Avenue
Duluth, Minnesota 55804

Dr. Eugene J. Aubert
Director, Great Lakes Environmental Research Laboratory
National Oceanographic and Atmospheric Administration
2300 Washtenaw Avenue
Ann Arbor, Michigan 48104

Professor Leonard B. Dworsky
Civil and Environmental Engineering
Cornell University
302 Hollister Hall
Ithaca, New York 14853

Mr. Alvin R. Balden
19 Alina Lane
Hot Springs Village, Arkansas 71901

Mrs. Evelyn Stebbins
Cleveland Citizens for Clean Air and Water Inc.
705 Elmwood
Rocky River, Ohio 44116

Dr. Herbert E. Allen
Assistant Professor
Department of Environmental Engineering
Illinois Institute of Technology
Chicago, Illinois 60616

Dr. L. J. Hetling (Resigned Nov/75)
Director, Environmental Quality
Environmental Research & Development
N.Y. Dept. of Environ. Conservation
50 Wolf Road, Room 519
Albany, New York 12201

Mr. C. M. Fetterolf (Resigned June/75 as Michigan State representative; appointed ex officio member June/75)
Executive Secretary
Great Lakes Fishery Commission
1451 Green Road
Ann Arbor, Michigan 48107

Professor Archie J. McDonnell (Effective Nov/75)
Department of Civil Engineering
Water Resources Research Center
The Pennsylvania State University
University Park, Pennsylvania 16802

Professor Joseph Shapiro (Effective Nov/75)
Geology and Ecology Department
University of Minnesota
Minneapolis, Minnesota 55455

Secretariat Responsibilities
Dr. Dennis E. Konasewich
Research Scientist
Great Lakes Regional Office
International Joint Commission
100 Ouellette Avenue, 8th Floor
Windsor, Ontario N9A 6T3

CANADIAN SECTION

Dr. A. R. LeFeuvre (Chairman)
Director
Canada Centre for Inland Waters
Environment Canada
P. O. Box 5050
Burlington, Ontario L7R 4A6

Mr. Arnold J. Drapeau
Professor
Ecole Polytechnique
Campus de L'Universite de Montreal
C. P. 6079 - Succursale "A"
Montreal, Quebec H3C 3A7

Mr. H. R. Holland
462 Charlesworth Lane
Sarnia, Ontario N7Y 2R2

Mr. Paul D. Foley
Coordinator
Development and Research Group
Ontario Ministry of the Environment
135 St. Clair Avenue West
Toronto, Ontario M4V 1P5

Dr. J. C. N. Westwood
Professor & Head of Microbiology
and Immunology
Faculty of Medicine
University of Ottawa
Ottawa, Ontario K1N 6N5

Mr. J. Douglas Roseborough
Director
Fish and Wildlife Research Branch
Ontario Ministry of Natural Resources
P. O. Box 50
Maple, Ontario L0J 1E0

Mrs. Mary Munro
3020 First Street
Burlington, Ontario L7N 1C3

Dr. M. G. Johnson (Resigned Nov/75)
Director
Great Lakes Biolimnology Laboratory
Canada Centre for Inland Waters
P.O. Box 5050
Burlington, Ontario L7R 4A6

Dr. J. R. Vallentyne (Effective Nov/75)
Senior Scientist to the
Asst. Deputy Minister
Ocean & Aquatic Affairs
Fisheries and Marine Services
Environment Canada
Fontaine Building
Ottawa, Ontario K1A 0H3

Ex Officio Member
Mr. Floyd C. Elder
Acting Head
Basin Investigation & Modelling Section
CCIW, Environment Canada
P.O. Box 5050
Burlington, Ontario L7R 4A6

Secretariat Responsibilities
Dr. Dennis E. Konasewich
Research Scientist
Great Lakes Regional Office
International Joint Commission
100 Ouellette Avenue, 8th Floor
Windsor, Ontario N9A 6T3

TERMS OF REFERENCE
STANDING COMMITTEE ON THE SCIENTIFIC BASIS FOR WATER QUALITY CRITERIA

The Scientific Basis for Water Quality Criteria Committee of the Research Advisory Board has a mandate to:

1. Selectively assess the status of ongoing research related to water quality criteria for the Great Lakes to:

   (a) Determine relationship of ongoing work to identified needs;

   (b) Identify opportunities for cooperative efforts.

2. Make recommendations to the Research Advisory Board concerning the above matters.

MEMBERSHIP LIST

Dr. William Brungs (Resigned as Chairman Feb/76 - remains member of Committee)
Environmental Research Laboratory
6201 Congdon Blvd.
Duluth, Minnesota 55804

Dr. Andrew Robertson (Acting Chairman - effective Feb/76)
Great Lakes Environmental Research Laboratory
National Oceanographic and Atmospheric Administration
2300 Washtenaw Avenue
Ann Arbor, Michigan 48104

Mr. Carlos M. Fetterolf
Executive Secretary
Great Lakes Fishery Commission
1451 Green Road
Ann Arbor, Michigan 48107

Dr. Rolf Hartung
School of Public Health I
University of Michigan
Ann Arbor, Michigan 48109

Mr. S. W. Reeder
Coordinator Water Quality Objectives and Standards, Water Quality Branch
Inland Waters Directorate
Environment Canada
Place Vincent Massey
Ottawa, Ontario K1A 0E7

Dr. I. Hoffman
National Research Council of Canada
Association Committee on Scientific Criteria for Environmental Quality
Ottawa, Ontario K1A 0R6

Dr. W. M. Strachan
Chemical Limnology Subdivision
Canada Centre for Inland Waters
P.O. Box 5050
Burlington, Ontario L7R 4A6

Secretariat Responsibilities
Dr. Dennis E. Konasewich
Research Scientist
Great Lakes Regional Office
International Joint Commission
100 Ouellette Avenue, 8th Floor
Windsor, Ontario N9A 6T3