PREDICTING THE SITE-SPECIFIC BIOAVAILABILITY OF ZINC USING THE INDICATOR SPECIES PROCEDURE: A CASE STUDY

THESIS

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National Water Quality Criteria intended to protect aquatic life and their uses from the adverse effects of pollutants may not be appropriate due to site-specific factors that alter chemical bioavailability. The Indicator Species Procedure may be used to derive site-specific criteria in order to account for differences in site-specific bioavailability. This procedure was implemented using zinc for three chemically different site (river) waters. The purpose of this study was to quantify the bioavailability of zinc in each site water and correlate results to water quality parameters and/or zinc speciation. Results demonstrated that national criteria for zinc accurately predicted the experimentally derived site-specific values within a factor of two when adjusted for water hardness. Particulate forms of zinc were shown to be biologically unavailable under conditions tested.

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CHAPTER I

INTRODUCTION

Background

Throughout the 1960's and 1970's the deleterious environmental consequences of our heavily industrialized society were ever-increasingly becoming apparent. Widespread fish kills following agricultural pesticide applications and incidents such as the industrial contamination of the James River with Kepone demonstrated the susceptibility of the nation's aquatic ecosystems to anthropogenic inputs (Carson, 1962). If the quality of the nation's water resources was to be protected, the necessity of a concerted national effort to curtail water pollution seemed paramount.

In 1972, Congress passed Public Law 92-500, the Federal Water Pollution Control Act (FWPCA), which set forth specific goals for improving national water quality, thus providing the foundation upon which current water pollution policy rests. The ultimate goal envisioned in this piece of legislation was the elimination of water pollution discharge by 1985 with an interim goal, wherever attainable, providing for the propagation of fish and wildlife and allowing body contact recreation by no later than 1983. These objectives were to be achieved by imposing technology-based effluent limitations on all point source dischargers. A permitting system, referred to as the National Pollution Discharge Elimination System (NPDES), served as the primary mechanism through which pollution abatement regulations, promulgated for attaining water quality goals, would be implemented and enforced. In addition, states were required to establish water quality standards for receiving waters based on a designated use (i.e., protection and propagation of aquatic life, recreation, public water supply, industrial or agricultural uses) and a scientifically derived criterion that would support that use. Furthermore, states were to identify all "water quality limited waters" that would not meet water quality standards despite prescribed effluent limitations. Subsequent studies were then to be performed to delineate the waste loading input that these systems could tolerate before exceeding water quality standards. Both effluent limitations for point source polluters and water quality criteria to aid states in establishing enforceable water quality standards were to be published and periodically updated by the U.S. Environmental Protection Agency. Provisions for pretreatment of industrial wastes being discharged into municipal treatment plants and for abatement of non-point source inputs were also included within the framework of the Act (Bregman and Beeland, 1976).

Although the strategy dictated by the Act was commendable, several underlying assumptions were highly tenuous. It was assumed that ultimately, zero discharge or the total elimination of point source pollution was (1) necessary in order to protect our aquatic systems (2) technologically achievable and (3) economically feasible (Cairns, 1980). In practice, the technological capability to remove all pollutants from all effluents simply does not exist. However, even in situations where high removal efficiencies are possible, the capital investments, energy and natural resource expenditures necessary to attain successively greater efficiencies rise exponentially resulting in unreasonable treatment costs. Even if advanced treatment technologies are employed, they will by no means eliminate the environmental problems that these wastes present, since such treatment processes merely extract these materials from the aqueous phase and then convert them into a different form that again presents some level of risk to society. Aside from the technological and economical considerations, a more critical issue centered around the assumption that any amount of waste discharged into an aquatic ecosystem was socially unacceptable. Cairns (1977) challenged this rationale arguing that each aquatic ecosystem has an innate capacity to tolerate waste inputs before the biological integrity of the system was jeopardized. That is, there exists some time dependent, site-specific loading rate or assimilative capacity for most chemicals, which if not exceeded, would prevent substantial adverse impacts. This does not imply that a certain degree of harm does not result, but rather this level of harm does not interfere with the intended uses of the aquatic system and is judged socially acceptable. Thus, the concept of assimilative capacity extends our societies' waste treatment operations into the receiving water adding an additional, but rarely stated, use to our nation's waters. More recently, Cairns (1981) reasoned that if the benefits of a highly industrialized nation are to be enjoyed without a concomitant deterioration in the quality of our nation's water resources then the rate at which wastes are introduced into aquatic systems must be synchronized with their ability to process these wastes. Using such a management approach, the benefits derived from industry and aquatic systems could be optimized, while avoiding unnecessarily stringent and costly discharge limitations which if met would provide little, if any, social merit.

In 1980, as a result of litigation with the National Resource Defense Council, the USEPA published water quality criteria for 64 toxic pollutants. These criteria which superceded previous criteria compiled in Quality Criteria for Water, often referred to as the "Red Book," reflected the latest scientific information for the protection of aquatic life, human health, and some recreational activities (Federal Register, 1980). Criteria supporting other designated uses such as agricultural or industrial uses were not specifically addressed. Nevertheless, USEPA suggested that the newly proposed criteria developed primarily for human health and aquatic life considerations were sufficiently stringent to protect other use designations. The fact that these criteria permitted limited pollution discharge revealed the Agency's new position regarding assimilative capacity.

First, support for this concept was evidenced in terms of the proposed criteria for the protection of aquatic life which provided both a maximum allowable instantaneous concentration as well as a maximum 24 hour average concentration that later evolved into a maximum monthly average concentration (Federal Register, 1984a). These concentrations, if not exceeded, would theoretically protect most aquatic life.

Secondly, USEPA rescinded their presumptive applicability policy that required states to adopt criteria for water quality standards at least as stringent as "Red

Book" criteria. This approach essentially mandated the incorporation of national criteria into water quality standards leaving little flexibility for states to modify standards to reflect local site-specific factors or designated uses requiring less stringent criteria. This rationale hinged on the presupposition that the variability in community response for different systems would not be large enough to warrant the development of criteria for individual sites (Cairns, 1981). Unfortunately, such reasoning neglected the system and time dependent nature of assimilative capacity. Although the agency revoked this policy thereby paving the way for the development of site-specific criteria, little guidance was provided to states elaborating the methodology by which national criteria were to be adjusted. As a result, national criteria or "single numbers" continued to be adopted into most state water quality standards.

Guidelines For Deriving National Water Quality Criteria

The 1980 guidelines, describing the procedures by which national criteria were derived, were developed in an attempt to provide a scientifically sound and standardized method for analyzing data upon which criterion development was based. Unlike standards, economic and technological considerations were not factored into criterion formulation.

In 1985, the Agency revised these guidelines in an attempt to improve the technical validity of this methodology (USEPA, 1985a). Briefly, acute toxicity data are collected for a minimum of eight different Families of aquatic organisms occupying an array of ecologically or commercially important niches. Genus Mean Acute Values (GMAV's) are computed by taking the geometric mean of the LC50's (or EC50's) reported for members of the same genus. The GMAV's are then ranked and used to estimate the Final Acute Value (FAV) which is calculated such that 95% of the species tested are protected (Erikson and Stephan, 1985). Since the FAV is calculated from point toxicity estimates that cause an adverse effect on the test population (i.e., 50% mortality) the Criterion Maximum Concentration (CMC) is calculated as the FAV divided by two in an attempt to provide a "safe" level to 95% of the species tested. This concentration on the average, if not exceeded over a one hour period more than once every three years, should protect the receiving water from acute toxicity effects.

The assessment of chronic toxicity data for aquatic animals and plants as well as information on bioconcentration of lipophilic chemicals in aquatic organisms yields a Final Chronic, Final Plant and Final Residue Value, respectively. The Final Residue Value is included to prevent aquatic organisms from obtaining body burdens that were believed to pose significant risk to human and wildlife consumers. The lowest of the above three values is designated as the Criterion Continuous Concentration (CCC) which provides an estimate of the highest four day average concentration, which if not violated with a frequency greater than once every three years, should protect aquatic organisms and their uses from being unacceptablly affected by chronic exposures. Additional data regarding the effects on community structure or functional processes (i.e., respiration, productivity, nutrient cycling) or behavioral responses (i.e., preference and avoidance, swimming endurance, cough response) are also reviewed and professional judgement is used to evaluate the reasonableness of the two-number criteria derived via the formal procedure. However, such data are usually limited or not available for many chemicals (Matthews et al., 1982). The current approach for deriving numerical National Water Quality Criteria is summarized in Figure 1 and is intended to protect 95% of the tested genera and all commercially, recreationally and socially important species (Stephan, 1985).

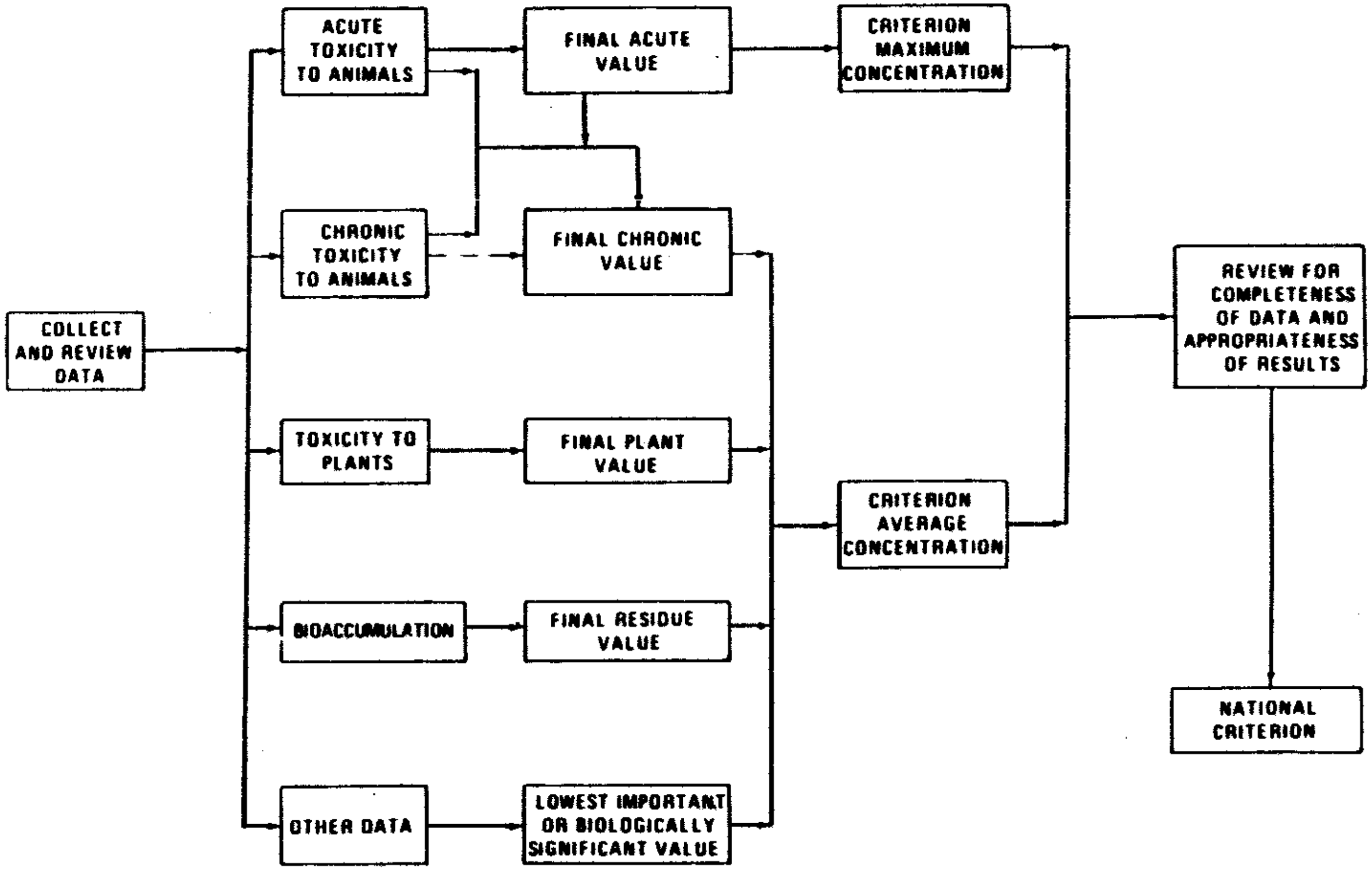

FIGURE 1 Schematic Diagram Describing the Derivation of National Water Quality Criteria

To date, the revised guidelines have been applied to ammonia, arsenic, cadmium, chlorine, chromium, copper, cyanide, lead mercury, aluminum, nickel, pentachlorophenol, chlorypyrifos selenium, parathion, toxaphene and zinc (Federal Register 1985, Federal Register 1986 a, b). One of the most important features of stating criteria in accordance with the revised procedures is that criteria are stated in terms of three statistical properties: (1) magnitude (2) duration and (3) frequency. Specifying criteria in such terms readily facilitates application of dynamic statistical models for determining waste load allocations in water quality limited situations rather than using the conservative and hence less cost effective steady state models based on a 7Q10 or other worst case design flow (Jaworski and Mount, 1985).

The extent to which these guidelines will yield criteria that accurately reflect the level of protection predicted will largely depend on the validity of the following underlying assumptions:

(1) It is not necessary to protect all the members of an aquatic community in order to protect the community and its uses as a whole. As an alternative corollary, if most of the component species are protected the overall biological integrity of the system will be maintained.

(2) Single species tests representing organisms from several functional niches, which may or may not be present in the actual receiving system, can be

utilized to adequately protect complex, interacting multispecies communities.

(3) Toxicity endpoints derived under laboratory conditions can be extrapolated to field situations without a significant introduction of error.

The first assumption is generally accepted by regulatory agencies on the basis that the loss of a few recreationally or commercially unimportant species is of little consequence if the function of the lost species can be compensated by existing species or new species that are able to fill this vacant niche. One exception might occur in ecosystems where certain key functions can only be carried out by a narrowly defined group of organisms, which if adversely impacted, would result in the demise of the ecosystem. Examples of communities containing "keystone" species have been cited (USEPA, 1985b). In addition, ecosystems once stressed often have the ability to recover. Cairns (1976) has discussed some of the factors which determine ecosystem elasticity. The U.S. Environmental Protection Agency suggests that three years would be an adequate recovery period for most ecosystems and therefore the Agency has incorporated a three year return period into their criteria formulation (USEPA, 1985c).

The second assumption postulates that if community structure is protected the integrity of community function will also be maintained. Some studies have indicated that functional responses may be more sensitive than structural end points and that functional end points should be required as part of the minimum data requirements for deriving national criteria. A further concern is that the toxicity data bases are themselves unrepresentative of the structure of the aquatic communities that are to be protected. Seegert et al. (1985) have shown that the majority of data contained in the national toxicity data bases reflect only a limited number of commonly tested species, and that many of these species are restricted to certain geographical locations. The authors conclude that more information on a wide variety of organisms is necessary to ensure that the range of sensitivities in the community is represented so that appropriate national criteria can be established.

Lastly, how well do our laboratory estimates of toxicity translate to the field? A variety of factors confound the direct extrapolation of lab results to field situations (Table I). In addition, upon entering a receiving water, a chemical exerts an affect on the aquatic system, but in turn the system has an affect on the chemical, potentially enhancing or ameliorating its harmful effects. The literature is replete with examples of physical, chemical and biological parameters that demonstrablly affect the bioavailability/toxicity of a variety of chemicals,

TABLE I

DIFFICULTIES ASSOCIATED WITH EXTRAPOLATING LABORATORY TOXICITY DATA TO THE FIELD

VARIABLE	LABORATORY	ENVIRONMENT
Type of toxicant	single/known	multiple/unknown
Toxicant concentration	constant	variable/intermittent
Exposure	single toxicant	multiple toxicants
Interspecific competition	absent	present
Disease/parasites	absent	present
Population density	extreme/controlled	variable
Space	constrained	adequate/unconstrained
Temperature	constant	fluctuating
Structure	glass/impoverished	plants/stones/cover

especially metals (Sprague, 1985). Since toxicity experiments are generally conducted in "clean" laboratory water where the potential for interactions that reduce toxicity are minimal coupled with the fact that sensitive species may be overrepresented in the national toxicity data bases, it is believed that the national criteria derived via the aforementioned procedures tend to be rather conservative estimates of environmentally safe concentrations.

Use Attainability and Site Specific Criteria

Presently, most of the nation's point source dischargers have already, or will in the near future, come into compliance with technology based effluent controls. Consequently, USEPA has advised states, which are required by section 303 of the FWPCA to revise their water quality standards every 3 years, to focus re-evaluations on water quality-limited segments rather than review all waters statewide. Such a strategy directs attention to receiving systems where water quality standards are not being met yet. Costs for going beyond best available technology are prohibitive. In an attempt to aid states in establishing water quality standards under these circumstances, the agency has provided guidance in the form of a revised Water Quality Standards Regulation (Federal Register, 1983). The new regulations encourage states to assess use attainability and develop site-specific criteria.

In the past, states based water quality standards on a designated use providing for the protection of aquatic life. Although consistent with the Act's interim goal, the actual attainability of this designated use was not examined. In certain cases, other non-water quality factors (i.e., flow, temperature, physical habitat, etc.) may preclude the attainment of uses despite improvements in water quality achieved through pollution abatement measures. Therefore, USEPA is advocating an assessment of the physical, chemical, and biological site-specific factors that influence the suitability of a given water for a particular use. Specifically a use attainability analysis is designed to aid states in answering: (1) what use(s) should be protected? (2) to what extent does pollution contribute to the impairment of the use(s)? (3) what are the levels of point source control necessary to restore, enhance or maintain the use(s) and (4) what is the level of nonpoint source pollution necessary to restore maintain or enhance the use(s)? (Gostomski, 1985). In addition, cost-benefit considerations are to be included in deciding whether a specific designated use is indeed appropriate for the receiving water in question. Using this information, states could identify unrealistic or unachievable uses thereby obviating false expectations for water quality improvements following the implementation of costly advanced treatment technologies. Technical guidance for conducting use attainability analyses has been provided by the Agency (USEPA, 1983b, 1984a, b).

The second aspect of the new regulations encourages states to develop site-specific criteria to reflect local conditions. Site-specific criteria aimed at protecting aquatic life may deviate from national criteria as a result of differences in the sensitivities of local aquatic communities, as well as differences in the capacity of natural waters to mitigate toxicant effects (Federal Register, 1983). USEPA has proposed three methods for establishing site-specific criteria based on the above two considerations, either individually or in combination, depending on prior information concerning the fate of the chemical of interest and the nature of the receiving system, as well as the availability of time and resources (USEPA, 1983).

The first alternative is the recalculation procedure which simply involves recalculating the criteria after omitting data for non-resident species that are included in the national data base. If elimination of data for unrepresentative local species prohibits attainment of the minimum data requirements, further acute toxicity testing in laboratory water with resident species is necessary. This approach is recommended where differences in sensitivities to a given toxicant are anticipated between organisms in the local community versus those comprising the national data base. For chemicals in which environmental variables are known or expected to alter bioavailability and differences in resident species sensitivity is thought to be insignificant, USEPA suggests utilizing the indicator species procedure. In this method, parallel acute toxicity tests using both laboratory and site dilution water are compared statistically for one, either resident or non-resident, fish and invertebrate species. If the LC-50's derived from these bioassays are significantly different then a water effects ratio is calculated as follows:

$$\text{Water Effects Ratio} = \frac{\text{Site Water LC-50}}{\text{Lab Water LC-50}}$$

Multiplication of this ratio with the national maximum concentration yields the site-specific Criterion Maximum Concentration. A Site-Specific Chronic Value which is used to calculate the site-specific Criterion Continuous Concentration is obtained by either direct calculation employing the national acute-chronic ratio or by additional toxicity testing involving chronic studies. The indicator species procedure would therefore provide a relatively simple methodology for normalizing water quality variables by using surrogate aquatic organisms to indicate the differences in toxicological effects that would be expected when extrapolating between laboratory and natural site waters.

The last prescribed alternative presented to states for site-specific criterion development is the resident species procedure. This approach integrates both differences in the sensitivity range of local aquatic communities and alterations in the biological availability/toxicity due to site-water- toxicant interactions. It entails redeveloping the minimum acute toxicity data set (i.e., testing organisms from eight different families) in site-water using representative, indigenous species. This information is then used to calculate a Site-Specific Final Acute Value which, when divided by two, yields the site-specific Criterion Maximum Concentration. The Criterion Continuous Concentration, as in the indicator species procedure, is either calculated using the national acute-chronic ratio or is experimentally derived through further chronic testing in site-water. The resident species method confers the greatest level of environmental realism and would be particularly useful in situations where prior exposure to elevated levels of toxicants has resulted in an acclimated community that is more resistant than would be predicted from laboratory toxicity data. However, the primary drawback of this procedure is the resource expenditures of time and money required. The three methods just outlined have been applied and have been found to be workable and to yield reasonable results (Sphear and Carlson, 1984; Carlson and Roush, 1985; Heath and Arthur, 1985). The assumptions and limitations of the three techniques for deriving site specific water quality criteria have been discussed (Utility Water Act Group, 1983; USEPA, 1983; American Petroleum Institute, 1983; Patten, 1984).

USEPA anticipates that by ensuring designated uses are realistic through use attainability analyzes, coupled with the development of site-specific criteria that optimizes the system's waste processing potential, states can establish enforceable water quality standards which will preserve the biological integrity of the nation's waters without the costs of unreasonable and scientifically unjustifiable pollution control restraints.

Water Quality-Based Toxics Control Policy

Although USEPA's Revised Water Quality Standard Regulation provides the necessary mechanisms to protect our aquatic resources from specific toxic constituents this aim may not be achieved in practice since (1) water quality standards apply to only single chemicals and cannot account for the combined effect of multiple toxicant exposures and (2) it may be impossible to identify and quantify all potentially toxic substances. In response to these difficulties USEPA has issued "A Policy for the Development of Water Quality Based Permit Limitations for Toxic Pollutants" (Federal Register, 1984b; Wise, 1985). The new policy calls for an integrated approach involving the regulation of both specific pollutants as well as whole effluent toxicity.

The whole effluent approach utilizes the principles of hazard assessment in which estimates of effluent acute toxicity are compared to effluent exposures that are anticipated, based on receiving water dilution. If the effluent's effects level exceeds the predicted exposure by a wide margin of safety then potential adverse effects are assumed minimal and additional toxicity data need not be collected. However, if the margin of safety is not acceptable, further tiers of testing are initiated so that the margin of safety can be more narrowly defined or until the specific problem can be identified. The technical aspects of this policy and guidance for its implementation are detailed in the Technical Support Document for Water Quality Based Toxics Control (USEPA, 1985c).

Kimerle et al. (1985) and Kimerle (1986) have suggested that a hazard assessment approach be extended to the development of water quality criteria, arguing that the $200,000 to $400,000 required to generate the data base necessary to develop a water quality criterion is not justified in cases where environmental exposure data is far below effects levels obtained from toxicity tests performed on a few commonly tested aquatic species. They propose that the magnitude of this safety margin be used as the criterion for judging the extent of the toxicity data base needed rather than the current practice of setting inflexible minimum data requirements before a criterion can be prescribed. They also point out that due to the large data base required under the current approach, development of water quality criteria for toxic chemicals has been greatly impeded and, in fact, definitive national criteria have been issued for only 17 out of the 65 priority toxic pollutants. In contrast, by applying hazard assessment concepts, chemicals whose exposures are posing the greatest threat to water quality can be identified and further data collection focused on these problem chemicals. It would appear that the current methodology for establishing national criteria will evolve to include features inherent in the hazard assessment approach.

The information provided in the foregoing introduction has been included in an attempt to provide an overview of USEPA's current strategy for regulating toxic water pollutants and how site-specific criteria fit into this regulatory framework. In water quality limited segments, pollutant-specific techniques (including site-specific criteria) are recommended if discharges contain a few well quantified pollutants where the interactions and effects of the pollutants are known and where human health hazards are a concern or toxic chemical bioaccumulation is expected. Whole effluent techniques are advocated in cases where effluents are complex or where multiple effluent discharges occur. Since these techniques are complimentary both approaches may be used in many cases (Wise, 1985).

CHAPTER II

LITERATURE REVIEW OF ZINC IN THE AQUATIC ENVIRONMENT

Uses and Sources of Zinc

Zinc is a flush-white heavy metal with an atomic weight of 65.37. It occurs in a single divalent oxidation state and is considered intermediate between hard and soft acid acceptors with respect to interaction with ligands. Consequently, zinc complexes with both hard (oxygen donors) bases and soft (sulphur donors) bases so that both carbonate and sulfide ores of this metal are found in nature (Moore and Ramamoorthy, 1984). Zinc has several distinguishing properties. When heated, zinc becomes ductile and easily workable. In addition, zinc is highly electrochemically active. Zinc also combines with a variety of other metals to form an array of alloys with specific desirable properties. The primary categories of zinc uses are galvanizing, brass and bronze products, castings, rolled zinc and zinc based chemicals, pigments and dusts (Cammarota 1980).

Galvanizing accounts for 90% of the corrosion resistant coatings on iron and steel products. These surface coatings enable sacrificial corrosion of the zinc while protecting against structural degradation of the substrate metal. Galvanized products find a wide range of applications including office equipment, appliances, industrial containers, agricultural equipment, construction materials for buildings, bridges and highways, and underbody autoparts. Galvanized sheet is the most common material used for air conditioning, heating and ventilation ducts and is often used in electrical conduits and telephone wires.

Brass, a zinc and copper alloy, and bronze, a copper, tin, and zinc alloy, find a variety of applications in architectural construction and building hardware. Brass fittings are used extensively in the plumbing, heating and auto industries. Other uses include coinage, ammunition, home appliances and jewelry. Another major use of zinc alloys is diecasts that are used in the manufacture of automobile parts such as door handles, carburetors and pumps, scientific instruments and electrical components. Rolled zinc finds use in the production of dry cell batteries.

A number of zinc chemicals are used commercially. Zinc oxide is used in the paint, rubber and chemical industries. Zinc oxide improves longevity and appearance of paints and serves as a catalyst in the vulcanization of rubber as well as in chemical syntheses involving alkylation, dehydrogenation and oxidation reactions. Zinc oxide also is used in ceramics, glasses, plastics, cements, cosmetics, lubricants, fungicides and pharmaceuticals. Zinc chloride finds application in the manufacture of rayon fabric and as a wood preservative. Organozinc compounds serve as polymerization catalysts (National Academy of Sciences, 1979).

Clearly zinc provides a host of beneficial uses to society and ranks fourth among the metals in world consumption. Canada is currently the largest producer of zinc, but substantial reserves are found in the United States, Australia and the Soviet Union. Understanding the use patterns of a chemical are prerequisite in assessing the hazard that a chemical poses to the environment. Knowledge of how a chemical enters an aquatic system is necessary since its route of introduction may influence its fate and ultimate effect as well as dictate abatement measures to be initiated, i.e., point vs. non-point source controls. Natural geological weathering of zinc containing minerals typically gives rise to zinc levels in surface waters in the range from 0.5 to 15 ug/l but concentrations may be much higher due to anthropogenic inputs (Moore and Ramamoorthy, 1984). Anthropogenic inputs may be broadly grouped into point source discharges, atmospheric deposition, land runoff and groundwater intrusion.

Many industrial effluents contain significant quantities of zinc. Metal refining, metal finishing and certain organic chemical industries provide effluents with high zinc concentrations typically ranging from 2-30 mg/l (Patterson and Mineau, 1975). However, tertiary treatment usually involving precipitation with lime may achieve final concentrations from 0.1 to 2.5 mg/l (National Academy of Sciences, 1979). Table II illustrates zinc concentrations in a variety of industrial wastewaters. Zinc concentrations are often the highest among the trace elements found in municipal wastewaters. One study based on 58 sewage treatment plants in Michigan found effluent concentrations ranging from 0.01 to 4.70 mg/l with most below 1.00 mg/l (Blakeslee, 1973).

An important distinction can be made between industrial and municipal effluents. Zinc occurs predominantly in dissolved form in industrial effluents whereas zinc is usually absorbed to microbial solids in municipal effluents (Wilbur et al., 1980). Consequently, zinc discharged to a municipal sewage treatment plants will most likely be removed from the water column as the microbial solids are sedimented in the final clarifiers. McDermott et al. (1962) found that primary treatment was ineffective at removing dissolved zinc (14%) but the activated sludge process was highly effective removing 74 to 95% of the zinc from the

TABLE II

ZINC CONCENTRATIONS (UG/L) IN INDUSTRIAL AND DOMESTIC WASTEWATERS

Source	Concentration
Meat Processing	460
Fat Rendering	3,890
Fish Processing	1,590
Bakery	280
Miscellaneous Foods	1,100
Brewery	470
Soft Drinks and Flavorings	2,990
Textile Dying	500
Fur Dressing and Dying	1,730
Miscellanous Chemicals	800
Laundry	1,750
Car Wash	920
Mining Operations[b]	8,500
Metal Finishing/Galvanizing	200-88,000
Pickling Dipping	10,000-35,000
Vulcanized Fiber	200,000-300,000
Primary Treated Municipal Effluent[c]	260-1,130
Secondary Treated Municipal Effluent[c]	100-1,300

[a] After Forestner and Wittman (1979) unless otherwise indicated.

[b] National Academy of Sciences (1979)

[c] Wilbur et al. (1980)

waste stream. Therefore, it is not surprising that elevated zinc levels have been reported for digested sewage sludges ranging from 500 to 50,000 mg/l (Chaney, 1973). In contrast, zinc levels in uncontaminated soils generally range from 10 to 300 ppm with a median value of about 80 ppm (National Academy of Sciences 1979).

The reduction of dissolved zinc in both municipal and industrial wastewaters afforded by incorporation into solid wastes may be of ephemeral benefit to aquatic systems if these wastes are then incinerated, releasing zinc into the atmosphere. Although limited data are available, zinc loadings contributed by both wet and dry deposition to natural waters appear considerable (Nriagru and Davidson, 1980). Beside waste incineration, smelting, wood, oil and coal combustion and wearing of automobile tires constitute significant sources of atmospheric zinc. As zinc is deposited as fallout on urban environments it can be washed into drainage systems and ultimately to receiving waters as stormwater runoff. In a comprehensive study, Wilber et al. (1980) calculated that 86% of the total annual load of zinc to an urban stream was attributable to stormwater runoff. Furthermore, approximately 70% of the zinc in the stormwater was associated with either the nonsettleable (>0.75 microns) or settleable solids fraction. Nienke (1977) reviewed atmospheric inputs of zinc to Lake Michigan and concluded that both dry fallout and rainfall exceeds that of tributary input. In another study, Peyton et al. (1976) found that aerial zinc may account for greater than 50% of the total zinc deposited in receiving waters.

Another important source of zinc contamination as well as a variety of other toxic metals to aquatic systems is acid mine drainage (Weatherly et al., 1979). Unearthed mine wastes exposed to atmospheric oxygen and moisture provide conditions favorable for growth of chemoautotrophic bacteria which oxidize ferrous iron to ferric iron which then initiates a series of reactions that dissolve ZnS containing ores with concomitant production of sulfuric acid. Zinc may also enter natural waters through groundwater seepage due to damaged or improperly constructed sewers or landfills that have not been properly sealed.

Aquatic Chemistry of Zinc: Fate and Transport

Metals released into aquatic systems undergo a variety of environmental fate and physical transport processes. These processes collectively determine the qualitative and quantitative distribution of metals in the environment. As Figure 2 illustrates, an aquatic system can be conceptualized as four primary abiotic compartments with which metals can associate: surface water, suspended

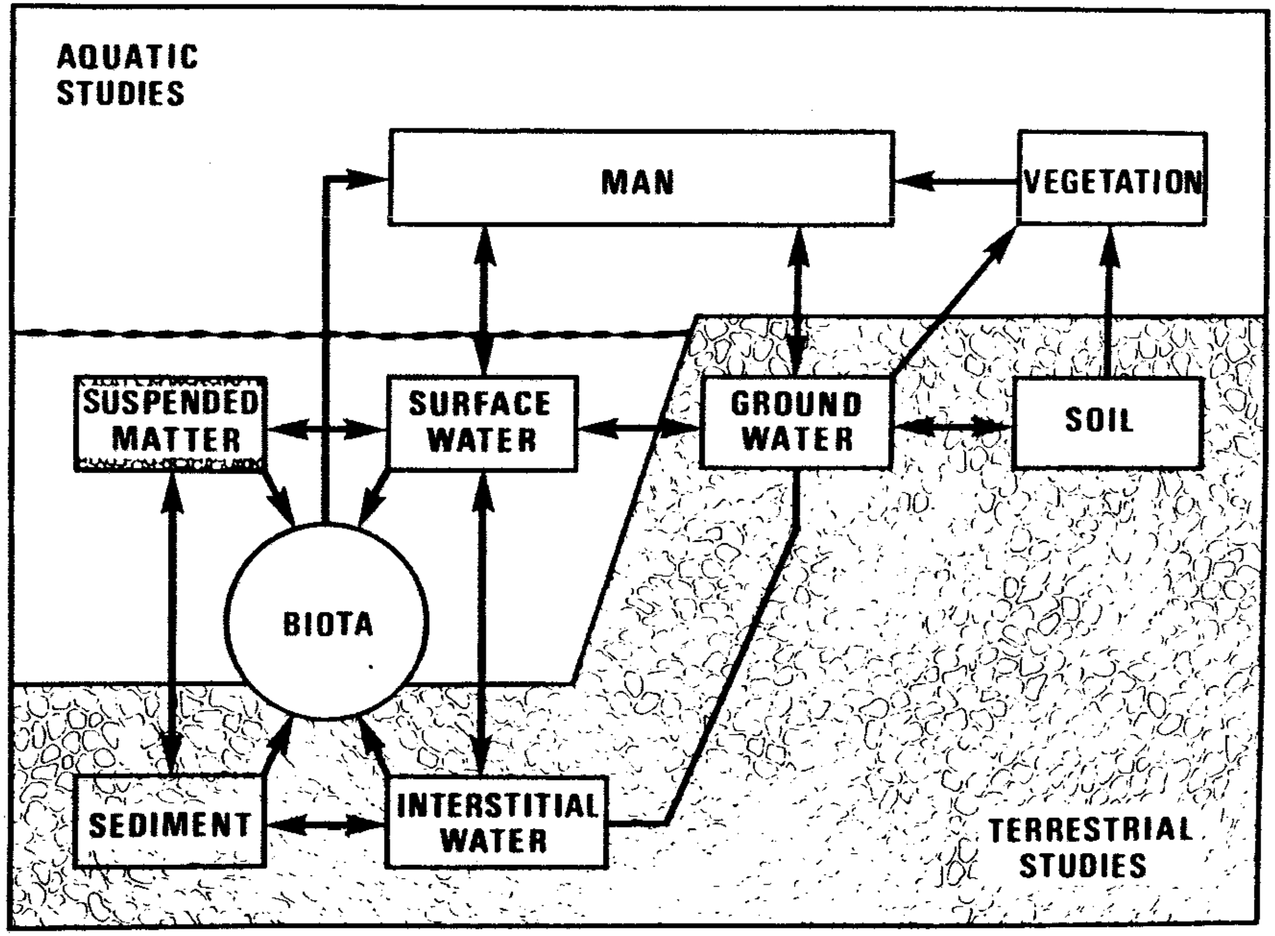

FIGURE 2 Interaction of Metals in the Environment from Salmons and Forestner (1984)

matter, sediment and interstitial waters. These compartments interact in response to environmental processes. For example, metal dissolved in the water column interacts with suspended material through sorption/desorption and precipitation reactions. The suspended material and sediment interact through sedimentation and resuspension. Diagenic changes that may occur in sediments over time may result in the uptake of or release of metals in interstitial waters. Likewise, interstitial waters interact with overlying waters through processes such as diffusion and bioturbation. The aquatic system is in turn influenced by its surrounding watershed through surface runoff, geology topography and other edaphic factors as described by Hynes' (1970). Metals in each of the various aquatic compartments impact aquatic life and, either directly or indirectly, man. Predicting the dynamic partitioning of metals among the various interacting compartments and quantifying the impact that these various metal reservoirs exert is the research challenge now being faced (Salomons and Forestner, 1984).

Unlike many xenobiotic chemicals, metals are usually conservative and in general are not directly affected by such processes as volatilization, photolysis and biodegradation. Rather the primary mechanism controlling the fate of metals is speciation.

A variety of environmental variables determine the nature and extent of the metal species formed. Vuceta and Morgan (1978) have discussed some of the more important variables: the specific metal of interest and its corresponding concentration, the nature and concentration of solids that provide surface binding sites, pH, the types and concentrations of both organic and inorganic ligands. These factors competitively interact so that the metal is partitioned into a variety of different forms and the system proceeds towards minimum free energy, i.e., thermodynamic equilibrium (Stumm and Morgan, 1980). Figure 3 illustrates metal speciation in the aquatic environment.

Adsorption is reported to be the most important fate process affecting zinc speciation in the aquatic environment (Hem, 1972; USEPA, 1979). Adsorption is a surface phenomena whereby soluble atoms or molecules are extracted from the aqueous phase and are accumulated at the surface of a solid phase. The electronic structure, small size and ionic charge of zinc all contribute its strong affinity to solids (Shuman, 1980). Bonding mechanisms between absorbate and absorbent vary in strength and may be physical, electrostatic or chemical in nature (Huang, 1980). Physical adsorption occurs as a result of Vander Waals Forces, i.e.,

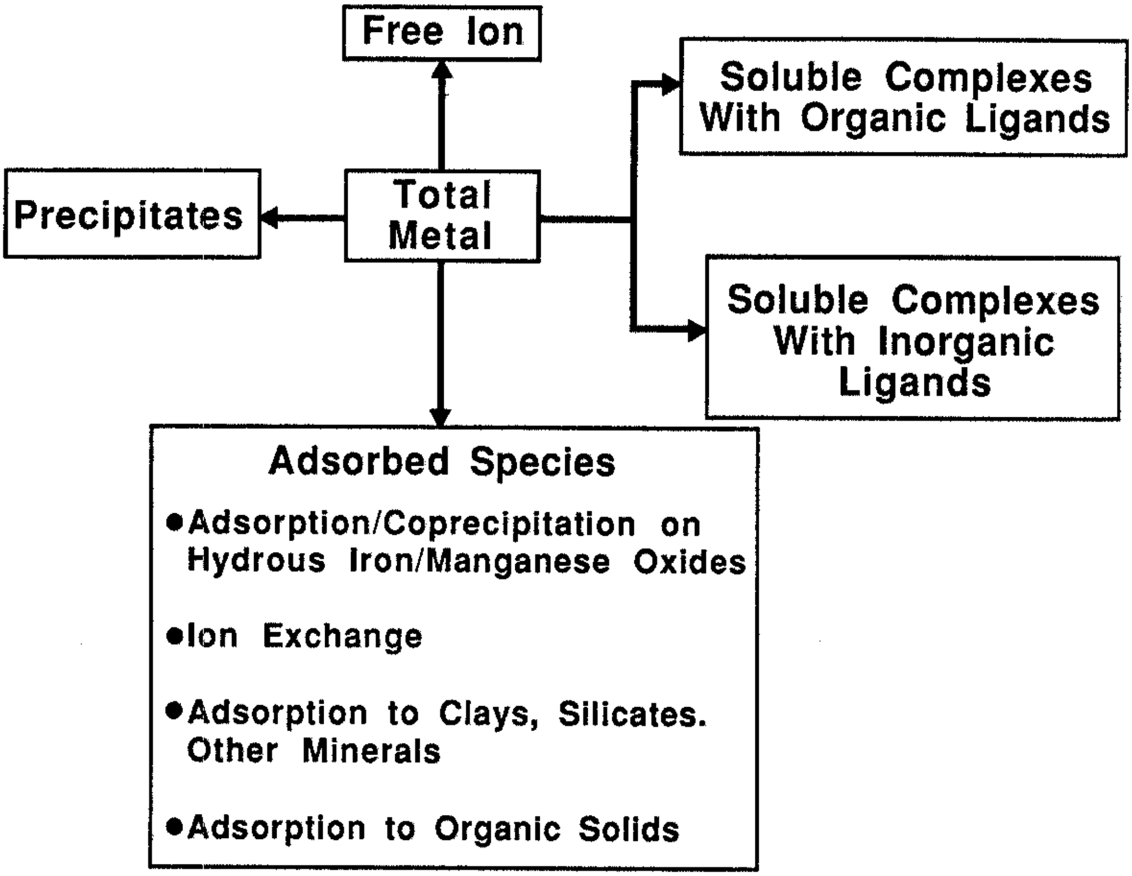

FIGURE 3--Metal Speciation in the Aquatic Environment

ion-dipole, dipole-dipole interactions and the absorbate is bound relatively weakly. Electrostatic adsorption results due to the attractive forces between oppositely charged species. Typically, most natural solids are negatively charged explaining their affinity for cations such as zinc. Unlike electrostatic adsorption, which is considered non-specific and reversible in nature, chemical adsorption or specific adsorption is usually irreversible and involves the formation of relatively strong covalent bonds. For example, hydrous oxides of AL, Fe and Mn adsorb zinc ions via this mechanism whereby zinc is bonded by covalent bonds via surface O and OH groups to the structural cations (Huang, 1980; Salomons and Forestner, 1984).

Adsorption processes are usually described by plotting the mass of chemical adsorbed per unit mass of solid versus the concentration of the material remaining in solution at constant temperature. If the resulting plot describes a rectangular hyperbola where the concentration of chemical on the solid phase asymptotically levels off as surface binding sites become saturated, the Langmiur isotherm equation is used. If however, an absorption maximum is not apparent and the plot is parabolic in nature, the Freundlich isotherm equation is used. A simplified method for characterizing adsorption phenomena involves calculating the slope or partition coefficient (kp) for the linear portion of the experimental adsorption isotherm. The partition coefficient represents the ratio of sorbed to soluble chemical on mass terms and is expressed in units l/kg (O'Connor and Connoly, 1980; Dickson et al., 1981). Table III summarizes zinc partition coefficients for a variety of solid phases. As indicated in this table equilibration has been shown or is assumed to occur rather quickly, ie., usually within 24 hours.

Hydrogen ion concentration is one of the most important factors controlling the extent of metal adsorption. At low pH, hydrogen ions protonate the surface of solids thereby outcompeting heavy metals cations while at higher pH's deprotonation of these surface sites augments interphasial metal reactions (Huang et al., 1977; Davis and Leckie, 1978). A characteristic narrow pH region has been demonstrated for a variety of heavy metals in which adsorption is enhanced markedly. For zinc this range occurs between pH 5.0 and 7.0 and is generally insensitive to the type of adsorbent (Huang et al., 1977; James and McNaughton, 1977). Ros Vincent et al. (1977) found that 33% of the adsorbed zinc was mobilized from marine sediment when treated with a pH 5.4 buffer. Kalabasi et al. (1978) in their study of zinc adsorption to Fe O and Al O found that

TABLE III

ZINC ADSORPTION STUDIES

Type of Solids	Solids Concentration (mg/L)	Equilibrium Concentration of Zinc (mg/L)	Equilibration Time (hrs)	pH	Partition Coefficient (L/kg)	Reference
Kaolinite	2,500	2.0	1	7.35	8.6×10^2	Bourg and Filby (1974)
Montmorillonite	2,500	2.0	1	7.35	7.4×10^2	
Illite	2,500	2.0	1	7.35	3.3×10^2	
Eight Natural Solids	20,000	<8.0	20	5.27-6.67	1.5×10^2 to 3.8×10^2	Shuman (1975)
Al_2O_3, SiO_2 and two soils	5,000	65.0	24	5.00	2.5×10^0 to 4.2×10^1	Huang et. al. (1977)
				6.00	7.5×10^1 to 1.8×10^2	
				7.00	8.6×10^2 to 3.8×10^3	
				8.00	$>9.8 \times 10^3$	
SiO_2	20,000	6.5	24	5.00	$< 1 \times 10^0$	James and McNaughton (1977)
				6.00	6.8×10^1	
				7.00	3.3×10^2	
MnOx	2,100	6.5	24	5.00	6.8×10^1	
				6.0	3.2×10^3	
				7.00	2.3×10^4	
FeOOH	2,000	32.5	24	5.00	1.0×10^1	
				6.00	5.6×10^1	
				7.00	6.2×10^3	

TABLE III --Continued

Type of Solids	Solids Concentration (mg/L)	Equilibrium Concentration of Zinc (mg/L)	Equilibration Time (hrs)	pH	Partition Coefficient (L/kg)	Reference
Al_2O_3	5,880	6.5	24	5.00 6.00 7.00	$<1.0 \times 10^0$ 2.0×10^0 9.8×10^2	James and McNaughton (1977)
Suspended Solids						
River	25	-	-	-	1.3×10^5	Grieve & Fletcher (1977)
Estuary	4-9	-	-	-	1.0×10^5	
Al_2O_3	4,000	80.0	24	6.42 6.70 6.92	3.3×10^1 1.0×10^2 3.8×10^3	Kalbasi et.al. (1978)
Fe_2O_3	4,000	80.0	24	5.93 6.66 6.96	6.5×10^1 2.6×10^2 4.2×10^3	
Illite + Humic Acid	267 - 400 100	8.6	24	6.00	1.8×10^4	Hatton & Pickering (1980)
Kaolinite + Humic Acid	433 - 500	8.6	24	6.00	6.8×10^3	
am - $Fe(OH)_3$	100	2.6	-	6.00 6.50 7.00	1.1×10^3 6.3×10^3 3.8×10^4	Leckie, et.al. (1980)
- Al_2O_3	1,000	2.6	-	6.00 6.50	2.0×10^2 6.0×10^2	

TABLE III --Continued

Type of Solids	Solids Concentration (mg/L)	Equilibrium Concentration of Zinc (mg/L)	Equilibration Time (hrs)	pH	Partition Coefficient (L/kg)	Reference
SiO_2	30,000	2.6	-	7.00 7.50 6.50 7.00 7.50	3.0×10^3 9.0×10^3 5.9×10^0 3.3×10^1 1.3×10^2	Leckie, et.al. (1980)
Montmorillonite and Two Lake	100 100 750 750	1.32 11.05 1.32 11.05	96 96 96 96	7.7 - 7.9 7.2 - 7.5 7.7 - 7.9 7.2 - 7.5	1.0×10^4 to 1.4×10^4 5.5×10^3 to 1.6×10^4 1.6×10^3 to 2.3×10^3 5.7×10^3 to 7.8×10^3	Dickson, et.al. (1981)
Lake Sediment	40,000	.017 - .068	1	7.80	1.7×10^2 to 3.3×10^2	Neinke & Lee (1982)
Lake Suspended Solids	1 - 100	-	-	-	5.0×10^4 to 5.0×10^6	Rygwelski et.al. (1984)
River Suspended Solids	250	.05	24	5.00 6.00 7.00 8.00	1.1×10^3 2.7×10^3 3.6×10^4 7.6×10^4	McIlroy et.al. (1986)
		2.00	24	5.00 6.00 7.00 8.00	4.0×10^2 7.6×10^2 3.8×10^3 2.1×10^4	
Clay	200,000	.03 - 6.5	48	6.70	6.8×10^2	Reddy & Dunn (1986)
Loam	200,000	.03 - 6.5	48	6.80	6.5×10^2	
Sandy Loam	200,000	.03 - 6.5	48	5.67	1.4×10^2	

60-90% of the zinc was specifically adsorbed while 10-40% was adsorbed via a non-specific mechanism. The ratio of specific to non-specific adsorption was shown to increase with increasing pH.

The nature of solids has also been shown to demonstrably affect adsorption. Guy and Chakarbarti (1975) found that different solids had varying adsorptive capacities and proposed the following sequence in order of decreasing heavy metal affinity: manganese oxides > humic acids > iron oxides > clay minerals. Among the clay minerals, zinc adsorption is strongly influenced by clay type and generally follows the order bentonite > montmorillionite > illite > kaolinite (Bourg and Filby, 1974; Reddy and Perkins, 1974; Farrah and Pickering, 1977). This sequence reflects corresponding reductions in cation exchange capacity (CEC) and surface area and increases in particle size. Particle size has been inversely correlated with metal concentrations in suspended sediments (Gibbs, 1977). Ferrous and manganese oxides may occur as discrete particles or as surface coatings on clay minerals. These oxides have a high affinity for metals and may play an important role in reducing aqueous phase zinc through either adsorption or co-precipitation mechanisms (Lee, 1975; Grieve and Fletcher, 1977; Bradley and Lewin, 1982). Naturally occurring organic materials in lake and river sediments have been correlated with zinc adsorption and accumulation (Shimp et al. 1971; Pita and Hyne 1975; Tada and Suzuki 1982). The presence of such organic materials typically leads to an increase in the CEC of solid substrates. Studies have also shown that the organic component of solids tends to bind zinc more tightly than inorganic bonding sites (Shuman 1980; Tada and Suzuki 1982).

O'Connor and Connolly (1980) observed an inverse relationship between partition coefficients and solids concentration for a wide range of compounds. Voice et al. (1983) rationalized these observations by suggesting that higher solid concentrations contribute higher levels of organic material to the aqueous phase through desorption processes than low solid concentrations. Elevated levels of desorbed material, coined the third phase, could then react with and thus stabilize aqueous forms, thereby explaining the reductions in partition coefficients observed at high solid concentrations. However, DiToro et al. (1985) conducted experiments with organic free glass spheres and found the same phenomena. Therefore, they postulated that increased particle interaction at high solid levels reduced the quantity of available binding sites or that the strength of the binding sites was reduced leading to increased desorption. This adsorbent mass effect for zinc is apparent from Table III.

A variety of other factors may influence adsorption processes as well. For example, adsorption is theoretically inversely proportional to temperature. O'Shea and Mancy (1978) reported adsorption to be an inverse function of water hardness. As hardness increased calcium and magnesium ions may outcompete heavy metals for surface sites. Likewise, solution equilibria, as discussed below, that govern the specific forms in which aqueous metal exist, affects metal partitioning.

Zinc in solution may form complexes with many inorganic ligands commonly found in natural waters. However, the relatively low concentrations at which these ligands usually occur and their correspondingly weak stability constants limit the number of aqueous forms found at significant levels. Thus, only a few dominant aqueous species constitute the majority of total aqueous metal. Below pH 7.0, the divalent hydrated cation is the predominant form. As pH increases zinc carbonate complexes are formed. Above pH 8.0, aqueous zinc occurs primarily as hydroxide species (Hem, 1972). The changes in aqueous forms associated with increasing pH are directly related to the increasing concentrations of complexing carbonate and hydroxide ligands. In general, complexation with inorganic ligands other than the hydroxide ion reduces metal adsorption (Jenne and Zachara, 1984).

Dissolved organic material may also complex heavy metals. Humic substances usually constitute the largest fraction of dissolved organic carbon and have provided the main focus in investigations on natural water metal-organic interactions (Reuter and Perdue, 1977). Metal organic complexation in natural water may be described using conditional equilibrium constants that are derived by metal titration experiments. The various methods used to calculate conditional equilibrium constants have recently been reviewed (Neubecker and Allen, 1983). Mantoura et al. (1978) report conditional stability constants (Log k) for zinc to range from 5.05-5.31 for Lakes, 5.36-5.41 for rivers and 4.99-5.87 for sediments. Generally, increased organic complexation of metals reduces adsorption by stabilizing aqueous forms (Vuceta and Morgan, 1978; Voice et al., 1983). Hatton and Pickering (1980) found a competition between the removal of zinc from solution by clays and the formation of soluble metal humates. In contrast, Huang et al. (1977) demonstrated that zinc adsorption in the presence of several organic anions including humic acid augmented adsorption. Apparently, either the anions were first adsorbed to solids and then scavenged zinc from solution or direct adsorption of zinc organic complexes occurred. Others have suggested that metal organic complexes may adsorb readily to solid surfaces (Davis and Leckie, 1978; Mouvet and Bourg, 1983). The role that organic ligands play in altering metal speciation is still not well understood but nevertheless is thought to be important. A summary of relevant environmental variables that have been reported to affect zinc adsorption is provided in Table IV while Figure 4 summarizes major processes and mechanisms of metal adsorption in aquatic environments.

Precipitation reactions provide another mechanism by which metals can be removed from solution. Under aerobic conditions zinc may precipitate as the hydroxide or carbonate. However, O'Connor et al. (1964) and Hem (1972) compared calculated solubilities with actual zinc measurements from numerous U.S. Rivers and concluded that zinc was usually well undersaturated with respect to either of these solids. Hem (1972) suggests that zinc silicate has very low solubility and may exert greater control over zinc solubility in natural surface and ground water. In spite of this fact, adsorption rather than precipitation is expected to be the most important control limiting solubility (USEPA, 1979). This may not be true in the case of toxicity

TABLE IV

FACTORS AFFECTING ZINC ADSORPTION INDICATING THE DIRECTION IN WHICH INCREASES IN EACH PARAMETER TYPICALLY INFLUENCES ADSORPTION

Parameter	Affect on Zinc Adsorption	Reference
pH	Increase	Huang et.al. (1977) James & McNaughton (1977) Kalbasi et. al. (1978)
Fe/Mn Oxides	Increase	Grieve & Fletcher (1977) Bradley & Lewin (1982)
Particulate Organic Matter	Increase	Pita & Hyne (1975) Tada & Suzuki (1982) Watanabe et. al. (1985)
Particle Size	Decrease	Forestner & Wittman (1979) Tada & Suzuki (1982)
Cation Exchange Capacity	Increase	Shuman (1976) Reddy & Dunn (1986)
Anions	Decrease	Huang et. al. (1977)
Competing Cations	Decrease	O'shea & Mancy (1978) Jenne (1984)
Salinity	Decrease	Grieve & Fletcher (1977) Leckie et. al. (1980)
Dissolved Organics	Decrease	Huang et. al. (1977) Hatton & Pickering (1980)
Solids Concentration	Decrease	Rygwelski et. al. (1984) McIlroy et. al. (1986)

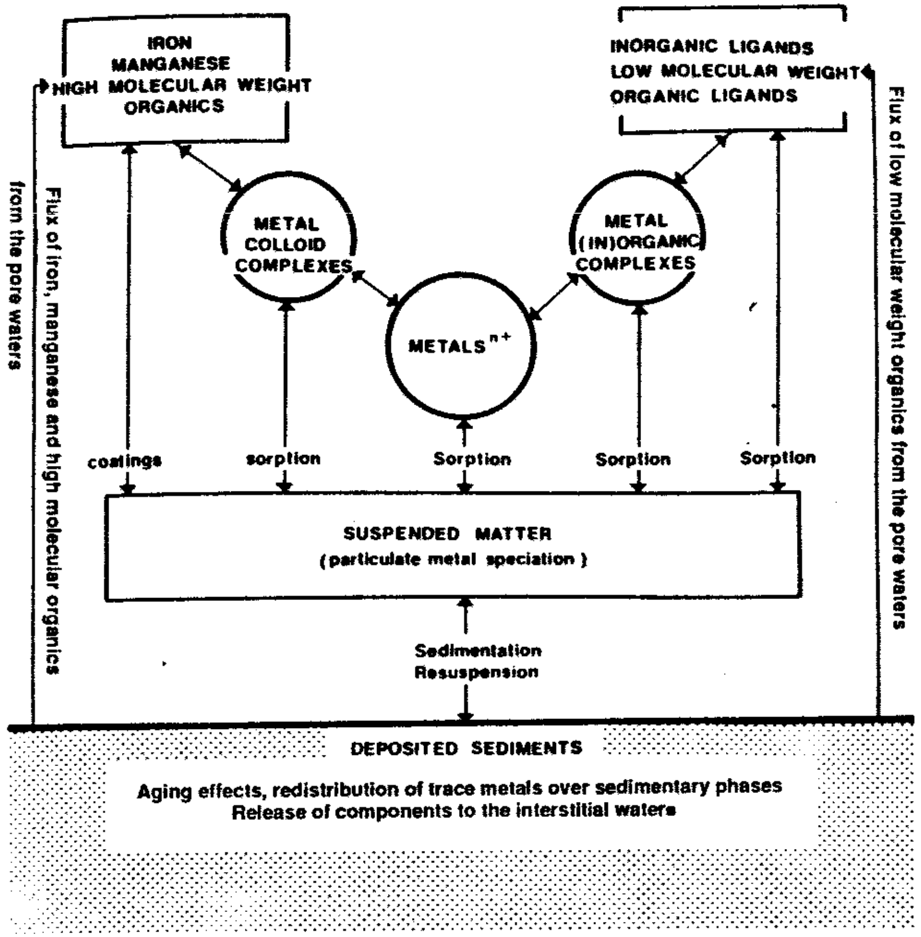

FIGURE 4 Processes and Mechanisms Influencing the Distribution of Metal Species Between Dissolved and Solid Phases from Salmons and Forestner (1984).

experiments where high levels of zinc must be used to elicit an organism response. Zinc solubility curves were prepared in a manner analogous to Hem (1972) but more recent thermodynamic data included in the MINTEQ geochemical model was used in calculations and are shown in Figure 5. These curves indicate that zinc carbonate will predominate as the solid phase except in waters with low alkalinity and extremely high pH. Under reducing conditions, as might occur in the hypolimnion of a lake during stratification or in a sewer, zinc may be precipitated as the sulfide. At pH 7 zinc solubility is less than 100 ug/l, 10 ug/l and 1 ug/l at hydrogen sulfide concentrations of 0.01 mg/l, 0.10 mg/l and 10.00 mg/l, respectively (Wilber et al., 1980).

Efforts aimed at quantifying metal speciation in aquatic environments can be broadly divided into two basic approaches. These approaches, as they apply to the determination of zinc speciation, have been reviewed by Florence (1980). The first approach involves direct measurement of various species using appropriate analytical methodologies. This can range from simple procedures, such as sample filtration to differentiate soluble from particulate forms, to advanced speciation schemes, such as proposed by Bately and Florence (1976) using Anode Stripping Voltammetry, which differentiates metals into eight

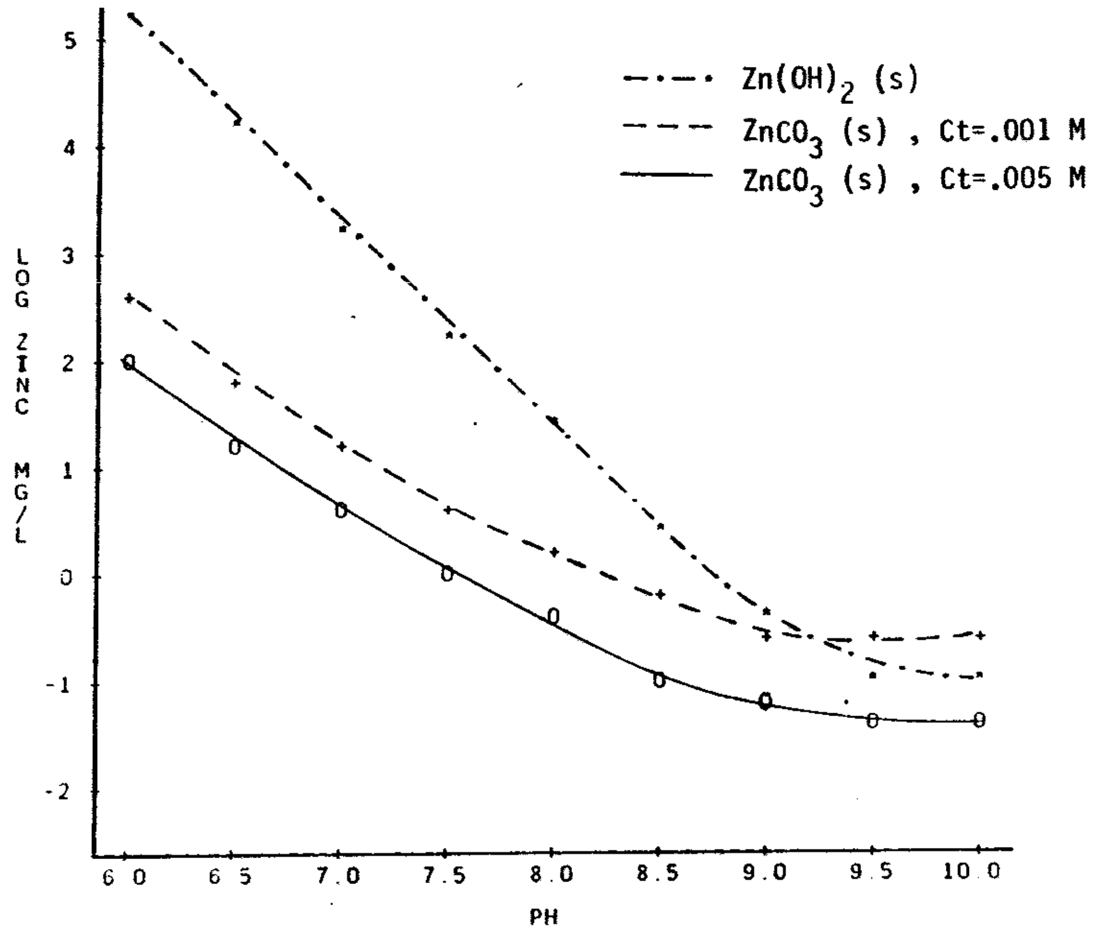

FIGURE 5 Solubility of Zinc Hydroxide and Zinc Carbonate as a Function of pH at Ionic Strength=0 and Temperature=25 C. Ct Refers to Total Inorganic Carbon Species and Corresponds to an Alkalinity of 50 and 250 as CaCO3 at pH=8.3 for Ct=.001 and .005 M, Respectively.

fractions. For instance, on the average, 40% of the zinc measured in polluted U.S. rivers was found to be in a particulate form (Kopp and Kroner, 1968) which compares closely to the 45% value provided by Salmons and Forestner for (1984) polluted rivers in Germany. Approximately 90% of the zinc in the Mississippi River was found to be in the particulate fraction (Trefry and Presley, 1976). Kubota et al. (1974) reported that 40% to 90% of the zinc in 12 New York streams was transported in a non-filterable form. Hart and Davies (1981) applied a speciation scheme to an Australian river and found that about one-third of the zinc was in a soluble, non-ion exchangeable fraction and the remaining two-thirds in a soluble, ion-exchangeable form, 80% of which was dialysable. Speciation of metals associated with solid materials is usually delineated with the aid of selective extractants. Solomons and Forestner, (1980), in their study of metal speciation in 8 river sediments, found that much of the anthropogenic zinc was associated with reducible sediment phases.

Due to the unavailability of conventional analytical methods that differentiate individual metal species, model predictions based on thermodynamic data provide a second simpler approach for estimating metal speciation. Long and Angino (1977) developed a speciation model to investigate a variety of inorganic ligand interactions. Model predictions agreed with earlier work concerning free ion dominances at low pH and the prevalence of aqueous carbonate and hydroxide species at higher pH's. Turner et al. (1981) applied an equlibrium model to two model freshwaters one at pH 6 and the other at pH 9. They found that in the pH 6 water, 98% of the zinc occurred as the free ion with the remaining 2% occurring as an aqueous $ZnSO_4$ complex. In comparison, 78% of the zinc occurred as $ZnOH^+$, 16% as $ZnCO_3$ and 6% as the free ion in the pH 9 water. Several sophisticated thermodynamic equilibrium computer models have been recently developed which predict both inorganic and organic complexation reactions as well as adsorption processes (Bourg, 1982; Felmy et al., 1983). Application of these models to natural waters has indicated that zinc may exist primarily in adsorbed forms (Mouvet and Bourg, 1983; Brown and Felmy, 1984). Speciation calculations for zinc in the Meuse River indicated that 82% of the zinc was in an adsorbed form, 12% existed as free ion, 4% existed as soluble carbonate complexes and 1% occurred as a humic acid complex. Other species constituted less than 1% of the total zinc (Mouvet and Bourg, 1983). Although equilibrium models can serve as useful predictive tools, they have a number of limitations. First, thermodynamic data are often unavailable for several important metal interactions including adsorption unto solids and colloids, complexation with dissolved organic materials and solubility of certain solids. Secondly, kinetic aspects of metal speciation are not considered (Jenne and Zachara, 1984). Moreover, these authors state that modeling attempts to predict the adsorption of metals from natural waters may result in large (perhaps orders of magnitude) errors. Even with site-specific model calibration, based on laboratory adsorption experiments, major discrepancies can result when compared to field data. This is particularly unfortunate in the case of zinc since adsorption is reported to be the dominant fate process.

The driving force that necessitates the understanding of metal speciation in the aquatic environment is its relationship to bioavailability. Various metal species differ in their toxicity or bioavailability and hence in their overall impact on receiving systems. Although current criteria and standards are based on total metal concentrations, this measurement may not be indicative of zinc that is biologically available (Florence, 1980). Thus, a system exposed to low levels of highly bioavailable species will be stressed to a far greater extent than a system receiving high levels of less bioavailable forms.

The toxicity and bioavailability of zinc will be subsequently discussed.

Aquatic Toxicity and Bioavailability of Zinc

Zinc is an essential element for both animals and plants and is the second most abundant of the trace transition elements in biological fluids exceeded only by that of iron. Zinc plays an important role in protein synthesis since it is a component of DNA and RNA polymerase. This element is reported to influence a variety of physiological processes including release of several pituitary hormones, prolongation of adrenoconticotropin and insulin activity, spermatogenesis, muscle, liver, eye and brain function (National Academy of Sciences, 1979). A number of zinc containing metalloenzymes have been identified which catalyze hydrolysis, hydration, oxidation-reduction and group transfer reactions. Zinc is a component in: carboxypeptidase which serves as a digestive enzyme; carbonic anhydrase which regulates the in-vivo carbon-dioxide bicarbonate equilibrium; alkaline phosphatase which hydrolyzes phosphate monoesters and is implicated in phosphate catabolism; alcohol dehydrogenase which is an enzyme in the degradative pathway of ethanol; superoxide dimutase which catalyzes the dismutation of the superoxide radical before it can exert a toxic effect; and aspartate transcarbamylase which is involved in pyrimidine biosynthesis. The zinc ions in these enzymes are usually coordinated to imadazole nitrogens, gamma-carboxyl groups of glutamic acid and/or the sulfhydryl groups of cysteine moities and may function either in the catalytic mechanism of the enzyme or in maintaining its structural conformation (Vallee, 1959; National Academy of Sciences, 1979). Zinc may form complexes with specific enzymes and enhance or reduce their activity and may also function in the maintenance of the structure of certain nonenzymatic proteins (Vallee, 1959).

Zinc is not particularly toxic to humans and in fact deficiencies pose more serious problems than probable over exposure. The recommended secondary drinking water standard is 5 mg/l and the recommended daily allowance is 15 mg/day for adults. In contrast, aquatic life can be quite sensitive to zinc. A large body of aquatic toxicity data has been compiled and reviewed (USEPA, 1980; Weatherly et al., 1980; USEPA, 1986). Several generalizations are apparent. First, toxicity values vary considerably between species in different phyla and species in different classes within the same phylum. Genus mean acute LC50 values range from 0.05 mg/l for Ceriodaphnia to 86.73 mg/l for Argia, the damselfly genus. Secondly, Rotifera, Crustaceans (cladocerans) and Mollusca (gastropods) are most sensitive with LC50's ranging from 0.1 ppm to several ppm. Certain fish species, notably stripped bass, longfin dace, tilapia and salmonids have also been shown to be sensitive to zinc. Furthermore, salmonids demonstrate avoidance behavior during ten minute exposures to extremely low zinc levels in the 5 to 50 ppb range (USEPA, 1986). Lastly, most aquatic insects are tolerant of zinc with LC50's often exceeding 10 mg/l. However, in the environment these organisms may be adversely affected since zinc has been shown to inhibit the colonization of allochthonous detritus by bacteria and fungi, thereby causing a reduction of food resources upon which macroinvertebrate communities depend (Weatherly et al., 1980). Although often insensitive to metals in acute laboratory toxicity tests, this community has been shown to be a sensitive biological indicator of adverse metal impacts (Lapoint et al., 1984; Van Hassel and Gaulke, 1986). This illustrates the difficulty in extrapolating laboratory toxicity results in an attempt to protect ecosystem structure while neglecting impacts on ecosystem function. Other functional processes may be sensitive to zinc. For instance, Hillman (1974) found that 0.05 mg/l of zinc was shown to inhibit photosynthesis of planktonic green algae. Similarly, Marshall et al., (1983) showed that primary productivity of plankton was retarded at levels as low as 0.015 mg/l.

The bioaccumulation of zinc in aquatic organisms, as the effects of zinc on ecosystem functions, is poorly understood. Reported bioconcentration factors (BCF's) may vary from 51 for the Atlantic salmon to 23,820 for the eastern oyster. An inverse relationship between BCF and zinc concentration in water has emerged as a generalization from several independent investigations (USEPA, 1986). No action limits for maximum acceptable levels of zinc in fish tissue are available. Interestingly, studies that have documented zinc acute to chronic ratios have demonstrated that this ratio is low. The Final Acute to Chronic Ratio for zinc was calculated to be 2.208 based on the geometric mean of this ratio obtained from several studies on different organisms (USEPA, 1986). This tends to indicate that emphasis should be placed on protecting aquatic systems against short term acute zinc exposures.

A number of factors affect toxicity test results and can explain reported variation in these data. Test procedures such as test design (i.e., static versus flow-through), number of replicates and statistical methods used for data analysis may influence results. Biotic factors include the biochemistry, physiology, behavior and overall health and life stage of the test organism as well as the degree of acclimation that may have resulted as a consequence of prior chemical exposure. Examples of abiotic factors that may modify zinc toxicity include dissolved oxygen levels, temperature, hardness, pH, suspended solids and chelating agents (Black et al., 1973; Sprague, 1985). For instance, zinc toxicity increases with increasing temperature (USEPA, 1986). However, the most common environmental parameter that has been used to account for toxicological differences observed with zinc is water hardness expressed as mg/l $CaCO_3$. Consequently, national water quality criteria for zinc have been formulated as a function of hardness and are plotted in Figures 6a,b. Water hardness generally reflects the levels of divalent calcium and magnesium ions present but other cations such as barium and strontium may also contribute to this measurement (APHA, 1980).

The mechanism through which hardness antagonizes metal toxicity has received attention. Three explanations have been postulated (USEPA, 1980). The first view is based on the assumption that hardness cations can out compete zinc ions for organism binding sites thereby causing a reduction in observed toxicity. A second hypothesis attributes this effect to increases in ionic strength and subsequent decreases in the activity of dissolved zinc species as

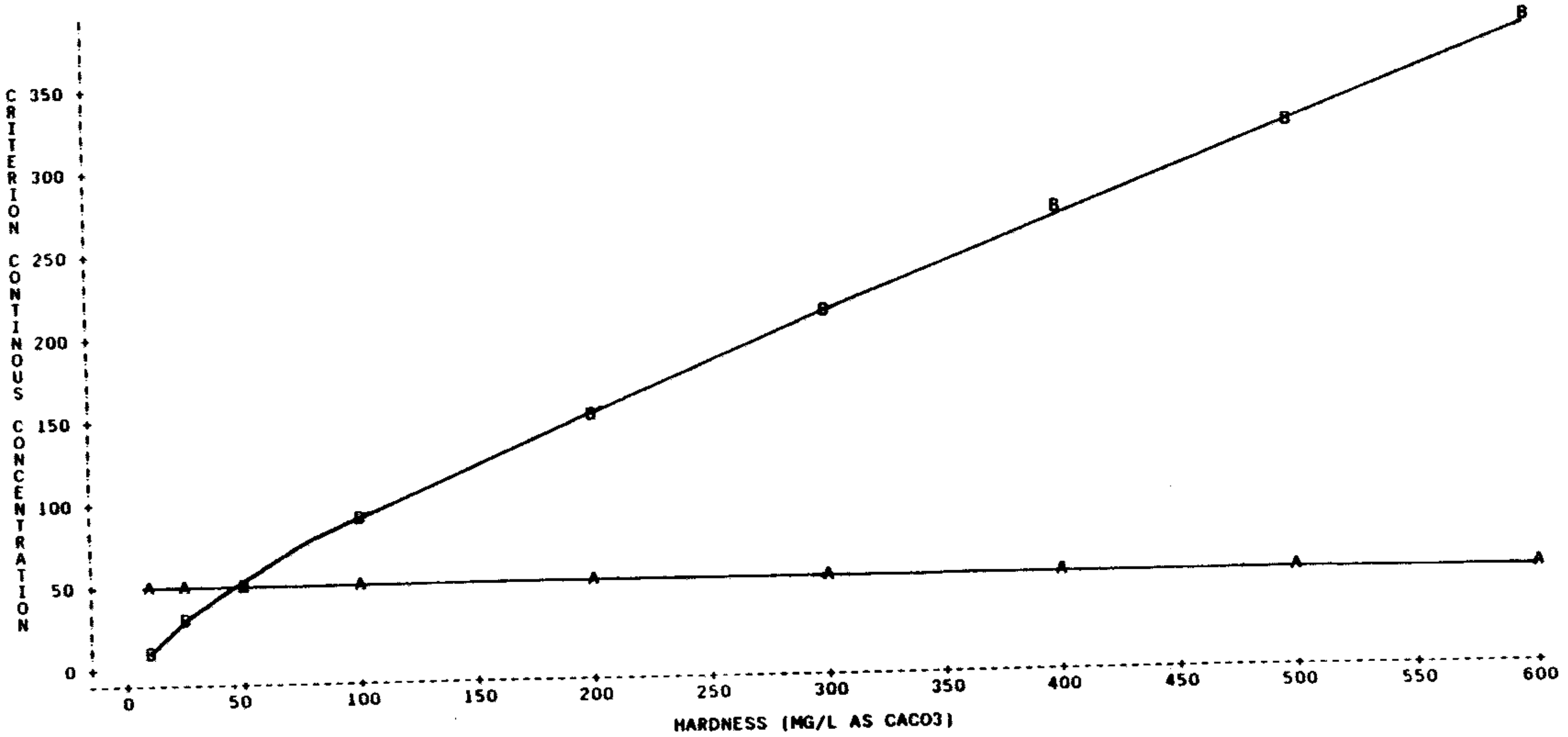

FIGURE 6a Comparison of previous USEPA (1980) National Water Quality Criteria for zinc (ug/L) to recently proposed criteria. Plot symbols A and B represent existing and proposed criteria, respectively.

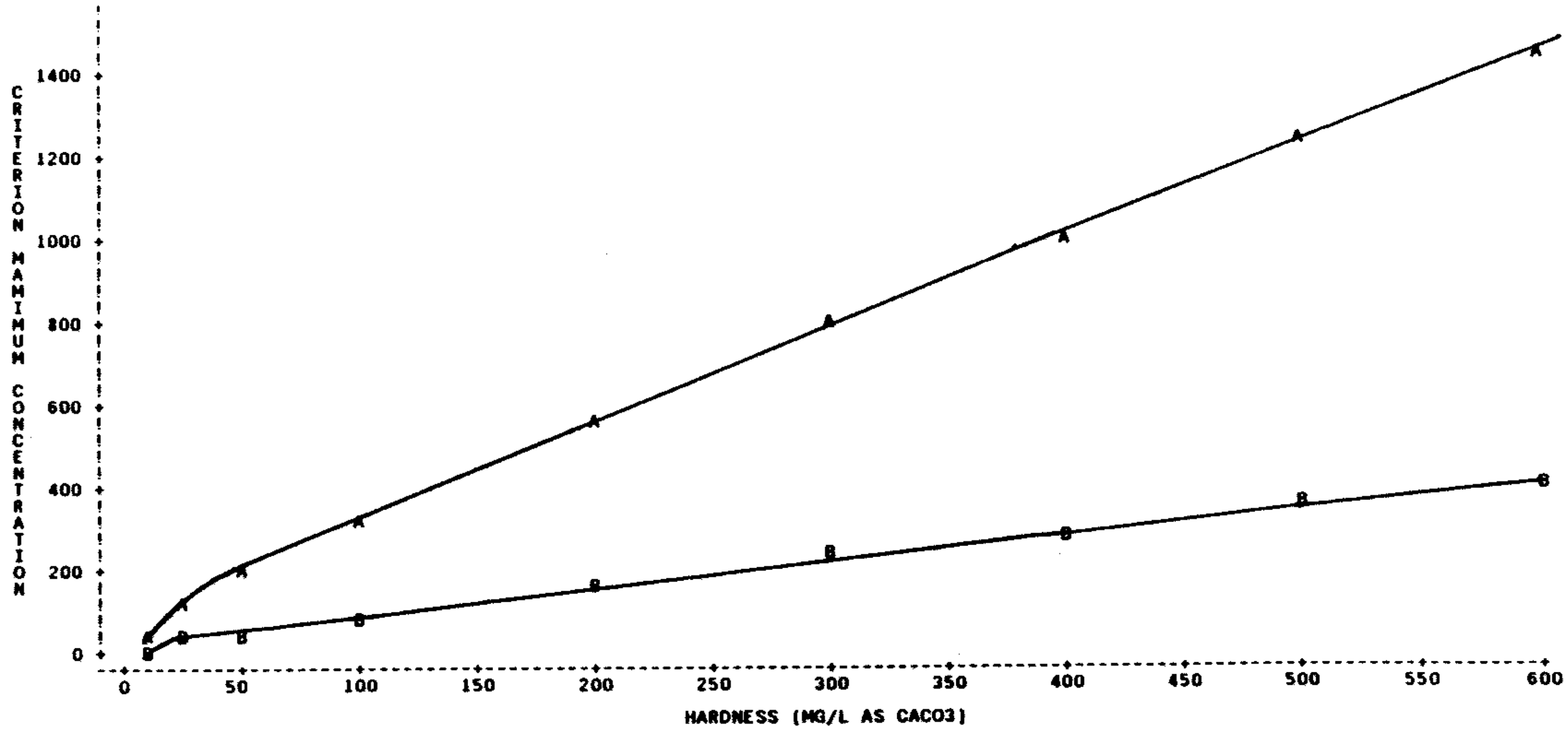

FIGURE 6b Comparison of previous USEPA (1980) National Water Quality Criteria for zinc (ug/L) to recently proposed criteria. Plot symbols A and B represent existing and proposed criteria, respectively.

hardness is increased. A third mechanism explains this effect in terms of changes in zinc speciation that accompanies changes in water hardness. The argument is made that since alkalinity and pH typically covary with hardness, reductions in toxicity are due to the formation of less toxic inorganic hydroxyl and carbonate complexes.

Although numerous studies have been conducted documenting the dependence of zinc toxicity on hardness, little information has been provided concerning the mechanism involved. This is due to the fact that, in previous studies, hardness was not varied independently of other water quality variables that could also influence the observed toxicity. Furthermore, results were expressed in terms of total rather than soluble zinc concentrations. Distinguishing between total and soluble zinc is necessary when conducting toxicity tests since the levels of zinc required in test solutions may be oversaturated with respect to zinc carbonate or hydroxide precipitates. In an elegant series of experiments using rainbow trout, Bradley and Sprague, (1985) demonstrated that changes in hardness were alone sufficient to cause changes in toxicity. Under the experimental conditions, zinc speciation, as determined by actual measurement as well as by thermodynamic calculations, remained fairly constant with the free ion prevailing as the dominant species. These same authors also varied pH independently and found that dissolved zinc was considerably more toxic as pH increased. This finding has often been obscured in earlier work because results were expressed as a function of total rather than dissolved zinc. At higher pH's a larger fraction of the total zinc is converted into precipitated less biologically available forms. Thus, the increasing toxicity of the dissolved zinc at higher pH's was often offset by the reduction in dissolved zinc concentrations that occur as a result of precipitation reactions. Using their data an excellent correlation can be obtained between the Log LC50 based on dissolved zinc (Zn^{+2} and $ZnOH^{+}$) predicted using a thermodynamic equilibrium model and adjusted to a water hardness of 50, and pH. In a subsequent study, Bradley and Sprague (1985a) tested two hypotheses concerning the mechanism by which pH and hardness altered zinc toxicity. They suggested that either the intrinsic toxicity of the zinc taken up by the organism was reduced at high hardness and low pH or alternatively the rate of zinc accumulation was reduced. Experimental findings showed that the intrinsic toxicity of zinc in fish gills was similar as evidenced by relatively uniform levels of zinc found in gills at the time of death. However, the time required for zinc accumulation in the gills was always longer in hard water and at lower pH's. Therefore, these authors concluded that the effect of hardness and pH was due to the alteration of the uptake and/or excretion dynamics of zinc accumulation in gill tissue. The actual mechanism causing the reduced rate of zinc accumulation into the gill was postulated to be either due to the competitive effect of calcium, magnesium and hydrogen ion for organism binding sites or due to changes in branchial permeability which control the rate of diffusion of electrolytes across gill membranes (Bradley and Sprague, 1985a). This first mechanism has served as the conceptual basis of a gill surface interaction model for trace metal toxicity to fishes (Pagenkopf, 1983). In this model the gill surface is treated as a ligand for which solute ions compete. In the absence of competing ions, zinc can more effectively interact with fish gills and hence exert a more pronounced toxic effect. In this context, the affinity of zinc for the gill bears a similar resemblance to the enhanced adsorption of zinc to solids as pH is raised. This model assumes that acute metal toxicity is caused by damage to the gills which in the case of zinc appears to be true (Skidmore and Tovell, 1972; Burton et al., 1972). However, less is known regarding zinc's toxic mode of action in other aquatic organisms.

Although differences in zinc toxicity may not be directly attributable to changes in zinc speciation, a number of studies have shown that alteration of chemical speciation may lead to changes in bioavailability. Borgman (1983) reviewed the toxicity of dissolved metal species to aquatic biota and found that carbonate, chloride and organic complexes were generally not toxic or at least less toxic relative to free ions and hydroxyl complexes. These conclusions were derived primarily from studies with copper although zinc and cadmium were suggested to follow a similar pattern. Two studies, one using an alga, the other using a daphnid, have confirmed that the addition of organic chelators reduces zinc toxicity and that toxicity appears to be dependent upon free zinc ion (Allen et al., 1980; Biesinger et al., 1974). Similar findings have been reported for cadmium (USEPA, 1983a). Gause and Winner (1985) have also shown that the toxicity of zinc to daphnids is reduced in the presence of humic acid. Using regression techniques they reported the relationship:

$$LC50(ug/L)=1.38(hardness) + 42.93(humic\ acid) + 72.09$$

$$(r^2=.95, p<0.05)$$

where hardness and humic acid are in units of mg/L. However, Borgman (1983) points out that some complexing agents, called ionphores, are readily adsorbed by cells and could potentially enhance toxicity. Laegreld et al. (1983) in their study of cadmium toxicity to phytoplankton concluded that free metal ion activity was not closely connected with toxicity, as has often been assumed since low molecular weight organic-metal complexes may enhance metal uptake potentially increasing bioavailability. Clearly more work is needed for identifying which species are the most toxicologically active. Cowan et al. (1985) have developed a methodology for determining toxic species by relating observed toxicity to equilibrium model calculations of speciation that are predicted to occur in the test exposure using multivariate statistical techniques.

Adsorption is suspected to be one of the most important fate processs affecting zinc. Consequently, the question arises as to the bioavailability of solid bound zinc. Generally, the association of zinc with a solid phase is considered to render zinc less biologically available to nektonic and planktonic organisms than dissolved zinc and might be viewed as an ecosystem detoxification mechanism. Dickson et al. (1984) showed that mortality generally decreased when daphnids were exposed to zinc in the presence of two suspended sediments as well as montmorillonite clay. The bioavailability of sediment bound zinc to benthic organisms is complex and not well understood (Luoma and

Bryan 1979). In a study on the bioavailability of zinc associated with different model substrates to a deposit-feeding clam, Luoma and Jenne (1977) found that the uptake rate of zinc followed the sequence in increasing order: exchangable fraction > carbonate bound fraction = Fe-Mn oxide bound fraction = organic detritus >> biogenic carbonate fraction (crushed clam shells). These authors also demonstrated that uptake from the various sinks was directly related to sediment-to-water desorption. Thus, the bioavailability of sediment bound zinc was directly related to the strength of the metal-sediment interaction. Sposito (1981) has pointed out the generalization emerging from recent metal toxicity studies, namely that as a general rule, strongly complexed forms of a trace metal are intrinsically less toxic than weakly complexed or free ionic forms. The bioavailability of zinc associated with various sediment sinks is suggested to follow the order: exchangeable fraction > carbonate bound fraction = Fe-Mn oxide bound fraction = organic carbon fraction >> residual fraction (Moore and Ramamoorthy, 1984).

Currently, it is not feasible to predict site-specific bioavailability of metals. This stems from the inability to accurately predict chemical speciation, as discussed previously, and the limited knowledge that is available for distinguishing the relative toxicities of different metal species. Since predictions may be subject to large margins of error the empirical Indicator Species Procedure has been developed by USEPA to account for site-specific differences in bioavailability by comparing actual toxicity observed in site water to that observed in laboratory reference water. Although studies utilizing this methodology are site-specific in nature, results from such investigations should provide additional information regarding the chemical fate and bioavailability of metals in general.

Study Objectives

The primary objective of this research was to provide guidance for determining under what circumstances site-specific criteria for zinc would be warranted. In other words, what are the general water quality characteristics of a receiving system which deem it amenable to a site-specific criterion modification process? Stated another way, under what water quality conditions would differences in the biological availability/toxicity of zinc be most likely expected? Additional applications of this research are expected. For example, an industry might ascertain apriori which location would be most appropriate for a new facility in order to minimize the environmental impact of its metal discharge. Furthermore, seasonal fluctuations in water quality data may be used to identify when an aquatic system is most vulnerable to zinc inputs. USEPA (1983) is encouraging states to explore seasonally dependent site-specific criteria to ensure that the "most sensitive" time of year dictates treatment design and NPDES permit specifications. Zinc was chosen as a model metal due to its ubiquitous occurrence in both municipal and industrial discharges as well as the reported importance that fate processes exert on zinc bioavailability (USEPA, 1979; Forestner and Wittman, 1979). The Indicator Species Procedure (ISP) was employed since it has been identified as potentially the most practical and technically defensible of the three procedures available for deriving site-specific criteria (Utility Water Act Group, 1983). The experimental approach used in this study for achieving the above objective involved obtaining three disparate natural waters, characterizing the chemical composition of these waters and then applying the ISP to each water. This information was then used to make generalizations concerning the utility of the ISP for zinc in other systems thus aiding water quality managers in deciding when this procedure might be successfully employed. It was hypothesized that toxicological differences could be related to differences in water quality characteristics and/or zinc speciation in test waters. Dissolved zinc measurements were taken during test exposures to differentiate between total and aqueous zinc forms. Based on the literature, it was assumed that toxicological differences would be reduced when toxicity results were expressed in terms of dissolved rather than total zinc concentrations. These analyses provided data describing the kinetic behavior of zinc during test exposures and the subsequent effects that zinc kinetics exerted on toxicity. In addition, comparisons between observed aqueous exposures were made with thermodynamic model predictions. As a secondary objective, a case study was initiated with a single river water to evaluate the ISP methodology in greater detail. These results will not be discussed since they have been presented elsewhere (Parkerton et al, 1986).

CHAPTER III

MATERIALS AND METHODS

Site Water

The Indicator Species Procedure as outlined in the *Water Quality Standards Handbook* (USEPA, 1983) was conducted for three rivers by performing acute toxicity tests using *Daphnia pulex* and *Pimephales promelas*. The rivers were chosen to provide systems that were chemically and hydrologically dissimilar. Selection was based upon review of extensive U.S. Geological Survey Water Quality monitoring data for Texas Rivers. As a result, the Elm Fork of the Trinity River near Sanger, TX, the Sabine River at Mineola, TX and the Red River near Gainsville, TX were chosen for sampling sites (Figure 7). Water quality monitoring data for each river at these stations are summarized in Table V. Boxplots are provided in Figures 8-13 for comparing the distributions of water quality variables between the three rivers. These data indicate that the Elm Fork of the Trinity River is a low order stream characterized by a relatively low but highly variable flow, intermediate hardness and high alkalinity and pH. In comparison, the Sabine River is a higher order stream with relatively low pH, hardness and alkalinity but with a slightly higher suspended solids load than the Trinity River. In contrast,

FIGURE 7 Location of Study Sites

TABLE V

US GEOLOGICAL SURVEY WATER QUALITY DATA
1968 to 1985

Parameter	Water	Mean	Median	Mode	SD[1]	CV[2]	Minimum Value	Maximum Value	Shape[3]	Number of Samples
hardness (mg/L as $CaCO_3$)	Trinity	213	225	240	65	30.5	6	340	B	112
	Sabine	102	86	100	60	58.8	16	460	+	298
	Red	643	650	1100	314	48.8	73	2100	+	351
alkalinity (mg/l as $CaCO_3$)	Trinity	237	237	230	84	35.4	4	443	N	112
	Sabine	52	57	64	21	40.4	6	125	B	298
	Red	136	130	115	40	29.4	41	286	N	332
conductivity (umho's)	Trinity	695	725	361	191	27.5	248	1130	–	114
	Sabine	817	400	212	1161	142.1	73	9000	+	301
	Red	3510	3510	1860	1860	53.0	300	11200	N	355
pH	Trinity	7.80	7.80	8.00	.35	4.5	6.7	8.5	N	114
	Sabine	7.03	7.00	7.20	.38	5.4	6.1	8.9	N	295
	Red	7.82	7.80	7.70	.39	5.0	6.9	9.0	N	352
Flow (CFS)	Trinity	140	26.5	18	489	349.3	.8	4450	+	88
	Sabine	1207	208	16	2616	216.7	1.2	17600	+	124
	Red	4793	795	122	10151	211.8	52	73800	+	519
Suspended Solids (mg/l)	Trinity	67.9	32.0	23.0	987	145.4	0	560	+	60
	Sabine	78.6	60.0	29.0	115.3	146.7	9.0	910	+	60
	Red	18.4	12.0	12.0	11.8	64.1	4.9	42	B	22

[1] SD = Standard Deviation

[2] CV = Coefficient of Variation

[3] + = positively skewed distribution
– = negatively skewed distribution
N = normal distribution
B = bimodal distribution

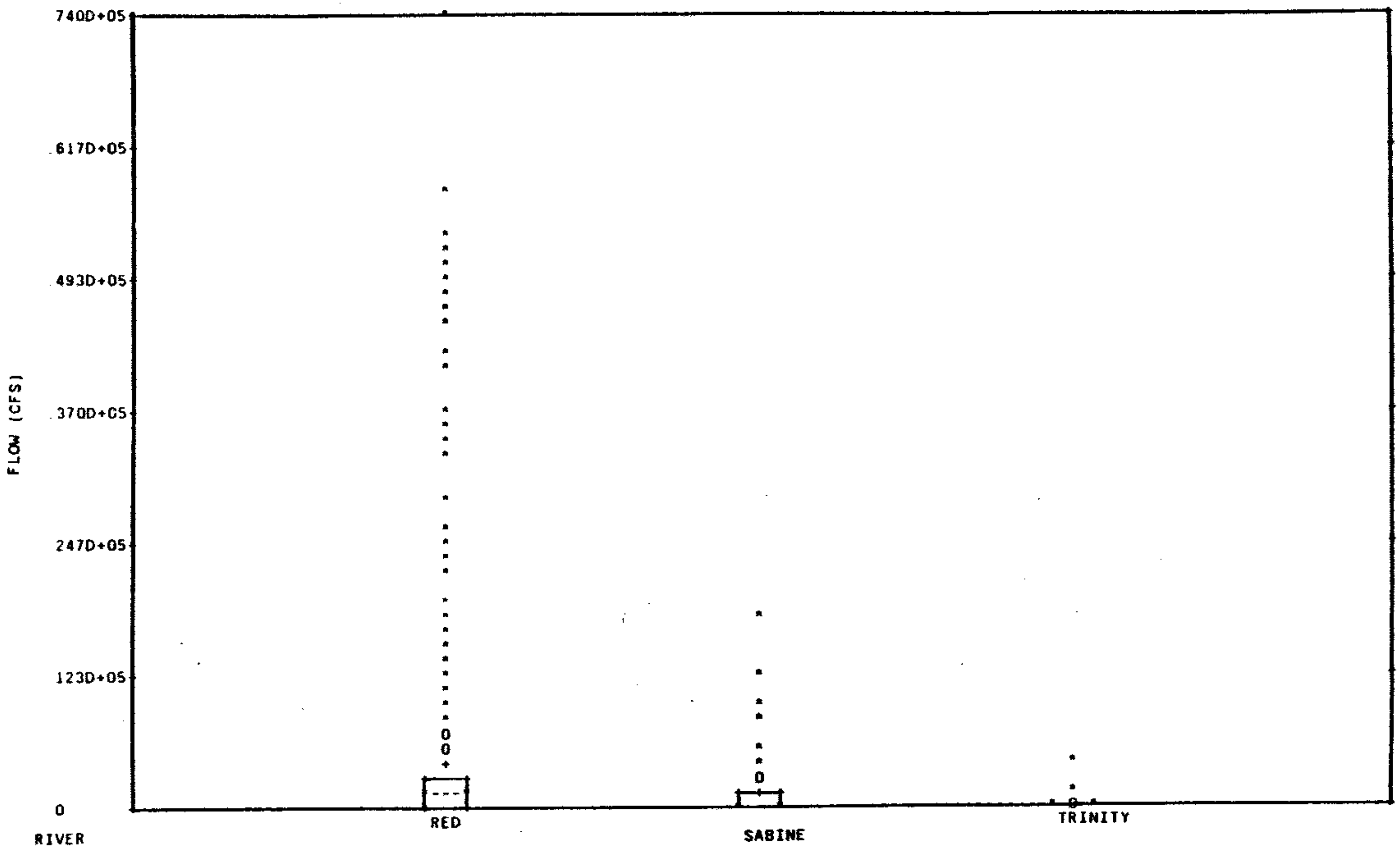

FIGURE 8 Boxplots of USGS Monitoring Data 1968-1985 for Flow in the Three Rivers Studied.

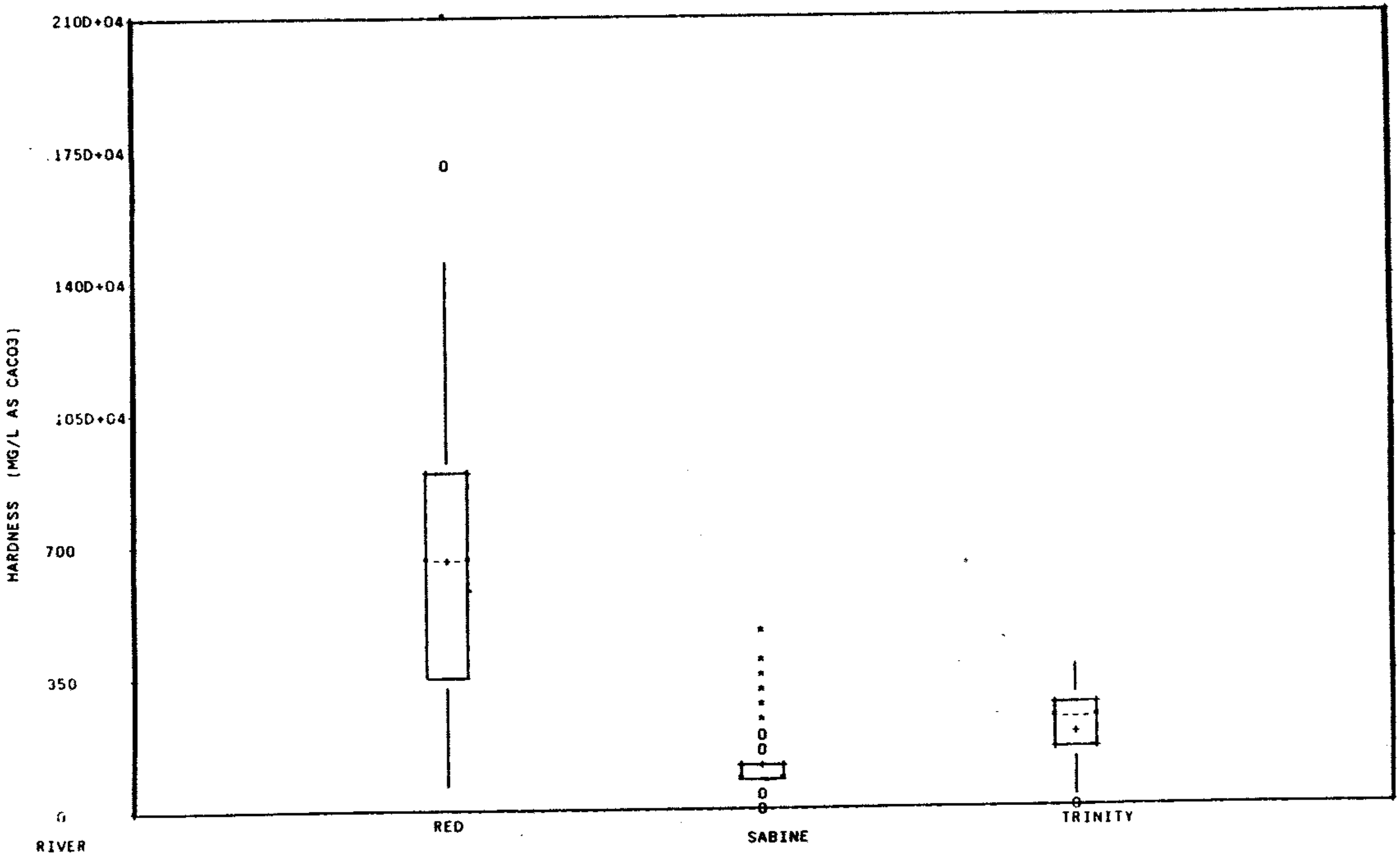

FIGURE 9 Boxplots of USGS Monitoring Data 1968-1985 for Hardness in the Three Rivers Studied.

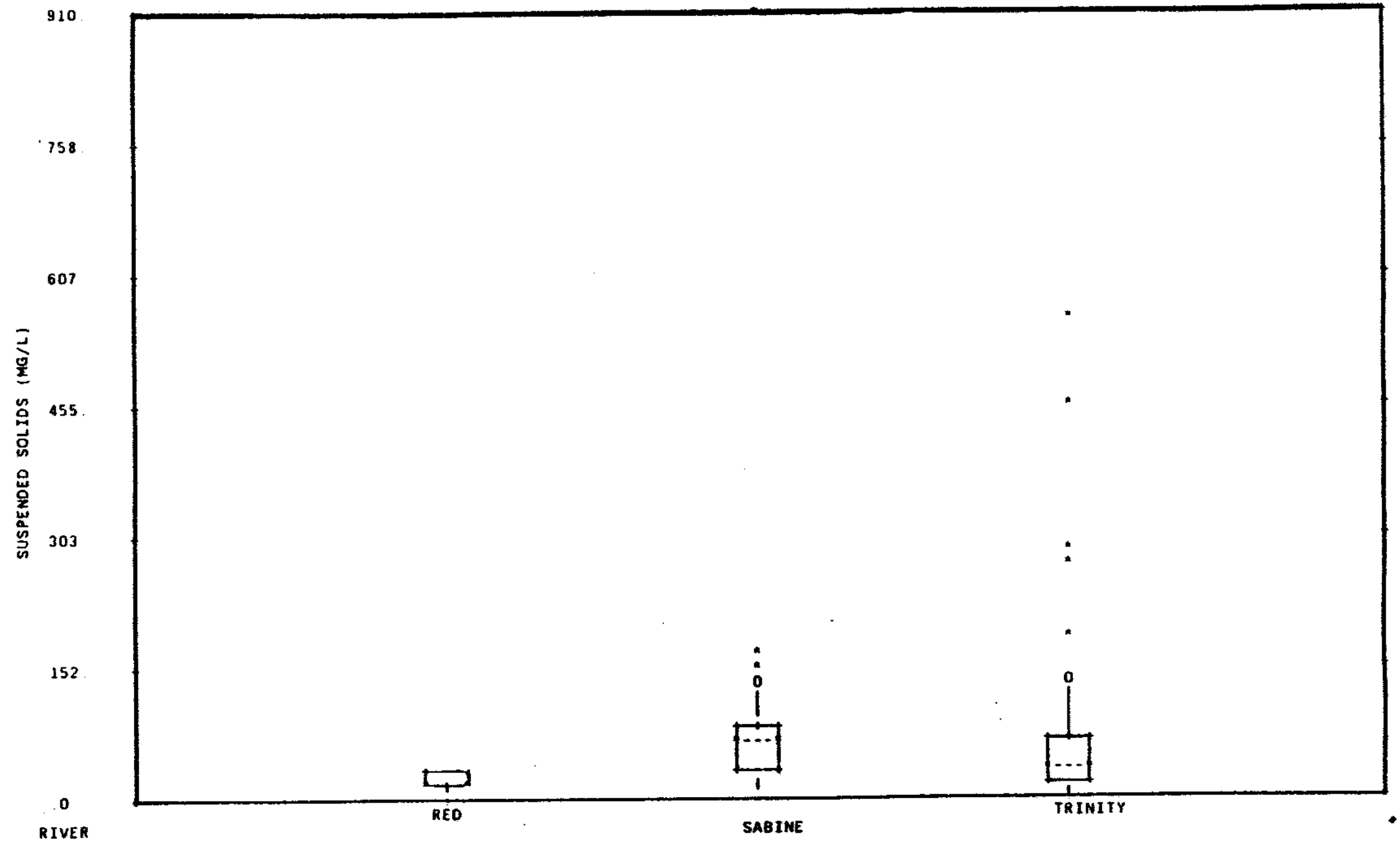

FIGURE 10 Boxplots of USGS Monitoring Data 1968-1985 for Suspended Solids for the Three Rivers Studied.

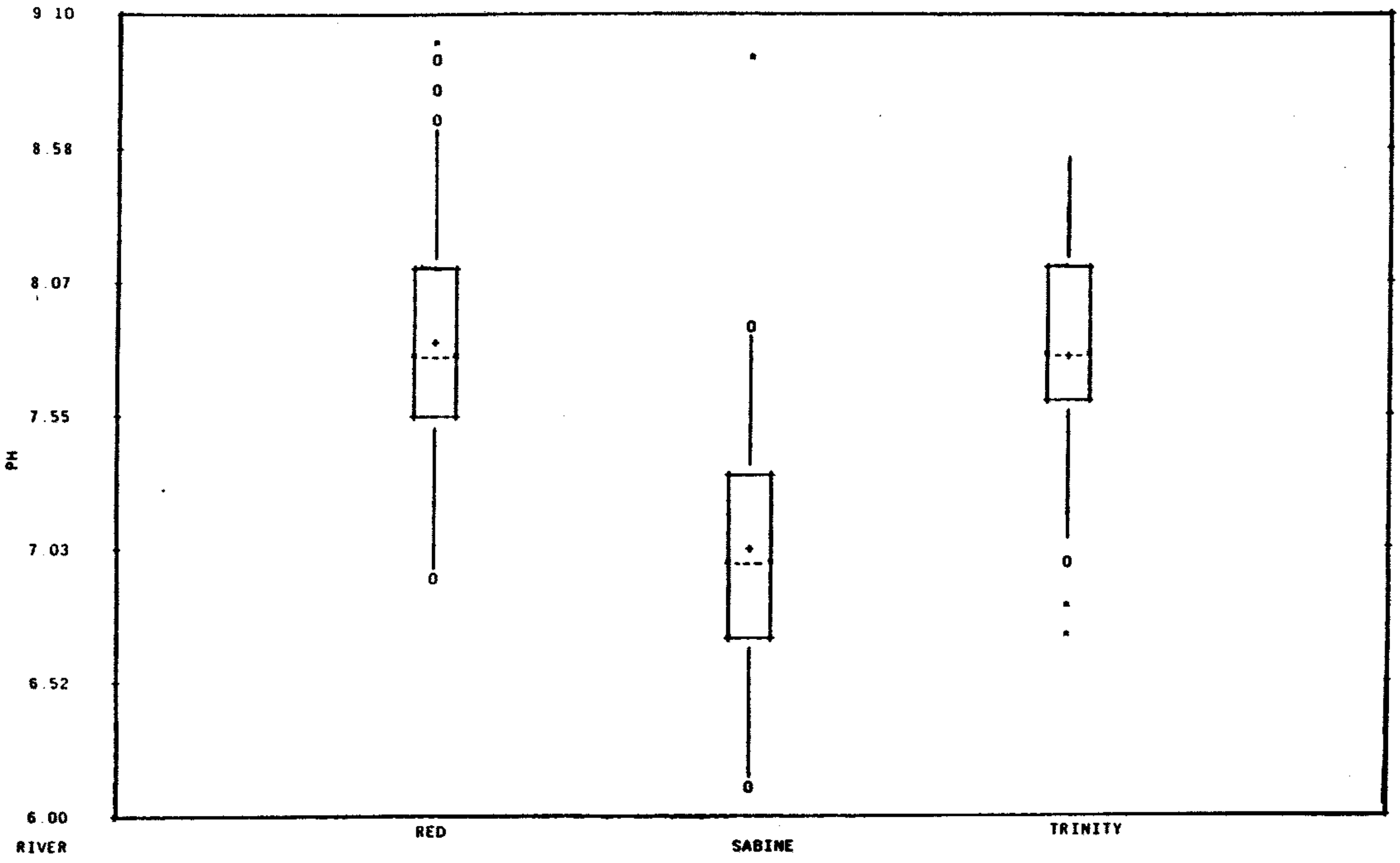

FIGURE 11 Boxplots of USGS Monitoring Data 1968-1985 for pH in the Three Rivers Studied.

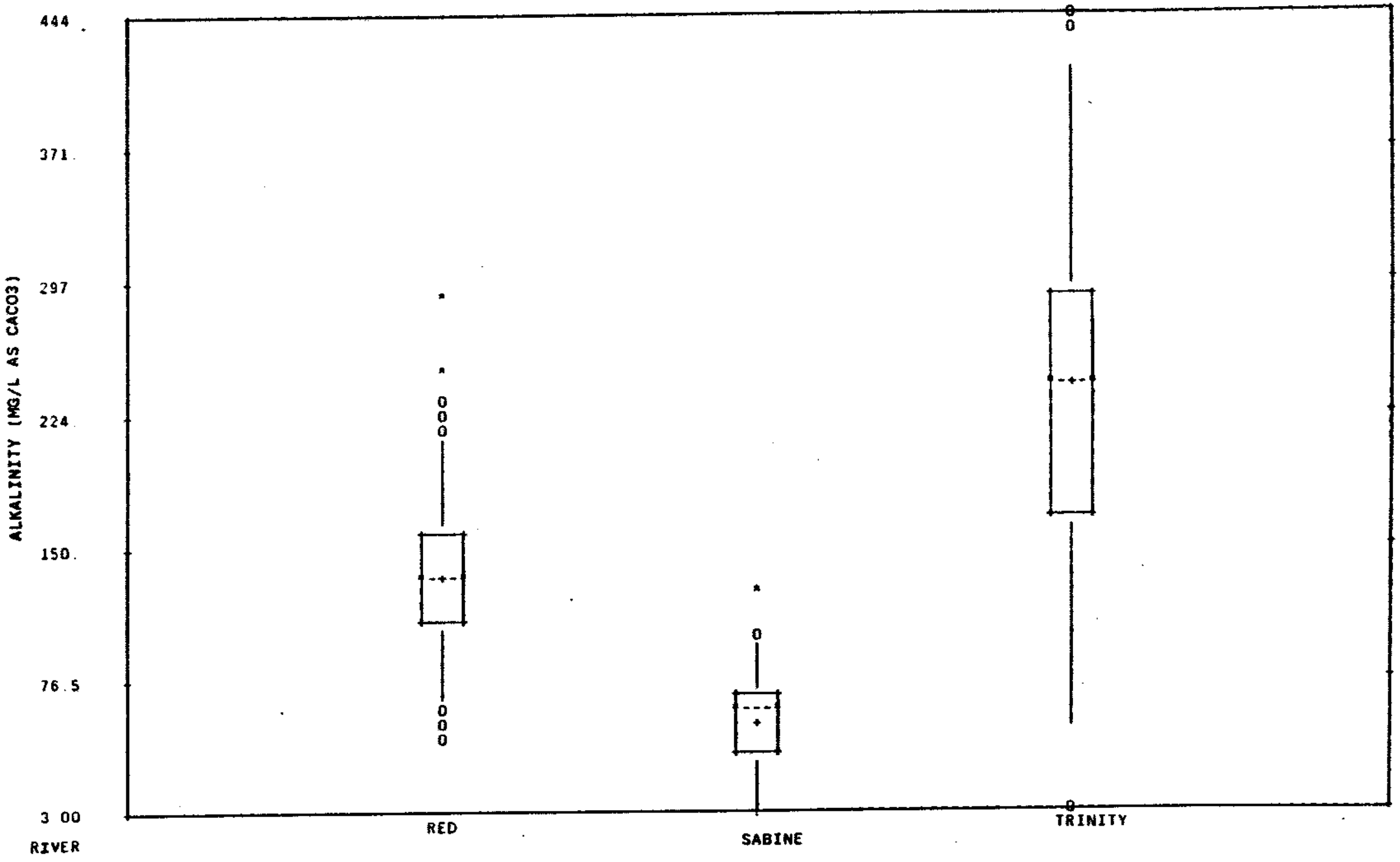

FIGURE 12 Boxplots of USGS Monitoring Data for Alkalinity in the Three Rivers Studied

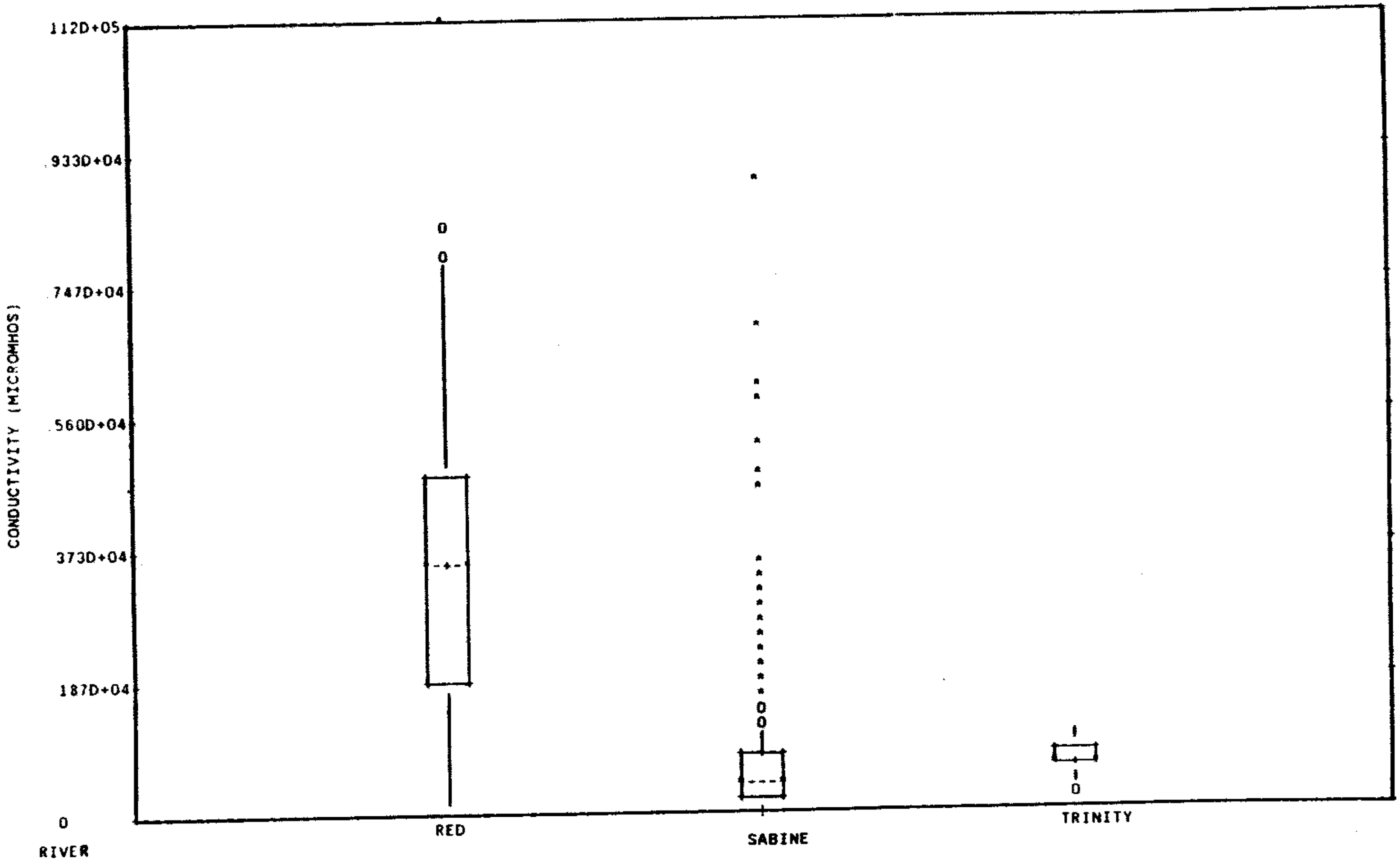

FIGURE 13 Boxplots of USGS Monitoring Data 1968-1985 for Conductivity in the Three Rivers Studied.

the Red River is the largest of the three rivers with an exceptionally high hardness, intermediate alkalinity and pH but with the lowest suspended solids load.

A single grab sample of each site water was collected in clean 30 gallon plastic garbage cans. Water was immediately returned to the laboratory and stored at 4 C until needed. Water quality characteristics for each site water were determined. Conductivity and pH were determined using an Electromark pH meter. Dissolved oxygen was measured with the aid of a model 54A YSI oxygen meter. Alkalinity and hardness were quantified titrametrically in accordance with APHA (1980). Dominant anions and cations were also determined following standard procedures (APHA, 1980). Non-carbonate hardness was calculated by subtracting the concentrations of calcium and magnesium (expressed in terms of mg/l as $CaCO_3$) from the hardness value obtained titrametrically. Suspended and volatile solids were determined by filtering a known volume of a well mixed sample of river water through a 0.45 micron filter (Schleicher and Schuell #30) and determining the weight of the residue retained after drying to constant weight at 105 ± 5 and 550 ± 25 C, respectively. Total dissolved and dissolved organic carbon were determined using a Dohrman DC-80 Carbon Analyzer according to the manufacturer's procedures. All analyses were conducted within a week of obtaining samples except in the case of dominant anions, cations and carbon measurements in which, depending upon site water, several months may have elapsed prior to analysis. Acute toxicity tests were conducted with each of the waters over a several month period following collection. Temporal changes in water quality that might have occured duing this time due to sample storage were assumed to be negligible in comparison to natural seasonal water quality fluctuations. In addition, periodic monitoring of pH, hardness, alkalinity and dissolved oxygen demonstrated that these parameters did not change appreciably over the course of the study.

Test Organism Culturing

Daphnia pulex cultures were maintained in growth chambers providing constant temperature (20 ± 2 C) and light conditions 550 Lux, 16:8 light/dark). Ten animals were reared in moderately hard reconstituted water (APHA, 1980) in 600 ml glass beakers and were fed approximately 3.75 x 10^6 cells of *Selenastrum capricornutum* daily. *Selenastrum* was batch cultured weekly in a 20 liter pyrex glass jug containing approximately 10 liters of medium and a stir bar. The container was inoculated from a stock culture and was placed on a stir plate in an environmental growth chamber maintained at constant temperature (24 C) and light intensity (cool white fluorescent 700 Lux). Air was pumped through a cotton filter and then introduced into the container via a pyrex glass diffuser using an aquarium air pump. Further details regarding the culture of *D. pulex* and *S. capricornutum* have been described by Price (1985) and Parkerton (1985).

Pimephales promelas were cultured in aquaria in a flow through system of carbon filtered, dechlorinated tap water at approximately 25 C and constant light conditions (325 Lux, 16:8 light/dark). Spawning fish were fed *ad libetum* and once daily with dry fish food. Eggs were removed daily and hatched in aerated 500 ml separatory funnels. Hatched fry were raised in aquaria and fed i .us ad libetum <24 hour old brine shrimp two or three times daily. Further details regarding the culture of *P. promelas* are provided by Stewart (in prep).

Zinc Analysis

Zinc solutions were prepared by dissolving reagent grade zinc chloride (CAS Reg. 7646-85-7) into deionized water after it had been passed through a Millipore Milli-R04 ultrapurification system. Zinc concentrations in toxicity tests were verified using a Perkin Elmer model 2380 atomic absorption spectrophotometer. The detection limit using this instrument was approximately 0.01 mg/l. Three methods were initially used to quantify zinc. Total zinc was determined using a HNO_3 digestion procedure following standard methodology (APHA, 1980). Total acidified zinc was determined by acidifying 50 ml of sample with 1 ml of conc HNO_3. Filterable zinc was quantified by passing the sample through a 0.45 micron filter (Schleicher and Schuell) and then acidifying with conc HNO_3. However, preliminary results such as provided in Table VI indicated that total acidified measurements were equivalent to total digested zinc values. Therefore, only total acidified measurements were used for quantifying total zinc concentrations in toxicity tests. Furthermore, quantification by this method should provide a better estimate of bioavailable zinc than that obtained by intense digestion. The effects of possible matrix interferences in each of the test waters was evaluated by preparing standards in each water matrix, acidifying, and then comparing to acidified standards prepared in millipore water. No interferences were observed, consequently the method of standard additions was not used.

Acute Toxicity Experiments

Static Acute toxicity tests were performed in general accordance with recommended test procedures (Peltier and

TABLE VI

ANALYTICAL DATA FOR ZINC DETERMINATION BY ATOMIC ABSORPTION SPECTROSCOPY

Experiment	Time	Total Digested	Total Acidified	Filterable	Particulate	Adsorbed[1] to Glass	% Recovery
1	0 hr	(Blank) .13	.04	.09	-	.07[c]	-
		.72	.58	.58	.00[a]	.10	-
		1.22	1.12	1.06	.06	.16	-
		1.86	1.78	1.61	.17	.19	-
		2.97	3.23	3.16	.07	.34	-
2	0 hr	(Blank) .33	.06	.06	0.0[a]	-	-
		1.01	.74	.68	.06	-	-
		1.65	1.48	1.35	.13	-	-
		2.27	2.58	1.96	.62	-	-
		2.96	2.87	2.65	.22	-	-
		3.73	3.73	3.43	.30	-	-
	48 hr	(Blank) -	.06	.03	.03[a]	.05	-
		-	.79	.53	.26	.07	-
		-	1.60	1.03	.57	.20	-
		-	2.30	1.42	.88	.25	-
		-	3.02	1.93	1.09	.16	-
		-	3.91	2.19	1.72	.37	-
3	0 hr	(Blank) -	.06	.06	.07[b]	-	-
		-	.77	.59	.22	-	96.1
		-	1.48	1.15	.26	-	90.5
		-	2.11	2.04	.36	-	110.4
		-	2.85	2.41	.41	-	96.5
		-	3.59	3.01	.57	-	97.8

[1] Measured by rinsing emptied glass vessel with dilute HNO_3 and then measuring zinc in the acid rinse

[a] Calculated by difference

[b] Calculated by digesting residue retained on filter

[c] After 48 hours

Weber, 1985) using five concentrations and a control. Daphnids were tested in 250 ml glass beakers using five replicates for each treatment level with ten <48 hour old neonates per replicate. Organisms of this age were chosen instead of <24 hour old individuals as usually recommended after preliminary experiments in reference water indicated that the sensitivity of 24 hour old neonates was not different than either 48 hour or 72 hour old organisms. However, the confidence intervals of the LC50's for tests using 72 hour olds were larger than for tests using younger individuals. Therefore, employing 48 hour old organisms seemed to provide the best compromise for yielding the large number of sensitive test organisms needed (300 animals per test). Three replicate exposures were used for fish tested in gallon glass jars with ten one month old fry measuring approximately 1 cm in length per replicate. Toxicity tests were conducted simultaneously for both site and reference waters so that a Water Effects Ratio (WER) could be determined. Reconstituted water and dechlorinated tap water served as the reference water for daphnid and fish tests, respectively. Daphnid (48 h) and fish (96 h) tests were conducted under similar conditions as those maintained for the stock cultures except without feeding. Test organisms were not acclimated to test waters prior to exposure since earlier work suggested that this was unnecessary (Parkerton et al., 1986). To assess the impact that alternate reference waters might impose on the results of the Indicator Species Procedure toxicity tests were conducted in which both organisms were tested concurrently in the two reference waters. Additionally, the effects of kinetics on zinc toxicity was explored by allowing the toxicant to interact for either 24 or 96 hours with the diluent prior to organism exposure. During this period spiked samples were agitated at 50 RPMS with the aid of a shaker table in an effort to enhance the attainment of equilibrium. One experiment was also conducted with daphnids to evaluate the role of suspended solids in altering zinc toxicity. Parallel acute zinc toxicity tests were performed using both filtered and unfiltered Trinity River water.

Daphnids were counted at the termination of each experiment (15X) by pouring each replicate beaker through a counting chamber constructed of petri plates and 250 micron mesh nitex TM screening. Organisms were examined under a dissecting microscope (15X) and endpoints were judged in terms of immobilization and lack of internal movement. Samples for total zinc were taken at the start of tests immediately after spiking. Samples for total zinc were taken at the termination of daphnid tests by acidification of an additional replicate carried through experiments without animals. Total zinc in fish tests was determined by directly acidifying one replicate from each treatment at the end of experiment after fish mortality had been recorded. Dissolved zinc (0.45 micron filterable) was also measured by taking composite samples from replicates at 0, 24 and 48 hours for daphnid tests and at approximately 0, 1, 3, 6, 12, 24, 48, and 96 hour intervals for fish tests. Dissolved oxygen and pH were monitored at the start and termination of each test.

Data Analyses

Data were first explored via graphical displays and univariate statistics as recommended (Peltier and Weber, 1985; Hartwig and Dearing, 1979). Toxicity data were analyzed using the probit procedure available in the SPSS-X statistical package (SPSS-X, 1985). Statistical differences between LC50's or EC50's were determined by the method suggested by Sprague and Fogels (1977). Kinetics of zinc sorption and/or precipitation was described by using multiple regression techniques. Analysis of covariance was employed to test if the rate of change of soluble zinc concentrations during toxicity tests was significantly different between the experimental waters. SAS (1982) programs were used for these analyses. Significance in all statistical tests was judged at the alpha=0.05 level. The geochemical model MINTEQ developed by Felmy et al., (1983) was used to predict zinc speciation based on equilibrium assumptions and to aid in interpretation of toxicity results.

CHAPTER IV

RESULTS AND DISCUSSION

Water Quality Analysis

The results of water quality analyses on the three river and two reference waters are provided in Table VII. The grab river water samples reflected the same relative differences in water quality as anticipated from monitoring data (Table IV). Using hardness as a single criterion one would expect that zinc would be least toxic in Red River water and most toxic in Sabine River water. The two laboratory reference waters were selected to provide similar hardnesses that were intermediate in comparison to the riverine waters.

Zinc Kinetics

Measurement of dissolved zinc during acute toxicity experiments with daphnids and fish revealed that zinc was not at equilibrium as evidenced by the decline of dissolved zinc concentrations during the course of toxicity experiments. As toxicity experiments progressed, a larger fraction of the total zinc was converted into particulate forms (Figures 14-18). Changes in dissolved zinc concentrations during test exposure were attributed primarily to precipitation rather than adsorption reactions,

TABLE VII

COMPARISON OF WATER QUALITY CHARACTERISTICS IN SITE AND LAB REFERENCE WATERS

Parameter	Trinity River	Sabine River	Red River	Lab^a Water 1	Lab^b Water 2
pH	7.7-8.0	7.0-7.6	7.7-8.5	7.5-7.9	7.8-8.1
Total hardness (mg/l as $CaCO_3$)	201±16	62±7	760±16	97±16	
Non-carbonate hardness (mg/l as $CaCO_3$)	38	6	82	0	53
Alkalinity (mg/l as $CaCO_3$)	176±10	47±3	160±9	44±6	96±6
Conductivity (uMhos / cm)	546	213	4370	323	321
Suspended Solids (mg/l)	32.5±3.3	90.6±7.6	77.7±5.3	-	-
Volatile Solids (mg/l)	6.4±.7	11.6±.2	15.5±1.7	-	-
Calcium (mg/l)	54.0	8.7	100.0	19.1^c	15.2
Magnesium (mg/l)	6.8	4.7	75.0	12.1^c	4.4
Sodium (mg/l)	43.0	31.0	570.0	26.3^c	41.0
Potassium (mg/l)	8.7	10.0	13.0	2.1^c	5.0
Manganese (mg/l)	.07	.04	.02	-	.03
Iron (mg/l)	.10	.50	.10	-	.05
Silicate (mg/l)	.96	.92	.90	-	.20
Sulfate (mg/l)	47.6	26.7	640.0	93.6^c	51.9
Chloride (mg/l)	50.3	21.3	1077.7	1.9^c	20.8
Dissolved organic carbon (mg/l)	4.58±.09	7.75±.19	5.01±.16	-	-
Total Carbon (mg/l)	87.5±.3	22.9 ±.2	46.7±3.4		
Dissolved oxygen (mg/l)	6.7-8.3	7.2-8.6	7.0-8.2	6.9-8.6	7.2-8.4

a Reconstituted moderately hard water used in daphnid experiments
b Dechlorinated tap water used in fish experiments
c Calculated based on nominal values

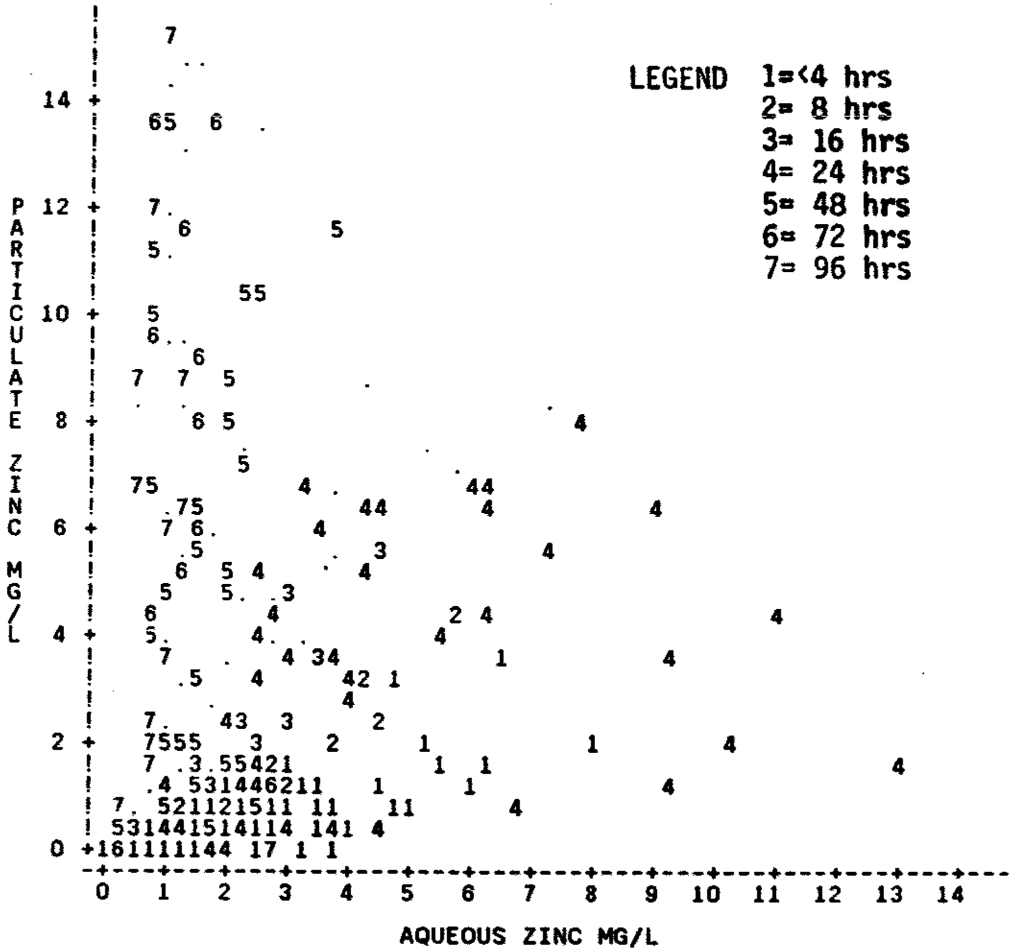

FIGURE 14 Scatterplot Showing the Relationship Between Particulate and Aqueous Zinc in Trinity River Toxicity Experiments.

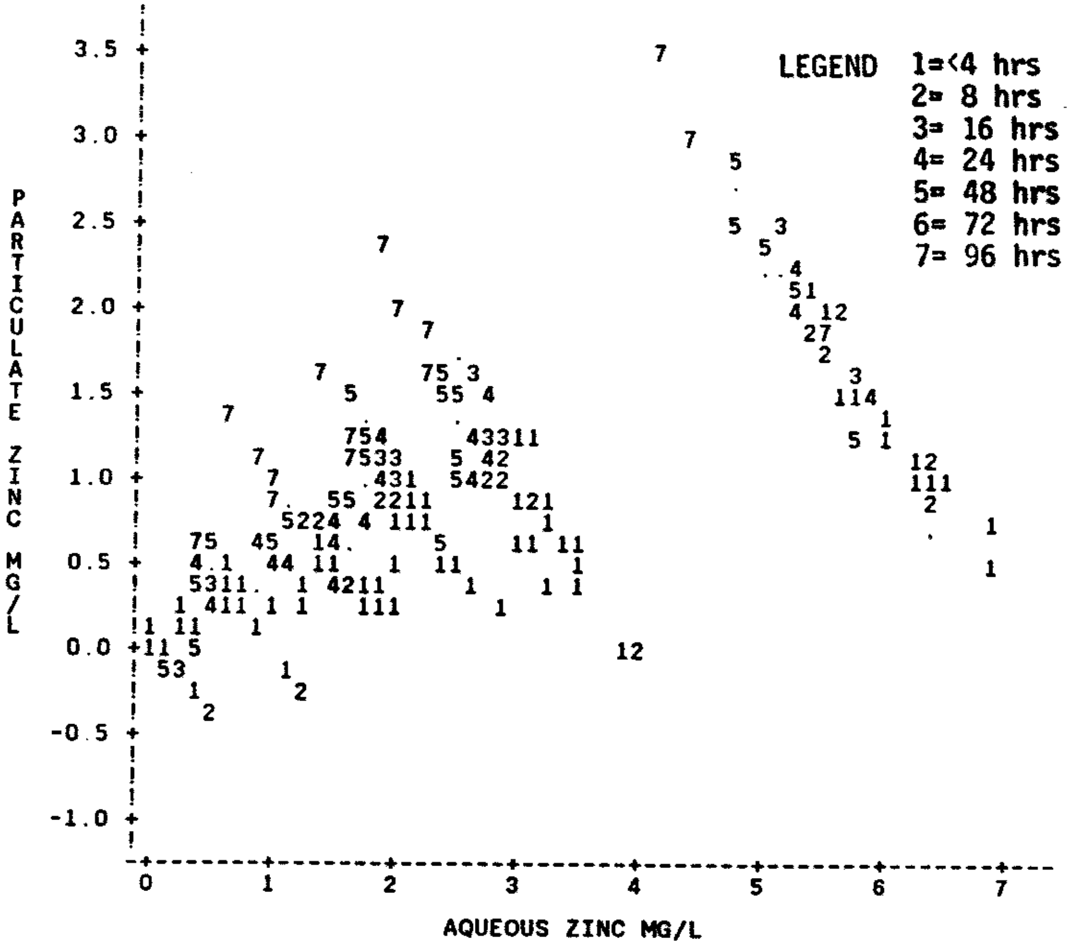

FIGURE 15 Scatterplot Showing the Relationship Between Particulate and Aqueous Zinc in Sabine River Toxicity Experiments.

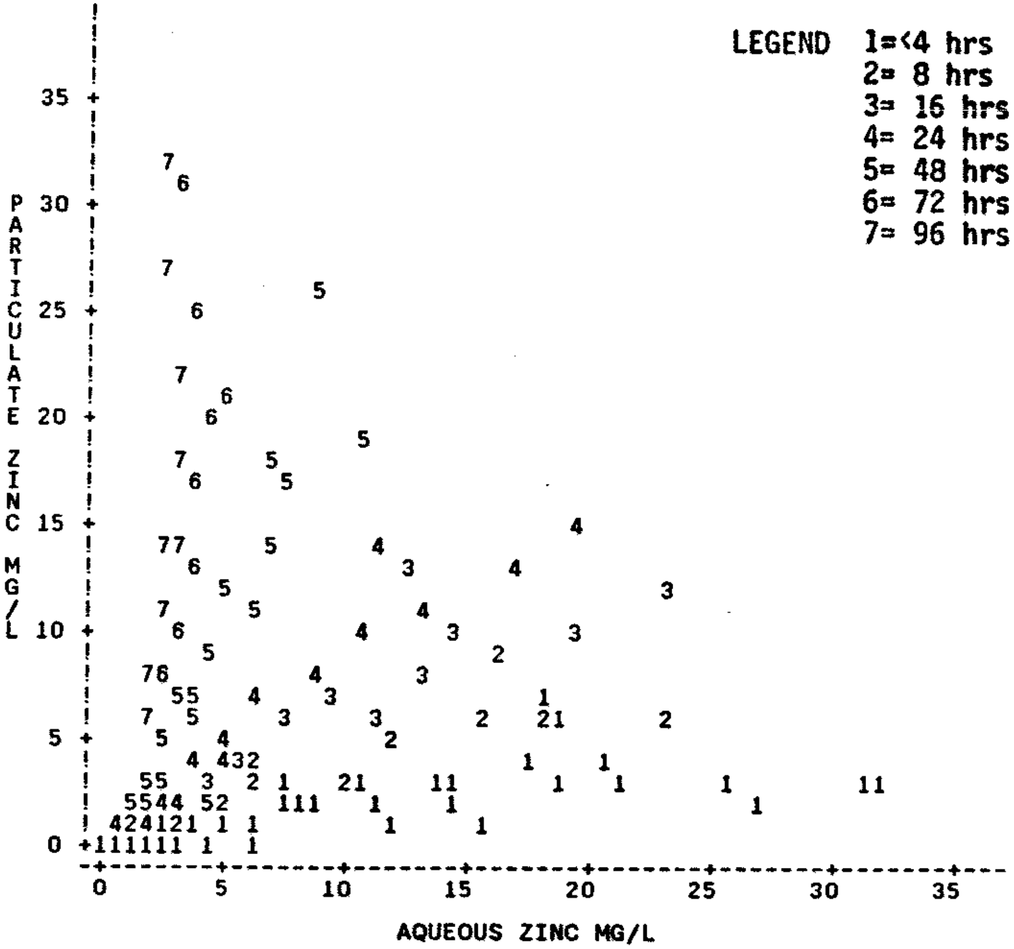

FIGURE 16 Scatterplot Showing the Relationship Between Particulate and Aqueous Zinc in Red River Toxicity Experiments.

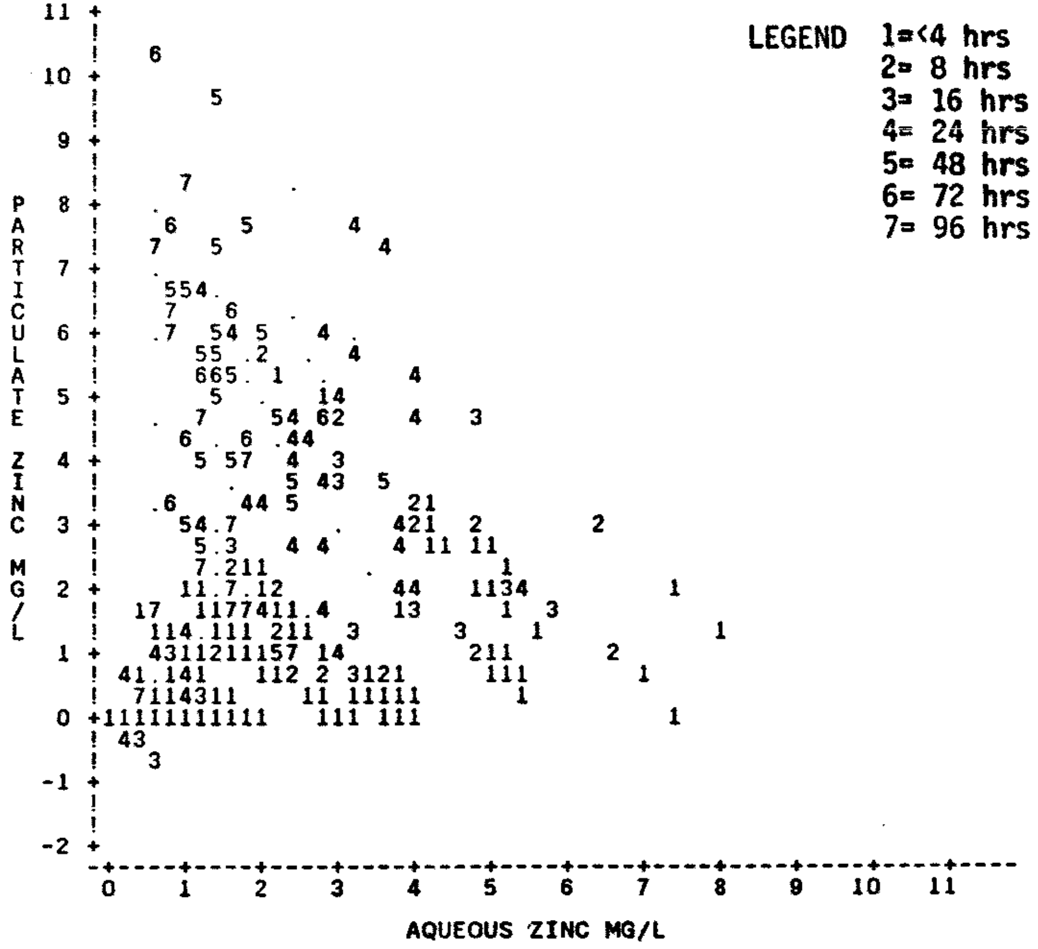

FIGURE 17 Scatterplot Showing the Relationship Between Particulate and Aqueous Zinc in Dechlorinated Tap Water Toxicity Experiments.

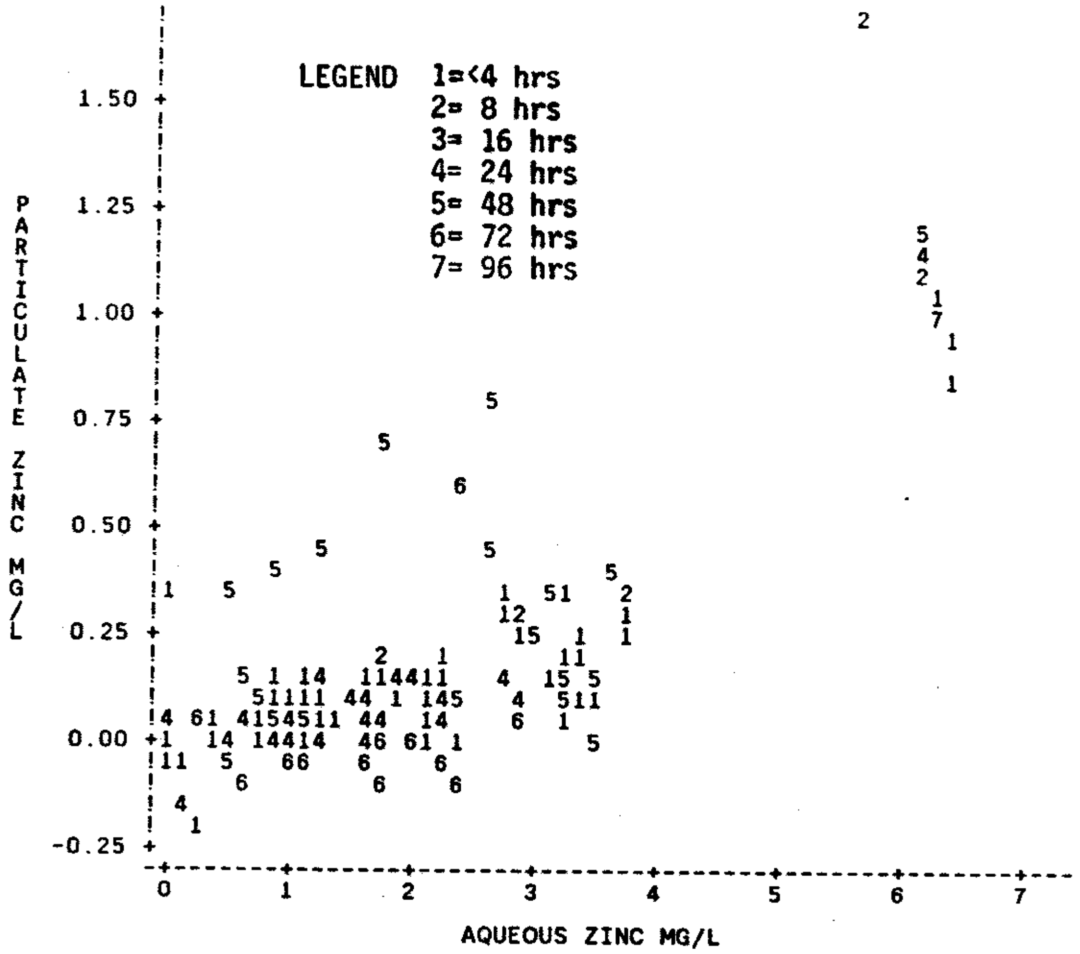

FIGURE 18 Scatterplot Showing the Relationship Between Particulate and Aqueous Zinc in Reconstituted Water Toxicity Experiments.

since changes in dissolved zinc for reference waters lacking suspended solids (Figure 17 and 18) followed a similar pattern as that observed for the river waters (Figure 14-16). Furthermore, most zinc adsorption is reported to be complete within a few hours (Fristoe and Nelson, 1983; Jenne and Zachara, 1984) and does not conform to the time dependent nature apparent in the data presented in Figure 14-16.

These experimental data did not, in general, obey classical first or second order kinetics. This may be attributable to the heterogeneous nature of the system under study. The observed data integrate a series of processes including adsorption to solids (and to the sides of glass test vessels), adsorption and uptake by test organisms and precipitation reactions. Consequently, these data were modeled empirically using multivariate statistical techniques.

The distributions of total and dissolved zinc were found to be positively skewed and a cuberoot transformation was necessary to normalize these variables. Stepwise regression was then performed to identify the best model for predicting aqueous zinc concentrations during test exposures based on total zinc concentrations, time and water quality variables. The best single model describing the data was:

$Y=.8449X1 + .0006X2X3 + .0392X4 - .1501X5 + .99 \quad r^2=.8991$

where:

Y = Cuberoot of aqueous zinc $(mg/l)^{.333}$

$X1$ = Cuberoot of total zinc $(mg/l)^{.333}$

$X2X3$ = interaction term

total zinc concentration(mg/l) x time(hrs)

$X4$ = pH

$X5$ = Log conductivity(Log umho's / cm)

However, analysis of covariance indicated that the regression coefficient for X1 and for the interaction term, X2X3, were significantly affected ($P = 0.0001$) by water type. Therefore, subsequent statistical analysis was performed on each water seperately. The best overall model describing the kinetics of zinc precipitation sorption in each of the experimental waters is summarized in Table VIII. The correlation coefficients indicate that these models account for 88 to 98% of the variability in the dependent variable (cuberoot aqueous concentration). These equations were used to generate Figures 19-23 which illustrate the kinetic behavior of aqueous zinc in each water for different values of total zinc. Figure 19 and 20 show that little change in aqueous zinc concentration occurs in either reconstituted water or Sabine River water. These results are in agreement with the zinc solubility diagram presented

TABLE VIII

EQUATIONS MODELING AQUEOUS ZINC CONCENTRATIONS DURING TOXICITY EXPERIMENTS

MODEL: $\text{cubrtac} = B_1(\text{cubrttc}) + B_2\ (\text{Time}) + B_3\ (\text{Time x tconc}) + B_o$

Water	B_1	B_2	B_3	B_o	R^2	Maximum ** Predictability
Trinity	.92030	.00111	-.00123	-.00845*	.90	16.02
Sabine	.89723	-.00119	-.00001*	.02720*	.97	7.55
Red	.90229	-.00172	-.00051	.05313	.98	34.76
Dechlorinated Tap	.81626	.00085	-.00108	.06976	.88	10.89
Reconstituted	.94102	-.00008*	.00003*	.04246	.98	7.28

* Coefficient not significantly different from zero at the α = .05 level

**Refers to the highest value of total zinc (mg/L) included in the data

cubrtac = Cube Root of Aqueous Concentration $(mg/L)^{1/3}$
cubrttc = Cube Root of Total Concentration $(mg/L)^{1/3}$
Time = time (hrs)
tconc x time = Interaction term (mg x hrs/L)

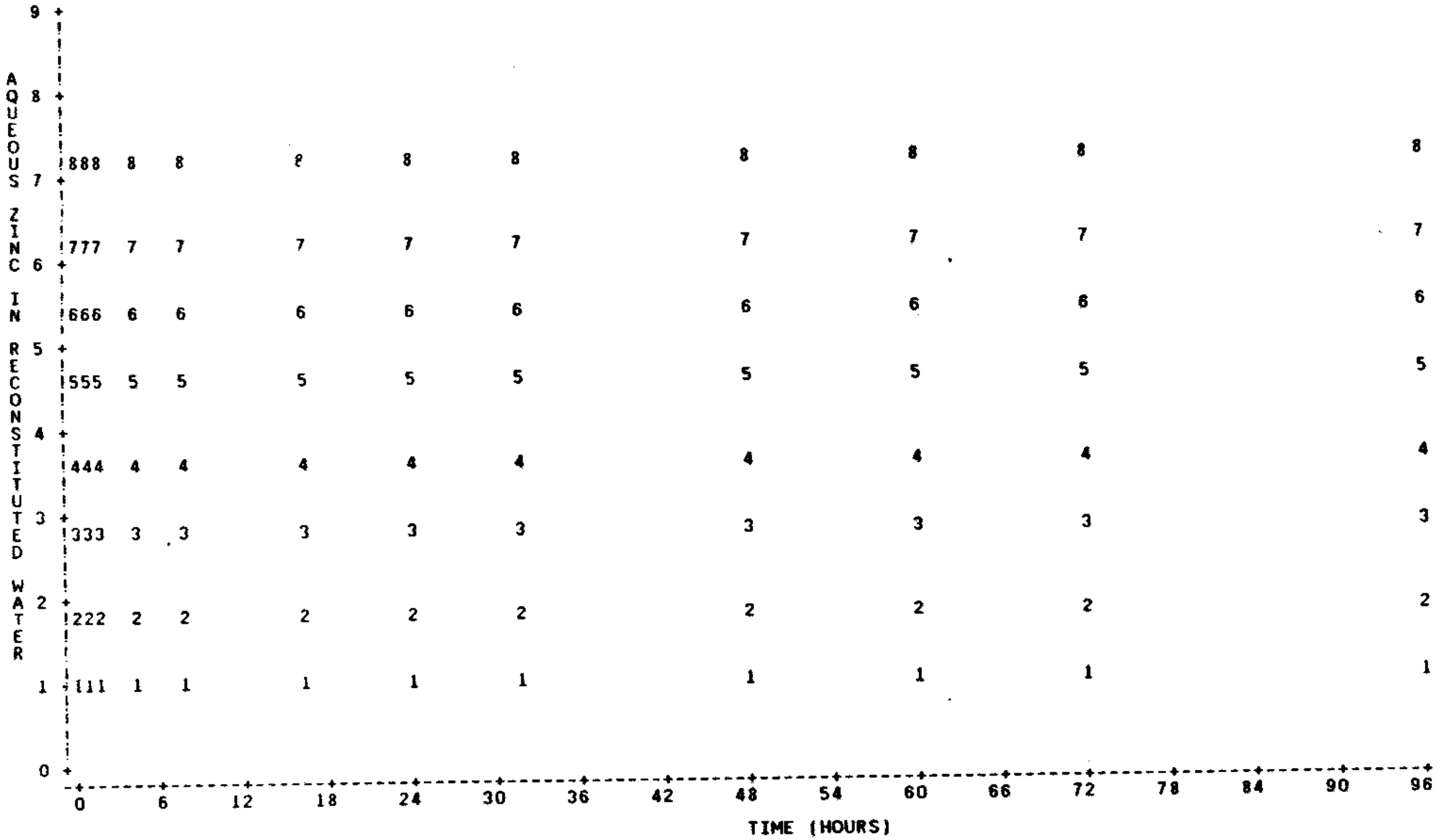

FIGURE 19 Kinetic Behavior of Aqueous Zinc(mg/L) during Toxicity Experiments in Reconstituted Water. Plot Symbols Represent Total Zinc(mg/L) Values.

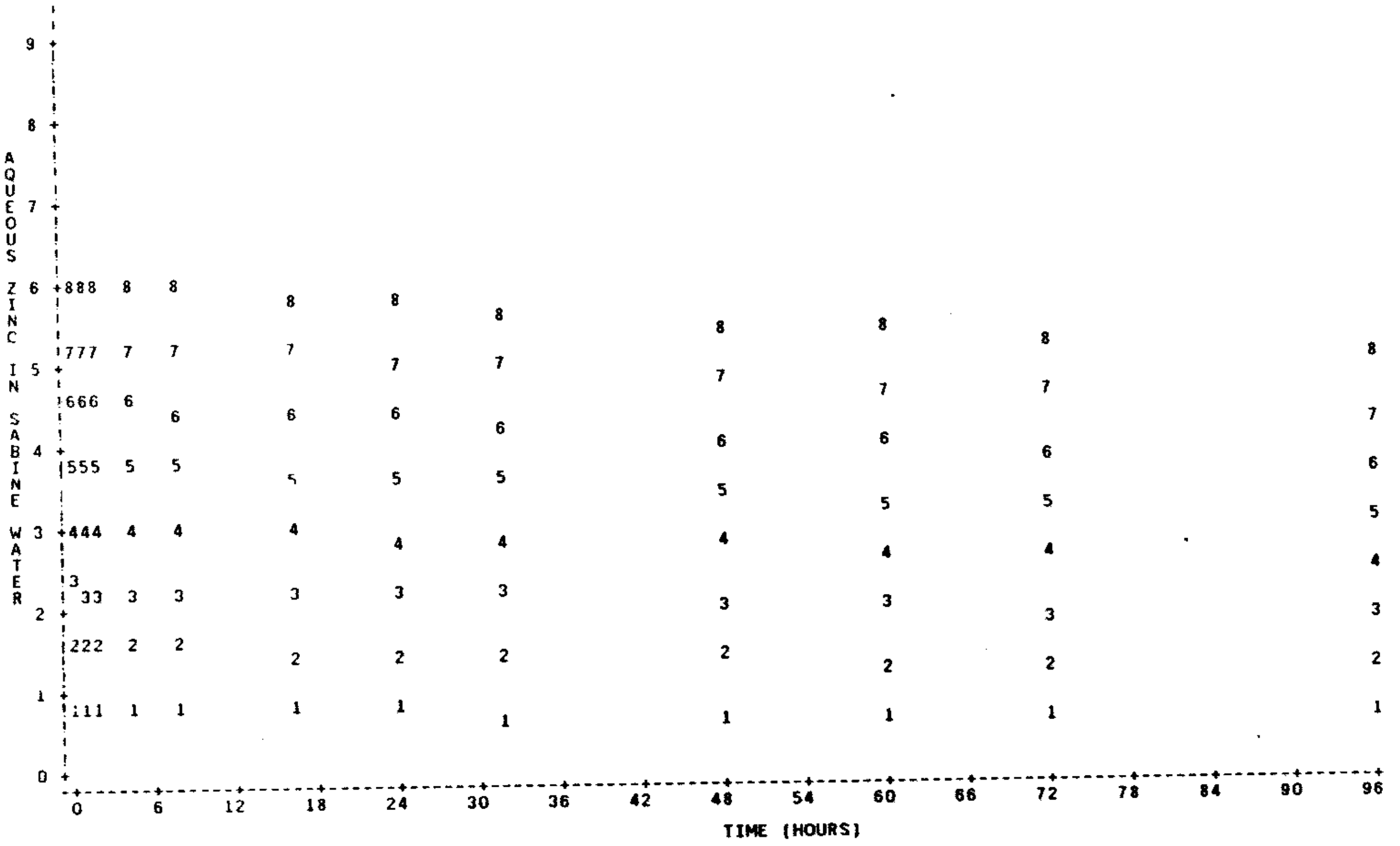

FIGURE 20 Kinetic Behavior of Aqueous Zinc(mg/L) during Toxicity Experiments in Sabine River Water. Plot Symbols Represent Total Zinc(mg/L) values.

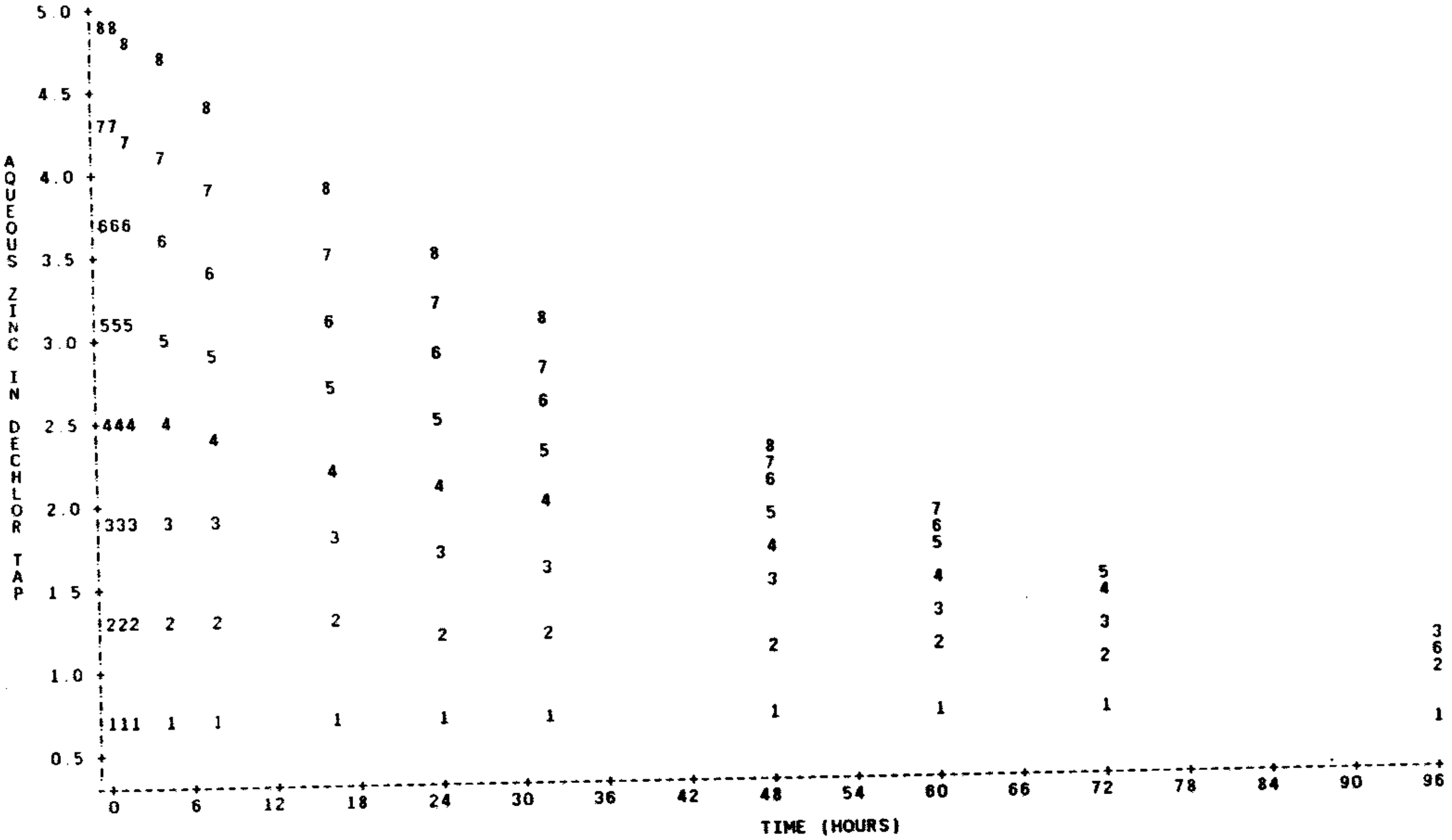

FIGURE 21 Kinetic Behavior of Aqueous Zinc(mg/L) during Toxicity Experiments in Dechlorinated Tap Water. Plot Symbols Represent Total Zinc(mg/L) Values.

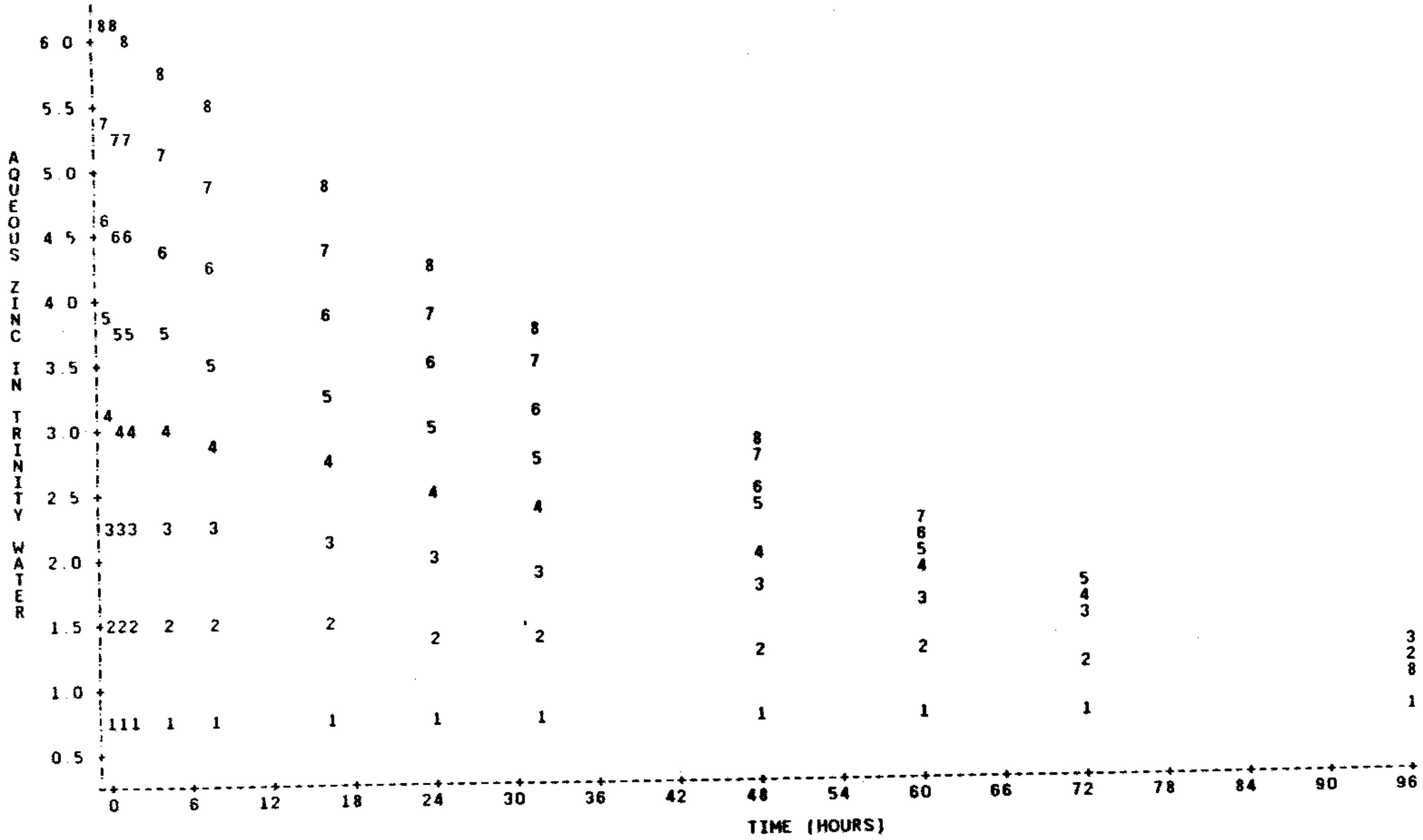

FIGURE 22 Kinetic Behavior of Aqueous Zinc(mg/L) during Toxicity Experiments in Trinity River Water. Plot Symbols Represent Total Zinc(mg/L) values.

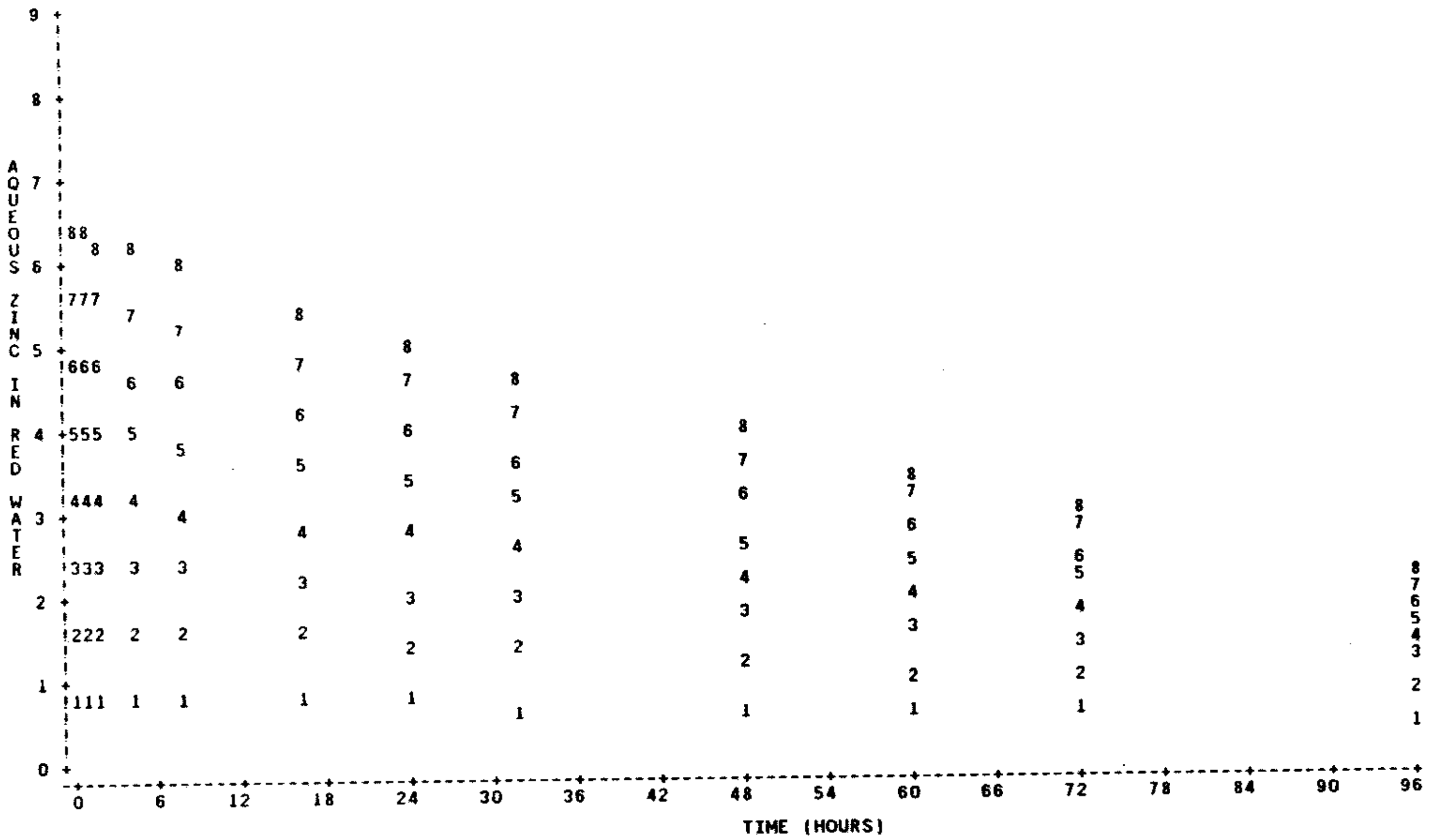

FIGURE 23 Kinetic Behavior of Aqueous Zinc(mg/L) during Toxicity Experiments in Red River Water. Plot Symbols Represent Total Zinc(mg/L) values.

in Figure 5 which predicts increased zinc solubility at the lower pH's of these two waters. In contrast, substantial reductions in aqueous zinc concentrations are observed in the higher pH, Trinity River, Red River and dechlorinated tap waters. Equilibrium appears to be reached after 96 hours in both dechlorinated tap and Trinity River waters for total zinc concentrations as high as 8 mg/l (Figures 21 and 22). The equilibrium concentrations of aqueous zinc observed in the Trinity and dechlorinated tap waters were approximately 1.2 and 1.0 mg/l, respectively, after 96 hours. In contrast, equilibrium in Red River water was kinetically hindered and was not attained after 96 hours (Figure 23). Comparison of predicted aqueous zinc concentrations in each of the waters at 1, 2, 5 and 8 mg/l are provided in Figures 24-27. These figures illustrate that the conversion of zinc to particulate forms in each water occurs most rapidly and to the greatest extent in the following order: dechlorinated tap > Trinity > Red > Sabine > reconstituted water. Figures 24-27 also indicate that a very rapid initial reduction in aqueous zinc occurs immediately after zinc is spiked since the initial aqueous concentration at the start of the experiment (time = 0 hrs) is usually lower than the total zinc concentration. However, this is probably an experimental artifact since

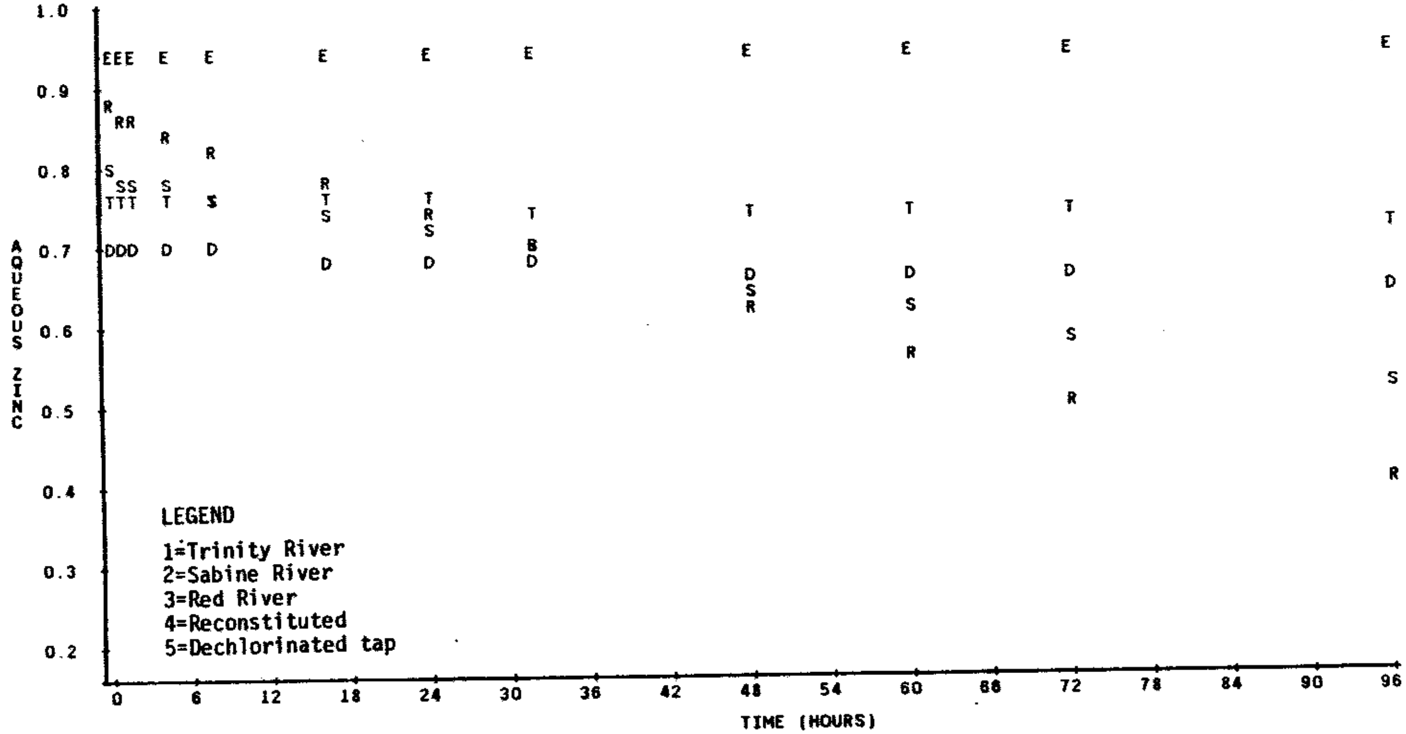

FIGURE 24 Comparison of Aqueous Zinc Kinetics Between the Five Experimental Waters. Total Zinc Concentration = 1.0 mg/L.

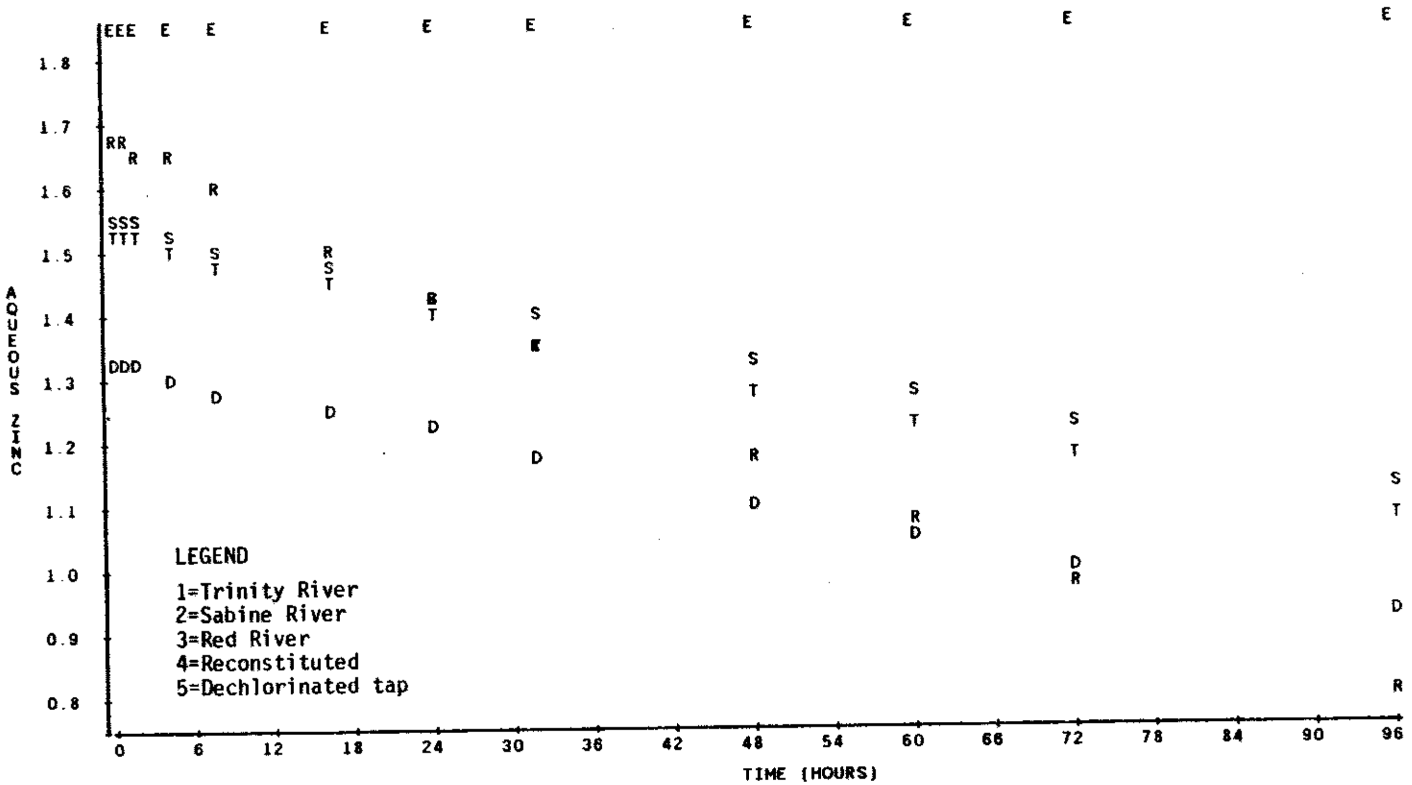

FIGURE 25 Comparison of Aqueous Zinc Kinetics Between the Five Experimental Waters. Total Zinc Concentration = 2.0 mg/L.

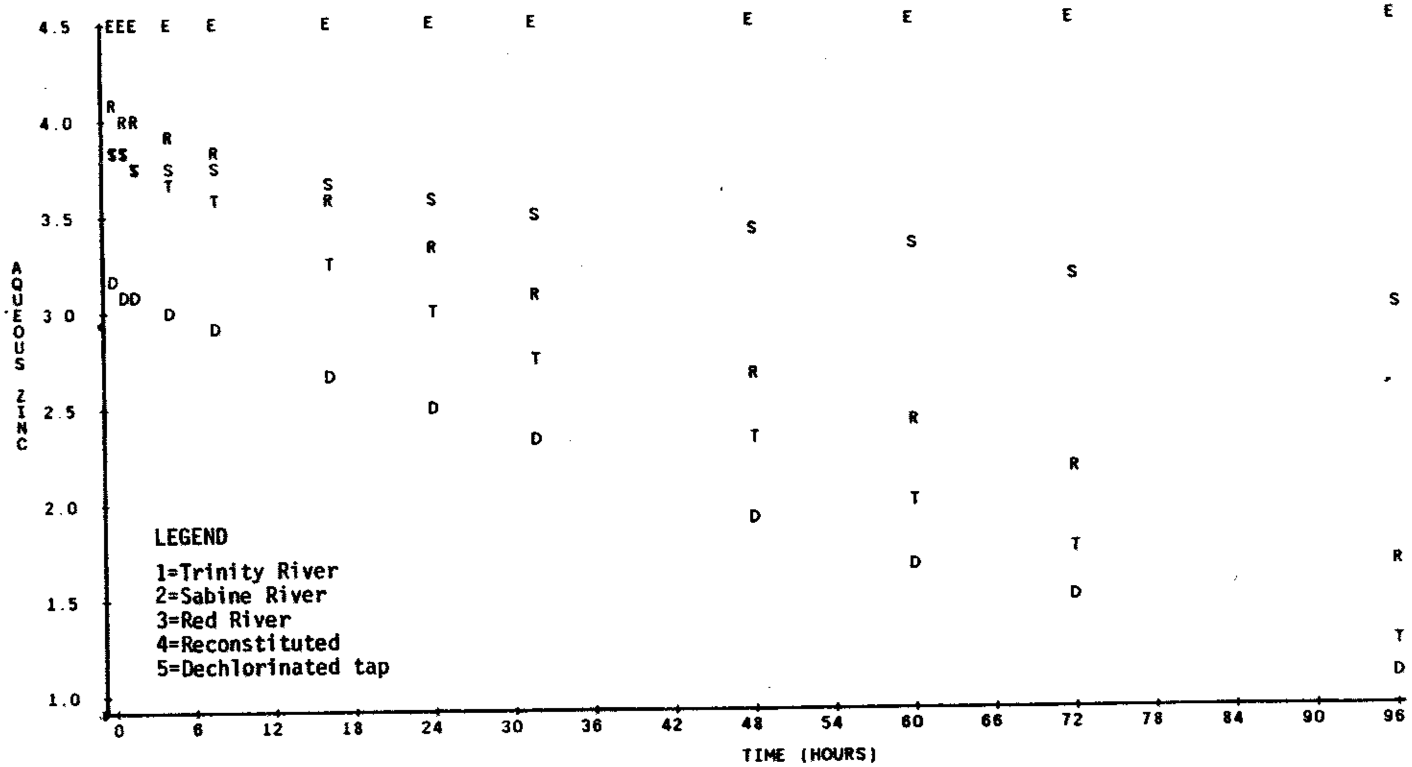

FIGURE 26 Comparison of Aqueous Zinc Kinetics Between the Five Experimental Waters. Total Zinc Concentration=5.0 mg/L.

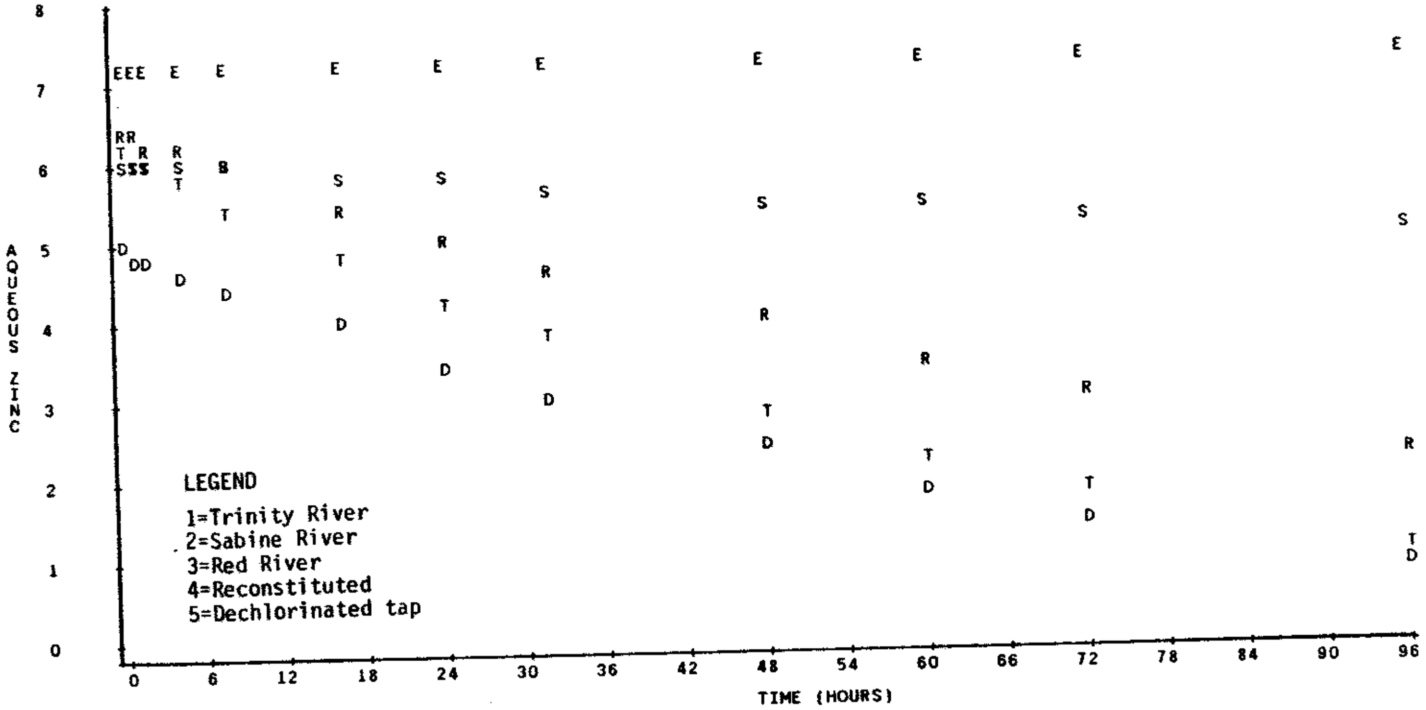

FIGURE 27 Comparison of Aqueous Zinc Kinetics Between the Five Experimental Waters. Total Zinc Concentration = 8.0 mg/L.

samples for dissolved zinc were not taken immediately after spiking, but rather were taken at the initiation of the toxicity tests after all organisms had been transfered to test chambers. Due to the large number of replicates used in the toxicity experiments, transferring animals into test vessels took up to an hour. Despite this caveat, these equations may be useful for predicting the behavior of aqueous zinc in other waters of similar quality if they are used within the bounds of the data upon which they were derived, i.e., given total zinc concentrations between 0.1 and less than the upper limit provided in Table VIII and within a 96 hour time frame.

Toxicity Results

Toxicity experiments were expressed in terms of total as well as mean aqueous zinc concentrations. Mean aqueous zinc concentrations were obtained by averaging time-series measurements for 0.45 micron filterable zinc during test exposures. Toxicity results are presented in Tables IX-XIV. These tables provide estimates for the probit slope and intercept and associated standard errors about these parameter estimates, a chi-square test to evaluate how well the data fit the probit model, as well as an EC50 value (or LC50 for fish) with 95% confidence intervals. In addition, a chi-square test for parallelism is provided. If

TABLE IX

PROBIT ANALYSIS OF TOXICITY DATA FOR THE ELM FORK OF THE TRINITY RIVER, SANGER, TX BASED ON TOTAL ZINC (mg/L)

			Total Concentration										
	Test	Water*	ß	SE ß	α	SE α	X^2model	p	LCL - EC50 - UCL	X^2slope	p	LCL - RMP - UCL	WER
Daphnia	1	EFT EPA	5.32 2.80	.68 .42	2.49 4.39	.31 .09	10.88 5.34	.01 .03	2.08 - 2.95 - 5.77 1.42 - 1.65 - 2.03	8.06	<.01	1.22 - 2.06 - 6.30	1.79**
	2	EFT EPA	4.49 2,88	.55 .42	3.25 4.80	.23 .10	12.60 6.01	<.01 .05	1.47 - 2.45 - 5.25 . - 1.17 - .	4.59	.03	1.19 - 2.25 - 9.96	2.09
	3	EFT EPA	3.67 3.59	.46 .37	3.58 4.70	.19 .10	5.99 22.69	.11 <.01	2.18 - 2.44 - 2.74 .33 - 1.21 - 4.29	.13	.72	1.21 - 2.02 - 5.38	2.02
Fish	1	EFT Dechlor	1.45 3.21	.37 .50	4.34 3.26	.20 .28	3.48 6.81	.32 .078	1.91 - 2.84 - 4.07 2.94 - 3.48 - 4.17	7.86	<.01	.35 - .80 - 1.55	.82
	2	EFT Dechlor	4.66 3.92	.88 .63	2.65 3.84	.58 .31	.56 2.12	.91 .55	2.48 - 3.19 - 3.70 1.55 - 1.98 - 2.35	.60	.44	1.17 - 1.52 - 2.23	1.61**

*EFT = Trinity River
EPA = Reconstituted Water
Dechlor = Dechlorinated tap water

**Indicates statistically different from 1.0 at α = 0.05

TABLE X

PROBIT ANALYSIS OF TOXICITY DATA FOR THE ELM FORK OF THE TRINITY RIVER, SANGER, TX BASED ON MEAN AQUEOUS ZINC (mg/L)

Mean Aqueous Concentration													
	Test	Water *	β	SE β	α	SE α	X^2model	p	LCL - EC50 - UCL	X^2slope	p	LCL - RMP - UCL	WER
Daphnia	1	EFT EPA	5.65 2.80	.70 .42	3.19 4.39	.22 .09	6.37 5.34	.10 .15	1.96 - 2.08 - 2.32 1.42 - 1.64 - 2.03	10.50	<.01	1.01 - 1.48 - 2.82	1.27
	2	EFT EPA	4.56 2.89	.59 .43	3.81 4.80	.18 .10	19.42 .05	<.01 <.01	. - 1.83 - . . - 1.17 - .	4.28	.04	.95 - 1.67 - 6.60	1.56
	3	EFT EPA	3.62 3.59	.41 .37	4.14 4.70	.12 .10	1.67 22.69	.64 .00	1.54 - 1.73 - 1.95 .33 - 1.21 - 4.29	.14	.71	.98 - 1.43 - 2.46	1.43
Fish	1	EFT Dechlor	1.63 2.78	.43 .40	4.67 4.60	.14 .12	4.25 4.27	.23 .13	1.10 - 1.58 - 2.17 1.15 - 1.40 - 1.74	3.81	.05	.82 - 1.12 - 1.62	1.13
	2	EFT Dechlor	5.47 4.76	1.00 .73	3.54 3.99	.42 .29	.66 2.61	.88 .46	1.50 - 1.85 - 2.10 1.33 - 1.63 - 1.88	.43	.51	.91 - 1.09 - 1.34	1.13

* EFT = Trinity River
EPA = Reconstituted water
Dechlor = Dechlorinated tap water

TABLE XI

PROBIT ANALYSIS OF TOXICITY DATA FOR THE SABINE RIVER NEAR MINEOLA, TX BASED ON TOTAL ZINC (mg/L)

						Total Concentration							
Test		Water*	β	SE β	α	SE α	X^2model	p	LCL - EC50 - UCL	X^2slope	p	LCL - RMP - UCL	WER
Daphnia	1	SAB EPA	5.45 3.27	.55 .36	4.98 4.73	.12 .11	1.84 5.89	.61 .12	.91 - 1.01 - 1.11 1.04 - 1.21 - 1.38	19.69	.00	.48 - .80 - 1.18	.83
	2	SAB EPA	4.16 3.09	.41 .31	4.95 5.08	.01 .09	11.86 4.50	.01 .21	.62 - 1.03 - 1.55 .82 - .94 - 1.08	1.81	.18	.75 - 1.05 - 1.49	1.09
	3	SAB EPA	3.31 4.13	.34 .47	4.92 4.54	.09 .13	3.96 10.78	.27 .13	.92 - 1.06 - 1.20 .69 - 1.29 - 1.80	1.91	.17	.57 - .84 - 1.14	.82
Fish	1	SAB Dechlor	3.09 2.41	.47 .40	4.24 4.10	.21 .20	9.45 7.98	.02 .05	.13 - 1.76 - 3.11 .57 - 2.36 - 5.54	.71	.40	.34 - .71 - 1.20	.75
	2	SAB Dechlor***	3.64 -	.59 -	4.29 -	.23 -	.68 -	.88 -	1.24 - 1.57 - 1.86 - - -	-	-	- - -	-
	3	SAB Dechlor	2.89 1.82	.46 .38	4.12 4.48	.21 .18	5.37 7.63	.15 .05	1.61 - 2.02 - 2.42 1.35 - 1.94 - 2.54	3.20	.07	.50 - .96 - 1.82	1.04

* SAB = Sabine River
EPA = Reconstituted water
Dechlor = Dechlorinated tap water
*** Data not included due to control mortality

TABLE XII

PROBIT ANALYSIS OF TOXICITY DATA FOR THE SABINE RIVER NEAR MINEOLA, TX BASED ON MEAN AQUEOUS ZINC (mg/L)

		Mean Aqueous Concentration											
	Test	Water*	β	SE β	α	SE α	X^2model	p	LCL - EC50 - UCL	X^2slope	p	LCL - RMP - UCL	WER
Daphnia	1	SAB EPA	5.23 3.26	.51 .36	5.96 4.83	.15 .10	2.09 5.82	.55 .12	.59 - .65 - .72 .98 - 1.13 - 1.29	17.30	.00	.27 - .57 - .89	.57*
	2	SAB EPA	3.73 2.99	.37 .31	5.56 5.13	.11 .09	10.36 4.36	.02 .23	.42 - .71 - 1.07 .78 - .90 - 1.04	.56	.45	.51 - .76 - 1.05	.79
	3	SAB EPA	2.77 3.71	.28 .42	5.50 4.98	.10 .09	5.35 13.26	.15 .00	.56 - .66 - .77 .39 - 1.01 - 1.53	3.26	.07	.36 - ..69 - 1.11	.65
Fish	1	SAB Dechlor	2.87 2.74	.45 .45	4.61 4.21	.17 .18	9.54 6.82	.02 .08	.07 - 1.35 - 2.50 1.58 - 1.95 - 2.35	-	-	.34 - .69 - 1.11	.71
	2	SAB Dechlor**	3.14 -	.51 -	5.01 -	.15 -	1.16 -	.76 -	.75 - .99 - 1.21 - - -	-	-	- - -	-
	3	SAB Dechlor	2.82 2.34	.46 .51	4.53 4.78	.16 .14	6.89 9.12	.08 .03	1.16 - 1.47 - 1.78 . - 1.24 - .	.59	.44	.68 - 1.15 - 2.33	1.18

* SAB = Sabine River
EPA = Reconstituted water
Dechlor = Dechlorinated tap water

***Data not included due to control mortality

TABLE XIII

PROBIT ANALYSIS OF TOXICITY DATA FOR THE RED RIVER, GAINSVILLE, TX BASED ON TOTAL ZINC (mg/L)

Mean Aqueous Concentration												
Test	Water*	β	SE β	α	SE α	X^2model	p	LCL - EC50 - UCL	X^2slope	p	LCL - RMP - UCL	WER
Daphnia 1	RED	2.49	.31	9.38	.12	9.21	.027	.95 - 1.78 - 3.64	9.58	.00	1.40 - 2.47 - 6.66	2.37**
	EPA	3.93	.46	5.48	.11	4.96	.18	.65 - .75 - .85				
2	RED	2.28	.28	4.72	.08	1.49	.68	1.14 - 1.33 - 1.58	3.34	.07	1.04 - 1.27 - 1.59	1.27**
	EPA	3.16	.34	4.93	.09	2.95	.40	.91 - 1.05 - 1.20				
3	RED	2.49	.32	4.35	.10	1.04	.79	1.57 - 1.82 - 2.18	4.53	.03	1.30 - 1.60 - 2.04	1.64**
	EPA	3.53	.35	4.84	.10	4.54	.21	.98 - 1.11 - 1.26				
Fish 1	RED	8.76	5.27	-3.02	3.96	3.42	.33	. - 8.23 - .	2.10	.15	4.38 - 9.71 -39.97	4.22
	Dechlor	2.74	.45	4.21	.18	6.82	.08	1.59 - 1.95 - 2.35				
2	RED	4.17	.71	.90	.65	3.27	.35	8.58 - 9.64 -11.38	.84	.36	4.25 - 9.04 -30.19	9.09**
	Dechlor	3.38	.56	4.91	.16	1.57	.67	.82 - 1.06 - 1.26				
3	RED	7.16	1.59	-1.86	1.70	12.52	-	. - 9.07 - .	-	-	- - -	5.56
	Dechlor	3.42	.52	4.27	.17	11.22**	.11	.19 - 1.63 - 4.32				

*RED = Red River
EPA = Reconstituted water
Dechlor = Dechlorinated tap water
**Indicates statistically different from 1.0 at $\alpha = 0.05$

TABLE XIV

PROBIT ANALYSIS OF TOXICITY DATA FOR THE RED RIVER, GAINSVILLE, TX BASED ON MEAN AQUEOUS ZINC (mg/L)

Total Concentration													
	Test	Water*	β	SE β	α	SE α	X^2model	p	LCL - EC50 - UCL	X^2slope	p	LCL - RMP - UCL	WER
Daphnia	1	RED	2.42	.31	4.18	.13	11.25	.01	1.00 - 2.19 - 5.79	10.85	.00	1.49 - 3.05 - 13.66	2.88**
		EPA	4.25	.49	5.49	.11	9.89*	.02	.42 - .76 - 1.11				
	2	RED	2.29	.29	4.22	.12	1.95	.58	1.87 - 2.19 - 2.59	3.38	.07	1.53 - 1.96 - 2.67	1.95**
		EPA	3.17	.34	4.84	.10	2.96	.40	.97 - 1.12 - 1.28				
	3	RED	2.41	.31	3.92	.14	1.37	.71	2.40 - 2.80 - 3.39	6.11	.01	1.78 - 2.33 - 3.28	2.39**
		EPA	3.62	.37	4.75	.10	4.46	.22	1.03 - 1.17 - 1.32				
Fish	1	RED	7.76	4.71	-4.21	4.72	3.48	.32	. -15.38 - .	2.02	.16	6.07 -16.80 -106.44	6.53
		Dechlor	2.41	.40	4.10	.20	7.98	.05	.57 - 2.36 - 5.54				
	2	RED	3.74	.63	.29	.74	2.10	.55	15.96 -18.20 -21.91	1.05	.31	5.61 -13.77 - 57.82	13.89**
		Dechlor	2.96	.49	4.65	.19	1.67	.65	.97 - 1.31 - 1.60				
	3	RED	7.55	1.50	-4.27	1.98	9.50	.02	. -16.74 - .	-	-	- - -	6.53
		Dechlor	3.13	.48	3.72	.24	9.34**	.02	.82 - 2.56 - 5.56				

*RED = Red River
EPA = Reconstituted water
Dechlor = Dechlorinated tap water
**Indicates statistically different from 1.0 at $\alpha = 0.05$

significant ($p < 0.05$), this statistic indicates that the slope of the concentration response curves for the natural and reference waters are statistically different. The relative median potency and associated 95% confidence limits is also reported and represents the ratio of LC50 values. If the 95% confidence intervals about the relative median potency do not include 1.0 then the potency of zinc between the two waters is judged statistically different. However, this assumes that the concentration response slopes are equivalent. The Water Effects Ratio (WER) also is shown for each experiment. Several generalizations are apparent.

First, the response of test organisms to zinc in reference water was shown to be reproducible during this study indicating that cultures were adequate for providing consistently healthy animals. The variability observed in zinc reference tests was about the same for both organisms and is summarized in Table XV. Secondly, toxicological differences expressed as a WER between natural and reference waters were generally less than a factor of 3 and often not significant. Similar conclusions were reached when these data were compared in terms of relative median potency, although this method indicated more toxicologically significant differences. Fish tested in Red River water provided the only exception yielding high WERs of 6.52 to

TABLE XV

VARIATION IN ZINC REFERENCE TOXICITY EXPERIMENTS

Organism	Reference Water	Zinc Exposure	LC50 (mg/l)		Probit Slope		Condition Factor[1]		Number of Experiments
			X[a]	C.V.[b]	X[a]	C.V.[b]	X[a]	C.V.[b]	
P. promeleas	Dechlorinated Tap Water	Total	2.30	29.2	2.91	22.6	11.1	5.3	7
		Mean Aqueous	1.46	20.5	3.27	23.9	10.6	3.6	7
D. pulex	Moderately Hard Reconstituted Water	Total	1.06	24.5	3.40	20.4	10.1	3.7	18
		Mean Aqueous	NA[c]	NA	NA	NA	NA	NA	-

[1] Condition factor calculated as the standard deviation of the Log tolerance distribution divided by the mean of the Log tolerance distribution x 100. This parameter has the advantage of utilizing both point and slope estimates of toxicity to assess organism response.

[a] X - mean

[b] C.V. - Coefficient of Variation = (Stnd. deviation/mean) x 100

[c] NA - Not applicable since total and mean aqueous zinc were equivalent in these tests.

13.89 and 4.22 to 9.09 for total and mean aqueous zinc, respectively (Tables XIII and XIV). Thirdly, in general WERs expressed in terms of mean aqueous zinc were reduced, i.e. approached unity, in comparison to WERs based on total zinc. This supports the hypothesis that particulate metal forms are less biologically available. In addition, WERs based on total and aqueous zinc were the same for both daphnids and fish tested in reconstituted water. This was expected, since total and aqueous zinc concentrations during these tests were similar. Although toxicological differences for daphnids tested in Sabine River water apparently became more significant when based on mean aqueous zinc concentrations, as reflected by WERs that deviate from unity, this also was probably attributable to the reduced toxicity of particulate zinc. In this case, dissolved zinc exerted greater toxicity in Sabine River water relative to reconstituted water. However, this difference was obscured when the WER was expressed in terms of total zinc concentration because the enhanced toxicity of the dissolved species was offset by the reduced bioavailability of particulate zinc forms included in the total zinc measurement.

Individual experiments comparing zinc toxicity in natural and reference waters shown in Tables IX-XIV were combined and reanalyzed. These results are given in Table XVI and XVII. Toxicity data are also provided for fish tested in reconstituted water and daphnids tested in dechlorinated tap water. It is interesting to note that the toxicities of zinc in the reference waters of similar hardness are statistically different. Moreover, zinc is approximately twice as toxic to daphnids but only half as toxic to fish when tested in dechlorinated tap water relative to reconstituted water (Table XVI). The differences in zinc toxicity between the two reference waters for fish are not observed when results are expressed in terms of mean dissolved zinc. However, the toxicity of dissolved zinc to daphnids tested in dechlorinated tap water appears to be greater than in reconstituted water (Table XVII).

Examination of the relationship between LC50 and slope estimates based on both total and mean aqueous concentration in Tables XVI and XVII reveal four general patterns (Figure 28). In the first case, both LC50 and slope estimates are the same regardless of whether results are expressed in terms of mean aqueous or total zinc concentrations. Toxicity results in reconstituted water fall into this first category. In the next three cases, the LC50 is reduced when expressed in terms of mean aqueous zinc. However, the slope

TABLE XVI

SUMMARY OF PROBIT ANALYSIS RESULTS BASED ON TOTAL ZINC (mg/L)

Organism	Water*	ß	SE ß	α	SE α	X^2model	p	LCL - EC50 - UCL	X^2slope	p	LCL - RMP - UCL	WER
Daphnia	EFT EPA	4.30 3.04	.31 .22	3.20 4.63	.13 .051	53.50 48.24	<.01 <.01	2.27 - 2.61 - 3.06 1.11 - 1.32 - 1.64	1.47	.23	1.51 - 2.07 - 3.24	1.98**
Fish	EFT Dechlor	4.66 3.92	.88 .63	2.65 3.84	.58 .31	.56 2.12	.91 .55	2.48 - 3.19 - 3.70 1.55 - 1.98 - 2.35	.60	.44	1.17 - 1.52 - 2.23	1.61**
Daphnia	SAB EPA	4.12 3.20	.23 .20	1.95 1.84	.060 .059	32.02 35.19	<.01 <.01	.92 - 1.03 - 1.15 .96 - 1.12 - 1.28	6.66	<.01	.74 - .89 - 1.05	.92
Fish	SAB Dechlor	3.14 2.10	.29 .27	4.22 4.30	.12 .13	22.95 18.21	.04 .02	1.46 - 1.76 - 2.05 1.46 - 2.15 - 2.91	7.28	<.01	.55 - .76 - 1.00	.82
Daphnia	RED EPA	2.35 3.44	.17 .21	4.11 5.00	.073 .057	20.69 43.40	.08 <.01	2.18 - 2.39 - 2.64 .85 - 1.00 - 1.16	14.64	<.00	1.81 - 2.41 - 3.48	2.39**
Fish	RED Dechlor	5.19 2.76	.46 .32	-1.27 4.28	.57 .14	20.14 39.18	.02 <.01	14.24 -16.18 -18.21 .97 - 1.82 - 2.56	23.58	<.00	2.90 - 8.27 -62.85	8.89**
Daphnia	EPA Dechlor	3.06 2.56	.22 .18	4.74 5.30	.061 .058	45.54 108.5	<.01 <.01	1.01 - 1.22 - 1.43 .46 - .77 - 1.06	6.00	.01	1.09 - 1.53 - 2.34	1.58**
Fish	EPA Dechlor	3.57 2.89	.61 .45	4.52 3.85	.23 .23	5.51 .729	.138 .886	1.03 - 1.36 - 1.64 2.04 - 2.49 - 2.98	-	-	.34 - .51 - .69	.55**

*EFT = Trinity River
SAB = Sabine River
RED = Red River

EPA = Reconstituted water
Dechlor = Dechlorinated tap water
**Indicates statistically different from 1.0 at α = 0.05

TABLE XVII

SUMMARY OF PROBIT ANALYSIS RESULTS BASED ON MEAN AQUEOUS ZINC (mg/L)

Organism	Water*	β	SE β	α	SE α	X^2model	p	LCL - EC50 - UCL	X^2slope	p	LCL - RMP - UCL	WER
Daphnia	EFT EPA	4.32 2.96	.31 .22	3.82 4.69	.09 .05	43.66 72.88	<.01 <.01	1.67 - 1.88 - 2.17 1.04 - 1.27 - 1.63	13.04	<.01	1.11 - 1.44 - 2.05	1.48**
Fish	EFT Dechlor	5.47 4.76	1.00 .73	3.54 3.99	.42 .29	.66 2.61	.88 .46	1.50 - 1.85 - 2.10 1.33 - 1.63 - 1.88	.43	.51	.91 - 1.09 - 1.34	1.13
Daphnia	SAB EPA	3.62 3.22	.20 .21	5.62 5.00	.06 .05	40.91 29.43	<.01 <.01	.58 - .68 - .78 .87 - 1.00 - 1.13	-	-	.53 - .67 - .81	.68**
Fish	SAB Dechlor	2.83 2.28	.26 .31	4.72 4.58	.09 .11	28.91 24.45	<.01 <.01	.98 - 1.13 - 1.50 .97 - 1.53 - 2.13	2.02	.15	.56 - .78 - 1.06	.74
Daphnia	RED EPA	2.33 3.39	.17 .20	4.51 5.05	.05 .05	22.49 34.45	.05 <.01	1.41 - 1.62 - 1.88 .83 - .96 - 1.10	18.10	<.01	1.35 - 1.69 - 2.20	1.69**
Fish	RED Dechlor	5.65 3.34	.50 .37	-.35 4.59	.49 .11	17.64 29.34	.04 <.01	7.92 - 8.85 - 9.79 .90 - 1.32 - 1.69	16.07	<.01	2.84 - 6.35 -25.45	6.70**
Daphnia	EPA Dechlor	3.31 2.94	.29 .22	4.94 5.65	.07 .06	37.30 141.90	<.01 <.01	.75 - 1.04 - 1.33 .34 - .60 - .83	2.67	.103	1.09 - 1.69 - 3.34	1.73**
Fish	EPA Dechlor	3.55 3.45	.60 .54	4.69 4.61	.20 .14	5.21 .248	.157 .970	.92 - 1.22 - 1.47 1.10 - 1.30 - 1.51	5.36	.616	.74 - .93 - 1.20	.94**

*EFT = Trinity River
SAB = Sabine River
RED = Red River

EPA = Reconstituted water
Dechlor = Dechlorinated tap water
**Indicates statistically different from 1.0 at $\alpha = 0.05$

of zinc concentration response when based on mean aqueous zinc is either unchanged (Case 2), increased (Case 3) or decreased (Case 4) relative to the slope expressed on a total zinc basis. The distance between these curves represents particulate zinc. In the second case, particulate zinc remains fairly constant with increasing zinc concentrations as would be expected if, for example, adsorption was the dominant process controlling zinc solubility. This pattern was observed for daphnids tested in Trinity and Red River waters. In the third case, particulate zinc increases with increasing zinc concentration as would be anticipated if a precipitation mechanism was in operation. Interestingly, this pattern was observed for fish tested in Trinity and Red River water. This pattern seems plausible since fish were less sensitive than daphnids so that higher levels of zinc were required. Consequently, the extent of oversaturation and thus precipitation would be greater in these tests. In the last case, particulate zinc appears to decrease as a function of increasing zinc concentration. Toxicity results for both daphnids and fish in Sabine River water conformed to this pattern. One possible explanation may be as follows. At the lower pH of this water, zinc adsorption sites are saturated and a larger fraction of zinc remains soluble as

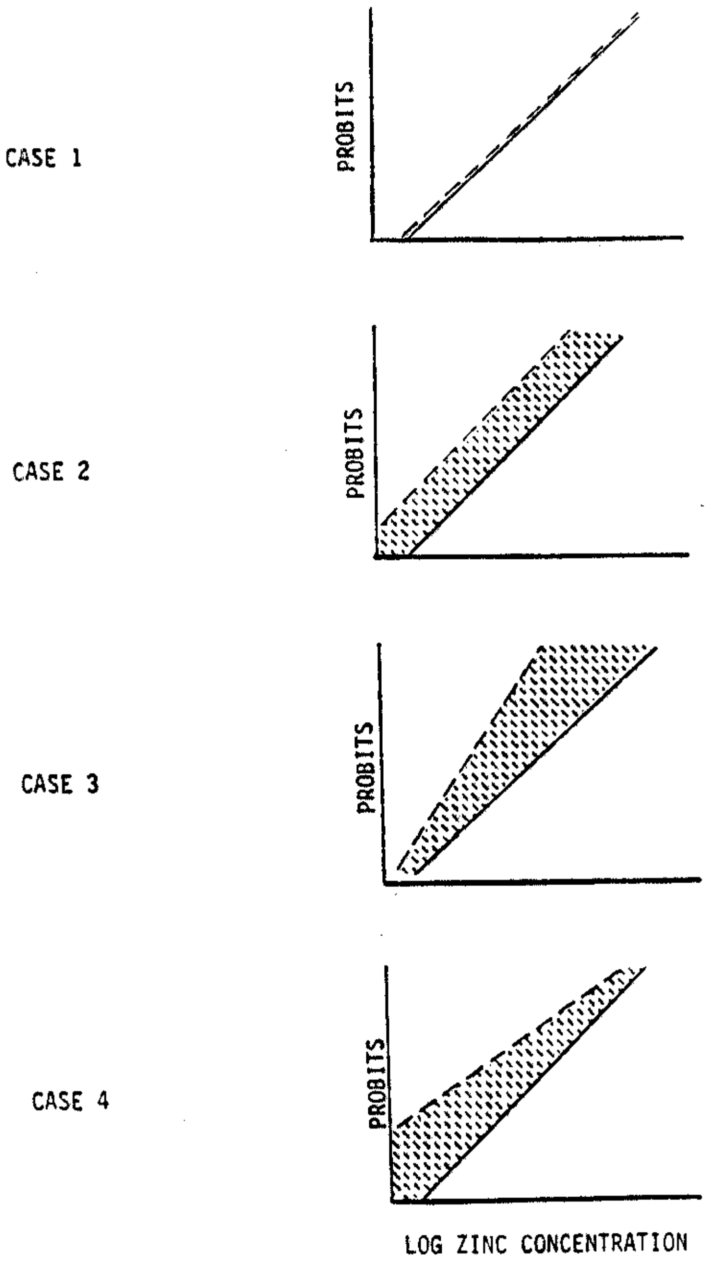

FIGURE 28 Four Observed Patterns Describing the Relationship Between Probit Slopes Based on Total Zinc and Mean Aqueous Zinc Concentrations. Solid Lines Represent Total Zinc while Dashed Lines Represent Aqueous Zinc. The Hatched Area Between Probit Lines Indicates Particulate Zinc.

zinc concentration is increased. Moreover, precipitation reactions that would decrease aqueous zinc levels do not come into play due to the lower pH. In addition, at higher zinc concentrations the formation of dissolved zinc-organic complexes may stabilize aqueous forms. The dissolved organic carbon content of this river was the highest of the waters used in this study (Table VII).

Using water quality data in Table VII and the total zinc LC50 concentrations provided in Table XVI, the speciation of zinc was predicted using MINTEQ. In order to predict adsorbed zinc species, partition coefficients for zinc adsorption to suspended solids in each of the natural waters were estimated by plotting the difference between total and aqueous zinc measurements i.e. particulate zinc versus aqueous zinc measurements that were observed within the first hour of toxicity experiments. Aqueous concentrations above 5 mg/l were excluded since levels in excess of this value were, in certain waters, not linear with lower, presumably undersaturated, dissolved zinc levels. The resultant kp's (L/kg) were $7.28 \pm .56 \times 10^3$, $1.81 \pm .15 \times 10^3$ and $1.56 \pm .11 \times 10^3$ for Trinity, Sabine and Red River suspended solids and agree with earlier work (Hall et al.,in press). Correlation coefficients for these relationships were .71, .60 and .74, respectively, and all were highly significant (p = 0.0001). These values represent worst case estimates and probably overestimate true kp's since rapidly precipitated zinc as well as zinc adsorbed to glassware and organisms is assumed negligible. The speciation of zinc in toxicity experiments is summarized in Table XVIII. Calculations presented in this Table indicate that zinc is predicted to occur predominantly as the free ion. However, in the case of the Red river water, precipitated zinc carbonate is predicted to be the dominant species. Zinc silicate is predicted to be an important component in Sabine River water.

Figure 29 indicates that the predicted aqueous zinc concentrations during toxicity experiments usually agree with the measured mean aqueous zinc exposures calculated from time-series measurements. However, the predicted dissolved zinc LC50 for fish tested in Red River water grossly underestimates the actual zinc exposure. This emphasizes the potential errors that may result when predicting metal exposures based on equilibrium models for systems that are not in equilibrium. In this specific instance, zinc exposure is underestimated by more than a factor of 20. These data also are in agreement with Bradley and Spragues' (1985) findings that the toxicity of dissolved zinc to fish is inversely related to water hardness and

TABLE XVIII

ZINC SPECIATION IN TOXICITY EXPERIMENTS*

				LC50 Total Zn	LC50 Mean Dissolved Zn	←Predicted Dissolved Species→								(Predicted Solid Species)		
Water	pH	Hardness	Organism			Zn+2	ZnOH+	Zn(OH)2Aq	ZnSO4	ZnCL+	ZnOHCL	ZnHCO3	ZnCO3Aq	ZnCO3 ppt	ZnSiO4 ppt	Adsorbed Zn
Trinity River	7.82	201	_D._ _Pulex_	2.61	1.88	1.66	.07	.04	.24	0	0	.04	.13	0	0	.41
			_P._ _promeleas_	3.19	1.85	1.57	.09	.07	.10	0	0	.05	.25	0	.64	.38
Sabine River	7.20	62	_D._ _pulex_	1.03	.68	.35	.01	0	.02	0	0	0	0	0	.57	.08
			_P._ _promeleas_	1.76	1.13	.88	.01	0	.04	0	0	0	0	0	.64	.20
Red River	7.94	760	_D._ _pulex_	2.39	1.62	.27	.02	.02	.08	.01	.01	0	0	1.88	.06	.04
			_P._ _promeleas_	16.18	8.85	.28	.02	.02	.09	.01	.01	0	0	15.66	.06	.05
Dechlorinated Tap Water	7.92	109	_D._ _pulex_	.77	.60	.43	.03	.03	.03	.0	.0	.02	.10	0	.13	0
			_P._ _promeleas_	2.11	1.44	1.33	.09	.09	.09	0	0	.05	.33	0	.13	0
Reconstituted Water	7.65	97	_D._ _pulex_	1.15	1.07	.91	.03	.02	.11	0	0	.02	.06	0	0	0
			_P._ _promeleas_	1.36	1.22	1.08	.04	.02	.13	0	0	.02	.07	0	0	0

The MINTEQ geochemical model developed by Felmy et al. (1983) was used to predict zinc speciation. Model calculations assumed equilibrium with atmospheric carbon dioxide. Zinc is expressed in units of mg/l.

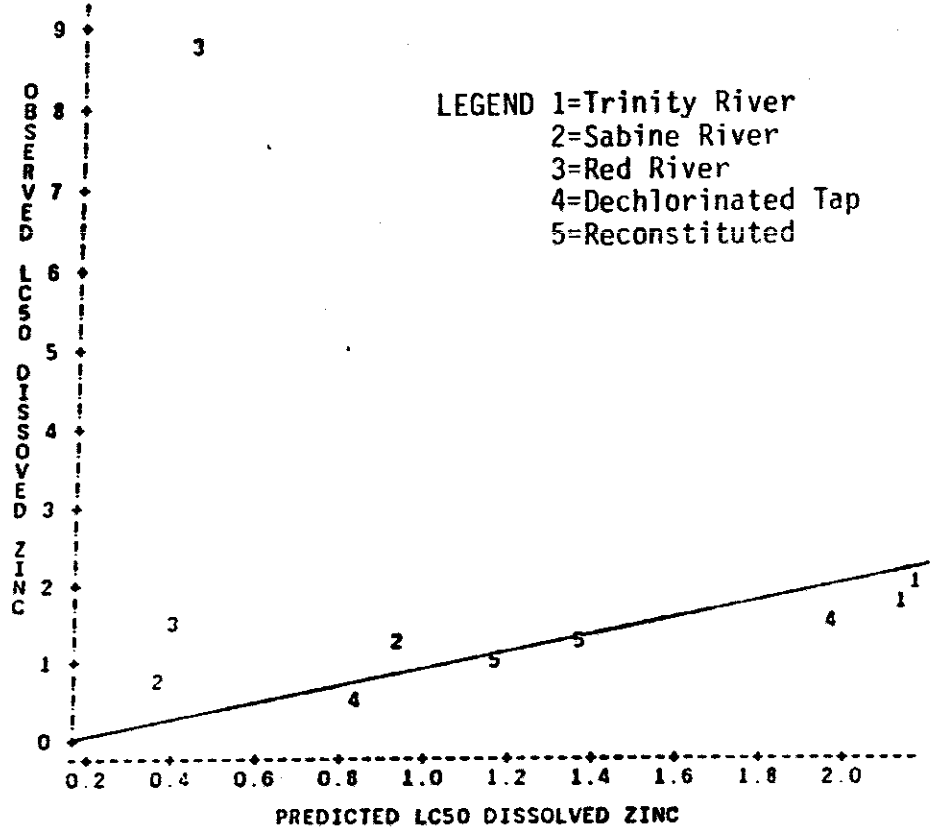

FIGURE 29 Predicted Aqueous versus Observed Aqueous Zinc Exposures. Observed Values calculated as the Mean of Time-Series Dissolved Zinc Measurements during Toxicity tests. Predicted Values obtained from the geochemical model MINTEQ.

directly related to pH. Using their data for rainbow trout the following equation was determined:

LC50 (dissolved zinc mg/l) = .0177 (hardness) - 1.171 (pH) + 8.11 (r^2 = .7824, p = 0.009)

and for my data the corresponding equation was LC50 (dissolved zinc mg/l) = .0018 (hardness) - .673 (pH) + 5.19 (r^2 = .9869, p = 0.007).

Toxicity experiments conducted with rainbow and brook trout by Holcombe and Andrew (1978) were used by Pagenkopf (1983) to study the effects of zinc speciation on toxicity. Using these data, excellent agreement is also observed when the data are fit to the above model:

LC50 (dissolved zinc mg/l) =

.0090 (hardness) - 1.935 (pH) + 1.466 (r^2=.9643, p=0.003)

brook trout

.0191 (hardness) - 2.721 (pH) + 21.48 (r^2=.9902, p=0.0005)

rainbow trout

Undoubtedly, the extremely high correlation coefficients for these models are in part, a consequence of violating the statistical assumption that the independent variables, hardness and pH, are uncorrelated. Nevertheless, this analysis appears to indicate that increasing hardness or decreasing pH reduces the toxicity of dissolved zinc to fish. However, this relationship was not adequate for describing zinc toxicity results with daphnids. This is partly related to the unexpectedly low LC50 value for daphnids, based on either total or aqueous zinc, that was obtained in Red River water (Figures 30,31). The hardness of this water was extremely high, yet zinc toxicity was not reduced to the extent predicted. This suggests that the antagonistic effect of hardness on zinc toxicity may reach some maximal level, after which, further increases in water hardness afford little additional protective effect. Toxicity differences between daphnids and fish may also reflect differences in zinc's toxic mode of action. Alternative models were tested for describing daphnid toxicity results as a function of speciation and water quality variables but attempts were unsuccessful.

Up to this point, data suggested that particulate zinc forms were biologically unavailable. To further investigate this observation, two zinc toxicity experiments, using Trinity River and dechlorinated tap water, were conducted in which fish were exposed after 0, 24 and 96 hours of zinc equilibration with test waters. As equilibration time increased, a greater fraction of the total zinc was converted into precipitated forms with a concomitant reduction in zinc toxicity. Test solutions with total zinc concentrations initially resulting in 100% mortality caused

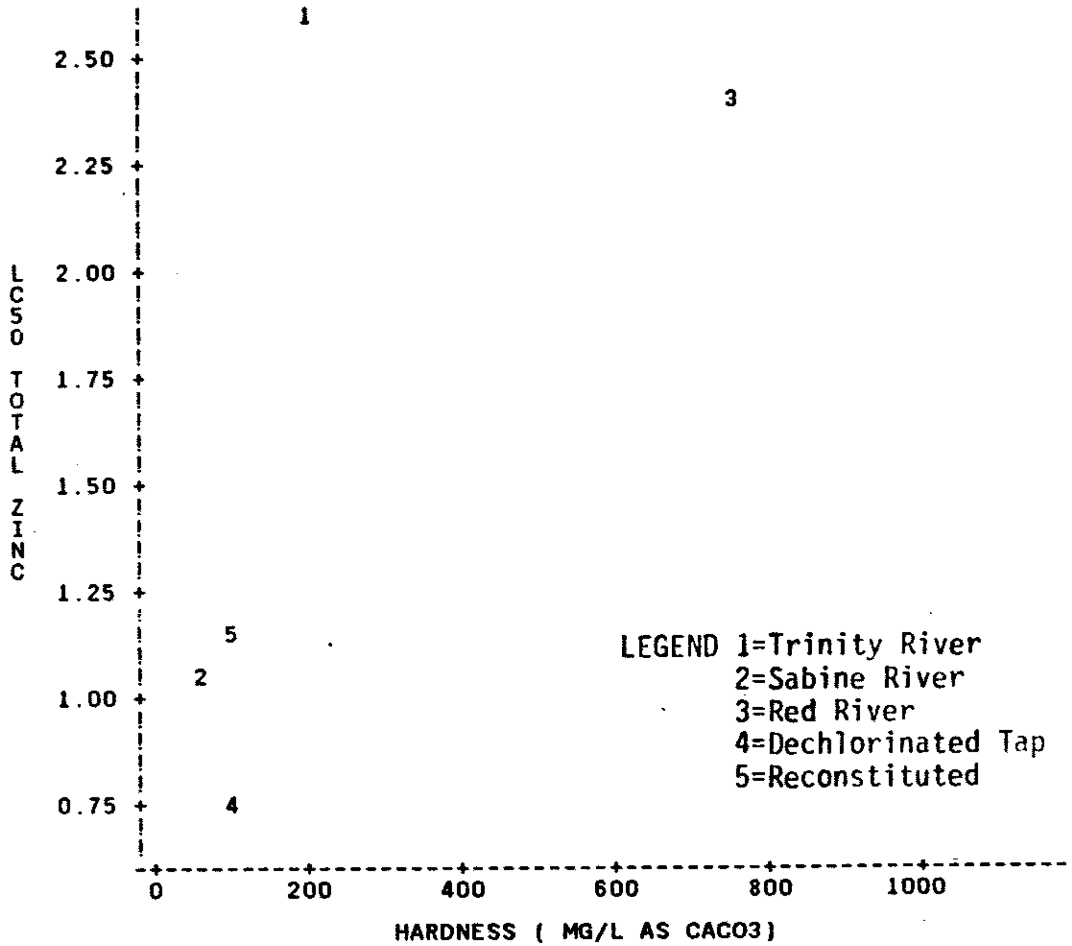

FIGURE 30 Daphnid LC50s based on Total Zinc(mg/L) as a Function of Test Water Hardness.

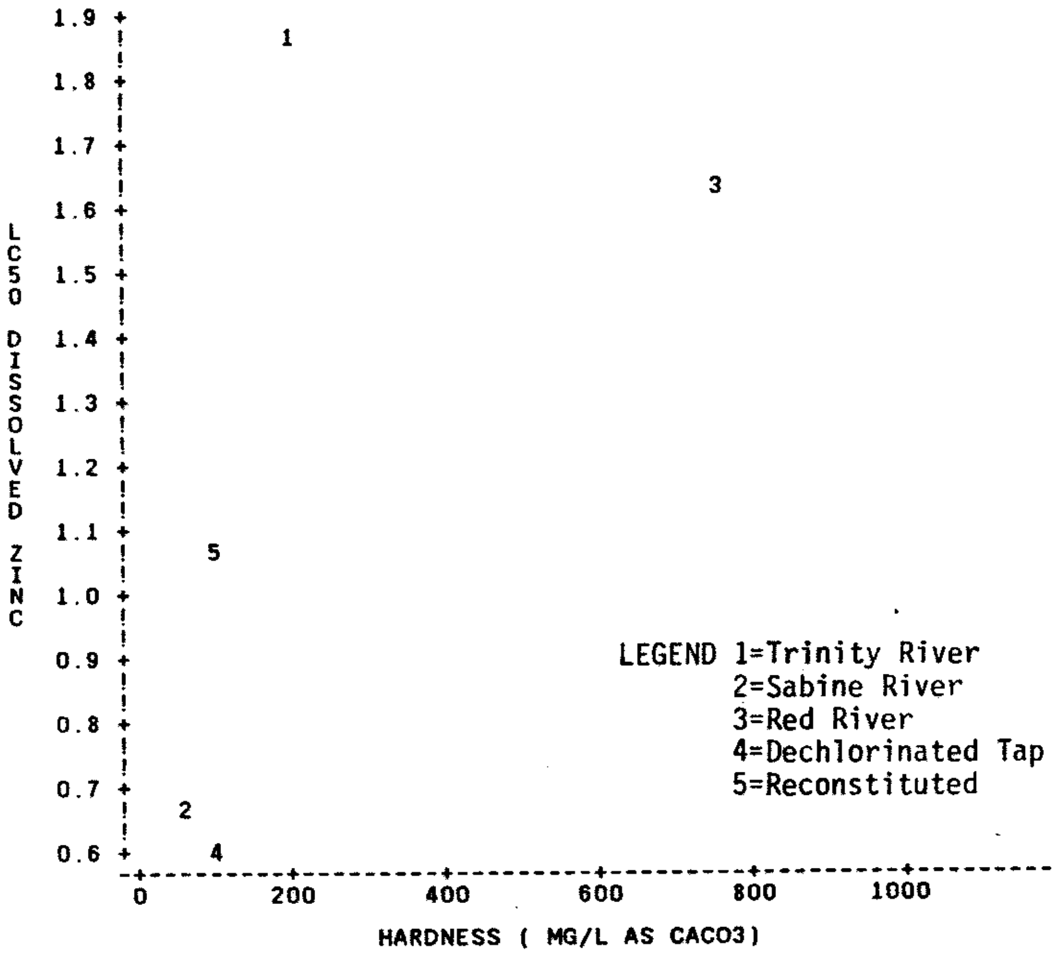

FIGURE 31 Daphnid LC50s Based on Mean Dissolved Zinc(mg/L) as a Function of Test Water Hardness.

no mortality after 96 hours of equilibration (Figure 32). These results were observed for both waters. Thus, under the given set of experimental conditions, precipitated zinc was demonstrated to be toxicologically unavailable to the fathead minnow.

In a second experiment, increasing concentrations of zinc were spiked to Trinity River water and were then allowed to equilibrate for 96 hours. Half of each solution was filtered through a 0.45 micron filter while the other half was left unfiltered. Daphnids were then exposed in filtered and unfiltered waters. LC50 values of 2.04 and 2.42 for filtered and unfiltered samples based on total zinc were statistically significantly different. In contrast, LC50 values based on dissolved zinc were 1.70 and 1.78, respectively, and were not statistically different. The differences between total and aqueous concentrations were believed to be primarily due to adsorption rather than precipitation mechanisms because most of the concentration levels used in this toxicity experiment were probably undersaturated with respect to solid precipitates. These results indicate that particulate zinc, in this case probably adsorbed forms, are not biologically available to daphnids under the conditions tested.

In order to provide some predictive ability as to

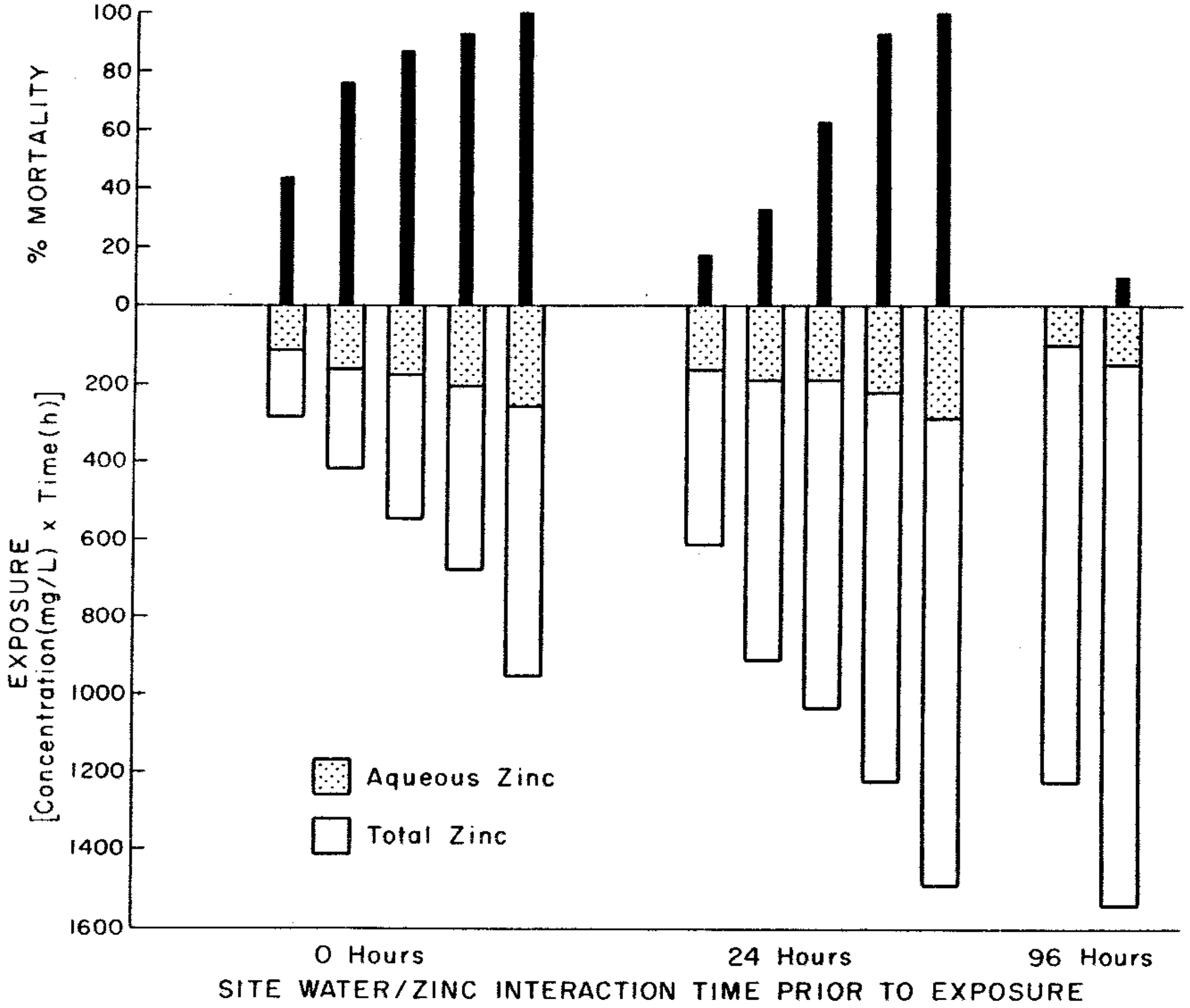

FIGURE 32 Effect of Time on Zinc Tocicity to *P. promel* in Trinity River Water after 96 hours of exposure.

whether suspended solids are likely to significantly influence zinc bioavailability, a simple model was constructed. The model assumes solids independent zinc partitioning and requires that the relationship between zinc toxicity response based on total and dissolved zinc be represented by Case 2 in Figure 28. In a natural system, where zinc concentrations typically will be undersaturated, adsorption processes should predominate. Consequently, this scenario (Case 2) would appear most appropriate. The model further assumes that only dissolved zinc exerts a toxic action. The model operates as follows: a zinc partition coefficient and a representative suspended solids measurement for the receiving system in question is input so that the fraction of zinc remaining in the aqueous phase can be calculated. This fraction represents the fractional reduction in the LC50 expected as a result of a zinc adsorption. Partition coefficients for zinc generally range from 1 to 10^6 for aquatic solids (Table III) and suspended solids typically range from 10^2 to 10^3 in most lotic systems (USGS, 1976). The determining factor which will govern whether or not this fractional reduction is significant is the tightness of the confidence intervals about the LC50 estimate. The confidence about the LC50 estimate will in turn depend upon test design and the test organism used.

Thus, the third input parameter for the model must provide a measure that relates to the confidence associated with experimentally derived toxicity end points. If confidence limits are tight, small reductions in dissolved zinc concentrations may translate into toxicologically significant differences whereas, the reverse is also true. If confidence intervals are wide even large reductions in dissolved zinc may not result in significantly different LC50's if laboratory toxicity tests are conducted in filtered and unfiltered water as in the experiment discussed above. To keep the model as general as possible, the third input parameter was formulated as the difference between upper and lower 95% confidence limits divided by the LC50. Typical values for this parameter range from .1 to 10. Sensitivity analyses using this model (Table XIX) revealed that the Log of the product of suspended solids (mg/l) x partition coefficient (L/kg) would have to exceed 5 in order for solids to significantly alter zinc bioavailability. Above this value, however, suspended solids are predicted to significantly reduce zinc bioavailability to water column organisms. Further details regarding the derivation of this model, which may be applied to any chemical, are included in Appendix 1.

In an attempt to evaluate how well natural zinc criteria

TABLE XIX

PREDICTED WATER EFFECTS RATIOS (LC50 WITH SOLIDS/LC50 WITHOUT SOLIDS) RESULTING FROM ZINC ADSORPTION TO SUSPENDED SOLIDS BASED ON PARTITION COEFFICIENT (L/KG) SUSPENDED SOLIDS CONCENTRATION (MG/L) AND CONFIDENCE INTERVALS ABOUT TOXICITY ESTIMATES.

(UCL-LCL)/LC50*	Log (Partition Coefficient x Suspended Solids)				
	5	6	7	8	9
.10	NS	2	11	101	1001
.25	NS	2	11	101	1001
.50	NS	2	11	101	1001
.75	NS	2	11	101	1001
1.00	NS	NS	11	101	1001
2.50	NS	NS	11	101	1001
5.00	NS	NS	11	101	1001
7.50	NS	NS	11	101	1001
10.00	NS	NS	NS	101	1001

*(Upper 95%Confidence Limit-Lower 95%Confidence Limit)/LC50

NS = Water Effects Ratio not statistically significantly different from one.

reflect site-specific toxicity differences found in this study, the observed WERs were compared to those that would be predicted in each of these experiments based solely on differences in water hardness by applying the equation provided in the proposed 1986 National Water Quality Criteria Document for Zinc (Figure 6). Table XX indicates that observed and predicted WERs show excellent agreement and differences are well within a factor of two. Thus, as a first approximation, water quality monitoring data for water hardness, and to a less extent suspended solids, can be used to predict the site-specific sensitivity of aquatic systems to zinc inputs.

TABLE XX

WATER EFFECTS RATIOS (LC50 SITE WATER/LC50 LAB WATER) BASED ON TOTAL AND MEAN AQUEOUS ZINC (mg/L)

		DAPHNIA PULEX	PIMEPHALES PROMELAS	GEOMETRIC MEAN
Trinity River	Total	1.05* (1.82)	1.61* (1.65)	1.82 (1.74)
	Dissolved	1.98*	1.13	1.24
Sabine River	Total	.92 (.69)	.82 (.63)	.87 (.66)
	Dissolved	.68*	.74	.71
Red River	Total	2.39* (5.40)	8.89* (4.91)	4.61 (5.15)
	Dissolved	1.69*	6.70*	3.36

* Indicates ratio is statistically different from one at =.05.

( ) Predicted ratio based on ratio of national criterion adjusted for site and lab water hardness. using the equation: Criterion Maximum Concentration (ug/l) = $e^{(0.8195[\ln \text{hardness}] + 0.7871)}$ USEPA (1986).

CHAPTER V

Conclusions and Recommendations

Conclusions

The following conclusions are evident based on the results of this work:

1. Kinetics of zinc precipitation/adsorption during toxicity tests varied considerably in the five experimental waters tested. Statistical models were shown to be useful for describing these kinetic data.
2. Thermodynamic equilibrium model predictions for dissolved zinc generally agreed with mean dissolved zinc exposures determined from time-series measurements. This indicates the utility of interpreting toxicity data using equilibrium models. However, model predictions may grossly underestimate exposures for systems that are not at, or readily approaching, equilibrium.
3. The toxicity of zinc to aquatic organisms is complex. Even after accounting for the speciation of zinc, variations in toxicity data are still apparent. In the case of fish, the toxicity of dissolved zinc appears to be inversely related to water hardness and directly related to pH.

4. Particulate forms of zinc were not biologically available to the two organisms under the conditions tested.
5. Application of the Indicator Species Procedure for deriving site-specific criteria based on total zinc may be unnecessary since national criterion appropriately adjusted for water hardness accurately predict, within a factor of two, actual toxicity differences observed in the laboratory.
6. Aquatic systems with elevated levels of water hardness and suspended solids will in general be the least vulnerable to deleterious zinc impacts. Assuming that the partition coefficients derived in this study are representative of other systems, suspended solids levels in excess of 100 mg/L are likely to alter the chemical bioavailability of zinc to water column organisms.

Recommendations

The Indicator Species Procedure has a number of major drawbacks when applied to the derivation of zinc site-specific criteria. First, the procedure assesses the impact of zinc to water column organisms, only. This may be inappropriate since fate processes will tend to cause zinc to be accumulated in the sediments. Thus, reductions in the bioavailability of zinc as demonstrated by the Indicator Species Procedure may overlook potential adverse exposures that may be imposed on sediment biota. Secondly, extrapolating the results from comparative acute toxicity tests to field situations may introduce another source of error. To elicit acute toxicity end points, relatively high levels of zinc must be employed. This in turn introduces precipitation reactions that are unlikely to be occuring in the natural system to the extent observed in toxicity tests. Thus, differences between laboratory and site waters may simply reflect differences in the ability of these waters to precipitate high levels of zinc and have little utility for predicting the site-specific bioavailability of zinc in the field. In addition, the results obtained using the Indicator Species Procedure will be highly subject to the reference water chosen for comparison. Based on these limitations, it would seem most appropriate to utilize chronic toxicity testing and to include at least one test organism that is associated with the sediment. In addition, choice of a standardized reference water would be necessary to ensure a valid baseline to which site-specific data could be compared. Additional research concerning the influence of pH on zinc toxicity in natural systems is warranted. Apparently, a tradeoff may exist between the enhanced toxicity of dissolved zinc at higher pH and the increased tendency of the dissolved zinc to adsorb to suspended solids at higher pH. Further attempts aimed at understanding the kinetics of zinc speciation and aquatic fate as well as illucidating zinc's mode of toxic action to aquatic life should aid in the development of models that predict site-specific zinc bioavailability.

APPENDIX I - Derivation of Sorption-Solids-Toxicity Model

Appendix I

Derivation of Sorption-Solids-Toxicity Model

$$FACR = 1 - \left(\frac{Kp \times ss/1E + 06}{(Kp \times ss/1E + 06)+1} \right)$$

where:

FACR = Fraction of Aqueous Concentration Remaining in Solution

K_p = Zinc Partition Coefficient (L/kg)

ss = Suspended Solids Concentration (mg/l)

The LC50 without solids may be formulated as:

LC50 without solids = LC50 with solids x FACR

rearranging

LC50 with solids = LC50 without solids/FACR

The difference in the LC50s with and without solids can be expressed as:

(LC50 without solids/FACR) - LC50 without solids

This difference can be compared to the difference between upper and lower 95% confidence limits that are typical for the organism and test chemical in question. If the difference between LC50s exceeds the difference between confidence limits then it is likely that the predicted difference would be statistically significant and could be demonstrated in laboratory experiments.

Dividing through by the LC50 without solids yields the following general relationship:

$$1/FACR - 1 = (UCL - LCL)/LC50$$

$$\text{If} \quad 1/FACR - 1 > (UCL - LCL)/LC50$$

then solids are likely to significantly reduce zinc bioavailability.

$$\text{If } 1/FACR - 1 < (UCL - LCL)/LC50$$

Then solids are unlikely to significantly reduce zinc bioavailability.

These equations formed the basis for the development of a simple BASIC computer program that was used for the sensitivity analysis.

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