

REVIEW PAPER

CHEMICAL ASPECTS OF BIOASSAY TECHNIQUES FOR ESTABLISHING WATER QUALITY CRITERIA

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Abstract—Increasing emphasis is being placed on the use of bioassay techniques to evaluate potential water quality problems in the aquatic environment. Although the physical environment is carefully maintained, often less emphasis is placed on maintaining the proper chemical environment in bioassay tests. Considerations of oxidation state, solubility, complexation, ionic strength, type and amount of solids, salt ratios and concentrations and organic content are essential in extrapolating the results from the test conditions to the environment. The chemical history of the test organism must be controlled to insure meaningful results, and analytical measurements should be made to insure that the proper chemical species is maintained throughout the test period. If the environmental conditions are not duplicated, studies are needed to determine how the results are dependent on the test environment used. This paper discusses examples in which changes in the chemical environment of a bioassay test may affect the results of the test and recommends procedures for minimizing problems of this type.

INTRODUCTION

INCREASING emphasis is being placed today on determining the effects of chemicals on water quality. For example, during the past 5 yr, the United States has established two national water quality laboratories, the freshwater quality laboratory in Duluth, Minnesota, and the marine laboratory in Kingston, Rhode Island, for the purpose of determining the critical concentrations of chemicals for various aquatic organisms. In addition an increasing number of papers are being published by governmental, university and industrial laboratories on the critical concentrations of chemicals to aquatic organisms. The procedures generally used to establish critical concentrations for an element or compound in natural waters involves acute and/or chronic bioassay where the organism is exposed to the chemical for a prescribed period of time. These tests result in a correlation between the concentration of the chemical and the detrimental effects on the organisms which is often the basis for establishing water quality standards by federal and state regulatory agencies. Although bioassays provide essential information for evaluating the significance of hazardous chemicals in the environment and for establishing control measures for their use, the accuracy with which the results of the bioassay can be extrapolated to environmental conditions depends largely on the knowledge of the state of the chemical in the environment and the ability to reproduce this chemical state under test conditions of a bioassay procedure.

One of the most significant findings in recent years is that the chronic sublethal toxicity of many elements and compounds occurs at concentrations which are 100 or more times less than the critical concentration for acute lethal effects on the same organism. In some circumstances water quality criteria based on chronic sublethal bioassay procedures are equal to or less than the apparent natural concentrations in some particular body of water without any apparent effects on the aquatic organisms

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present in the waters. While it is possible that the natural conditions are having subtle detrimental effects on the organism under question it is more likely that the particular form of the element that was found to be toxic under laboratory bioassay conditions is different than the form of the element present in the natural waters. Therefore, the apparent contradiction is resolved due to a difference in the chemical environment of the bioassay procedure and the natural waters where the results of the laboratory procedures are being applied. Also, there may be inadequate data available to determine the existing concentrations in the water or the analytical and sampling methods that have been used for establishing existing concentrations may not be reliable. For example, the proposed standard for zinc in Lake Superior was apparently lower than the natural levels of zinc in Lake Superior (KOPP and KRONER, 1967; MOUNT, 1966; FWQA, 1969) though there were no data to suggest that fish reproduction was being hampered by these apparently high zinc concentrations. A study was initiated at the University of Wisconsin Water Chemistry Program to determine the existing zinc concentrations in eastern Lake Superior and the forms of the zinc present in the water at various locations in the lake. NIENKE (1971) found in the summer of 1969 that the zinc concentration in the waters of eastern Lake Superior did not exceed the proposed water quality criteria of the Environment Protection Agency, Duluth Laboratory for the reproduction of fish. Therefore, the apparently anomalous situation of the naturally occurring zinc concentrations being sufficiently high to inhibit natural reproduction, i.e. the water quality standard being lower than existing natural concentrations without any apparent effects, may be due to errors in the analysis of zinc by the previous investigators or due to a reduction in the zinc input from local sources near the sampling station used by the Environmental Protection Agency as a basis for monitoring water quality in eastern Lake Superior.

Another important reason for this type of problem is that there is a significant difference between the testing procedures that were used in the laboratory studies to establish the critical concentrations of the chemical and the conditions that exist in natural waters. Of particular concern are differences in the chemical environment between the two conditions. Often toxicologists place considerable emphasis on maintaining the same physical environment with respect to temperature and light in the test conditions; however, the chemical conditions may markedly differ from those found in many natural waters. The chemical environment of a bioassay procedure includes consideration of each of the major types of chemical reactions of it that can occur in natural waters. Included within this group are various types of oxidation-reduction (redox), precipitation, gas transfer, sorption, both abiotic and biotic, and complexation including chelation. Not only must the position of equilibrium which is determined by the thermodynamics of the reactions be considered but also consideration must be given to kinetics of the reactions since frequently the primary factor which controls the chemical concentrations in natural waters is the rate that this reaction proceeds toward equilibrium. This paper is devoted to discussing the various aspects of the chemistry of natural waters which may influence the bioassay procedures being used to establish water quality criteria.

THE CHEMICAL ENVIRONMENT-FORM OF THE ELEMENT

The forms of elements or compounds that occur in natural water govern to a large extent the biological availability of the element. Therefore, efforts to duplicate the

natural environment in laboratory tests and to extrapolate the results in establishing standards must consider the processes which determine the predominant form of the element in the study. A brief review of the type of chemical reactions that govern the chemical environment of a bioassay procedure is presented below.

Redox

The first question that should be asked with respect to chemical environment is whether the redox conditions involved in the bioassay simulate those found in the natural environment of interest because the oxidation-reduction conditions in a culture or bioassay procedure may be critical to obtaining meaningful results. Oxygen is the driving force governing whether oxidizing or reducing conditions are prevalent in most natural waters, and the redox conditions present, in turn, determine the state of an element in the aqueous environment. For example, the predominant factor governing the aqueous environmental chemistry of iron is the relative amounts of the iron in the ferrous or ferric form. For ferric iron there are mass law equilibria which govern the chemistry of the element, the most important being the precipitation as hydroxide. The ferrous ion on the other hand, behaves differently and generally has less tendency to form hydroxide precipitates and complexes.

Since most bioassays are conducted under aerobic conditions, it may be incorrectly assumed that all ions will be in the highest oxidation state possible for aqueous solutions of near-neutral pH. Considerations of thermodynamic stability alone are insufficient. For example, HOOD and SLOWEY (1964) in a study of the aqueous environmental chemistry of manganese in the Gulf of Mexico, found that the ^{54}Mn derived from atmospheric fallout behaved differently from the manganese normally present in the water. Further studies by Hood and Slowey revealed that the ^{54}Mn derived from atmospheric fallout was in a higher oxidation state than the manganese normally present in sea water and therefore the ^{54}Mn derived from atmospheric fallout was a poor tracer for what happens in the environment. DELFINO (1968) has shown that most surface waters should contain Manganese (IV) in the form of solid MnO_2 as well as Manganese (II). Mn(II) appears to be derived by the uptake of organisms of MnO_2 which consequently is reduced to Mn(II) and released upon death of the organism. The Mn(II) , although thermodynamically unstable in natural waters, has been shown by MORGAN and STUMM (1965) to be very slowly oxidized to Mn(IV) at the pH of natural waters. Studies by Delfino using Lake Mendota water, as well as the studies of EHRlich (1971) have found that the Mn(II) present in lake waters is oxidized at a higher rate than would be predicted by Morgan and Stumm's work on abiotic systems in a well defined media. At this time, it is not possible to predict whether the greater rates of oxidation result from microorganisms or the influence of some other chemicals on the kinetics of oxidation by abiotic means. However, it is certain that the rates of oxidation are sufficiently slow so that Mn(II) is found in natural water where MnO_2 is expected, based on thermodynamic considerations.

There is no simple method by which the investigator can precisely determine the redox conditions in his bioassay system. A possible general indication of this can be obtained from oxidation-reduction potential; however, the measurement of the oxidation reduction potential does not yield reliable information on the distribution of various chemical species present in solution. The discrepancy between the measured oxidation-reduction potential and the distribution of various chemical species present

in solution. The discrepancy between the measured oxidation-reduction potential and the distribution of various redox couples is due in part, to the slow kinetics of the reactions such as those described for the $\text{Mn(II)}\text{--MnO}_2$ system. Another example of this problem is the work of BREZONIK and LEE (1966) concerned with the denitrification reaction in natural waters and waste water systems who found that anaerobic fermentation proceeded in a normal manner in a waste water culture system provided that nitrate ion was absent. On the addition of nitrate to the system the formation of methane was immediately stopped until all of the nitrate had been denitrified to nitrogen gas and other products. The methane producers in the system were not killed by the nitrate but merely inhibited from further methane production since immediately after the disappearance of the nitrate ion, the methane production resumed. The nitrate increases the effective oxidation-reduction potential in the system to sufficient levels to inhibit methane production.

Biochemical transformations

A special case that must be considered is the fact that the critical environment with respect to the toxicity of a chemical that may occur in natural waters may not be the one that is readily measured by routine chemical and biological parameters that are normally used to assess water quality. For example, the intestinal tract of some organisms such as zooplankton and fish represents a markedly different environment than the normal lake, streams, and oceans. Therefore, consideration must be given to the possible release of materials under conditions on the inside of organisms.

In addition to organisms creating special environments where with certain types of chemical transformations might take place which would not ordinarily take place based on the bulk characteristics of the water, consideration must be given to organisms changing the forms of a particular element in the bioassay or natural water system which in turn changes its availability. For example, CLESCERI and LEE (1965) found in a study of the hydrolysis of condensed phosphates in natural waters and algal culture media that consistently the algae developed to approximately one-half of the total yield in cultures in which the initial phosphorus supplied was a condensed phosphate as compared to when the initial phosphorus was the same concentration of phosphorus supplied as orthophosphate. It is speculated that as part of the hydrolysis reaction converting the condensed phosphate to orthophosphate, some of the phosphorus is converted to an organic form which does not become available during the periods of the test.

The use of toxaphene as a piscicide in lake rehabilitation programs demonstrates the significance of biological processes in the observed toxicity of some chlorinated hydrocarbons to aquatic organisms. Toxaphene is a mixture of approximately 20 unidentified chlorinated terpenes containing 68 per cent chlorine. With the exception of endrin, toxaphene is one of the most lethal of the chlorinated hydrocarbon pesticides to fish on an acute basis (HENDERSON *et al.*, 1958). The median tolerance limit (TL_m) for bluegills is 7.5, 3.8, and $3.5 \mu\text{M l}^{-1}$ for exposure times of 24, 48 and 96 h, respectively. Considerable confusion has arisen regarding the factors controlling the rate of lake detoxification after the deliberate addition of toxaphene for rough fish control because some lakes detoxified within 6 months while others remained toxic for several years after treatment. Moreover, the toxaphene residues in restocked fish which spawned were often greater than in those fish killed by the initial toxaphene (LEE *et al.*,

1968). In addition to the lower levels of toxaphene in the blood which likely accompanied the slow uptake of toxaphene in the restocked fish, the evidence indicated that the toxaphene accumulated by stocked fish was less toxic than that used to treat the lakes.

VEITH and LEE (1971) have shown that in excess of 50 per cent of the toxaphene added to unstratified lakes is transported to the sediments of the lake. To examine the possibility that toxaphene loses biological activity in the environment, a bioassay of toxaphene residues in lake sediments was conducted using small bluegills (3–4 in.) as test animals.

Approximately 65 kg (dry wt) of sediment were dredged from Ottman Lake, Wisconsin 9 months after treatment and were extracted for 2 days with the azeotrope of hexane and acetone in a Soxhlet extractor. The extracts were cleaned up on multiple acid-Celite and Florisil columns, and the final volume was reduced to a stock solution in 1 liter of acetone.

The purpose of this experiment was the comparison of the toxicity of the sediment extract with that of the Coopertox formulation of toxaphene which had been added to the lake 9 months before. To accomplish this, a 1500 gal tank was fitted with four 195-l. tanks. The toxicity of 5, 20 and 50 $\mu\text{g l}^{-1}$ of the Ottman Lake sediment extract (plus control) was compared with that of similar concentrations of Coopertox No. 6 toxaphene (plus control) using 10–15 fish per tank.

The results of the bioassay using the Coopertox formulation of toxaphene (VEITH, 1968; HUGHES, 1970) substantiated the high toxicity of this formulation used in the lake treatment in that all fish exposed to 70 $\mu\text{g l}^{-1}$ were killed within 8 h, and those exposed to 17 $\mu\text{g l}^{-1}$ were killed within 18 h. All of the control fish were alive after 96 h. The toxaphene concentration in the bluegills exposed to 70 $\mu\text{g l}^{-1}$ averaged 8 $\mu\text{g g}^{-1}$ while that in the fish exposed to 17 $\mu\text{g l}^{-1}$ averaged 9 $\mu\text{g g}^{-1}$ —demonstrating the significance of the rate of uptake and blood levels of toxicants compared to the observed body residue level.

The results of the bioassay using the toxaphene extracted from Ottman Lake sediments after 9 months in the environment showed no mortality in the test fish in the tanks receiving initial doses of 4 and 17 $\mu\text{g l}^{-1}$. The initial concentration of 37 $\mu\text{g l}^{-1}$ killed five of the test fish over the 96-h period. Fish in water containing 4 $\mu\text{g l}^{-1}$ accumulated an average concentration of toxaphene of 3 $\mu\text{g g}^{-1}$. In water dosed at 17 $\mu\text{g l}^{-1}$, bluegills on the average accumulated 4 $\mu\text{g gm}^{-1}$ toxaphene. Bluegills which died in water dosed at 37.0 $\mu\text{g l}^{-1}$ accumulated an average of 10 $\mu\text{g gm}^{-1}$, while the lone survivor contained 14.0 $\mu\text{g g}^{-1}$.

The fact that the fish exposed to the high concentrations of toxaphene from lake sediments died slowly over a 96-h period while those exposed to Coopertox toxaphene the formulation added to the lake died within 18 h indicates that the toxicity of toxaphene is attenuated in the environment. Although this conclusion is substantiated by data from field studies and by changes in the chemical composition of toxaphene as evidenced by gas chromatography, the possibility that the organic material co-extracted with the toxaphene from the sediments may interact with toxaphene and produce a less toxic solution is not precluded by the above data. A similar bioassay using Coopertox toxaphene mixed with the organic material extracted from untreated lake sediments did not show a decrease in toxicity. This example of the selective degradation of the toxic components to fish when the material is placed in the environment

points to the importance of measuring specific toxic components in the analysis of the formulation. Also this type of result demonstrates the need for bioassay procedures to evaluate the significance of a particular residue of a compound in natural waters.

Complexation

The interactions of many of the toxic transition metals with naturally occurring organic complexing ligands are of great importance to the processes involved in biological availability and subsequent growth, whether it be stimulatory or inhibitory. SIEGEL (1969) presented a review of metal-organic interactions in marine systems and concluded that the availability of metals to phytoplankton is determined by the complexing species and concentration present in solution. An example demonstrating the importance of the presence of chelates on the interaction between metal ions and aquatic organisms is the use of ethylenediamine tetraacetic acid (EDTA) along with the iron micronutrient requirements in culture media for productivity studies. When ferric iron is added to aerobic natural waters, hydrolysis leads to rapid precipitation of the iron as ferric hydroxide, thereby removing this essential micronutrient from biological availability and further growth. When a few $\mu\text{moles l}^{-1}$ of a chelator such as EDTA is added to the water, the iron-EDTA chelate is of sufficient stability to prevent the removal of iron by precipitation under the culture conditions and yet allows the iron to be incorporated into the cell for growth. However, if a ligand such as diethylenetriamine pentacetic acid (DPTA) which has greater stability with iron than does EDTA is added to the solution, the iron is prevented from precipitating as ferric hydroxide and is also no longer available for growth because of the high stability of the metal-organic interaction (SIEGEL, 1969).

The chemistry of complexation in natural waters and reviews of the nature of organic matter in the environment have also been presented by HOOD (1970) and LEE (1966). Although many of the naturally occurring organic compounds are capable of complexing with trace metals, one of the potentially more important classes of natural complexing agents is the "humic-like" acids which result from metabolic processes in natural waters. When a toxic metal such as copper or cadmium is introduced into a bioassay test water, detailed chemical studies are needed to estimate the extent to which the metal ion has been complexed as well as the significance of the interaction in extrapolating the test results to water quality standards. The practice of testing with stream or lake water which has been filtered through carbon beds may produce misleading results in bioassay with metal toxicants since much of the organic material which can alter the form, hence the toxicity, of the metal is removed from the natural water. This is particularly important in the long-term, continuous flow bioassays conducted with metals at or near the $\mu\text{g l}^{-1}$ level. Associated with the use of activated carbon beds in "cleaning" test water is the release of peroxides which may affect the bioassay results.

An example of the unavailability of some soluble forms of chemical species was provided by the work of FITZGERALD and FAUST (1965), where they investigated the toxicity of copper to algae in the presence of various organic compounds. Copper (II) is fairly toxic to aquatic organisms such as algae as the aquo ion. Copper citrate, a soluble complex of Cu(II), also shows high toxicity to aquatic organisms and is sometimes used to prevent the rapid precipitation of the added copper as a basic carbonate

in natural waters with a high alkalinity. Copper (II) EDTA is also a soluble complex of Cu(II) which showed little or no toxicity to aquatic organisms such as algae. Therefore, while all three species are soluble, their behavior with respect to aquatic organisms is strikingly different.

Another factor that influences the behavior of chemical species in the environment and further complicates the development of meaningful water quality criteria is the fact that the chemical species in the particular discharge or in a sample of water may change with time. Laboratory and field studies have shown that the use of citric acid as a complexing agent to prevent the precipitation of copper as a basic carbonate in hard waters generally is of limited term value since the bacteria present in laboratory cultures and natural waters degrades the citrate, thus removing the copper resulting in its precipitation as a basic carbonate. In this case, a toxic chemical species to many algae would be converted to a non-toxic species and would be removed by deposition in the sediments.

The problems of complexation of transition metals affecting the results of a bioassay test are particularly important in long term (multi-day) batch bioassay procedures in which the test organisms may excrete sufficient concentrations of complexing chemicals to completely change the toxicity of the test compound to the organism.

Sorption

The presence of surfaces in the form of colloidal materials, detritus, and other particulate matter is extremely important in determining the availability and/or toxicity of compounds in natural waters, and bioassay techniques must assure that the particulate matter of the type found in natural waters is present. Some controversy exists today with respect to the use of NTA in detergents in that low levels of NTA could potentially solubilize copper and other potentially toxic transition metals from lake sediments and make them available to aquatic organisms. Studies by SANCHEZ (1971) using Lake Monona sediments have shown that increasing the concentration of NTA in the water results in a decreased concentration of copper. Although the copper-NTA chelate is stable, the chelate is readily sorbed onto the particulate matter. If particulate matter were not present, the NTA could solubilize sufficient quantities of copper to be toxic to primary productivity. However, the relatively rapid biodegradation of NTA in natural water essentially rules out any potential problems of this type from the use of NTA in detergents.

The most recent work on chronic toxicity of such elements as cadmium is taxing the ability of the analytical chemist to determine these compounds accurately in natural waters. Mount has found that the chronic toxicity levels for cadmium with respect to inhibiting certain fish food organism reproduction is in the order of $0.5 \mu\text{g l}^{-1}$ (FWQA, LAKE SUPERIOR ENFORCEMENT CONFERENCE PROCEEDINGS, 1969). It is extremely difficult to determine total cadmium in Lake Superior at these levels, much less the particular species that may be present. In order to determine whether or not the $0.5 \mu\text{g l}^{-1}$ proposed limit for cadmium in Lake Superior is reasonable, it is necessary to determine what actual species exist in the water. At these concentrations, it is highly likely that the concentrations of cadmium found would be primarily associated with colloidal matter present in the water in the form of either clay or other minerals or remains of dead organisms. It also is probable with an element like cadmium that the ion could be associated with various organic compounds in the form of a complex.

Therefore, cadmium concentrations exceeding the critical concentrations proposed for this element in Lake Superior water does not mean that these concentrations are toxic to aquatic organisms.

Numerous studies have found that there is a magnification of the concentrations of some compounds as one proceeds to higher trophic levels. This biomagnification is being shown to be of particular significance to the top predators such as fish-eating birds. Particular problems are present in attempting to examine this biomagnification in the lower trophic levels due to the inability of investigators to separate the non-living and living particles present in natural waters. Some investigators tend to lump together the amounts of a toxic chemical present in natural waters in true solution, within microorganisms such as algae, bacteria and fungi with the amounts of the chemical associated with the non-living particles. This type of lumping should not be done since each of these categories may have a markedly different effect on the uptake of a compound by higher trophic levels.

The sorption reactions with the walls of the vessels used in the laboratory bioassay test may be significant in influencing the results of the test. This is especially true for trace amounts of transition metals and high mol. wt organics. The sorption characteristics of the walls of the bioassay vessel will likely change with time during the course of the test due to sorption and accumulation of other compounds at present in the solution on the walls of the vessel which in turn would influence the sorption of the compound of interest. For example, WANG (1967) found that parathion was poorly sorbed by certain clay minerals. These same clays that have been exposed to certain organics in solution showed enhancement or depression of parathion sorption. Numerous studies have shown that the activity of microorganisms is proportional in many instances to the total surface area present in the system. For example, the rate at which BOD is exerted in a bottle system as well as possibly in the environment is dependent on the surface area of the particles in solution as well as the walls of the vessel. Much greater rates of BOD exertion are noted whenever the test is conducted in the presence of glass beads or other solids in the bottle as compared to their absence. It should also be noted that not only is the amount of surface important, but also the degree of agitation in the system. Typically well-mixed systems proceed at greater rates than non-mixed systems. Mixing tends to reduce the thickness of boundary layers for liquid-solid reactions which may influence the rate controlling step in some processes. Stimulation of organism activity in the presence of solids or by mixing should not be interpreted as the organism deriving energy from the solids. The solids may simply serve as a platform for the accumulation of nutrients from solution and thereby stimulate growth by increased concentrations at the surface of the solids.

Although the conditions for conducting bioassays with chlorinated hydrocarbons and other toxicants have been established with respect to biological and statistical criteria (DOUDEROFF *et al.*, 1951; AMERICAN PUBLIC HEALTH ASSOCIATION *et al.*, 1965; FEDERAL WATER POLLUTION CONTROL ADMINISTRATION, 1968), little emphasis has been placed on the chemical nature of the toxicant in the test water. Test waters are normally dosed by adding the toxicant as a solution of a solvent which is miscible with water, the assumption being that the toxicant becomes dispersed under these conditions and represents the dissolved state. Because of the small quantities of toxicants present in many bioassays, few studies have reported actual concentrations of the toxicant in the water. While the use of continuous-flow bioassay techniques allow the

steady-state concentration of the toxicant to be determined throughout the test, the nature of the toxicant-water interaction remains dependent on the techniques used in dosing the water.

In the previously discussed evaluation of the toxicity of toxaphene extracted from lake sediments, the extract was introduced as an acetone solution and the test water was analyzed throughout the 96-h bioassay. Despite efforts to minimize the removal of toxaphene from the water through sorption by maintaining a large ratio of volume to surface area in the tank design and a low concentration of suspended solids, the toxaphene concentration in the tanks receiving higher doses decreased by 40–50 per cent after the initial dosing and mixing. The more rapid decreases in the tanks containing the higher doses of toxaphene suggest that the low solubility of toxaphene may be an important factor in addition to sorption processes in removing the toxicant from the test water.

GUNTHER *et al.* (1968) have critically reviewed the solubility data for pesticides as well as the procedures for solubility determinations and have discussed the operationally-defined nature of the term "solubility" when applied to hydrophobic non-electrolytes. COHEN *et al.* (1960) reported that the solubility of toxaphene in water is less than 0.4 mg l^{-1} rather than the 3 mg l^{-1} value reported by the manufacturer. The data from this bioassay substantiates this in that a large percentage of the toxaphene at the $37 \text{ } \mu\text{g l}^{-1}$ concentration was removed either through sorption or precipitation.

The common procedure of adding acetone to bioassay solutions in order to solubilize pesticides may lead to incorrect appraisal of toxicity of pesticides to aquatic organisms. The acetone should alter the rate and possibly the extent of uptake of the pesticide by the test organism. The use of acetone in the control does not correct for this problem. Furthermore, in most natural waters some pesticides which are apparently in solution, are most probably associated with colloidal particles. There is likely to be very little relationship between the rate and extent of pesticide uptake in most laboratory bioassay situations to their behavior in natural waters.

Total salts and ionic balance

An important part of the chemical environment of a bioassay procedure is the total concentration of salts present in the medium. In addition to direct osmotic effects on aquatic organisms, total salts play an important role in influencing the distribution of the chemical species present. Any chemical reactions which are dependent on mass law equilibria will be dependent on the total ionic strength of the solution. Changing the total salt content will change the ionic strength which, in turn, will decrease or increase the activity (effective concentration) of the chemicals present in solution. While most bioassay workers are concerned with the concentration of a particular component and its effects on an aquatic organism, the activity of the particular component may be the determining factor. To achieve a significant difference between activity and concentration between two different test conditions, it is necessary to add large amounts of inert salts. However, the effects of the "inert salts" on various aquatic organisms are not well understood today.

Ion pair formation may be a significant process by which the availability of an ion in waters of higher ionic strength is altered. Although ion pair formation may be only an artifact of non-ideal deviations from the Debye-Huckel limiting law rather than

formation of a new chemical species, it is certain that some type of phenomenon occurs in solution which, in effect, reduces the effective concentration of certain chemical species to a considerable extent at relatively large concentrations such as in sea water. For example, the normal activity coefficient for a divalent ion in sea water is in the order of 0.3. However, the effective carbonate concentration present in sea water samples is approximately a factor of 10 less than the effective concentration based on conventional Debye-Huckel limiting law corrections. This may be due to ion pair formation with some of the more common cations present in sea water such as calcium, magnesium and sodium. Thus, in typical sea water, and estuaries, there may be a factor of 50 difference between the effect of a certain carbonate concentration on aquatic organisms as compared to the measured concentration.

There is a tendency by some investigators to attempt to use bioassay data developed for fresh water organisms for the marine environment. In general, fresh water bioassay data should only be used as a guide. Significant difference occurs with respect to the aqueous environmental chemistry of many elements in marine and fresh waters. Studies are needed on the marine environment in order to ascertain the potential significance of these differences. In some cases, due to complex formation with chloro species, the toxicity might actually be increased over what might be found in fresh waters. In others there is a strong likelihood that toxicity might be decreased. Any pH dependent toxicity such as that for ammonia should be carefully checked since the activities of the various species in solution will be changed significantly in fresh and marine waters. It is recommended that in those situations where the only data available for the potential toxicity of a particular element to aquatic organisms is fresh water data that this data be used as a guideline to potential problems in the sea water system and that the regulatory agency and the alleged polluter should jointly conduct bioassay tests to ascertain whether or not the particular element under investigation does in fact cause toxicity to selected key organisms.

Some of the problems associated with interpreting the results of algal growth studies may stem from the fact that total salts present in the algal media were many times greater than the total salt concentrations found in most natural waters. Not only must increased attention be paid to the total concentrations of charged and uncharged species in bioassay solutions, but also consideration must be given to the ratios of the dominant ions present, i.e. monovalent to divalent species. While it is not clear at this point what the significance of these ratios is except in possibly influencing membrane transport processes, it is reasonable to expect that certain chemicals will behave differently in a solution composed primarily of monovalent species as compared to divalent species. It is doubtful, however, that minor changes in the monovalent to divalent ratios would have significant effects on the bioassay process and further study is needed to better define this relationship.

Hardness

For some compounds the effects of hardness, alkalinity, total salt, etc. is understandable to some extent based on chemical interactions of the toxicant and Ca, Mg, HCO_3^- , CO_3^{2-} , etc. For example, the effects of hardness on copper toxicity to aquatic organisms may be due in part to the precipitation of a basic carbonate complex formation with inorganic or organic components for the effects of mono and divalent

ions on membrane transport processes. Considerable confusion exists regarding the effect of calcium and magnesium concentrations on the toxicity of many compounds. It is often found that the toxicity decreases with increasing hardness. For example, zinc was found to be less toxic to trout in brackish or salt waters than in freshwaters (HERBERT and WAKEFORD, 1964). The effectiveness of the lampricide, 3-trifluoromethyl-4-nitrophenol (TFM) now being used in the Great Lakes is generally greater in soft waters (KANAYAMA, 1963). The difficulty in determining which mechanism controls the system stems from the fact that the calcium and magnesium concentrations cannot be varied without varying other important parameters in the system. Hard waters generally contain greater alkalinities and total dissolved salts than do soft waters. Since the pH also affects the toxicity of most toxicants the greater pH often associated with hard waters precludes detailed mechanistic studies. Furthermore, since the increase in calcium and magnesium may cause physiological changes in organisms, variations of toxicity with hardness cannot be interpreted as direct interactions between the toxicant and the calcium, magnesium, or bicarbonate ion without detailed studies of the nature of these interactions.

Acid-base

Many of the toxicants which enter into acid-base reactions exhibit a pronounced variation of toxicity with changes of pH. Weak acid such as TFM mentioned previously has a pK of 6.07 (THINGVOLD, 1970) and the toxicity of the compound decreases with increasing degree of dissociation as the pH is raised. The toxicity of TFM should change significantly as a function of pH in natural waters due to the fact that the pK of the acid dissociation constant is near the pH of natural waters. Ammonia as NH_3 is toxic to fish while NH_4^+ shows less toxicity (WUHRMANN and WOKER, 1948). ELLIS (1940) found 2.5 mg l^{-1} total ammonia-N at pH of 8.0 to be acutely toxic to fish. However, this level of total ammonia-N at lower pH values may not produce adverse effects. Thus, the pH of bioassay waters and equilibrium constants for dissociated toxicants must be specified and monitored continuously if the results of bioassays are to be meaningful.

Solubility

Bioassay procedures must consider whether the chemicals being tested are in solution or in particulate form. In some instances, this may be extremely difficult to do. For example, iron in aerobic waters readily forms colloidal ferric hydroxide with particulate sizes less than $0.10 \mu\text{m}$ dia. The normal criteria used to distinguish between soluble and particulate forms is the $0.45 \mu\text{m}$ pore size membrane filter which may lead to erroneous results for those elements which tend to form colloidal particles.

Furthermore there is considerable debate among the regulatory agencies on whether water quality criteria should be established for soluble or total concentrations of the transition metals. The problem arises from the fact that the interactions of some transition metals with certain types of particulate matter result in making the potentially toxic species unavailable to the aquatic organism. On the other hand the interactions with the same metals but other types of particulate matter, resulting in the formation of an insoluble species, may have little effect on the availability of the element to the organism. At the present time our understanding of the aqueous environmental chemistry of most elements in natural waters is such that it is impossible

to make *a priori* predictions of availability without detailed chemical studies. Consideration must be given to both the abiotic and biotic chemistry of the element in the specific waters under consideration.

It is felt that the most reasonable approach for establishing meaningful water quality standards is one where the standards are based on the total of the chemical species. In those instances where the alleged violator of the standard feels that it is inappropriate to apply the standard based on the total composition of its waste, he should be given the opportunity to conduct in cooperation with a regulatory agency bioassay tests to determine in fact whether or not a substantial part of the total is currently unavailable to key aquatic organisms in the receiving waters. The chemical environment of the receiving waters must be considered from not only the current conditions but those that might be encountered in the reasonably foreseeable future.

Perhaps the most significant problem that an experimentalist in the eutrophication area has in trying to establish meaningful water quality criteria is a problem of duplicating the conditions that exist in natural waters in terms of available forms of nutrient elements. Generally, only a fraction of the total input of nutritive elements becomes available in the aquatic environment. Suggestions that water quality criteria be based only on soluble species are unrealistic in the sense that some soluble species are released from particulate forms in the environment. For example, the nitrogen and phosphorus that are present in atmospheric dust fall, storm water drainage from urban areas, and agricultural runoff is predominantly particulate in nature. At the present time, there is insufficient data to determine the percentage of the particulate forms that will become available in a lake water environment. It can only be reasoned that the amounts that will become available for the growth of algae and other aquatic plants would in general be greater than the soluble orthophosphates but less than the total phosphorus present in the source. Similarly with nitrogen, the forms of ammonia and nitrate represent immediately available forms, while the total nitrogen represents the potentially available nitrogen nutrient. A possible exception with regard to the lower limits of availability would be the presence of large amounts of particulate matter which sorb the nitrogen and phosphorus from the water and therefore make it unavailable to aquatic organisms. In order to make meaningful predictions on the amounts of algae and macrophytes that might develop in a given aquatic environment, information regarding the fraction of the total key aquatic plant nutrients such as nitrogen and phosphorus that will become available in that particular environment is needed. This will require a combination of chemical and biological studies, including short and long term bioassay procedures as well as chemical and biochemical leaching tests.

Today there is considerable emphasis on chronic vs. acute toxicity. Chronic toxicity for various chemicals and aquatic organisms involves long term testing programs usually involving one or more generations of the organism. In much the same way there is an equivalent type of testing with regard to availability. The short term availability is usually within a few days to a few weeks vs. the long term availability which may take many months to a year. The work of SPEAR (1970), BORTLESON (1970), WALTON (1971) and AUSTIN (1970) are typical examples of the importance of doing long term testing with regard to availability of various chemical species to influence water quality. Spear found that phosphorus under aerobic conditions was not available within a few hours; however, over a period of several months large amounts of phos-

phorus were released from lake sediments which could stimulate the growth of algae. Austin, under similar conditions using Lake Mendota and Trout Lake sediments, found that the significant part of the organic nitrogen present converted into forms which are readily available for algal growth during the period of several weeks to several months. Short term testing such as that done by FITZGERALD (1970) indicated that phosphorus was not available in Lake Mendota; however, the longer term testing showed the contrary where, given sufficient time, the phosphorus present in Lake Mendota sediments under aerobic conditions can become available. As discussed by LEE (1970), with respect to the availability of phosphorus from lake sediments, a controlling factor influencing availability is not the chemistry or biochemistry of the release reactions but is the hydrodynamics of the mixing of the sediments and the interstitial water associated with the sediments with the overlying waters. A bioassay procedure based on a short term test of a few days to a week gives an unrealistic picture of what will actually happen in the same lakes. Similarly long term bioassay tests probably grossly overestimate the potentially available phosphorus and nitrogen from deep water sediments due to differences in the hydrodynamics of the test conditions compared with those present in deep waters of the lake.

Often the rate of growth of attached organisms is directly dependent on the rate of transport of water to the organism. For example, the *Cladophora* growth in streams is often more prolific in areas where the average velocity of the stream is greater than its slack water areas. Part of the factors contributing to this effect of velocity on growth is the transport of the limiting nutrients to the organism. The organisms in relatively static conditions may deplete the nutrients to the point where growth will be inhibited.

In recent years there has been considerable emphasis placed on developing a bioassay procedure to evaluate the potential fertilizing ability of a waste water for a particular lake or stream. These efforts have resulted in the development of the current Provisional Algal Assay Procedure. Basically this procedure involves taking a sampling of water, adding various nutrients to the water, and determining, using the standard test organism, the stimulation of growth by the various added nutrients. From these results, it is possible to make an assessment of the nutrient or nutrients present in the water that may limit the growth of the test organism, and if the test organism behaves somewhat similar to the natural populations present in the water under consideration then it is possible to extrapolate to what may be limiting algal growth in natural water being investigated. While results of this type are of interest, they do not provide the type of data that is needed to initiate corrective action programs involving limiting nutrient input to a particular lake or stream since the results tell you what may be limiting algal growth at the particular time of the test and do not give any information on what can be made limiting by reducing the nutrient input for the various nutrients that could influence algal growth. This point has been discussed further by LEE (1971) and demonstrates the importance of designing a bioassay for a particular purpose.

Recent studies by SANCHEZ (1971) have shown that the one and a half million pounds of copper sulfate that have been added to Lake Monona, located in Madison, Wisconsin, have become incorporated into the sediments, resulting in no evident water quality problems. Sanchez found that sulfide controls copper concentrations in the anoxic hypolimnion of this lake, while the basic copper carbonate controls the concentrations in the epilimnion and in the waters of the unstratified lake.

Another example of the importance that must be placed on the role of particulate and soluble species in establishing water quality criteria is provided by the work of SHEARER and LEE (1964) on the leaching of radium from uranium mill wastes. They found that large quantities of radium 226 relative to the maximum permissible concentrations could be leached from various types of uranium mill wastes. In this case, however, the chemical environment and time of leaching had no influence on the amount of radium leached. The leaching of radium was essentially instantaneous and remained primarily controlled by the amount of water in the system. The fact that the particulate radium in uranium mill tailings is the radioactive species with some of the parent and daughter species being strong gamma emitters further complicates the problem since the particulate uranium mill wastes could still be a significant hazard to aquatic organisms even though the uranium is not leached. Therefore, radioactive species require special consideration with respect to bioassay techniques.

ANALYTICAL PROBLEMS OF BIOASSAY PROCEDURES

Often analytical methods for various chemicals are not specific for a particular form of the element or compound. The lack of specificity causes problems in interpretation of data with respect to the potential effects of chemicals on aquatic organisms. For example, there has been a controversy in limnological literature within the past few years regarding the interpretation of the results from the molybdenum blue orthophosphate procedure. RIGLER (1966) has reported that a substantial part of the orthophosphate measured in some natural waters are not available for algal growth. CHAMBERLAIN and SHAPIRO (1969) stated that the discrepancy is due to interferences in the analytical procedure principally caused by arsenic being measured as orthophosphate. WALTON and LEE (1971), using a combination of molybdenum blue orthophosphate procedure and bioassay procedure found that the soluble orthophosphate found in Lake Mendota waters by count or other biomass measurement techniques are in agreement. The results recorded by Rigler and those of Walton and Lee may be compatible due to the different concentration ranges used. At very low concentrations, the likelihood of interferences in analytical procedures caused by other phosphorus compounds which may hydrolyze to soluble orthophosphate in the chemical test and yet not be available for algal growth, is much greater; however, at higher concentrations, possible hydrolysis is insignificant and no error is observed.

Further studies of the availability of nutrient forms have revealed significant analytical errors in the analysis of condensed phosphates. LEE *et al.* (1965) studied the hydrolysis of condensed phosphates in algal cultures and natural waters and evaluated the phosphate analytical procedures that are normally used for orthophosphate in a few week old culture of *Chlorella* and of *Microcystis*. Immediate analysis of the algal cultures after the addition of the orthophosphate revealed that the orthophosphate in culture filtrate in which *Microcystis* had been grown could not be recovered. No problems of this type were encountered with the filtrate from a *Chlorella* culture. It was found that heating the sample in the presence of hydrochloric acid at pH 1 in an autoclave for approximately 15 min destroyed this interfering substance. Therefore, in a culture in which *Microcystis* is grown, orthophosphate will be measured as condensed phosphate. It is not known at this time whether this problem occurs to a significant

extent in natural waters although the work of WALTON and LEE (1971) suggests it does not. The concentrations of *Microcystis* present in the cultures which were tested were much higher than those that occur in the environment. Also the concentrations of phosphorus added (in the order of $1 \text{ mg l}^{-1} \text{ P}$) are generally higher than those encountered in the environment. It is results such as these which greatly complicate the interpretation of data involving the use of analytical procedures for the potential effects of chemicals on aquatic organisms.

As demonstrated in the discussion of the chemistry of toxaphene in natural waters, analytical procedures may produce misleading results with respect to toxicity if detailed separation techniques are not employed. Although stocked fish in treated lakes may contain more toxaphene than the fish initially killed by the toxaphene treatment based on determinations of the toxaphene "finger print" by gas chromatography, some of the toxic components are likely to be absent in stocked fish (HUGHES, 1970; VEITH, 1968).

Failure to conduct detailed trace analyses for contaminants in the chemicals employed in bioassays may also produce erroneous results. Contaminants in reagent grade chemicals have to be considered because toxic metals may produce effects in aquatic organisms after prolonged exposure to concentrations several orders of magnitude lower than those needed to kill the organisms. Many American Chemical Society reagent grade chemicals contain concentrations of transition metals that exceed the proposed chronic toxicity levels in reagents prepared from them. Some industrial and agricultural chemicals contain trace contaminants which are more toxic than the parent compound. The chlorodibenzo-*p*-dioxins are associated with the production of chlorophenols which are the precursors to chlorinated herbicides such as 2,4-D and 2,4,5-T, and have produced egg mortality at the $30 \text{ } \mu\text{g kg}^{-1}$ level (UNITED STATES SENATE, 1970). There are indications that compounds of similar toxicity, the chlorinated isomers of dibenzofuran exist as contaminants in the commercial preparations of chlorobiphenyls (PCBs) which are widespread contaminants in the environment. Analytical procedures capable of detecting trace contaminants in chemicals to be tested are required to preclude the possibility of erroneous results from bioassays.

There is a tendency, and in some cases a statutory requirement, that regulatory agencies use the Standard Methods for the Examination of Water and Waste Water as the method for analysis of a given sample of water to determine whether or not the water exceeds a standard. Some of the procedures in Standard Methods do not measure the specific discrete forms of an element but measure some property that is in some way related to the total amount of the element present. Often this relationship is unknown. Without such information, it may be impossible to translate in a meaningful way the results of a chemical analysis of a water sample based on a Standard Methods procedure into the critical concentrations established for a particular compound as listed in a water quality standard. Water quality standards should be written in such a way as to allow the regulatory agency the option of using analytical procedures which enable them to investigate the particular forms of an element and use the results of these analyses to tell whether or not the sample exceeds a particular water quality standard.

It is extremely important that everyone who uses a Standard Methods procedure appreciates the fact that the widely held concept that the use of the same methods leads to comparable results is incorrect. The use of the same analytical procedure for

a particular element or compound in two different waters does not lead to comparable results. The primary reason why this is not the case is due to the fact that one of the waters may contain a substance which interferes in the chemical test while the other water does not or contains a different amount of the interfering substance. For example, the use of the unmodified Winkler test for dissolved oxygen in waters which contain small amounts of nitrite leads to erroneous results, while the same test on a water which contains no nitrite gives in general a reliable estimate of the dissolved oxygen content of the water. Therefore, an investigator or different investigators using unmodified Winkler on waters which contain nitrite and those which do not, do not have comparable results. The use of Standard Methods procedures is a step in the right direction toward obtaining comparable results; however, the use of standardized procedures does not relieve the user of the responsibility of ascertaining whether or not the particular procedure selected is in fact applicable to the water under investigation.

Often an investigator conducting bioassay tests is forced to use analytical procedures to measure gross characteristics of certain chemicals rather than specific forms. Such measurements may lead to situations which would cause apparent contradictory or confusing data with respect to the relationship of the apparent concentrations of the compounds under question to their toxicity to certain types of aquatic organisms. For example, the recent findings that chlorinated sewage effluents show fairly high toxicity to various aquatic organisms falls into this category of potential problems with the chemical analysis involved. Most investigators in this area are using the amperometric method for measurement of chlorine residuals. This method does not measure a specific form of the element but measures some part of the oxidizing chlorine present in solution that will react with iodide at specific pH conditions. Studies have shown that some of the chloramines formed from the chlorination of ammonia and other related compounds react with iodide to varying degrees. The degree to which they react with iodide may be altogether different than the degree with which a specific component might be toxic to a particular aquatic organism. Since the kinetics of the reactions between chlorine and ammonia are extremely complex, with a number of competing series and parallel reactions proceeding at one time leading to the formation of mono-chloramine di-chloramine, and nitrogen trichloride, it is likely that the toxicity of a residual chlorine measured by the amperometric procedure will be different as a function of the time of contact between the chlorine and the test water. It is highly likely that the toxicity per unit combined chlorine, as measured by the amperometric procedure, would be different at one hour after chlorination as compared to 10 h or several days, due to the relatively slow kinetics of the interchange of the various chloramine species. This situation is much more complicated in waste waters since the chlorination of typical domestic sewage produces not only chloramines but also chloramino acid, chloropeptides and proteins, chloro-urea compounds, etc. The kinetics of these various reactions and the inter-reaction among these compounds is very poorly understood at this time. Further complication in this type of system is the fact that the various tests for available chlorine, such as the amperometric procedure, *o*-tolidine procedure, etc. will give different results for the residual present at any time. It has been known for many years that there is still significant bactericidal properties in chlorinated waste water effluent under conditions where the *o*-tolidine chlorine residuals are zero, yet the iodide residual is still present.

Any investigator conducting a bioassay procedure on specific chemicals should have a good knowledge of what forms of the chemicals are present as a function of time in his test solution and what forms of the various chemicals are measured by the analytical procedures used in his studies. Without this information, the investigator may find himself in an almost impossible situation of trying to sort out what is apparently a "hodgepodge" of no response tests which in fact reflect the complexity of the chemical systems involved.

Organism previous chemical environment. Not only must consideration be given to the chemical environment of a bioassay solution; in addition, consideration must be given to the previous chemical environment of the test organism. For example, in the case of algal assay procedures, the result obtained depends to some degree on the culture techniques used for the algae prior to the actual bioassay test. If the algae are cultured under conditions where there is a surplus of phosphorus, then attempting to evaluate the availability of phosphorus from various types of compounds at low levels may be fruitless since the algae are able to carry over sufficient quantities of phosphorus from the stock culture to the test conditions, and therefore grow essentially to the same degree in the controls as those which are receiving the additional supplementary phosphorus from the source. This is particularly important when dealing with very small changes in the chemical composition of various chemical species.

It is also important for users of certain types of assay techniques such as short term measurements of metabolic activity to be aware that the short term test may not evaluate the long term availability. The ^{14}C technique is sometimes used as a measure of potential stimulatory effects of a chemical on algae. They may be stimulated very little if they already have surplus amounts of phosphorus or nitrogen which were acquired from the stock culture. On the other hand, the techniques which are based on total yield involving culture for longer periods of time normally would yield valid results under these conditions. These minor variations in the phosphorus present in the organisms at the time of transfer would have little effect on the overall yield of the organisms. A further example of this type of problem is with regard to enzymatic activity of certain organisms. FITZGERALD *et al.* (1968) have shown that organisms cultured under limiting phosphorus conditions possess phosphatase enzymes which can be used to make available condensed inorganic and organic phosphorus compounds for algal growth. On the other hand, organisms which are cultured in a surplus orthophosphate environment do not produce these enzymes with the result that phosphorus starved cells behave differently in the bioassay than cells that had surplus phosphorus.

Thermodynamic and kinetic models of the water chemistry of compounds. The thermodynamic approach to develop models for the aqueous environmental chemistry of an element such as used by STUMM and MORGAN (1970) must be viewed as a very crude model of the expected behavior of the element in natural waters. The pH potential and pH-pE diagrams that are frequently used to present the thermodynamic equilibrium conditions that exist in a particular natural water are directly dependent on the ability of the experimenter to properly select the forms of the element present in a given system. In addition, such an approach assumes that the system is at thermodynamic equilibrium. One of the most difficult situations that exists today with respect to construction of diagrams of this type is our inability to properly select the chemical species that actually exist in the aquatic environment. Particular problems develop

with respect to the solid phases present. The formation of well-defined solid phases is often an extremely slow process. There are numerous examples of what would be predicted for a particular solid phase in fact does not develop in natural waters. Often thermodynamically metastable species are formed which may persist for long periods of time and only after years or more may evolve into predictable solid phases. Often the solid phases formed in a particular reaction show little or no crystallinity, i.e. are more or less amorphous to X-ray diffraction. Also, many of these precipitates are badly contaminated with other compounds which have coprecipitated with the primary compound. Aging of a precipitate will generally improve the crystallinity. Aging is also generally accompanied by a lowering of the degree of contamination of the precipitate by other species.

An investigator attempting to develop a model for the expected behavior of a certain element frequently encounters situations for which there is no known equilibrium constant, for example, the case of the chemistry of trace transition metals with the dissolved organic matter present in natural waters. Considerable evidence exists that a substantial part of this dissolved organic matter can act as ligands to complex trace transition metals; however, since the nature of this organic matter is virtually unknown at this time with respect to specific chemical species present in natural waters, it is impossible to develop any type of meaningful thermodynamic description of these equilibria and therefore a pH potential diagram is generally constructed which ignores complexation with natural organics. Because of the fact that often the toxicity of a particular element to an aquatic organism is highly dependent on the degree of complexation with various elements, the model based on a system which ignores complexation then, is, in fact, a model which has little or no applicability to some natural water systems.

Of particular concern today is the problem with respect to formulation of sorption reactions between dissolved species and particulate matter such as detrital minerals, remains of organisms, etc. It is impossible at the present time to develop meaningful equilibrium models for most sorption reactions which have any general applicability to wide range concentrations of the elements under consideration in many natural water systems.

The use of kinetic models to describe the expected chemical species present in a particular environment is often met with great difficulty due to the fact that these kinetic models are based on laboratory studies and have involved the use of "pure water" for the study of this particular compound, and the rates of reactions are highly dependent on the presence or absence of other chemicals which tend to catalyze or inhibit the rate of reaction. For example, in the studies of the oxygenation of ferrous iron by dissolved oxygen, it was found by STUMM and LEE (1961) that the presence of very small amounts of copper greatly accelerated this reaction. In fact almost everything else that was added to this solution would tend to accelerate the reaction over that which would be obtained in a relatively pure system. Therefore, from a natural water point of view, it is extremely difficult if not impossible, to predict *a priori* what the rate of reaction of ferrous iron with oxygen would be given the pH and initial iron and oxygen concentrations due to the presence of various catalysts in the solution. It is known that the rate of some precipitation reactions are highly dependent on the presence of an ion. For example, magnesium is known to inhibit calcite formations as well as hydroxyapatite formations. Therefore, the rate which calcium or phosphorus

precipitates in either calcite or hydroxyapatite will be dependent on the amounts of magnesium in the solution.

The degree of mixing in natural waters may often further complicate the use of equilibrium models as representative of natural water conditions. The rate of exchange of materials between the water and sediments is highly dependent on the mixing of the sediment and water at the sediment water interface. HAWLEY (1967) found that in the case of Lake Mendota, the waters immediately above the sediment water interface during the summer may be a factor of 10 undersaturated with respect to the calcium carbonate content of the water. High degree of undersaturation occurred when sediments only a few inches away contained 30 per cent by weight calcium carbonate. Studies by HAWLEY (1967) have shown that this degree of undersaturation was dependent upon the relatively slow mixing of the water in contact with the sediments since taking samples out of this water into the laboratory and mixing them resulted in a rapid equilibration of the sample with respect to calcium carbonate solubility. Equilibrium and kinetic models should be used as a guide to the expected behavior of the compounds in the bioassay water. However, they cannot be relied upon to predict behavior because of the relatively poor understanding of the aqueous environmental chemistry of most compounds.

WATER QUALITY STANDARDS

Development of bioassay procedures for establishing water quality standards must give detailed consideration to the aqueous environmental chemistry of the elements or compound being tested. Many bioassays have failed to produce useful data due in part to a lack of knowledge concerning the chemical environment created in the test or present in natural waters. The use of bioassay procedures to establish water quality standards for chemicals in natural water requires a cooperative effort of chemists and biologists in order to produce meaningful results.

Perhaps the first step in developing standards for a compound is the development of analytical methods capable of distinguishing between the different oxidation states, free and complexed species, and dissolved and particulate forms, etc. Where these objectives are unattainable, the bioassay results must be qualified accordingly.

Establishing standards requires that statistically reliable estimates be available for the concentrations which exist in the natural water of interest. Equally important is the need for a statistically reliable sampling program in the environment. A few chemical analyses conducted with questionable analytical procedures for near shore waters should not be used to establish criteria for open waters for large lakes such as the Great Lakes. The establishment of maximum concentrations without knowledge of the natural sampling and analytical variability may lead to unenforceable standards.

In addition to monitoring the concentration of the chemical being tested in the bioassay a complete chemical analysis of the water must be made in order to simulate the environment of interest to a maximum extent. The analysis should include estimates of total dissolved solids, ratios of dominant ions, pH, and trace metals. For those parameters such as pH, calcium and magnesium concentrations, and suspended solids which may be expected to vary with time studies to evaluate the effect of this variation on toxicity or growth should be conducted. Since the availability of the test material determines the biological activity, studies of the rates of chemical

transformations from unavailable forms such as particulate or complexed species to the available forms are needed to guide the time-span of the bioassay.

Whenever an alleged violation of a water quality standard has occurred the following questions should be asked: 1. What actual chemical species of the compound under question are present in the natural waters? 2. Are there any characteristics of the natural water system under investigation that could alter the chemical environment of the compound under question and that of the test conditions? 3. What were the chemical conditions in the bioassay procedure that were used to establish the critical concentrations of the element? Specifically, 4. What were the forms of the chemicals added in the bioassay procedure and were detailed chemical analyses of the solutions made at several times during the test for specific forms of the test compound? Most of the literature reports of bioassay procedures fail to provide this type of information. Therefore, someone reviewing a particular standard is often in the position of not really knowing whether or not the standard is truly applicable to a particular natural water.

Under conditions where the concentrations of the element used in a particular standard were based primarily on acute toxicity at relatively high levels, this caused few problems. However, today with increasing emphasis being placed on using chronic, sub-lethal toxicity and the associated much lower concentrations of the elements, there must be greater concern given to the chemistry of the elements involved.

CONCLUSION

With increasing emphasis being placed today in establishing water quality standards at lower concentrations, much greater care must be given to the chemical environment that exists in the bioassay tests which are used as a basis for these standards. The individual designing such tests must have a good knowledge of the characteristics of the test organism, the physical environment of the test, and also give consideration to the chemical environment. This consideration of the chemical environment must include a detailed description of the types of the chemical reactions possibly occurring within the test system which could affect the forms of the elements throughout the course of the bioassay test.

Also, a review of the redox, acid base, precipitation, complexation, gas exchange, sorption (biotic and abiotic) and biochemical transformation reaction thermodynamics and kinetics should be made based on the information available in the literature. The literature value should be used to develop a chemical model for the expected aqueous environmental chemistry of the test compound in the bioassay procedure. It is important to emphasize that the models of this type can serve only as an approximate guide to the expected chemical behavior of the test compound. Measurements must be made on the specific species of the compound to be certain that the chemical is in fact behaving according to thermodynamic and kinetic considerations.

The agency responsible for developing water quality standards should carefully review the conditions of the bioassay procedures being considered as a basis for the proposed standard with particular attention given to the chemical environment of the test to be certain that the environment of the test was similar to the conditions that will exist in natural waters where the standard will be applied. It is important that when an alleged violation of a standard occurs that the regulatory agency and the alleged discharger should conduct joint studies to ascertain whether or not a particular form of

the compound under question is in fact present in the water to affect the organisms or to cause some other deleterious effect on water quality before any enforcement action is taken.

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