

G. Fred Lee¹ and R. Anne Jones¹

Translation of Laboratory Results to Field Conditions: The Role of Aquatic Chemistry in Assessing Toxicity

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ABSTRACT: One of the most significant problems facing the water quality management field today is the translation of laboratory-derived aquatic toxicology criteria and standards to natural water field conditions. This problem is caused, in part, by the markedly different chemical environments that frequently exist in laboratory toxicity tests and the field. Normally, laboratory-developed water quality criteria are derived from bioassays in which the contaminant is presented to the organism in a completely or nearly completely available form or forms; however, under field conditions, many contaminants exist in a variety of forms only some of which are available to aquatic organisms.

Guidance is provided on how to use knowledge of the aqueous environmental chemistry of the contaminant or contaminants of concern to detect situations in which there may be markedly different toxicity levels under field and laboratory conditions. A hazard-assessment approach is outlined by which maximum use can be made of existing laboratory bioassay data and environmental chemistry in site-specific water quality evaluation.

KEY WORDS: aquatic chemistry, bioassay, aquatic toxicology, bioconcentration, water quality criteria, water quality standards, hazard assessment

There is considerable concern today regarding the application or translation of laboratory bioassay results to field situations. This concern arises, in large part, out of the use of U.S. Environmental Protection Agency (EPA) water quality criteria in the development of state water quality standards. The EPA criteria [1,2]² are generally equivalent to chronic exposure, safe concentrations of completely available forms of the contaminants. Until

¹Professor and lecturer, respectively, Department of Civil Engineering, Texas Tech, Lubbock, TX-79409. G. Fred Lee & Associates, El Macero, CA www.gfredlee.com

²The italic numbers in brackets refer to the list of references appended to this paper.

November 1980 [2] the EPA, in its review of state standards, followed a policy of presumptive applicability; that is, the EPA water quality criteria were presumed to be applicable to essentially all waters. In accord with this policy, if a state were to adopt a water quality standard for a particular parameter, the standard would, in general, have to be at least as strict as the EPA numeric criterion for that parameter; the standards have typically been applied to the total contaminant concentrations. In enforcing this policy, the EPA tacitly assumed that in all situations an organism could receive a chronic or lifetime exposure to the concentration of a contaminant measured and that all contaminant forms measured are available to adversely affect aquatic life. Thus, the results of standard laboratory bioassays that were used for criteria development were being directly applied to field situations without regard to factors, such as the availability of contaminants or durations of organism exposure, that affect an organism's response to a contaminant in the field.

The presumptive applicability policy of the EPA caused states either to choose not to adopt water quality standards for many parameters or to adopt the worst-case EPA criteria as numeric standards, or both. The use of such worst-case standards could, in some instances, result in waste load allocations and effluent limitations that would entail large-scale expenditure of public funds with limited and, in some instances, no improvement in water quality over that which could be achieved with a significantly reduced expenditure.

Partly in response to the increasingly widespread opposition to the states' adoption of the numeric water quality criteria as enforceable standards, the EPA abandoned its presumptive applicability policy [2]. This has, in essence, shifted the responsibility for water quality protection from the federal government to the state governments. It will now be up to the states to work with municipalities, industry, agricultural interests, and so forth to formulate water quality standards, and waste load allocations based thereon, that are technically valid, cost-effective, and protective of the environment. It is imperative that those involved in this effort understand the appropriate use and limitations of single-compound, laboratory bioassays in evaluating the significance of contaminants in the impairment of beneficial uses of a water body and, most important, the appropriate application of laboratory bioassay results to the wide variety of field conditions to which these results are applied. The use of these criteria in the development of state water quality standards and, indeed, in the proper application and interpretation of laboratory bioassay results, in general, requires an understanding of how the physical, chemical, and biological factors characteristic of the test system compare with the conditions that prevail in the field situation of concern. Other papers in this volume will discuss physical and biological factors; chemical factors will be addressed in this paper.

Chemical Forms of Contaminants: Considerations for Bioassays

In the early 1970s, Lee [3] reviewed the current state of knowledge on the chemical factors influencing aquatic organism bioassay results. Until that time, little regard had been given to the chemistry of bioassay systems. In fact, there were some who questioned whether the aqueous chemistry of contaminants was an important factor in conducting water quality bioassays. Throughout the 1970s an increasing number of papers appeared indicating that aquatic chemistry may be the most important factor governing the translation of laboratory bioassay results to field conditions. However, it was not until recently that widespread interest developed in defining how knowledge about the aqueous environmental chemistry of contaminants can and should be used in translating laboratory bioassay information and in developing state water quality standards.

Aqueous environmental chemistry describes the thermodynamics and kinetics of the transformations that a chemical contaminant may undergo in aquatic systems. With few exceptions, most contaminants exist in aquatic systems in a variety of forms, only some of which may influence water quality. Frequently, although not always, these transformations act to reduce or eliminate the parent contaminant's toxicity, producing nontoxic, "unavailable" forms. (The term "toxicity" is used in this paper in its broadest sense; it includes not only acute and chronic toxicity but also such phenomena as bioconcentration.) As discussed later, there are some notable exceptions in which the aqueous environmental chemistry of a contaminant is such that nontoxic forms are converted to more toxic forms. It is rare, however, that the more toxic transformation products are not converted to less toxic forms given sufficient time in the environment.

As discussed by Lee [3], the aqueous environmental chemistry of a contaminant may be conveniently divided into the following types of reactions: acid-base, precipitation, complexation, gas transfer, oxidation-reduction (redox), hydrolysis, photolysis-phototransformation, and abiotic and biotic sorption. Many of these reactions can significantly affect the availability and, therefore, the toxicity and the potential for bioaccumulation of a chemical within an organism's tissue. The rate and extent to which each of these reactions occur depend on the contaminant and the specific environmental conditions and, therefore, should be evaluated for every situation of concern.

In examining the aqueous environmental chemistry of a contaminant, it is usually best to first determine its oxidation state, which determines the degree to which the chemical participates in the other types of reactions. For example, iron exists in aquatic systems principally in two forms, the reduced ferrous and the oxidized ferric iron. At natural water pHs, the concentration of the ferric iron aquo species is very low because of its tendency to precipitate with hydroxide. Ferrous iron, though largely soluble as the aquo form in natural waters, is unstable in waters containing dissolved oxygen, being oxidized to ferric iron; it

is not normally present at readily measurable concentrations under those conditions. Therefore, under oxic conditions, the iron form of concern is ferric iron, and its aqueous environmental chemistry is dominated by reactions with hydroxide.

While all of the aforementioned reactions are important in determining the water quality impacts of certain contaminants, they differ in their amenability to evaluating toxicity. Reactions such as volatilization, hydrolysis, and phototransformation all tend to remove the contaminant from the system. The analytical methods that are frequently used normally can detect this change in concentration. The same situation exists for many chemicals which undergo oxidation-reduction reactions. However, there are some instances in which redox reactions change a functional group but that may not detectably change the concentration measured by the normally used analytical procedures. Thus, there would be an appreciable change in toxicity without a readily discernible change in concentration. Acid-base reactions can generally be readily handled by simple mass law equilibria.

Precipitation, complexation, and sorption reactions (especially if the sorption is on colloid-size particles) are, in general, the most difficult to handle. It is often difficult to detect analytically a change in concentration brought about by one of these reactions using the normal analytical procedures, which, in most instances, measure the total concentration of the contaminant, since these reactions may not result in a change in the total concentration of the chemical. Precipitation and sorption result in the formation of insoluble forms of the contaminant. Often, insoluble forms do not affect water quality to the extent that soluble forms can. However, it is possible that, for certain kinds of organisms such as filter feeders, contaminants associated with particulates might be equally or more available. This situation could be especially important for those contaminants that tend to bioaccumulate in higher trophic-level organisms through the food web. There are many examples in the literature of the reduced availability-toxicity of contaminants associated with sediments. Jones et al [4] have recently published a review of work on the toxicity to aquatic life of waterway sediments in the United States. Brungs and Bailey, in 1967 [5], were among the early workers to demonstrate that the presence of suspended solids reduced the toxicity of contaminants to aquatic life. They found that the toxicity of endrin was significantly reduced in the presence of clays. Jones et al [4] found similar results in that only a small part of the total concentrations of a wide variety of contaminants such as heavy metals and pesticides present in the waterway sediment were available, that is, in forms toxic to aquatic test organisms.

In the water quality management field, distinguishing between soluble and insoluble forms is often based on filtration with a $0.45\text{-}\mu\text{m}$ pore-size membrane filter. This filter specification was not selected on the basis of its proper separation of the two forms, but rather, primarily because its resistance to flow of water is sufficiently low that the laboratory analyst can

collect an adequate amount of filtrate within a sufficiently short period of time so that analyses are not significantly delayed. It is well-known that appreciable quantities of some particulate contaminants, such as ferric iron, can pass through a $0.45\text{-}\mu\text{m}$ pore-size filter and be measured as soluble when, in fact, they are colloidal particulates. Such material may be as small as $0.001\text{ }\mu\text{m}$ in diameter. At this time, essentially nothing is known about the availability of contaminants present as colloidal precipitates or sorbed on colloidal particles. While such contaminants are measured, at least in part, as soluble forms, only a small part of the colloidal-particulate concentration measured is likely to be available to impact on water quality. This is one reason that this method of differentiating available and unavailable contaminant forms is often not reliable for certain contaminants. Filtration through membrane and certain other filters is also often not a satisfactory means of distinguishing between contaminants present in true solution and those present as colloidal suspensions, because almost all such filters contain trace amounts of metals that are leached to the filtrate. Nienke and Lee [6] found that it was impossible to determine reliably the soluble zinc in Lake Michigan water using membrane filtration with Millipore filters because the zinc present in the filters leached into and contaminated the samples. Such filters can also sorb contaminants such as certain organics, resulting in incorrect chemical measurements. Further, colloid-size materials, as mentioned previously, may be sufficiently small to pass through $0.45\text{-}\mu\text{m}$ pore-size filters. Although high-speed centrifugation may be more appropriate for separating soluble from insoluble contaminant forms, it is usually too time-consuming for routine use. If there is a question regarding the suitability of filtering a sample through a $0.45\text{-}\mu\text{m}$ pore-size filter for the evaluation being conducted, high-speed centrifugation should be checked.

Another cause of discrepancies between soluble and toxic forms of some elements, especially heavy metals, is the formation of chemical complexes. The formation of complexes or chelates, which are complexes composed of two or more ligand groups within the complexing molecule, is frequently invoked as a reason for some contaminants' not being toxic to aquatic life. Generally, much of what are labeled as complexes can also be readily explained by sorption onto finely divided particulate matter. In general, the behavior of contaminants sorbed on colloids or present as complexes is the same. About the only way to discern, at environmental concentrations, whether the toxicity of a metal ion is due to its presence as a soluble complex or a sorbed colloidal form is by evaluating the toxicity of a filtered water before and after high-speed centrifugation. If there is an appreciable decrease in toxicity after centrifugation, then it is possible that a large part of the metal was present as colloidal species and that this fraction was responsible for at least part of its toxicity.

It must not be inferred, however, that because a heavy metal is present as a complex it is necessarily toxic. It has been known for many years that copper

complexes with citrate are toxic to algae whereas copper complexes with ethylenediaminetetraacetic acid (EDTA) are nontoxic. Further work is needed to determine whether a relationship exists between the magnitude of the stability constant for metal ion complexes and the toxicity of the complex.

Another aspect of complexation which must be considered is that many of the important complexing agents for heavy metals in aquatic systems are organic compounds that are subject to biotransformation. Such transformations can significantly alter the ability of the organic compound to form a complex. This behavior is readily demonstrated in the use of copper citrate for algae control in water supply reservoirs. The addition of copper sulfate (an aquo species) to reservoirs for algae control is not very effective in hard-water, high-alkalinity lakes and reservoirs, because copper in this form precipitates as a basic carbonate. This precipitation will not occur for a period of time if the copper is added as the complex copper citrate. Eventually, as bacteria transform the citrate, the effectiveness of the copper diminishes because of precipitation of the released aquo species of copper with hydroxide and carbonate.

In the mid-1970s the International Joint Commission (IJC) for the Great Lakes held a workshop devoted to the toxicity of metal forms to aquatic life in natural water systems. The proceedings of this workshop [7], and in particular the paper by Andrew [8] devoted to the toxicity of various forms of copper to aquatic life, clearly show that the toxicity of copper, as well as the toxicity of several other heavy metals, is best correlated with the concentration of the ionic form. Precipitated, sorbed, or complexed forms are usually much less toxic than the free metal ion. It is clear from the proceedings of this IJC symposium, as well as from numerous other studies, that the total concentration of a contaminant which is frequently measured for comparison with water quality standards may be a poor measure of a contaminant's potential toxicity to aquatic life.

An Example of How Water Quality Characteristics Impact on Toxicity

The Study Characteristics

The results of the Morrison et al [9] study, as reported by Pitts [10], concerning the toxicity of copper in the Cache la Poudre River (Poudre River) water to fish, demonstrate the impact certain water characteristics can have on toxicity. This river originates in the Rocky Mountains as a high-quality mountain stream and, as shown in Fig. 1, it flows past Fort Collins, CO, and Windsor, CO, eventually emptying into the South Platte River near Greeley, CO. Morrison et al [9] conducted a series of static toxicity tests in which aquariums were filled with Poudre River water taken at various locations, shown in Fig. 1. Copper was added to the aquariums, and the 96-h LC₅₀ values for copper were determined for both rainbow trout and fathead minnows.

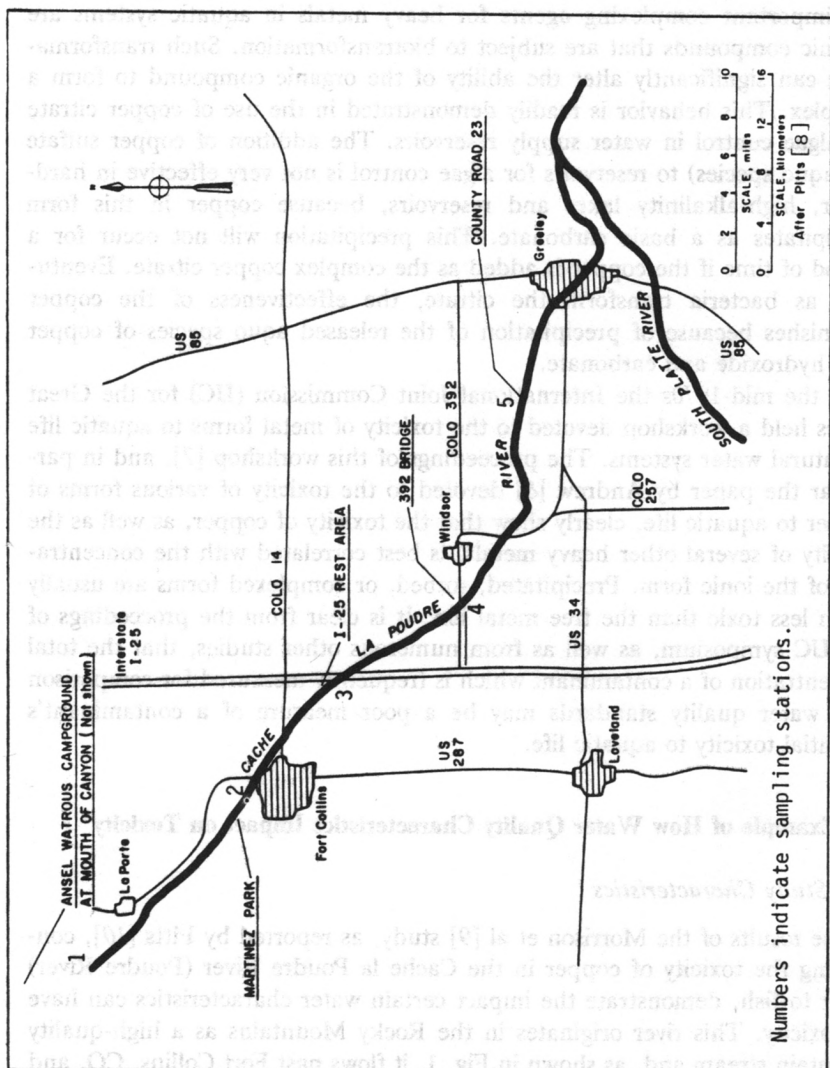


FIG. 1—Collection sites for river water bioassays in Morrison et al study [9].

Typical results, as reported by Pitts [10], are presented in Tables 1 and 2 and show that the toxicity of copper to these fish was dependent on the source of water used in the test. Water obtained at Site 1, which represents mountain stream water before significant impact by man, showed the greatest toxicity. Site 2 is located within the city of Fort Collins and receives a small amount of urban and agricultural drainage. It is evident from Tables 1 and 2 that, in general, copper added to water from Site 2 is much less toxic to both types of fish. Sites 1 and 2 are about 24 km (15 miles) apart on the river. Copper added to water from Site 3, which is 16 km (10 miles) downriver from Site 2, is even less toxic. Between Sites 2 and 3, the river receives substantial inputs of irrigation return water, and, at some times during the year, the discharge of well-treated secondary effluent from a domestic wastewater treatment plant which serves 50 000 to 60 000 people.

Between Sites 3 and 4, a distance of about 8 km (5 miles), some irrigation return water is added to the river at certain times during the year. It is evident from Tables 1 and 2 that there is little difference in the copper toxicity between the water from Site 3 and that from Site 4. Between Sites 4 and 5, which are about 16 km (10 miles) apart, the river receives irrigation return water and, most important, municipal wastewater from the town of Windsor, CO, and industrial wastewater from the Kodak of Colorado facility. It appears from the data available that copper is somewhat more toxic to fathead minnows in Site 5 waters than in waters collected from Site 4.

In reviewing these results it should be pointed out that the toxicity to the test fish of copper added to Site 1 water was typical of that reported in the literature. Therefore, the surface and groundwater inputs to the Poudre River that occur after the river leaves the mountain significantly alter the acute toxicity of copper to trout and fathead minnows.

TABLE 1—Acute lethal toxicity of copper in Cache la Poudre River water to rainbow trout.^a

Site	Temperature, °C	pH	96-h LC ₅₀ , mg/L	95% Confidence Limit, mg/L
1	15	6.9	0.10	0.064 to 0.14
	10	7.2	0.017	0.005 to 0.060
2	15	8.2	0.21	0.15 to 0.29
	10	8.2	0.18	0.14 to 0.21
3	15	8.3	0.16	0.13 to 0.19
	10	8.2	0.46	0.39 to 0.52
4	15	8.4	0.29	0.24 to 0.35
	10	8.2	0.61	0.54 to 0.70
5	15	8.2	0.27	0.17 to 0.43
	10	7.9	0.57	0.48 to 0.68

^a After Pitts [10].

TABLE 2—*Acute lethal toxicity of copper in Cache la Poudre River water to fathead minnows.^a*

Site	Temperature, °C	pH	96-h LC ₅₀ , mg/L	95% Confidence Limit, mg/L
1	24	6.9	0.14	0.12 to 0.15
	20	6.9	0.10	0.091 to 0.12
2	25	8.3	0.68	0.60 to 0.76
	20	8.3	1.1	0.91 to 1.3
3	25	8.4	5.1	1.9 to 14
	20	7.8	1.5	1.1 to 2.1
4	25	8.4	5.1	4.1 to 6.4
	20	8.3	7.2	4.9 to 11
5	25	8.2	2.5	2.0 to 3.1
	20	8.0	1.7	1.3 to 2.2

^aAfter Pitts [10].

Factors Affecting Copper Toxicity in the Poudre River

There are a variety of factors that can contribute to varying toxicity of a contaminant such as was found in the Morrison et al [9] study, as reported by Pitts [10]. It is well-known that the toxicity of copper is dependent upon the hardness-alkalinity of the water. The state of Colorado Water Quality Control Commission [11] has proposed water quality standard guidelines for copper which are a function of water hardness or alkalinity. For a hardness or alkalinity of 0 to 100 mg/L as calcium carbonate (CaCO₃), a copper guideline of 5 µg/L has been established. For waters containing between 100 and 300 mg/L as CaCO₃ of hardness or alkalinity, the copper guideline value is 10 µg/L. For waters having alkalinity or hardness values between 300 and 400 mg/L as CaCO₃, the copper guideline value is 20 µg/L; and above 400 mg/L as CaCO₃, the copper guideline value is 40 µg/L. Therefore, based on the Colorado Water Quality Control Commission guidelines for copper [11], there is almost a factor of 10 difference in the toxicity of copper to aquatic life in low hardness or low alkalinity waters compared with that found in extremely high-hardness or high-alkalinity waters.

The EPA November 1980 [2] water quality criteria for copper established maximum allowable copper concentrations in freshwater systems of 12, 22, and 43 µg/L for waters having hardness values of 50, 100, and 200 mg/L as CaCO₃, respectively; the EPA maximum concentrations for copper as a function of hardness are somewhat higher than those established by the state of Colorado Water Quality Control Commission [11]. In the EPA [2] criteria there is a factor of about 4 between allowable copper concentrations in low-hardness and high-hardness waters, as compared with the Colorado factor of 10.

The only characteristics of the test waters measured by Morrison et al [9] were temperature and pH; they did not measure alkalinity or hardness. The Morrison et al [9] water samples were collected in fall 1978 and winter-spring

1979. During 1978 to 1980, the U.S. Geological Survey (USGS) measured hardness and alkalinity of Poudre River water from this area. Table 3 presents the average concentrations for these parameters found by the USGS. While most of the data were collected in 1979 to 1980, a year after the Morrison et al [9] sample collection, the average concentrations of hardness and alkalinity reported generally indicate the concentrations that would be found in any year at or near the sites indicated.

Examination of Table 3 shows that at or near Site 1 the hardness and alkalinity values were between 40 and 50 mg/L as CaCO_3 ; the most stringent state of Colorado copper water quality guideline would, therefore, be applicable to this water. In the Poudre River at Lincoln Street near Site 2, the hardness and alkalinity ranged from 100 to 150 mg/L as CaCO_3 , and therefore the second most stringent copper water quality guideline would be applicable. Near Site 3 the hardness and alkalinity values were approximately 800 to 900 mg/L as CaCO_3 ; therefore, the least stringent copper water quality guideline would be applied to the water. Site 5 hardness and alkalinity ranged from 200 to 551 mg/L as CaCO_3 , which would cause the copper water quality guideline corresponding to hardness values of 300 to 400 or 400+ mg/L as CaCO_3 to be applied, depending on whether alkalinity or hardness were used. The decrease in hardness and alkalinity between Sites 3 and 5 may account for the decrease in copper toxicity found by Morrison et al [9] for samples of water taken from these locations.

It is evident that the markedly different toxicities of copper to fathead minnows and trout found by Morrison et al [9] cannot be completely accounted for by the differences in hardness and alkalinity found in the Poudre River. It is logical to suggest that this difference may be due, in part, to complexing agents added to the river from agricultural drainage and irrigation return flows, urban storm-water drainage, and domestic and industrial wastewater inputs. It may also be due to the input of both inorganic and organic par-

TABLE 3—Average alkalinity and hardness for Cache la Poudre River, 1979–1980.^a

Location	Alkalinity, mg/L CaCO_3	Hardness, mg/L CaCO_3
Mouth of canyon (Site 1)	48	45
Lincoln Street (Site 2)	103	143
Tinnath Road (near Site 3)	192 ^b	915 ^b
Near Greeley (below Site 5)	215	551

^aBased on U.S. Geological Survey data provided to Fort Collins, CO, Department of Public Works.

^bData from 1978–1979.

ticulate matter to the river from these sources that would sorb copper and make it unavailable to the test organisms during the period of the test. Morrison et al [9] did not make measurements of suspended particulate matter or turbidity in their test aquariums. Therefore, it is impossible to correlate suspended solids levels with toxicity.

Morrison et al [9] conducted similar types of tests for ammonia, zinc, cadmium, and silver. In general, patterns of toxicity similar to those reported for copper were found for the zinc, cadmium, and silver with as much as an order of magnitude or more difference in the toxicities of these metals to trout and fathead minnows at the downstream study sites, compared with the study site near the mouth of the canyon just above the area where man starts to have a heavy impact on the characteristics of the river. It was interesting to find that ammonia showed little change in toxicity as a function of type of water when assessed in terms of un-ionized ammonia. Ammonia added to water from the mouth of the canyon (Site 1) showed approximately the same toxicity to fathead minnows and trout as it did when added to water from Sites 3 and 4. The lack of dependence of ammonia toxicity on type of water would be expected based on ammonia's relatively simple aqueous environmental chemistry compared with that of many of the heavy metals. While ammonia tends to form complexes with some metals, the complexes are weak and unimportant in affecting aquatic organism toxicity under most normal environmental conditions. The heavy metals generally of water quality concern all tend to form strong complexes with a variety of organic and inorganic compounds. Heavy metals also tend to sorb strongly on natural water particulate matter while ammonia sorption tendencies are, in general, much weaker. Therefore, it may be concluded that the Morrison et al [9] data on the acute lethal toxicity of copper, zinc, cadmium, silver, and ammonia are in accord with the aqueous environmental chemistry of these elements in the waters of concern.

Implications of Morrison et al Study Results on Copper Waste Load Allocation

It is evident from the results of the Morrison et al [9] study, as reported by Pitts [10], that appreciably greater concentrations of total copper can be allowed in the lower plains reaches of the Poudre River than in those parts of the river that are in the mountains. This conclusion assumes that the chronic toxicity of copper to aquatic life varies in a manner somewhat proportional to the change in acute toxicity of copper to aquatic life observed for different water sources. Work needs to be done to verify that this assumption is appropriate. Their studies also showed what appears to be strong justification for site-specific standards, and waste load allocations based on these standards, that consider the aqueous environmental chemistry of the contami-

nant of concern in the particular receiving water. Failure to develop such site-specific standards could easily result in unnecessarily strict water quality standards being applied to certain waters. In practical terms this means that an industry, such as a printed circuit industry, may choose not to locate in a certain area because it could not meet worst-case standards (for example, for copper) required of its effluent when, in fact, the effluent may not adversely affect the beneficial uses of the waters to which it would be discharged. By using site-specific standards, aquatic life-related beneficial uses can be protected without making unnecessary treatment demands of industry, municipalities, agricultural interests, and other sources of contaminants.

Other Studies on Translation of Laboratory Results to Field Situations

Toxicity of Chloramines

Lee et al [12] and Newbry et al [13] conducted a study on the persistence and toxicity of chlorine (chloramines) used for partial disinfection of domestic wastewaters in several Colorado Front Range streams. By conducting in-stream, caged fish bioassays and measuring chlorine concentrations over time, it was determined that the acute lethal toxicities (96-h LC_{50}) of the river waters below the wastewater treatment plants (where chlorine appeared to be the primary toxicant present) were essentially the same as toxicities determined in laboratory bioassays of mixtures of domestic wastewater and river water in Michigan, as well as in standard laboratory bioassays with chloramine, reported in the literature. This finding would be expected based on the aqueous environmental chemistry of chloramines. All of the transformations that chloramines tend to undergo in aquatic systems (such as phototransformation, volatilization, and demand) produce forms that are not measured by the analytical procedures frequently used for residual chlorine and that are not highly acutely toxic to aquatic life.

It is important to note that the work of Lee et al [12] and Newbry et al [13] focused on the toxicity of chlorinated municipal wastewater treatment plant effluents that contained high levels of ammonia (on the order of 20 mg N/L). If the wastewater had been nitrified prior to chlorination, somewhat different results would have been expected. The chlorination of domestic wastewater having low ammonia levels would be expected to result in the formation of a wide variety of organic chloramines which would have markedly different toxicities to aquatic life from the monochloramine typically present in non-nitrified effluent. Therefore, while the translatability of results between the Lee et al [12] study sites was good, it is likely that their results would not be directly applicable to situations involving chlorine residuals arising from the chlorination of nitrified domestic wastewater effluents.

Bioconcentration of Organics

Although most of the information available on problems of translating laboratory bioassays to field situations focuses on heavy metals, there is growing evidence that similar problems occur with many organic contaminants as well. An example of inappropriate translation of laboratory bioassays of organic compounds to field situations is provided by the bioconcentration factors and subsequent standards developed for some chlorinated hydrocarbon pesticides and polychlorinated biphenyls (PCBs). It is now apparent that the bioconcentration factors established for PCBs, based primarily on work with Lake Michigan and Lake Superior water, are not applicable to many other waters of the United States. The work of Jones and Lee [14] has shown that PCBs are present in some waters of the United States at concentrations a factor of 10 to 20 above the EPA July 1976 Red Book criterion for PCBs; yet the concentrations of PCBs in the flesh of fish living in these waters are far below the Food and Drug Administration (FDA) action limit of 5 ppm. This discrepancy appears to arise from the fact that PCBs tend to become strongly sorbed onto natural water particulate matter. Such sorption significantly reduces the availability to fish. Both fish and suspended sediment, which compete for the PCBs present in the water, are sinks for PCBs. Lake Michigan and Lake Superior waters are atypical of most natural waters in the United States in that they contain unusually small amounts of particulate matter. Therefore, bioconcentration factors and criteria or standards based thereon, developed through work on Lakes Michigan and Superior water, will be unnecessarily strict for most waters containing particulates because the PCB sorption reactions were not accounted for; such bioconcentration factors will also have limited capabilities for predicting the uptake of PCBs by fish in waters having more normal suspended sediment concentrations.

The results of studies on the impact that suspended particulate matter has on the uptake-bioconcentration of organic compounds in natural waters by aquatic organisms have serious implications for the use of a laboratory procedure such as that proposed by ASTM Committee E-47 on Biological Effects and Environmental Fate for developing bioconcentration factors. It is clear that such procedures will not generate data with widespread applicability for predicting the bioconcentration of contaminants in natural water. In addition to the approach specified by these procedures, part of the problem is that the analytical procedures generally used for measurement of PCBs and other contaminants of this type do not distinguish forms in true solution (and therefore available for bioconcentration) from those sorbed or otherwise associated with particulate matter, and thus having a different availability and impact mechanism for aquatic life. Care must be exercised in applying laboratory-derived bioconcentration factors to the total concentration of the contaminant in the water when estimating bioconcentration in the field.

Throughout this paper, emphasis has been given to those transformations that occur in the field which tend to reduce the toxicity or availability of contaminants to aquatic life. In general, transformations of this type are the most common; however, there are other important and notable transformations which also must be considered in any assessment of the potential hazard that a particular contaminant represents to beneficial uses of water on a site-specific basis. For example, several chlorinated hydrocarbon pesticide degradation products are more toxic to aquatic life than their parent compounds. The testing of Aldrin in laboratories would not, in general, detect the toxicity caused by dieldrin, a transformation of Aldrin. Similarly, heptachlor epoxide, which would be formed in aquatic systems, is much more toxic to aquatic life than its parent compound, heptachlor.

The photodecomposition of iron cyanides, which leads to the formation of hydrogen cyanide (HCN), is another example of a reaction in which a compound of fairly low toxicity to aquatic life is converted in the environment to a much more toxic chemical. Similar types of conversions can occur with heavy metals or other contaminants which are either complexed or sorbed by organic materials. This would be especially true in those wastewaters which, at the point of discharge, may be relatively nontoxic because of sorption or complexation reactions. However, in the environment, the organic fraction which is rendering the chemical of concern nontoxic would be decomposed, that is, biotransformed, thus releasing the contaminant in its toxic form. Consideration must be given not only to the behavior of potentially significant contaminants such as heavy metals, but also to contaminants such as strong complexing agents which, while nontoxic or essentially nontoxic to aquatic life, could, in the environment, mobilize highly toxic contaminants from sediments. For example, there has been concern for many years about the use of nitrilotriacetic acid (NTA) as a substitute for phosphate in detergent formulations. It is conceivable that the discharge of NTA into a poorly treated domestic wastewater could result in sufficient quantities of NTA entering the aquatic environment so that, when in contact with sediments, it could complex, that is, solubilize, heavy metals from the sediments making them available. Even if the solubilized complex of NTA is nontoxic, the NTA will ultimately be degraded in the environment and the heavy metal, at that point, would be free in the form of the aquo species. An example of this would be the heavy metals associated with domestic wastewater discharges in which the heavy metals are primarily in a particulate form. One must be certain that heavy metals which are nontoxic at the point of discharge do not become toxic downstream because of biotransformation of the chemicals that render them nontoxic.

Another situation that must be considered in evaluating the hazard that a contaminant represents to beneficial uses of aquatic systems, based on laboratory bioassays or toxicity tests, is a marked change in the environmental characteristics of the receiving waters from those of the waters used in the

toxicity testing. A classic example of this kind of situation occurs with ammonia. When associated with municipal wastewater discharges, ammonia may show relatively low toxicity since such waters tend to have a much lower pH than receiving waters, especially those rich in algal or other aquatic plant growth. A wastewater with relatively large amounts of total ammonia may be nontoxic at the point of discharge. However, in the receiving waters, even after dilution, because of the higher pH arising from photosynthesis especially during the late afternoon, the wastewaters may show appreciable toxicity to fish and some other forms of aquatic life.

Certain heavy metals, such as manganese and iron, tend to be much more soluble under reducing than oxidizing conditions. This is readily demonstrated in the environment by the relative concentrations of iron and manganese in the epilimnia and anoxic hypolimnia of lakes and reservoirs. This means that, since toxicity tests with aquatic organisms must be conducted under oxic conditions in order for the organisms to survive, it is possible that a contaminant which is unavailable in the presence of dissolved oxygen could become more available in the absence of oxygen, that is, in the anoxic hypolimnia of lakes. However, in evaluating this situation, one must consider the fact that most aquatic life requires dissolved oxygen; therefore, the issue is not whether a contaminant is more toxic in a reduced form but whether, upon contact with dissolved oxygen under suitable habitat conditions for fish and other aquatic life, the contaminant would remain in a reduced form or would be readily oxidized or removed under oxidizing conditions, that is, in the presence of dissolved oxygen. Jones and Lee [14] have found, in their examination of the behavior of a variety of contaminants present in U.S. waterway sediments, that the only two contaminants of the group they studied (which included heavy metals and chlorinated hydrocarbon pesticides—30 contaminants in all) that did not rapidly convert to insoluble forms upon exposure of the sediment extract to waters containing dissolved oxygen were ammonia and manganese. In the case of ammonia, this conversion is biochemically mediated. Similar results have been found for manganese by Delfino and Lee [15].

Discussion of Recommended Approach

A wealth of information has been developed from the EPA water quality criteria development efforts concerning the toxicity and bioconcentration responses of numerous types of organisms to exposure, under laboratory conditions, to a wide variety of chemical contaminants that could significantly impact on water quality. Most of this information was produced through artificial, usually worst-case, testing conditions. It is known, however, that such worst-case conditions rarely exist in natural water systems. Because of the behavior of many aquatic organisms and the contaminant dilution-dispersion patterns and variable concentrations of many contaminant

sources, organisms of concern often do not receive chronic lifetime exposure to contaminants. Many organisms are known to be able to tolerate even potentially acutely toxic levels of contaminants, provided the duration of their exposure is kept sufficiently short. Chemicals can undergo a variety of chemical and physical transformations which alter their toxicities. It is thus inappropriate, in many instances, to take the results of a worst-case laboratory bioassay (such as the "safe" concentration of a contaminant under worst-case conditions previously defined) and apply them to a water body (for example, the concentration in a water therefore shall not exceed the "safe" level). It behooves those in the water quality management field to develop a system whereby this wealth of toxicity-bioconcentration information can be used effectively for cost-effective, environmentally sound water quality management.

One of the major problems faced in translating laboratory bioassay data is how to account for the chemical transformations that will occur in the field but that did not occur in the laboratory. This problem has its roots in analytical deficiencies that are not readily overcome. Some transformations, such as photolysis and hydrolysis reactions, destroy the parent compound and create products which are generally analytically distinguishable from the parent compound even when the total concentration of the parent compound is measured. For other transformations, such as sorption, complexation, and precipitation, however, the transformation products are not generally analytically distinguishable from the parent compound, especially if the total concentration is measured. Although sample filtration or centrifugation can aid in making this distinction, in addition to the problems inherent in those procedures, they do not necessarily adequately separate available from unavailable forms. It is therefore not possible, in many cases, to translate laboratory bioassay results to field situations by measuring contaminant concentrations in the field.

The philosophy of the hazard-assessment approach can be employed in data translation. This approach has been described by Lee et al [16,17] and has also been the subject of several recent conferences and workshops (Cairns et al [18] and Dickson et al [19]). It involves a selective sequential (tiered) testing program designed to provide the technical input necessary to answer specific questions. With each subsequent tier, testing and evaluation become more sophisticated and expensive, but the answer derived is more accurate; the specific tests conducted in each tier depend on the question to be answered. Testing can be terminated at any level once an answer of sufficient reliability can be gleaned. For evaluating the impact of a particular discharge on water quality, each tier addresses key aspects of the environmental chemistry-fate of the contaminant or contaminant mixture and potentially significant transformation products, and the aquatic toxicology of the contaminant or contaminants and transformation products, in light of desired beneficial uses of the water.

Tier 1

The first tier of such an assessment for translation of laboratory bioassay data to field conditions when a point-source discharge is being evaluated should have basically two components. One is a review of the components of the discharge that may cause water quality problems, their forms, and what is known about their aqueous environmental chemistry. The second component is an evaluation of certain characteristics of the receiving water. For example, if the component of the discharge of concern is a metal, focus should be directed on the tendency of the forms of the metal present to undergo complexation, precipitation, and sorption reactions under the pH, temperature, hardness, alkalinity, and turbidity conditions that exist in the receiving waters and the forms and solubilities of the metal likely to be produced. For organic chemicals, primary attention should be given to their tendency for sorption and, depending on the specific organic compound, to its tendency to undergo photolysis, volatilization, and so on. It is also important to determine the solubility of the organic compound; some may be so insoluble under the conditions being considered that the levels in the water would be insignificant compared with any "critical" level. Consideration must be given not only to the transformation reactants and products but also to the rates of the transformations likely to be encountered. As indicated earlier, the receiving water pH, temperature, alkalinity, hardness, and turbidity should be determined as well as flow.

If, based on the aqueous environmental chemistry of the contaminants of concern it is likely that the contaminants will be in forms available to aquatic life, if the turbidity of the receiving water is less than 0.1 nephelometric turbidity units (NTU) (or there is essentially no tendency for sorption), and if the effluent-water mixing is such that the organisms of concern would likely be forced to spend critical life stages or substantial portions of their lifetimes in an area influenced by the discharge, the evaluation may be terminated and the worst-case bioassay results applied directly to the waters. Because of the importance of turbidity in making Tier 1 evaluations, the authors have provided a recommended, somewhat arbitrarily determined turbidity value cutoff. It is likely that this 0.1 NTU value will be changed as more information is gathered on turbidity-sorption relationships, but it should be adequate at this time for Tier 1 evaluations. This turbidity level can be reliably measured using a Hach 2100 A turbidimeter.

An alternative to measuring turbidity may be to measure Secchi depth. In deeper waters, if the Secchi depth is less than about 2 m, there may be enough particulate matter to result in sufficient sorption to be of concern. This value would also be subject to change as more information becomes available.

If, at the end of the Tier 1 evaluation, there are significant gaps in information regarding the effluent or receiving water characteristics, or about the

aqueous environmental chemistry-fate of the contaminants of concern, then Tier 2 testing should be undertaken. Tier 2 testing should also be undertaken if it is determined in Tier 1 that worst-case laboratory bioassay data are not appropriate for direct application to the field site of concern.

Tier 2

For the purposes of this general discussion, Tier 2 testing will be divided into two parts: Tier 2A for those situations in which data are lacking on the environmental chemistry, and so on, and Tier 2B for those in which it has been determined that worst-case laboratory bioassay results are not appropriate for the field site. The specific evaluations made in Tier 2A will depend on the voids left in Tier 1. Examples of the types of tests that may need to be run are presented here.

The complexation tendency of certain heavy metals is likely to be related to the dissolved organic carbon (DOC) concentration in the water. Although the exact relationship is currently inadequately defined, waters with higher DOC would be expected to cause greater complexation of heavy metals. Waters with high DOC levels also tend to have a greater sorption potential. The reason for this is that the dissolved organics coat suspended particulates, generally increasing their sorption capacity for some contaminants. Where indicated, DOC should be measured on samples that have been filtered through a 0.45- μm pore-size filter or centrifuged at high speed. If filtration were to be used, blanks must be run to ensure that the filters do not add significantly to the DOC content of the sample. The DOC should be measured after removal of inorganic carbon, using high-temperature catalyst-assisted or pressurized persulfate oxidation techniques, in which the carbon dioxide (CO_2) produced is measured. In general, chemical oxygen demand techniques involving oxidation with a chromic acid solution are not reliable for this purpose.

If a contaminant's sorption tendency is not known, it is suggested that it be determined on several standardized sorbents. A standard clay, a freshly prepared ferric hydroxide, and a standard organic particulate polymer, such as polystyrene beads, should be used in a testing procedure of the type being developed by ASTM Committee E-47 on Biological Effects and Environmental Fate. Because of the nonlinear and nonextrapolatable nature of many sorption reactions, the sorption test should be conducted at or near the expected environmental concentrations for the contaminants of concern, using suspended solids concentrations typical of what might be expected in the environment. Since many sorption reactions are not completely reversible, care must be taken to simulate, as closely as possible, the conditions that will prevail at the site of concern. If these conditions are unknown, then sorption-desorption testing must be conducted under the range of conditions that are expected to exist in the environment.

The characterization of the sorption tendencies of organic and inorganic contaminants evaluated for their aquatic toxicity-bioconcentration potential would be a major step toward providing the kinds of information needed to detect potential problems in translating laboratory results to field situations. In time, as more information becomes available, it may be possible to reduce the amount of testing to that of one type of solid. It is important to emphasize, however, that this testing is not designed to predict the amount of sorption that would occur in an aquatic system. Instead, it is to be used as a screening mechanism for detecting those chemicals which have a high sorption tendency. If an assessment of the suitability of worst-case laboratory results for the site of concern cannot be made at this level of testing, Tier 3 testing would be necessary.

If it is determined in Tier 1 that worst-case bioassay results are highly conservative for direct application to the site of concern, it is recommended that the worst-case results be used in Tier 2B as a screening tool. Based on expected or measured mixing patterns, the area below the discharge in which the chronic safe contaminant concentration is exceeded should be defined. This could easily be the starting point of a hazard assessment of the chemical such as that briefly described previously. It may be that the extent of this area is insignificant in terms of adversely impacting on aquatic life or other beneficial uses of the water. If the areal extent wherein the chronic safe level is exceeded is extensive and of concern, then Tier 3 testing would be indicated.

It should be mentioned that for some contaminants, notably chloroamine [20], environmental chemistry-fate models have been developed which can be used to estimate the areal extent and duration of contaminant persistence in a particular receiving water. In general, these models are in their infancy and are largely unverified. They should be applied with caution and verified for the system to which they are being applied before their outputs are accepted.

Tier 3

Tier 3 testing would involve conducting in-stream bioassays following the approaches outlined by Lee et al [16]. This tier can provide information that will assist in making future translations of laboratory results to the field. By the time Tier 3 testing is indicated, the discharger may find it more economical to use worst-case results than to continue testing but will more likely conduct a hazard assessment of the chemical or discharge. In the latter case, in-stream bioassays will undoubtedly have to be included. The previously described hazard assessment scheme is one that, based on experience, the authors feel will be cost-effective in developing the necessary information. Other schemes would also provide the desired information at approximately the same cost.

Additional Considerations

In addition to the large amount of worst-case laboratory bioassay results available, there is also a considerable amount of information available from more site-specific or less conventional bioassays. In using this information, the same considerations as outlined in this paper must be given to the comparability and translation of the results to a site before the results are used in assessing water quality impacts.

While the example provided in this paper was for a contaminant discharge, the same basic approaches would be used regardless of the contaminant source, be it nonpoint, natural, or unknown. These approaches can also be used for applying laboratory results to a reach of a river.

Little has been said about actual translation of laboratory results to field situations in terms of the modification of a bioassay result to fit a particular set of field circumstances. At this time, this type of translation cannot, in general, be reliably made. It may be possible to do this with some confidence when more information is available of the type derived by Lee et al [12] and Newbry et al [13] on chloramines, that is, the comparison of actual in-stream toxicity responses of fish with knowledge of pH, turbidity, hardness, alkalinity, and temperature to laboratory 96-h LC₅₀ values, chronic safe exposure levels, and so on. Tier 3 studies, if properly conducted, will be instrumental in obtaining this type of data.

Conclusions and Recommendations

The aqueous environmental chemistry of a contaminant plays a major role in governing its toxicity and impact on aquatic organisms in natural water systems. Because such chemical reactivity is often neglected in laboratory bioassays and because the results of many standard bioassays are for worst-case conditions, it is generally inappropriate to directly use worst-case laboratory bioassay results for field conditions, especially when considering total concentrations of contaminants. The chemical reactions generally of greatest concern in the translation of laboratory results to field situations are the formation of metal ion complexes and of finely divided precipitates or sorbed species.

A hazard-assessment approach should be used to identify those field situations for which worst-case laboratory bioassay results are not generally appropriate. Attention needs to be given to the contaminant characteristics and reactivity as well as to certain pertinent characteristics of the water at the site of concern, especially its turbidity. Such an approach in water quality management will allow maximum utilization of available information on contaminant toxicity and bioconcentration as well as contaminant aqueous environmental chemistry.

Although, in general, it is not yet possible to readily translate laboratory-derived information to field conditions, the capability of water quality managers to make this translation would be greatly facilitated with information developed from the recommended evaluation procedures presented.

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RESEARCH: Cairns, J. B., "Changes in the Fish Community of the Western Lake Erie Basin," *Journal of Great Lakes Research*, Vol. 1, 1975, pp. 1-10.

Abstract: The changes in population trends of fish in the Western Lake Erie Basin from the introduction of chemical effluents were examined for a 15-year period from the standpoint of whether the observed changes could have been predicted from the results of laboratory determinations of chemical resistance. It was concluded that such changes in community structure could have been predicted but the degree to which such species populations and the community as a whole were affected would require further detailed information about (1) the chemical and chemical combinations in the effluent, and (2) the response of the resident biota to various chemical exposures.

ABSTRACT: The changes in population trends of fish in the Western Lake Erie Basin from the introduction of chemical effluents were examined for a 15-year period from the standpoint of whether the observed changes could have been predicted from the results of laboratory determinations of chemical resistance. It was concluded that such changes in community structure could have been predicted but the degree to which such species populations and the community as a whole were affected would require further detailed information about (1) the chemical and chemical combinations in the effluent, and (2) the response of the resident biota to various chemical exposures.

KEY WORDS: aquatic ecology, chemical effluents, chemical resistance, community structure, laboratory data, toxicity, hazard assessment.

Usually using death as the physiological end point, the toxicologic tolerance test is the most direct means of obtaining information about the effect of a particular substance or factor on a specific population. Such determinations of tolerance for selected species of organisms are fundamentally important to the understanding of potential ecological effects, but extrapolating the results of such tests with single "representative" species to complex natural ecosystems is a hazardous step. Some impairment to the laboratory data base may occur by extending the test to more than one species and to more than one life stage, usually adult or juvenile, and also by