83-31-06

Joint Action of Toxicant Mixtures on Daphnids
Literature Review

by

Cynthia A. Parks
Research Assistant

August 1987

December 1987
Env. Eng. Report No. 100-87-8

Technical Report

Joint Action of Toxicant Mixtures on Daphnids
Literature Review

by

Cynthia A. Parks
Research Assistant

Environmental Engineering Program
Department of Civil Engineering
University of Massachusetts
Amherst, MA 01003

Submitted to the

Massachusetts Department of Environmental Quality Engineering
Division of Water Pollution Control
S. Russell Sylva, Commissioner
Thomas C. McMahon, Director

ACKNOWLEDGEMENTS

Appreciation is expressed to the Massachusetts Division of Water Pollution Control (MDWPC) for providing support through the Research and Demonstration Program for this literature review (Project Number 83-31). The following individuals are also extended a special thanks: James W. Male and Daniel J. Wagner for reviewing the report; Rebecca Scherer for drafting; and Karen Thatcher of Words-Worth for word processing.

EXECUTIVE SUMMARY

Most wastewater discharges and natural waters contain mixtures of toxicants. The national water quality criteria, however, are based on laboratory data derived for single toxicants. A pollutant's toxicity can be altered by its interaction with other toxicants and abiotic factors including pH, temperature, hardness, and dissolved oxygen. Both the United States Environmental Protection Agency and the European Inland Fisheries Advisory Commission are conducting research to develop water quality criteria for combined pollutants.

The types of joint effects which may occur when chemicals are mixed can usually be described by the concentration-addition or response-addition models. With the concentration-addition model, the toxicants produce similar yet independent effects. If the relative proportions of the constituent toxicants are known, the toxicity of the mixture can be predicted directly. Common indices used in this model include the toxic unit and the Marking Additive Index. The concentration-addition model is widely used for water quality pollution studies.

The response-addition model describes mixtures where the toxicants act independently, but contribute to a common response. The correlations between the susceptibilities of individual test organisms to each component toxicant determine the toxicity of a mixture. The response-addition model has been used extensively in pesticide development, in which the prime concern is maximum lethality.

Experimental design and data analysis are important factors which cannot be ignored in toxicity studies. The appropriate choice of trans-

formation for dose and response data and careful analysis of variance are two aspects of data analysis often overlooked. Multi-factorial designs are recommended for determining the importance of biotic and abiotic factors which may influence mixture toxicity.

Joint effects of a number of toxicant mixtures on species of fish, invertebrates, plants, and bacteria are presented. For common constituents of sewage and industrial wastes, the concentration-addition model appeared to be additive in acute toxicity tests with fish and invertebrates. Pesticide mixtures exhibited more-than-additive effects on both organism groups. With plants and bacteria, the interactions were variable. This was observed with mixtures of heavy metals where the results varied according to the species tested. Data from chronic studies were contradictory regarding joint interactions for all groups of organisms tested.

A small number of studies have been conducted using daphnids to assess the joint action of various chemical mixtures. A chelating agent complexed with copper and zinc greatly reduced the toxicity of the two metals and may have application in the attenuation of accidental spills. An antagonistic interaction was observed with the effects of copper and zinc mixtures on Lake Michigan zooplankton, which included several daphnid species. Binary mixtures of metal salts caused significant decreases in daphnid reproductive success at concentrations which showed no effect when the single metals were tested. Using metals mixed at multiples of the LC50 values, joint action was additive in both acute and chronic tests with daphnids.

Daphnids have been used as test organisms in toxicity tests with coal conversion effluents and landfill leachate. A systematic procedure for the hazard evaluation of coal conversion effluents using daphnids has been developed. Because of the considerable variation in the chemical composition of effluents, the results obtained in one study may not be typical for all coal conversion effluents. Toxicity tests with daphnids proved to be good indicators of landfill leachate toxicity and compared favorably with parallel tests using the standard static 96-h fish bioassay. The use of toxicity tests with organisms from different trophic levels should be considered when assessing the potential impact of a pollutant discharge on an aquatic ecosystem.

Water quality parameters can interact with pollutants and modify their toxicity. These parameters include temperature, pH, dissolved oxygen, carbon dioxide, hardness, and organic ligands. Toxicity has been shown to be inversely related to hardness.

The applicability of laboratory toxicity data to field conditions is not well understood. The selection of test species and dilution water is critical in influencing the relevance of laboratory results to natural conditions. Nutrition, acclimation, and genetic selection of the test organisms influence the accuracy of predictions based on laboratory data. If test parameters are appropriately chosen, the laboratory and field data will provide good agreement.

TABLE OF CONTENTS

FIGURES

TABLES

1. INTRODUCTION

National water quality criteria have been prepared to allow state and federal agencies to meet their responsibilities in evaluating the potential effects of chemical substances discharged into receiving waters in accordance with the Federal Water Pollution Control Act Amendment of 1972 and the Clean Water Act of 1981. Environmental testing guidelines have been promulgated under the Toxic Substances Control Act and the Federal Insecticide, Fungicide, and Rodenticide Act (Doherty, 1983). Most of the data used in the development of the national water quality criteria are from standard toxicity tests of single chemical compounds. Several physical and chemical factors including temperature, pH, dissolved oxygen, carbon dioxide, and hardness can modify the toxicity of a pollutant. Interaction with other chemicals in a complex effluent can also alter a pollutant's toxicity to aquatic organisms. These interactions have generally not been considered in the development of water quality criteria, despite the fact that pollutants in the natural environment rarely occur in the absence of other pollutants.

A working party of the European Inland Fisheries Advisory Commission (EIFAC, a regional commission of the United Nations Food and Agriculture Organization) has proposed water quality standards based on the critical review of all pertinent data on the toxicity of selected chemicals to fish (Lloyd, 1986). It has been suggested that the proposed standard for each chemical may need to be made more stringent if other pollutants are present. One of the main objectives of toxicity research in Europe is to determine the extent to which water quality standards set for single chemical compounds are valid when other toxic substances are present (Lloyd, 1986).

Daphnids are used extensively in toxicity tests because of their high sensitivity to chemical pollutants and the simplicity of their breeding and use (Leeuwangh, 1978). The purpose of this literature review is to compile information available on the joint action of toxicant mixtures tested with daphnids. The various models employed in evaluating the effects of mixtures of toxicants are presented in Section 2. A compilation of the results of multiple toxicity studies using aquatic organisms other than daphnids is contained in Section 3. A discussion of the limited number of joint action studies using daphnids is presented in Section 4. The subjects of interacting physical and chemical factors and the applicability of laboratory results to field conditions are discussed in Sections 5 and 6.

2. MODELS AND TERMINOLOGY

2.1 Mechanisms of Interaction

When a potentially toxic substance is present in water, several processes may be involved before an aquatic organism exhibits a response. These mechanisms may be categorized into three groupings as follows (EIFAC, 1980):

1. Chemical and physicochemical processes, which involve the interaction of the toxic substance with constituents of the water, make the toxic substance biologically available.

2. Physiological processes affect the amount of toxicant and its metabolites present in the organism's fluids, tissues, and organs and therefore, the quantity available at the site of action. Examples of these processes include absorption through gills, gut, and skin; transport and distribution by the circulatory system; metabolic transformation; and excretion.

3. Interaction between different physiological processes within the organism may occur when the organism is exposed to two or more potentially toxic substances. The interactions contribute to the response of the whole organism by affecting the chemicals' absorption; binding to plasma proteins; distribution; transport to and release from tissues; action on receptor sites; metabolism; and elimination.

The group 1 processes occur within the environment. Group 2 interactions occur within a kinetic phase while the group 3 interactions take place during a dynamic phase (Connell and Miller, 1984).

2.2 Terminology

There are several important types of pollutant interactions. If two or more pollutants in a mixture exert a combined toxic effect on an organism, the interaction is additive. When the overall effect on an organism is greater than when either pollutant acts alone, the interaction is labelled as synergism (Mason, 1981). Antagonism is the interaction resulting when the pollutants interfere with one another to lessen their impact. Connell and Miller (1984) outline several types of antagonism, including:

1. Competitive antagonism--antagonist displaces agonist from its site of action, e.g. displacement action of additional oxygen uptake in carbon monoxide intoxication.

2. Chemical antagonism--antagonist inactivates agonist through chemical interactions, e.g. chelation of metals.

3. Noncompetitive antagonism--antagonist interferes with induction of the effect by the agonist without reacting directly with the agonist or with specific agonist receptors, e.g. atropine blocks specific receptors of acetycholine which accumulates after the inactivation of acetylcholinesterase by organophosphorus compounds.

4. Functional antagonism--two agonists act on the same cell system, but contribute in opposite ways to the response of the cell.

5. Physiological antagonism--agonists act on different cell systems and produce opposing effects in these systems.

Figure 1 illustrates the types of interactions discussed above which may occur when an active substance (A) is combined with substance (B), which is inactive when applied alone.

2.3 Models

Various models and methods of data analysis have been suggested for describing the types of combined effects that occur when chemicals are present simultaneously. A model should ideally describe (Calamari and Alabaster, 1980):

1. Whether the effect of the mixture of chemicals is additive, synergistic or antagonistic.

2. The extent to which synergism and antagonism exist.

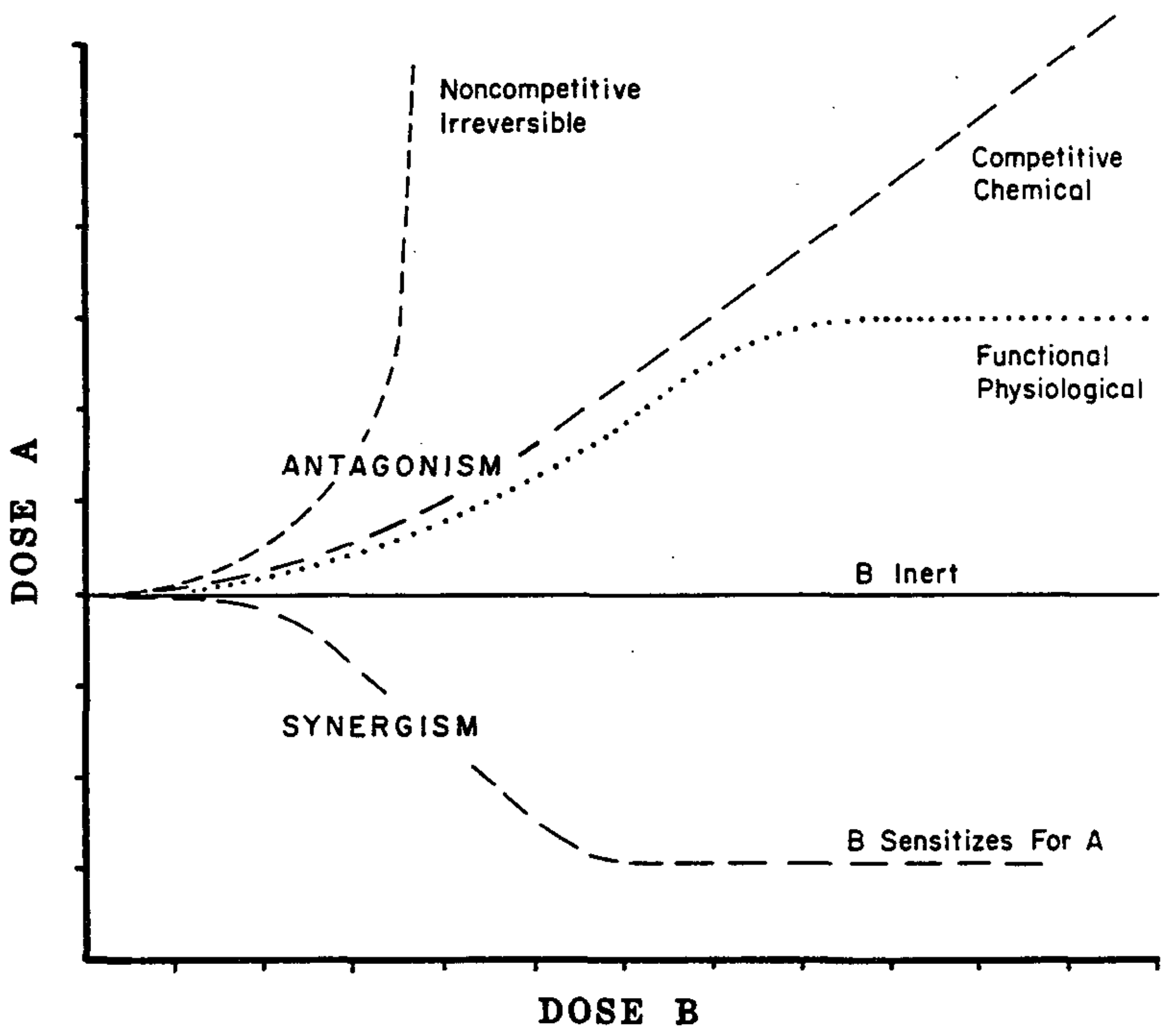

Figure 1. Isoboles (curves of equal biological response) for Substance A (active) Combined with Substance B (inactive alone, but influences A)

Source: Connell, Des W. and Gregory J. Miller. 1984. Chemistry and Ecotoxicology of Pollution. John Wiley & Sons, New York. 444 p.

3. Whether the observed response is valid for a wide range of concentrations and for different proportions of chemicals in a mixture.

4. Whether the threshold of action for independent components is affected and to what extent.

5. The minimum concentration or proportion of a chemical in a mixture that produces an effect in the presence of a given quantity of another chemical.

Concentration-Addition Model

There are two deterministic models commonly used for assessing the type of interaction in multiple toxicity studies: the concentration-addition and response-addition models. Additive joint effects of toxicant mixtures have qualitatively identical modes-of-actions for each toxicant, even though an effect of the same magnitude is produced by a different concentration of each. Bliss (1939) referred to this type of interaction as "similar-joint-effect" where the toxicants produce identical, but independent effects allowing one toxicant to be substituted at a constant proportion for the other. The toxicity of the mixture can be predicted directly from that of the constituents if their relative proportions are known (Bliss, 1939). Anderson and Weber (1975) renamed Bliss' empirical model the "concentration-addition" model.

In the concentration-addition model, the concentration of the individual mixture components is expressed as a proportion (p) of the respective median lethal concentration (LC50), approximate to either a threshold time (t) or fixed time exposure (e.g., 48 or 96 h) (Alabaster, 1981). Concentrations of mixtures can be expressed as the summation of the proportions of the median lethal concentration multiplied by the associated threshold or fixed time, e.g. ΣptLC50 or Σp48hLC50. The concentrations for other quantal responses and graded responses can be expressed in this manner as well (Alabaster, 1981). Sprague and Ramsay (1965) coined the term toxic unit (TU), which is used in the concentration-addition model. A toxic unit is equal to the concentration of pollutant which produces 50 percent mortality of test organisms within a specified time (usually 48 to 96 hours). The strength of a given toxicant expressed in toxic units is calculated as the proportion of its LC50 value. The joint action of a two-component mixture (A + B) can be expressed as $1\ TU_{(A\ +\ B)} = X\ TU_A + Y\ TU_B$ where $TU_{(A\ +\ B)}$ is the LC50 of the mixture multiplied by the specified time over which the mortality occurs. The coefficients X and Y represent the strengths of toxicants A and B, which are calculated as the proportion of their respective LC50 values. As summarized in Alabaster (1981), the joint action of the mixture can be defined as:

1. antagonistic if X or $Y \geq 1.0$
2. less than additive where $X < 1.0$ and $Y < 1.0$ and $X + Y > 1.0$
3. additive where $X + Y = 1.0$
4. more than additive when $X + Y < 1.0$

Marking has developed a similar additive toxicity index based on the empirical toxic unit method (EIFAC, 1980). Additive toxicity is indicated by the value of zero with synergism expressed by positive values and antagonism by negative values. The Marking Additive Index (MAI) is calculated with the following equation (Flickinger, 1984):

$$Am/Ai + Bm/Bi = S.$$

where Am, Bm = LC50 for A and B in the mixture

Ai, Bi = LC50 for A and B individually

S = sum of biological effects

If $S < 1.0$, then $MAI = (1/S) - 1.0$ and if $S > 1.0$,. $MAI = 1.0 - S$.

The empirical toxic unit method and the Marking Additive Index method have been subject to criticism. The predictive value of these indices has been limited by the difficulty of determining effective toxic concentrations with chemical analyses (Brown, 1968). Heavy metals are a good example, because a large proportion of the concentration determined to be present in polluted waters may be complexed or bound and unavailable for biological use. It should not be assumed that the sensitivity of a bioassay organism to a particular toxicant remains constant as evidenced by high inter- and intra-laboratory variability for acute toxicity tests using the same species (Flickinger, 1984). A priori it may be implausible to expect toxicants with different toxicological properties and concentration response curves to summate in the manners described above. The proportional toxicity of each toxicant comprising a mixture would perhaps be better determined from the actual percent mortality produced by the toxicant rather than by simply calculating the arithmetic fraction of the metal's LC50 value (Flickinger, 1984).

Different concentrations of the two components in a mixture can be described by the toxic unit method as illustrated in Figure 2. The theoretical relationship between dose and effect are shown graphically with isoboles of equivalent responses. If the fractions of doses sum to

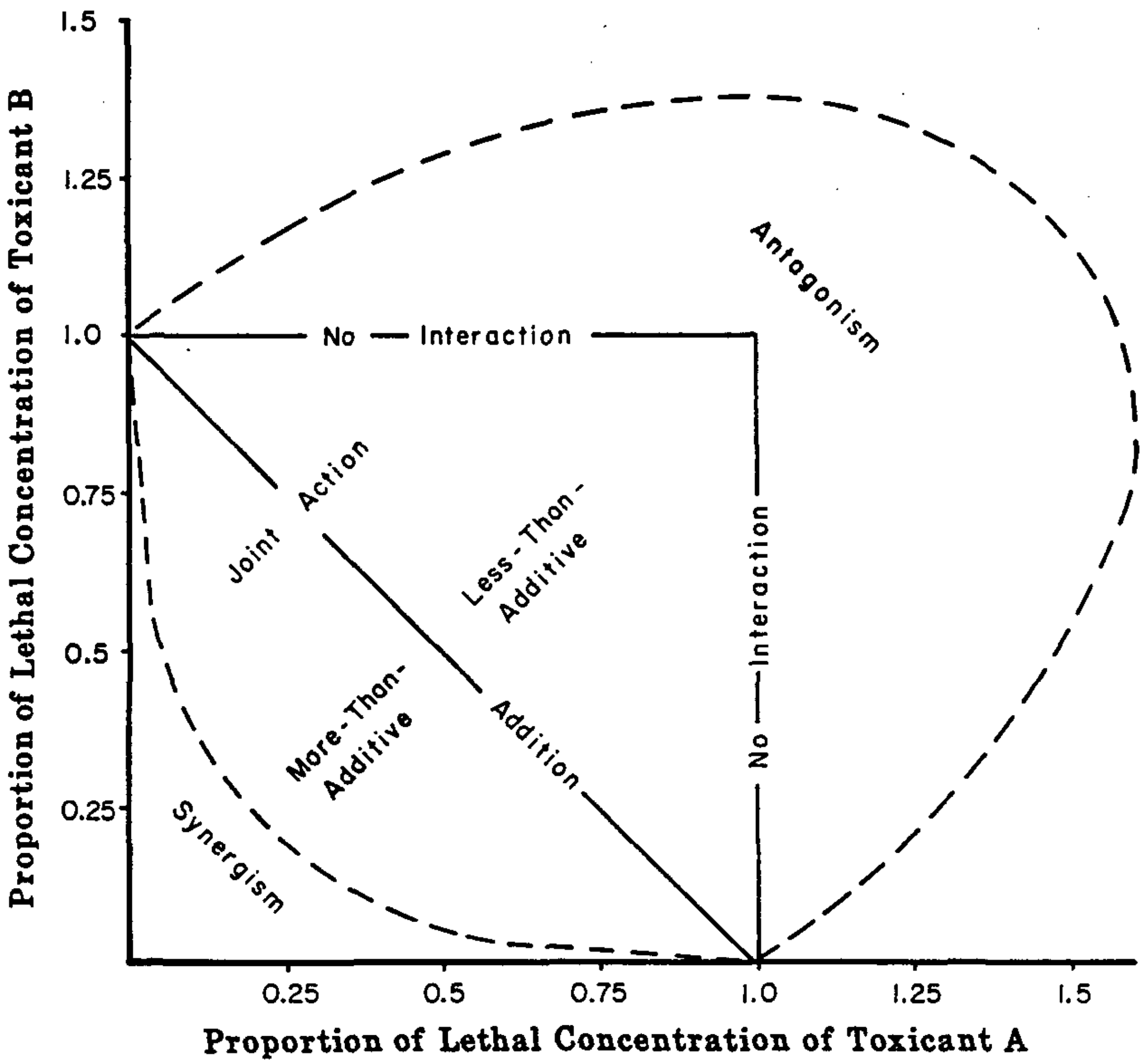

Figure 2. Types of Joint Actions Between Two Active Toxicants

Amended Source: Mason, C.F. 1981. Freshwater Pollution. Longman Group Limited, New York. 250 p.

unity (i.e., (concentration A in solution)/(LC50A) + (concentration B in solution)/(LC50B) = 1.0), and a 50 percent mortality is produced, the effect is additive and the response will fall on the diagonal line shown in Figure 2. Should the mortality occur at a sum of fractions greater than unity, the joint effect is antagonistic and the response falls above the diagonal line. Synergistic effects result when the mortality occurs with a sum of fractions less than unity. A diagram similar to Figure 2 can be drawn for any kind of quantitative response (i.e., LC90) and can be applied to qualitative (graded) responses as well (Calamari and Alabaster, 1980).

Response-Addition Model

The response-addition or independent-joint-action model is applied when toxicants act independently and have different modes of intoxication, but contribute to a common response (Bliss, 1939). The toxicity of the mixture to a given species depends on the correlation between the susceptibilities of the individual organisms to each constituent toxicant. The lowest response results when susceptibilities are positively correlated and the highest when susceptibilities are negatively correlated (i.e., acting independently). When an organism is exposed simultaneously to two independent toxicants, A and B, the organism may be killed by a lethal dose of either A or B or by a combination of both. To predict the proportion of organisms killed by the mixture, the extent to which the tolerance of the individual organism to toxicant A is correlated with its tolerance to toxicant B must be known. If there is complete negative correlation, all the organisms susceptible to A are tolerant to B and all organisms susceptible to B are tolerant to A.

Anderson and Weber (1975) have noted that a prerequisite for concentration-addition is parallelism between the response curves of the different toxicants in a mixture. This phenomenon cannot be relied upon, however, to distinguish concentration-addition from response-addition.

With mixtures containing more than two toxicants, the pattern of joint action between certain combinations of the respective constituents may differ. Anderson and Weber (1975) reported the lethal effects of copper and nickel in binary mixtures to be concentration-additive while those of dieldrin and pentachlorophenate in binary mixtures were response-additive. In a mixture containing all four toxicants, the copper and nickel were shown to remain concentration-additive between themselves, but interacted collectively as response-additive agents with the two organic constitutents.

The choice of the most suitable model for studying the joint effects of toxicant mixtures on test organisms is dependent on the type of information required. The response-addition model has been widely used in pesticide development where maximum lethality is the primary concern. The concentration-addition model is more pertinent for water pollution control studies and the application of water quality standards.

2.4 Experimental Design and Data Analysis

Difficulty in interpreting results reported in the literature, some of which are presented in Sections 3 and 4, has been encountered. This difficulty is due primarily to a lack of consistency in experimental design and to a deficiency of proper statistical analysis. The EIFAC Working Party report (1980) notes that two important aspects of data analysis often poorly handled by researchers are: the choice of appropriate transformations for dose and response data; and the testing of statistical significance of the differences between measured and predicted results. Sprague (1969) presents an excellent discussion of data analysis and error estimation.

Various transformations have been suggested for the units of dose and response, but in choosing those that appear to fit the observed data, additional relevant information about the mechanisms of action should not be ignored (EIFAC, 1980). The log-normal probability density function and corresponding probit analysis of dose-response curves is one of the most commonly used approaches to transformations. The logistic function, which was originally developed for population growth studies, is another frequently used function. The treatment based on this function, logit analysis, has gained wider acceptance recently. A third transformation analogous to the probit and logit transformations is the Weibull model. Analyzing dose-response curves from aquatic toxicity tests with macroorganisms and algae using both the Weibull and probit models showed the Weibull model to generally provide at least as good a fit to the experimental data as the probit model did. The Weibull model provides an especially useful basis for the treatment of multiple toxicity data (Christensen, 1984).

Several factors may affect the results of experiments designed to test the additivity of the effect of toxicants in a mixture. These factors include the type of response (longterm or shortterm, lethal or sub-lethal); the magnitude of response; and the type of and proportion between chemical constituents. Biological (age, size, acclimation, diet, sex) and environmental (water hardness, pH, etc.) variables also affect the experimental results.

Multi-factorial studies are used to assess the impact of factors influencing the toxicity of chemical mixtures. In theory, multi-factorial studies allow for the measurement of all joint interactions without the necessity of examining every possible combination. Voyer and Heltshe (1984) reviewed data sets from toxicity tests of metal mixtures on aquatic organisms and found that the assumption of factor interaction could not be accepted _a priori_. They recommended employing experimental designs such as the factorial requirement to directly evaluate factor interactions. With the factorial design, toxicant levels need to be lower than what would be expected to affect 50% of the organisms treated, otherwise, 100% mortality could occur in treatments with several toxicants and mask interactive effects that may be present (Voyer and Heltshe, 1984). If there is no evidence of interaction, standard testing procedures may then be employed.

The EIFAC Working Party paper (1980) makes reference to an experimental design which combines the multi-factorial approach with the quantal response method for binary mixtures used by Anderson and Weber (1975). This method assumes that any substance concentration can be expressed as an equi-effective concentration of another when the dose-response lines are non-parallel. The maximum attainable joint effect may be predicted at a particular ratio of the concentrations of the two components (EIFAC, 1980).

3. MULTIPLE TOXICITY STUDIES WITH ORGANISMS OTHER THAN DAPHNIDS

3.1 Fish

Abundant information derived primarily from laboratory experiments with supportive field data exists regarding the joint effects of various toxicant mixtures on fish. Several representative studies are indentified in Table 1. Roush et al. (1985) provides a comprehensive listing of references addressing the effects of pollutants on fish and other freshwater organisms. In fish, the extent that the joint effect may deviate from an additive effect depends upon several factors including the particular response measured; the magnitude of the response; the type of toxicant and its proportion to the mixture; water quality characteristics; the species; life cycle stage; prior toxicant acclimation; and the size of the dosage (Alabaster, 1981). Comparatively little attention has been given to the effect on joint toxicity of the relative toxic proportions of the chemicals present, which is considerable in some instances. It has been shown that the presence of one toxicant at concentrations below a certain proportion of the threshold LC50 may not contribute to the toxicity of the mixture (Alabaster, 1981).

Only a few experiments have been conducted on the longterm joint lethal toxicity of mixtures. Evidence for the interaction of sublethal concentrations of toxicants in mixtures is contradictory (Flickinger, 1984). Eaton (1973) demonstrated interaction, but not strict addition of chronic individual toxicant responses by fathead minnows exposed to a mixture of copper, cadmium, and zinc. Atlantic salmon exhibited an additive avoidance reaction to sublethal levels of copper and zinc. Cadmium and

Table 1. Multiple Toxicity Studies with Fish

Toxicant	Species	Exposure Period and Response	Joint Action	Reference
Ammonia + Cyanide	Rainbow Trout	96-h LC50	Additive	Broderius & Smith (1979)
Ammonia + Copper	Rainbow Trout	48-h LC50	Additive	Herbert & Vandyke (1964)
		LC25	Synergistic	
		LC10	Synergistic	
Ammonia + Zinc	Rainbow Trout	Threshold LC50	Additive	Herbert & Shurben (1964)
		Hard Water	Additive	
		Soft Water	Antagonistic	
Ammonia + Phenol + Zinc	Rainbow Trout	48-h LC50	Additive	Brown, Jordan, & Tiller (1969)
Phenol + Copper	Rainbow Trout	48-h LC50	Antagonistic	Brown & Dalton (1970)
Phenol + Copper + Zinc	Rainbow Trout	48-h LC50	Antagonistic	Brown & Dalton (1970)
Cyanide + Zinc	Fathead Minnow	96-h LC50	Synergistic	Broderius & Smith (1979)
Cyanide + Chromium	Fathead Minnow	96-h LC50	Antagonistic	Broderius & Smith (1979)
Copper + Zinc	Rainbow Trout	3-d LC50 (hard water)	Additive	Lloyd (1961)
		7-d LC50 (soft water)	Additive	
	Atlantic Salmon	7-d LC50 (soft water)	Additive	Sprague & Ramsay (1965)
	Longfin Dace	96-h LC50	Synergistic	Lewis (1978)
	Guppies	———	Synergistic	Flickinger (1984)
	Bluegill	96-h LC50	Additive	Thompson *et al*. (1980)
Copper + Zinc + Nickel	Rainbow Trout	48-h LC50	Additive	Brown & Dalton (1970)
Copper + Zinc + Cadmium	Fathead Minnow	96-h LC50 (Cu)	Synergistic	Eaton (1973)
		90% reduction in eggs	Synergistic	
		50% reduction in eggs	Antagonistic	
Copper + Manganese	Longfin Dace	96-h LC50	Antagonistic	Lewis (1978)
Copper + Surfactants (ABS + LAS)	Rainbow Trout	96-h LC50	Synergistic	Calamari & Marchetti (1970)
Copper + Surfactant (NP)	Rainbow Trout	96-h LC50	Antagonistic	Calamari & Marchetti (1970)
Mercury + Surfactant (LAS)	Rainbow Trout	96-h LC50	Additive	Calamari & Marchetti (1973)
Zinc + Nickel	Zebrafish	———	Synergistic	Flickinger (1984)
Selenium + Mercury	Carp eggs	% hatched	Synergistic	Pelletier (1985)
Cadmium + Chromium + Nickel	Rainbow Trout	6 months	———	Calamari *et al*. (1982)
Forest Insecticides	Rainbow Trout	96-h LC50	Additive and Synergistic	Marking & Mauck (1975)

zinc mixtures at these levels antagonistically affected the survival of flagfish (Flickinger, 1984). A limited number of studies with mixtures of toxicants indicate that the effect on the growth of fish is less additive than the corresponding effect on survival (Alabaster, 1981). One study showed that the joint effect of toxicants on both growth and production of fish was less than additive. It is possible that as concentrations of toxicants are reduced towards the "no observed effect concentration" (NOEC), their potential for addition is reduced (Alabaster, 1981). While there are few data available to directly compare the joint effect of a toxicant mixture on more than one species of fish, there exists no strong evidence for large inter-specific differences (Alabaster, 1981).

Due to the complexity of toxicity data and the difficulty encountered in interpreting results reported in the literature, the references cited here should be consulted for detailed information. The EIFAC Working Party report (1980) presents an excellent review of the results of laboratory and field studies with toxicant mixtures using fish as test organisms. Alabaster's paper (1981) provides a critical review of the EIFAC report (1980) and includes more recent data that have become available.

3.2 Aquatic Invertebrates

There is a very limited amount of data available on the joint effects of toxicant mixtures on aquatic invertebrate species (refer to Table 2). The results of studies conducted with daphnids are presented in the next section of this report. In general, the data for the joint effects of toxicant mixtures with constituents commonly found in sewage and industrial wastes show the concentration-addition model to be approximately

Table 2. Multiple Toxicity Studies with Invertebrates

Toxicant	Species	Exposure Period and Response	Joint Action	Reference
Copper + Zinc	Rotifer	Immobility	Additive	EIFAC (1980)
Zinc + Chromium	Rotifer	17-d No. remaining	Synergistic	EIFAC (1980)
Chromium + Chlorine	Rotifer	Immobility	Synergistic	EIFAC (1980)
Chromium + Fluoride	Rotifer	Immobility	Additive	EIFAC (1980)
Chromium + Copper	Rotifer	Immobility	Additive	EIFAC (1980)
Parathion + Herbicides	Mosquito	24-h % Mortality	Synergistic	EIFAC (1980)
DDT + Herbicides	Mosquito	24-h % Mortality	Additive	EIFAC (1980)
Nitrosalicylanilide + 4-nitro-3-trifluoromethyl Phenol	Ostracod	96-h LC50	Synergistic	EIFAC (1980)
Zinc + Cadmium	Freshwater Shrimp	96-h LC50	Antagonistic at concentrations below 1 TU. Additive at higher concentrations.	Thorp & Lake (1974
Copper + Mercury	Amphipod	96-h LC50	Antagonistic	Moulder (1980)

additive in invertebrate studies. This was also shown to be the case with fish (EIFAC, 1980). In studies with pesticide mixtures, the joint effects are generally more than additive (synergistic) with invertebrates.

A report by Borgmann et al. (1980) describes the chronic effects of toxicant mixtures on freshwater copepods. The effects of fourteen binary mixtures of cadmium, copper, mercury, lead, and arsenic on copepod growth and mortality rates were determined. There was a slight, but statistically significant synergism indicated by a drop in growth rate with the binary mixtures. The author concluded that the synergism was probably not biologically significant and could be accounted for by the composite of noninteractive, single metal effects. Daphnids and copepods differed much less in chronic toxicity effects than in acute toxicity results.

3.3 Plants and Bacteria

The joint toxicity data for aquatic plants and bacteria exhibit a variety of interactions. Alabaster (1981) reported that the data for aquatic plants generally show a slightly less than additive joint action with metals. With heavy metals, the results may vary according to the species tested. A specific metal-metal combination may be synergistic towards the growth of one species, but antagonistic towards the growth of another (Babich and Stotzky, 1983). The interaction between metals is also dependent on the relative concentrations of the toxicants and the sequence of exposure to the toxicants. The effect of the mixture of mercury and nickel on the cyanobacterium Anabaena inequalis was synergistic when both metals were added simultaneously or when the mercury was added first, but was antagonistic if nickel was added before mercury (Babich and

Stotzky, 1983). For pesticides, joint effects were inconsistent and unquantifiable (EIFAC, 1980).

Both the EIFAC paper (1980) and the article by Babich and Stotzky (1983) present excellent reviews of interaction studies with bacteria and aquatic plants. Table 3 contains highlights of some of the multiple toxicity data collected for plants and bacteria.

Many of the heavy metal interaction studies have been performed on algae with the type of interaction evaluated on the basis of effects on algal growth rates. Bartlett et al. (1974) studied the effects of copper, zinc, and cadmium on Selanastrum capricornutum. These three metals were reported to be synergistic in combination. Combinations of the copper and cadmium resulted in greater growth than equal concentrations of copper, which suggested that the cadmium inhibited the toxicity of the copper.

The Selanastrum capricornutum and Chlorella stigmatophora were examined for effects resulting from manganese, copper and lead mixtures by Christensen, Scherfig, and Dixon (1979). Combination experiments showed synergism between manganese and copper, antagonism between manganese and lead, and antagonism between copper and lead. Gotsis (1982) found selenium-mercury and selenium-copper mixtures to interact antagonistically in experiments with the alga Dunaliella minuta.

Using algae communities from three different natural sources, Wang (1985) determined the effect of iron and zinc on algal growth. With one sample, the iron and zinc interaction was antagonistic while with a second sample, the two metals were synergistic. No interaction was exhibited with the third community sample.

Longterm enclosure studies were conducted in Switzerland using mix-

Table 3. Multiple Toxicity Studies with Plants and Bacteria

Toxicant	Species	Joint Action	Reference
Manganese + Copper	Selanastrum capricornutum (alga) Chlorella stigmatophora (alga)	Synergism	Christensen et al. (1979)
Manganese + Lead	Selanastrum capricornutum (alga) Chlorella stigmatophora (alga)	Antagonism	Christensen et al. (1979)
Copper + Lead	Selanastrum capricornutum (alga) Chlorella stigmatophora (alga)	Antagonism	Christensen et al. (1979)
Copper + Cadmium	Selanastrum capricornutum (alga)	Antagonism	Bartlett et al. (1974)
Copper + Zinc	Klebsiella pneumoniae (bacterium)	Synergism	Babich & Stotzky (1983)
Selenium + Mercury	Dunaliella minuta (alga)	Antagonism	Gotsis (1982)
Selenium + Copper	Dunaliella minuta (alga)	Antagonism	Gotsis (1982)
Cadmium + Zinc	Lemna valdiviana (duckweed)	Synergism	EIFAC (1980)
Cadmium + Nickel	Physarum polycephalum (fungus)	Antagonism	Babich & Stotzky (1983)
Cadmium + Iron	Chlorella sp. (alga)	Antagonism	Babich & Stotzky (1983)
Nickel + Zinc	Anacystis nidulans (cyanobacterium)	Additive	Babich & Stotzky (1983)
Lead + Mercury	Cristigera sp. (protozoan)	Synergism	Babich & Stotzky (1983)
Zinc + Chromium	Klebsiella pneumoniae (bacterium)	Synergism	Babich & Stotzky (1983)

tures of cadmium, zinc, copper, mercury and lead at concentrations allowable for Switzerland's waters (Stokes, 1983). Depressed photosynthetic activity, reduction in species number and algal biomass, and changes in species composition were observed. Longterm changes including initial decreases in algae numbers followed by increasing density illustrate that caution must be exercised in extrapolating shortterm experimental results into generalities applicable to the field. The validity of existing water quality standards was evaluated in the study and the conclusion made that toxicant limits should be lowered.

A very limited number of joint toxicity studies have been performed with aquatic macrophytes. The growth of the duckweed Lemna valdiviana was inhibited in the presence of a mixture of zinc and cadmium (EIFAC, 1980). Jana and Choudhuri (1984) studied senescence in three submerged macrophytes (Hydrilla sp., Vallisneria sp., and Potamogeton sp.) caused by combinations of heavy metal pollutants. The senescence was exhibited by decreasing chlorophyll, RNA, DNA, protein, and dry weight, and increasing tissue permeability and free amino acids. Synergism between the heavy metal pollutants (mercuric chloride, lead acetate, cadmium chloride, and cupric sulfate) caused the degree of senescence to be much higher than with the individual toxicants.

4. MULTIPLE TOXICITY STUDIES WITH DAPHNIDS

There is a very limited amount of information available on the joint effects of toxicant mixtures on daphnids. Currently, an effort is being made by the U.S. E.P.A. and regulatory agencies in Europe to derive water quality criteria for combined pollutants recognizing that toxicants rarely occur singly in the natural environment. Research conducted to date on multiple toxicity has primarily employed fish as the test organisms. Only a limited number of investigations have used daphnids in multiple toxicity studies.

4.1 Heavy Metal Mixtures

Species richness and community biomass of crustacean zooplankton have been observed to be greatly reduced in metal-contaminated lakes near Sudbury, Ontario (Yan and Strus, 1980). The greatest reductions were observed in the lake with the highest metal levels. This example serves to illustrate that cladoceran zooplankton, which include the water fleas or daphnids, are severely impacted by heavy metal contamination. Much research has been performed on the acute and chronic toxicity of various metals to several species of Daphnia. Articles by Maki (1979), Biesinger and Christensen (1972), Baudouin and Scoppa (1974), Nebeker (1982), and Winner (1981, 1984) provide excellent references describing the acute and chronic effects of individual heavy metals on daphnids.

One of the first joint action studies on daphnids was performed by Biesinger, Andrew, and Arthur (1974) using metal-nitrilotriacetate (NTA) complexes to assess the impact of this compound on the aquatic environment.

Concentrations of NTA to be expected in the aquatic environment resulting from detergent usage would probably be less than 0.05 mg/l. Biesinger et al. (1974) performed chronic toxicity tests with three-week exposures to Daphnia magna using NTA alone and found the results, which varied tenfold, to be largely dependent on the chemical characteristics of the test water. There was a strong negative correlation between water hardness and NTA toxicity.

Another researcher had found that NTA chelated with copper and zinc greatly reduced the toxicity of these metals (Biesinger et al., 1974). The NTA was recommended as a possible antipollutant for accidental spills of copper and zinc.

Table 4 summarizes the toxicities of copper, zinc, and iron (III) as determined individually (Biesinger and Christensen, 1972) and in combination with NTA. The copper-NTA and zinc-NTA complexes were considerably less toxic than the metals alone. The toxicity of the iron (III)-NTA complex was only slightly decreased using a tenfold excess, by weight, of NTA.

These results suggest that copper and zinc were toxic only when they were present in molar excess nearly equal to or greater than the LC50 of the metals alone. NTA chelation did not significantly change the toxicity of iron. The differences in toxicity between iron and copper-zinc chelates may be related to the differences in valence of the metals. Based on their findings and those of other researchers, Biesinger et al. concluded that certain NTA-metal chelates would not be harmful to daphnids or other aquatic organisms.

Flickinger (1984) reported on research performed on the effects of

Table 4. Toxicity of Metals and Metal-Nitrilotriacetate Complexes to *Daphnia magna* per Biesinger *et al.* Study (1974)

Test Substance	Concentration of Metal Ions (mg/l)	
	Adult 3-wk LC50	50% Reproductive Impairment
Copper[1]	0.044	0.035
Copper + 2 mg/l Na_3NTA	0.26	0.26
Zinc[1]	0.158	0.115
Zinc + 2 mg/l Na_3NTA	0.48	0.45
Zinc + 8 mg/l Na_3NTA	1.7	1.6
Iron (III)[1]	5.9	5.2
Iron (III) + Na_3NTA (1:10 weight basis)	9.7	6.4

[1]Biesinger and Christensen, 1972.

binary mixtures of copper, cadmium, and chromium on _Daphnia magna_. The test organisms were exposed to metal concentrations selected on the basis of the percent mortality the metals induced rather than on fractions of their LC50 values. Using this experimental methodology, the resultant mortality following exposure to binary metal mixtures was consistent with the summation of the percent mortality produced by each metal. The Marking Additive Index failed to accurately predict the acute lethal toxicity of the mixtures.

Because Lake Michigan is being contaminated by metals from several sources, Marshall _et al_. (1981) studied the chronic toxicity of metal mixtures on resident zooplankton to provide a more realistic assessment of potential longterm effects. Their study aimed to assess the combined effects of cadmium and zinc on a zooplankton community using zinc to cadmium ratios prevalent in the lake. Observed effects were also evaluated in terms of the concentrations of cadmium and zinc sorbed by different particle size fractions. Two three-week experiments were performed _in situ_ with small enclosures in Lake Michigan. The daphnid species present were _Daphnia galeata mendotae_, _D. retrocurva_, and _D. longiremis_.

Combined additions of 2 μg/l cadmium plus 100 μg/l zinc and 1 μg/l cadmium plus 50 μg/l zinc caused significant reductions in the total crustacean density, species diversity, two community similarity indices (coefficient of community (CC) and percentage similarity (PS)), and final dis solved oxygen concentrations. Separate additions of 2 μg/l cadmium and 100 μg/l zinc were also observed to produce the same results. The effects of the 100 μg/l zinc were generally more pronounced than those of the 2 μg/l cadmium, but the responses of the zooplankton to cadmium and zinc were qualitatively similar. There were decreases in the numbers of the three daphnid species in response to the addition of the 100 μg/l zinc. This result was consistent with the reported reproductive impairment of *Daphnia magna* from additions of zinc with concentrations as low as 70 μg/l (Biesinger and Christensen, 1972). The effects of the separate addition of zinc are probably identical with those of cadmium addition at a zinc to cadmium molar ratio of 30:1 as suggested by the results obtained from adding the two metals individually.

The effects of the combined addition of 2 μg/l cadmium plus 100 μg/l zinc were similar to those obtained with zinc alone and were probably due mainly to the zinc. The two metals exhibited an antagonistic interaction when combined, producing effects that were less severe than would have been anticipated if their separate effects had been additive. The effects of the second zinc-cadmium mixture were also primarily due to the zinc.

Zinc probably governs the combined effects of cadmium and zinc, because it reduces the cadmium sorption by particle fractions. Increased concentrations of cadmium and zinc tended to increase sorption of both metals. Because the observed effects of combined mixtures of cadmium and zinc were regulated primarily by the zinc, the authors suggest that a less than ten-fold increase in Lake Michigan's zinc concentration, which is presently ~5 μg/l, could have negative impacts on the plankton community.

The effect of chelation on the short term toxicity of cadmium and copper to *Daphnia magna* was examined (Pommery *et al.*, 1984). The two metals were complexed with EDTA and dipotassium hydrogen phosphate (HK_2PO_4). The EDTA-complexed toxicity was found to be directly dependent on the concentration of the free metal. No such correlation was evident for the

HK_2PO_4 complexes, perhaps because of the labile nature of the substances formed.

Biesinger et al. (1986) conducted a study on the effects of toxic metal salt mixtures (chlorides of cadmium, mercury, and zinc) on the reproductive success of Daphnia magna. Three binary mixtures were tested with a complete block design at one-half, once, and twice the previously determined 16% reproductive impairment concentration for the individual metals (Biesinger and Christensen, 1972).

The binary mixtures for all combinations caused significant decreases in daphnid reproductive success at concentrations which did not have an impact on reproductive success when the metals were tested individually. Mercury did not affect reproductive success at a concentration of 3.5 µg/l nor cadmium at concentrations of 0.09, 0.19 and 0.35 µg/l. When mercury and cadmium were tested together at these concentrations, reproduction was significantly reduced.

In a mixture of cadmium and zinc, cadmium did not affect reproduction at all concentrations tested. By adding 74 µg/l of zinc to the cadmium with concentrations of 0.19 and 0.35 µg/l, significant decreases in reproduction were noted. Zinc reduced reproduction at a concentration of 140.3 µg/l alone and with all concentrations of cadmium and mercury tested. Zinc-mercury mixtures caused significantly less reproduction at a mercury concentration of 3.5 µg/l and zinc concentrations of 37.9 and 74.0 µg/l. Significant reproductive impairment was not observed when the two metals were tested individually.

As the results above indicate, mixtures composed of individual metals at "no effect" concentrations can still exert toxic effects in both chronic and acute tests. Water quality criteria and standards based on single toxicant results may be inadequate for protecting aquatic species in the presence of toxicant mixtures. The authors recommend the inclusion of provisions in water quality criteria which state that the toxicity of individual pollutants may be increased by the "safe level" presence of other pollutants.

An effort is being made by the E.P.A. to derive water quality criteria for combined pollutants. The objectives of studies performed by Spehar and Fiandt (1986) at the U.S. E.P.A. Duluth Research Lab were to determine: (1) whether the E.P.A.'s single-chemical water quality criteria were sufficient to protect aquatic organisms when mixtures of chemicals were present; and (2) the effects of mixtures at multiples of the LC50 and maximum acceptable toxicant concentration (MATC) using acute and chronic toxicity tests.

During acute toxicity testing, Spehar and Fiandt found that *Ceriodaphnia dubia* experienced nearly 100% mortality with arsenic, cadmium, chromium, copper, mercury, and lead combined at water quality criterion maximum concentrations. Production of young in the daphnids after 7 days in reconstituted water was significantly reduced following exposure to the above metals, combined at criterion average concentrations.

The acute tests were performed with the above-named metals mixed at multiples of the LC50 values. Joint action was additive in the acute tests with daphnids, based on toxic units calculated from individual toxicants in the mixture. Additive joint action was also exhibited in chronic tests. At mixture concentrations of one-half to one-third the MATC, production of young in the daphnids was significantly reduced.

Components of mixtures at or below no effect concentrations on an individual basis may contribute significantly to the chronic toxicity of a mixture.

Flickinger (1984) performed his doctoral research on the chronic toxicity of individual and bimetal mixtures of copper, cadmium, and zinc to Daphnia pulex (de Geer) in waters of differing hardness, alkalinity, and humic acid concentrations. The effects of the metals singly and in combination were measured by survival, the instantaneous rate of population growth, and reproductive indices. The reproductive indices included brood size, percent of eggs aborted per brood, age at maturity, and age at first reproduction. The accumulation of metals in mixtures by 7-day-old daphnids was also measured in medium water containing 0.0 and 0.75 mg humic acid/l.

The survivorship data indicated variable interaction between copper and zinc in soft water with 0.15 mg humic acid/l. The soft water had an average hardness and alkalinity of 52 and 54 mg/l as $CaCO_3$, respectively. The average hardness and alkalinity of the medium water was, respectively, 103 and 102 mg/l as $CaCO_3$. In medium water with 0.0 and 0.75 mg humic acid/l, the interaction between copper and zinc was largely independent based on survivorship. The toxicity of a mixture of copper and zinc could, therefore, be predicted from the toxicities of the individual metals. In accumulation experiments, the zinc and humic acid appeared to have no effect on copper accumulation in the medium water.

The interaction between cadmium and copper was synergistic in medium water containing 0.0 and 0.75 mg humic acid/l as evidenced by daphnid survival. The accumulation of cadmium in the medium water was enhanced by the copper and unaffected by humic acid. Cadmium had no observed effect on copper accumulation.

Cadmium and zinc exhibited significant synergistic toxicity in medium water as evidenced by survivorship data when a no-effect concentration of cadmium was combined with two different no-effect concentrations of zinc. Independent interaction between cadmium and zinc was evidenced by daphnid survival at effect levels of cadmium concentration combined with both effect and no-effect concentrations of zinc. In medium water containing 0.75 mg humic acid/l, an antagonistic interaction was observed when a no-effect level of cadmium was combined with an effect level of zinc concentration. Neither zinc nor humic acid affected the accumulation of cadmium.

4.2 Coal Conversion Effluents

To decrease modern society's dependence on oil, the increased use of coal is being promoted. Coal conversion technologies produce liquid, gaseous or solid fuels. These fuels contain less sulfur and produce less ash than coal, but the conversion processes produce large quantities of chemically complex solid and liquid wastes.

A systematic evaluation of the acute toxicity of the aqueous wastes from chemical conversion processes is described by Parkhurst *et al*. (1979). The following procedures were employed in the hazard evaluation:

1. Using standard analytical techniques, major chemical constituents of the effluent were analyzed.

2. The toxicity of each of the constituents was measured using a 48-hr LC50 test with *Daphnia magna*.

3. The 48-hr LC50 of the original effluent was also determined.

4. Using reagent grade chemicals, a synthetic effluent was produced, and its toxicity determined.

5. The contribution of the individual effluent constituents to the total effluent toxicity was determined with the following formula which assumes additivity between toxicants:

$$\text{toxic contribution of component A} = \frac{(C_i)/(LC50_i)}{\sum_{i=1}^{n} [(C_i)/(LC50_i)]}$$

where i = the particular component

C = the component concentration at the 48-h LC50 of the effluent

6. The presence of interactions between effluent constituents was determined using the Marking Additivity Index. If synergism or antagonism were indicated, the non-additive chemicals could be identified by comparing the chemicals' percentage concentration in the effluent to their calculated contribution to the effluent's toxicity. If significant differences were noted, the chemicals were assumed to exert a non-additive toxicity on the effluent toxicity.

7. A comparison was made between the toxicities of the original effluent and the synthetic effluent. If the acute toxicities of the two effluents differed significantly, steps one through seven were repeated.

Two effluents from a hydrocarbon (HCZ) coal conversion process at Oak Ridge National Laboratory were identified and evaluated using the procedure outlined above (Parkhurst et al., 1979). One of the effluents was untreated while the second had been treated by biological oxidation in a fluidized bed reactor. The major toxic constituent of the untreated effluent was phenol while ammonia was the principal toxic component of the treated effluent. The toxicity of the treated effluent was 1% of the untreated effluent's toxicity.

Acute toxicities of components of the untreated effluent were simply additive, but the component toxicities were less-than-additive in the treated effluent (Parkhurst et al., 1979). Ammonia appeared to be exhibiting an antagonistic toxicity, because it was present at 86 mg/l at the 48-hr LC50 dilution of 1.06 ml/liter in the treated effluent. The 48-hr LC50 of ammonia to Daphnia magna is 25 mg/l at pH 8.2, therefore, three times the concentration of ammonia necessary to kill 50% of the Daphnia was present in the treated effluent. The pH of the effluent was 7.3. This lower pH value may have reduced the toxicity of the ammonia.

Data obtained from systematically evaluating the toxicity of coal conversion wastes can be used to:

1. rapidly assess the effectiveness of treatment processes in reducing the acute toxicity of an effluent.

2. quickly identify and evaluate chemical interactions between effluent components. The manner in which effluents should be treated for toxicity reduction can be influenced by these interactions.

3. allow treatment plant operators at coal conversion operations to anticipate acute toxicity problems.

Considerable variation exists in the chemical composition of effluents from different coal conversion processes. The results obtained in the study by Parkhurst et al. (1979) may not be typical of all coal conversion effluents. The presence of variability underscores the need for using rapid biological and chemical techniques to assess the acute toxicity of effluent components.

Neufeld and Wallach (1984) examined the chemical composition and toxicity to Daphnia of leachates from a variety of coal conversion solid waste ashes and sludges. Values of the 48-hr LC50 data from Biesinger and Christensen (1972) for E.P.A. primary drinking water heavy metals were plotted against the corresponding primary and secondary drinking water standards on a log-log plot. A linear relationship was shown to exist between the published LC50 values for Daphnia magna and the drinking water standards. Because of this linear relationship, the authors suggest that toxicity measurements could perhaps be used as a regulatory tool for developing effluent discharge standards for specific leachates.

A linear log-log relationship was also found between the LC50 data and the weighted sum of metals data for the E.P.A. Extraction Procedure leachates generated from fly ash and bottom solid wastes. The weighting procedure for the metals consists of applying a linear equation for correlating toxicity with E.P.A. drinking water heavy metals. Synergistic effects were not considered in the weighting procedure as well as pH and non-toxic, yet chemically significant, species. Phenol concentrations for the leachates generated from lime and alum wastewater sludges also showed a linear log-log relationship with the LC50 data.

Phenols and arylamines are two major classes of contaminants found in coal liquefaction effluents, which are very difficult to remove from the effluents. Using mixtures of resorcinol and 6-methylquinoline to represent the two major contaminant classes, Herbes and Beauchamp (1977) investigated the compounds' potential toxic interaction in acute toxicity tests with Daphnia magna. Values for the 48-hr LC50 toxicities for several mixtures of the two compounds were determined by probit analyses. The concentrations of the two compounds in each LC50 mixture were calculated in terms of toxic units with one toxic unit defined as the 48-hr LC50 of each compound tested individually.

Herbes and Beauchamp (1977) found the mixtures of the two compounds to be less toxic than either compound tested alone. The data indicated a strong deviation from an additive interaction between the two compounds.

The results suggest that antagonism occurred when resorcinol was the major toxic component in the mixture while an intra-additive interaction resulted when 6-methylquinoline was the major toxic component. Intra-additive interaction is intermediate between non-interaction and strictly additive interaction. The authors recommended further experimentation to determine whether the observed pattern of toxic interaction was characteristic of organic bases and phenols in general.

4.3 Landfill Leachates

Atwater *et al.* (1983) examined the suitability of using daphnids to monitor municipal landfill leachate toxicity. Reproducible results were obtained with the daphnid bioassay procedures. Toxicity tests using *Daphnia pulex* compared favorably with parallel tests using the standard static 96-hr fish bioassay. The daphnid tests did not compare well with the residual oxygen bioassay (ROB), but this may have been due to problems with the ROB testing rather than to limitations with the daphnid tests.

Zinc was shown to play an important role in determining leachate toxicity to daphnids. Simple and multiple regressions of the daphnid toxicity data indicated that variations in zinc concentration could account for over 90% of observed variations in leachate effects on the daphnids. The zinc's presence and significance as a toxin to *Daphnia* may have masked other more subtle organism responses.

The effects of pH on daphnid survivorship were similar to those encountered with fish bioassay in that neutralizing acidic municipal landfill leachate reduced the apparent toxicity. The magnitude of these pH effects was somewhat reduced with the *Daphnia*, however. Advantages of total cost and test simplicity were realized with the daphnid bioassay test as compared to the standard fish bioassay procedure. No disadvantages were identified by the authors in using daphnids rather than fish as test organisms for landfill leachate toxicity studies.

Toxicity tests on landfill leachate were conducted with test organisms from different trophic levels (i.e., fathead minnow, zooplankton (*Daphnia magna*), green alga, and bacteria) by Plotkin and Ram (1984). Filtered leachate was tested with *D. magna*, since turbidity in the unfiltered leachate prevented confirmation of daphnid mortality. The 48-hr LC50 value was determined graphically to be equal to 62-66%. Based on these results, the leachate was considered to be moderately toxic to *D. magna*.

The concentrations of specific leachate compounds were compared with literature acute toxicity values for daphnids. The volatile organic and pesticide concentrations were several orders of magnitude below literature LC50 values. The silver concentration was 27 times greater than the 48-hr LC50 value reported for *Daphnia* in soft water (LeBlanc, 1980). The leachate mercury concentration was equal to a reported 48-hr LC50 value. The lead, cadmium, and manganese levels in the leachate were at 42%, 38%, and 37-81%, respectively, of the reported LC50 values for daphnids in soft water. Other metals present in the leachate were at levels at least one order of magnitude below the LC50 values for daphnids found in the literature. Based on hydrological data and toxicity test results, the leachate concentration in the receiving stream may reduce aquatic species diversity.

There was considerable variation between toxicity test results obtained using the four test species. The importance of conducting several toxicity tests with organisms from different trophic levels to assess the potential impact of a pollutant discharge on an aquatic ecosystem was shown with this study.

Morris and Buckley (1984) described a case study in Great Britain which employed the Daphnia bioassay test to monitor and control discharges of infiltration and surface drainage at a toxic waste landfill. The site runoff was stored in a lagoon and discharged to a nearby receiving water. Immobilization of any of the Daphnia during the 24-hr bioassay test with the runoff served to prevent discharge of the lagoon contents, necessitating treatment and alternative disposal. On one occasion, the bioassay test results were explained by a cell wall adjacent to the runoff lagoon fracturing and releasing leachate from the landfill.

The Daphnia bioassay test was described by the authors as being quick, simple, and effective in monitoring effluent discharges. Applications of the test recommended by the authors include: assessment of industrial discharges for impact on receiving water organisms and microorganisms in biological treatment operations; and monitoring of agricultural chemicals during normal use and accidental spills. The Daphnia bioassay test could be used to detect damage following spills of unknown toxic chemicals, since the results are provided promptly while chemical analyses may take days to complete. The authors further suggest that the use of the Daphnia bioassay test be included as a condition in effluent discharge permits.

4.4 Miscellaneous Mixtures

Sugatt et al. (1984) studied the toxicity of aqueous solutions saturated with mixtures of hydrophobic organic liquids in comparison to the toxicity of solutions prepared from solids. Toxicity was measured as the median lethal <u>time</u> (LT50) rather than as the more conventional median lethal concentration (LC50). The mixture of aqeuous solutions consisted of diethyl phthalate (DEP) and diisononyl phthalate (DINP).

The toxicities of compounds in liquid mixtures were influenced by the mixture composition. Neglecting biological interactions, the toxicity of a compound in a saturated liquid mixture tended to be lowered by the presence of other less toxic compounds.

Compound toxicities in solid mixtures were relatively independent of mixture composition. The toxicity of a saturated solution derived from a mixture of solids was shown to be equal to or to exceed that of a corresponding saturated solution of the mixture's most toxic component. As the complexity of the mixture increased, the toxicity increased. The authors state that the conclusions represent broad generalities and should be used with caution.

Canton <u>et</u> <u>al</u>. (1984) compared the joint effects of a mixture of 14 aquatic pollutants on mortality and inhibition of reproduction in *Daphnia magna*. These pollutants, which consisted of various chemical structures and different modes of action, included benzene, phenolic, and arylamine compounds, potassium dichromate, lindane, and malathion. Mortality was measured as 48-hr LC50 values and the reproduction inhibition was quantified as 16-day EC50 values.

The experimental results were evaluated using the Mixture Toxicity Index (MTI) proposed by Könemann (1981). The MTI is defined as:

$$MTI = 1 - (\log M / \log Mo)$$

where $M = \Sigma fi$ at 50% mortality

f = concentration of a compound in a mixture expressed as a fraction of the compound's LC50 value

$Mo = M/fmax$

$fmax$ = highest f-value in a mixture

The MTI values provide a classification scheme for the type of interaction in a mixture. This scheme is outlined as follows: MTI < 0 denotes antagonism; MTI = 0 indicates no interaction; MTI = 1 is concentration addition; and MTI > 1 represents supra-addition. The MTI is normalized for the number of chemicals in a mixture and their relative concentration, allowing comparison between various MTI values.

The MTI value associated with the 48-hr LC50 obtained by Canton _et al._ for the mixture was 0.95. This MTI value is almost equal to one, indicating that the acute mortality toxicity represents concentration addition. The authors explain that the relatively high joint toxicity to the _Daphnia magna_ results from a non-specific complex system of joint actions.

The MTI value determined for the reproduction inhibition experiment with _D. magna_ was 0.60, which indicates deviation from concentration addition. The reduced joint toxicity results from the reproduction inhibition being based on a number of specific modes of action by the compounds. These modes are simple similar or independent joint actions in comparison with the complex system associated with mortality. Independent actions generally result in a lower joint toxicity of mixtures. Even though the joint toxicity of the mixture at sublethal levels is lower than at lethal levels, the mixture toxicity is still higher than that of the individual chemicals and near concentration addition.

5. INTERACTIONS WITH WATER QUALITY PARAMETERS

The toxicity of pollutants can be altered by interaction with water quality parameters including pH, inorganic complexes, carbon dioxide, hardness, and organic ligands. A limited number of experiments have been conducted to assess the impact of these factors separately on the toxicity of heavy metals to daphnids.

Organic substances including natural compounds (sewage effluent, humic acids and amino acids) and synthetic chelators (EDTA and NTA) reduce the toxicity of metals to aquatic invertebrates. It has not been determined whether the reduced toxicity results from: 1. a reduction in the concentration of free metal with only the free metal being toxic or 2. the toxicity being caused by the complexed metal, with the complexed metal having a lower toxicity than the free metal. Borgmann (1983) found that the addition of amino acids increased the 48 hr. LC50's for D. magna with total copper, but decreased the free copper LC50 values. Winner (1985) collected data showing that humic acid significantly reduced both the acute and chronic toxicity of copper to D. pulex.

At constant pH and alkalinity, free metal concentrations are proportional to the concentration of various inorganic complexes, any of which may be toxic. The data collected to date seem to indicate that complexation by inorganic ligands (chloride, carbonate, and hydroxide) results in nontoxic complexes (Borgmann, 1983).

The effects of pH and hardness on metal toxicity are not well studied with invertebrates. Andrew et al. (1977) noted that increased pH resulted in an increase in the free copper toxicity to D. magna. Borgmann (1983) measured 24-hr. LC50 values for D. magna using copper and found that, on a total copper basis, toxicity was greatest at intermediate pH values. The free copper becomes more toxic at higher pH values with the effect being more pronounced above pH 7 (Borgmann, 1983). The toxicity of free zinc and free cadmium ions appears to increase with higher pH values as with free copper. Increased toxicity at higher pH values can be explained by the assumption that metal complexation, and consequently uptake by the organism, increases with pH through reduced competition of metal ions with hydrogen ions (Borgmann, 1983).

In general, the toxicity of metals to D. magna has been shown to increase with decreasing hardness (Bellavere and Gorbi, 1981). Winner (1985), however, found that hardness had little effect on either the acute (3-day) or the chronic (42-day) toxicity of copper to D. pulex. The acute toxicity of chromate, DDT, PCP, TPBS, and zinc to D. magna cultured in both hard and soft water was examined by Berglind and Dave (1984). The hardness of the culture water did not affect the toxicity of the PCP, TPBS, chromate, and zinc. However, the DDT was forty times more toxic in the soft as compared to the hard dilution water.

Because pH, water hardness, and complexation can influence the toxicity of free metal ions, examination of the effect of each of these factors must be performed under conditions where the other two variables are held constant. If this is not done, misinterpretation may result due to several factors cancelling (e.g. pH and complexation or pH and hardness) or reinforcing (e.g. hardness and complexation) one another. If all three factors are considered, toxicity appears to be most closely related to the free metal concentration (Borgmann, 1983).

Temperature is another factor which must be considered in toxicity tests. Elevated temperatures increase the toxicity of most pollutants to fish (Smith and Heath, 1979). McGinniss *et al*. (1977) found that the acute toxicity of two simulated effluents to *Daphnia* increased at higher acclimation temperatures. Stephenson and Watts (1984) examined the effects of different food and temperature regimes on the survival, reproduction, and growth of *D. magna* in chronic toxicity tests. Their results showed that temperatures of 20 or 23°C and a diet of *Chlorella* alone were best for meeting the EPA guidelines for a valid chronic toxicity test.

6. APPLICABILITY OF LABORATORY RESULTS TO FIELD CONDITIONS

Laboratory bioassays have been employed for obtaining the toxicological data used in establishing water quality criteria and for conducting aquatic impact assessments of chemicals. The need exists to confirm how well these data predict the toxicity of a chemical in the natural environment. Results may differ under actual field conditions, because of factors such as predation, competition for food and space, physical and chemical variables, and chemical interactions (Martin, 1973).

Laboratory toxicity test results can predict the responses of organisms under field conditions with varying degrees of accuracy. Only a limited number of field studies on toxicity have been conducted, since it is difficult to compile dose-response data in the field due to complex exposure patterns, multiple pollutants, and migration of organisms in and out of study areas (Chapman, 1983).

If factors that cause different dose responses in laboratory and natural organisms can be identified, their impacts can be determined in laboratory toxicity tests, thereby eliminating the need for field studies. External factors which have been identified include surrogate species, surrogate dilution water, and differing exposure regimes. A number of internal factors have also been recognized, including life stage, size, prior exposure, natural selection, nutrition, disease state, handling, and precision of toxicity tests (Chapman, 1983). Selection of the test species and dilution water can greatly influence the applicability of the laboratory data to field conditions.

Chapman's article provides a good review of literature addressing the applicability of laboratory data to field situations. The author identifies factors to which the organisms are more sensitive in the laboratory than in the field and vice versa. While the various factors most certainly interact, an overall variability term based on these factors cannot be computed, because a single phenomenon may be responsible for the observed response. Chronic response levels are probably less influenced by internal and external factors than acute response levels are. Nutrition, disease state, and behavioral factors are, however, more important in chronic tests than in acute tests.

The test species, dilution water, and relative nutritive state chosen for laboratory tests tend to be optimized. Chapman states that the use of these optimal variables has yielded laboratory responses which are more sensitive than field responses. Temperature, disease, variable exposure, and other stresses have probably led to more sensitive responses from field organisms, however.

The relative importance of factors influencing the responses of organisms in the laboratory and in the field is dependent on whether the goal of the laboratory tests is to determine relative toxicity, to estimate safe levels or to predict actual effects. If appropriate test parameters are chosen, the response of the laboratory organisms is a reasonable index of the response of the same species under natural conditions. According to Chapman, the apparent inaccuracy of laboratory toxicity test data is attributable not to an unnatural response of the laboratory test organism, but to extrapolation from a relatively narrow set of test conditions to a much broader range of environmental conditions.

Adams _et al_. (1983) conducted parallel field and laboratory toxicity tests using accepted laboratory methodologies for *D. magna* acute and chronic tests and fathead minnow acute and partial life-cycle tests. Commercial phosphate ester was the material tested. The field study was conducted in a simulated pond environment.

Good agreement was indicated between laboratory and field toxicity values for both acute and chronic data. The 48-h EC50 values for *D. magna* were: 343 μg/l for the laboratory test; 202 μg/l for filtered pond water; and 289 μg/l for unfiltered pond water. For the fathead minnows, the laboratory 96-h LC50 value equalled 3400 μg/l while the field 96-h LC50 values were greater than or equal to 647 μg/l (Adams *et al.*, 1983). The chronic MATC for *D. magna* in a laboratory flow-through test was determined to be 40-100 μg/l. Using filtered pond water in a chronic renewal test, the MATC results were 40-93 μg/l. Filtered and unfiltered pond waters produced chronic MATC values for caged daphnids of 60-136 μg/l and 60-226 μg/l respectively.

The authors concluded that laboratory acute and chronic toxicity tests with *D. magna* and fathead minnows provide realistic estimates of a chemical's toxicity to the same species in a simulated natural environment. The tests' results should provide reasonable estimates of toxic effects on populations in natural aquatic environments. The toxicity of a chemical in the natural environment can be predicted if the impact of the environment on the exposure concentration is understood.

Miller *et al.* (1986) studied a stream, which was the site of a former gold-mining operation. The purpose of the study was to explain why healthy biota were present when the national water quality criteria for toxicant metals were exceeded. Two hypotheses were offered to explain the presence of sensitive species under these conditions:

1. metals can be chelated by organic and inorganic compounds in effluents and receiving streams and, therefore, become biologically unavailable.

2. fish are able to acclimatize to sublethal metal concentrations thereby allowing them to tolerate potentially toxic ambient levels.

Toxicity tests were performed by Miller et al. with brook trout and the mayfly, Ephemerella grandis. Nonacclimated hatchery trout were more sensitive to metals than the resident trout. The hatchery trout were able to acclimate to the metals in the effluent after ten days so that they were no more sensitive to the metals than were the resident trout. Species used for toxicity tests should be chosen with care to determine site-specific metal criteria. Resident benthic macroinvertebrates may reflect changes in ambient metal levels with greater sensitivity than fish (Miller et al., 1986).

Effluent toxicity tests can be used to predict in-stream biological impacts from metal discharges. Sensitivity of the test species, seasonality, and variations in community structure response all must be taken into account. Annual and seasonal variations in community structure must be understood prior to establishing site-specific criteria. Seasonal variations in flow, temperature, rainfall, and melt which affect the interrelationships between physical/chemical environmental factors and the resident biota must be defined. With this knowledge, "critical seasons" may be established during which time the effluent releases may be modified (Miller et al., 1986).

Standard LC50 values do not predict "no effect" acute criteria values.

The absence of sensitive species in an aquatic ecosystem may stem from avoidance behavior to sublethal concentrations, because significant toxicological impacts can occur at concentrations much less than LC50 values. For this reason, safe effluent criteria or standards for acute toxicity should be based on the presence or absence of sensitive or desirable species in the receiving system rather than on standard LC50 determinations alone (Miller *et al.*, 1986).

Marshall (1979) studied the toxicity of cadmium to daphnids in the laboratory using Lake Michigan water and by conducting *in situ* toxicity tests in the epilimnion of Lake Michigan. The 48-h EC50 value determined using both the laboratory and natural populations was 40 µg/l cadmium. Because the initial toxicity effects were due primarily to increased mortality with most of the compensatory increase in reproduction occurring later, Marshall felt the EC50 values could be compared to the LC50 values reported for acute cadmium toxicity with other species of *Daphnia*. The 48-h EC50 value obtained compares well with the 48-h LC50 of 65 µg/l cadmium reported for *Daphnia magna* by Biesinger and Christensen (1972) and the 48-h LC50 of 55 µg/l obtained with *D. hyalina* by Baudouin and Scoppa (1974).

Lee and Jones (1983) examined the chemical environment as a major factor in the difference between laboratory toxicity tests and natural field conditions. As an example, the toxicity of copper as well as the toxicity of other heavy metals is best correlated with the concentration of the ionic form. Precipitated, sorbed, or complexed forms are usually less toxic than the free metal ion. The total concentration of a contaminant which is frequently measured for comparison with water quality standards may be a poor gauge of a contaminant's potential toxicity to aquatic life.

A hazard assessment approach was recommended by Lee and Jones to identify those field conditions for which worst-case laboratory bioassay results are not generally appropriate. Attention should be given to the contaminant characteristics and reactivity as well as to water quality characteristics at the site, especially turbidity. With this approach to water quality management, maximum utilization of available information on contaminant toxicity, bioaccumulation, and contaminant aquatic chemistry will be made.

7. CONCLUSIONS

1. To protect aquatic organisms against specific potential toxicants, national water quality criteria were formulated by the United States Environmental Protection Agency (U.S. E.P.A.) in 1984. These criteria were developed using toxicity data for single pollutants and multiple genera of aquatic life. The criteria do not address multiple pollutants, which occur in most discharges and natural waters.

2. Results of multiple-toxicant studies indicate that the water quality criteria may be modified through the influence of other toxicants present and water quality characteristics. The U.S. E.P.A. has made some allowance for toxicant mixtures by developing a whole effluent approach.

3. Further study is needed to determine the type and degree of interaction between toxicants on both an acute and a chronic basis and to differentiate the possible effects of water quality characteristics on these interactions. A research effort is currently underway by the E.P.A. to derive water quality criteria for combined pollutants. The European Inland Fisheries Advisory Commission has also been conducting research to determine the extent to which water quality standards assigned for single chemicals are valid when other toxicants are present.

4. Several different aquatic organisms have been used in toxicity tests with chemical mixtures. In acute toxicity tests of mixtures using fish and invertebrates, the concentration-addition model appears to be additive

for constituents commonly found in sewage and industrial wastes. Joint effects of pesticides are generally more-than-additive in tests with both organism groups. A variety of interactions are indicated in the joint toxicity data for aquatic plants and bacteria. Evidence for interaction of sublethal concentrations of toxicant mixtures is contradictory for all organism groups.

5. A limited number of joint toxicity studies have been performed with daphnids. Additive joint action in acute and chronic tests with heavy metals has been observed. Daphnids have proven to be good indicator species in assessing the toxicity of coal conversion effluents and landfill leachate to aquatic organisms. Results of chronic studies with daphnids suggest limitations in the single pollutant water quality criteria.

6. Difficulty is encountered in interpreting the results of many of the multiple toxicity studies. Reasons for this difficulty include: a lack of consistency in experimental design; deficiency of proper statistical analysis; and vague and contradictory nomenclature.

7. The toxicity of a mixture of pollutants can be modified by a number of physical and chemical factors including temperature, pH, dissolved oxygen, carbon dioxide, and hardness. The influence of hardness has been well documented. Toxicity is observed to decrease when hardness increases.

8. A need exists to determine how well toxicological data obtained from standard laboratory toxicity tests predict the toxicity of a pollutant

under natural conditions. Selection of test species and dilution water can greatly influence the applicability of laboratory data to different situations. The accuracy of predictions based on these data is influenced by nutrition, acclimation, and genetic selection of the test organisms. The impact of the environment on the toxicant exposure concentration must be understood to accurately predict the toxicity of a chemical in the natural setting. If appropriate test parameters are chosen, the response of the test organisms provides a reasonable index of the response exhibited by naturally occurring organisms.

8. REFERENCES

Adams, William J., Richard A. Kimerle, Barbara B. Heidolph, and Paul R. Michael. 1983. Field Comparison of Laboratory-derived Acute and Chronic Toxicity Data. In: Bishop, W.E., R.D. Cardwell, and B.B. Heidolph (eds.). Aquatic Toxicology and Hazard Assessment: Sixth Symposium. ASTM Special Publication 802. American Society for Testing and Materials, Philadelphia: 367-385.

Alabaster, John S. 1981. Joint Action of Mixtures of Toxicants on Aquatic Organisms. Chemistry and Industry. 15:529-534.

Anderson, P.D., and L.J. Weber. 1975. The Toxicity to Aquatic Populations of Mixtures Containing Certain Heavy Metals. In: Proceedings of the International Conference on Heavy Metals in the Environment, Canada, October 27-31. Toronto, University of Toronto, Institute of Environmental Studies: 933-953.

Andrew, R.W., K.E. Biesinger, and G.E. Glass. 1977. Effects of Inorganic Complexing on the Toxicity of Copper to Daphnia magna. Water Research. 11(3):309-315.

Atwater, James W., Susan Jasper, Donald W. Mavinic, and Fred A. Koch. 1983. Experiments Using Daphnia to Measure Landfill Leachate Toxicity. Water Research. 17(12):1855-1861.

Babich, H. and G. Stotzky. 1983. Influence of Chemical Speciation on the Toxicity of Heavy Metals to the Microbiota. In: Nriagu, Jerome O. (ed.). Aquatic Toxicology. John Wiley & Sons, New York: 1-46.

Bartlett, Larry, Fred W. Rabe, and William H. Funk. 1974. Effects of Copper, Zinc and Cadmium on Selanastrum capricornutum. Water Research. 8:179-185.

Baudouin, M.F. and P. Scoppa. 1974. Acute Toxicity of Various Metals to Freshwater Zooplankton. Bulletin of Environmental Contamination & Toxicology. 12(6):745-751.

Bellavere, C. and J. Gorbi. 1981. A Comparative Analysis of Acute Toxicity of Chromium, Copper and Cadmium to Daphnia magna, Biomphalaria glabrata, and Brachydanio rerio. Environmental Technology Letters. 2(3):119-128.

Berglind, Rune and Göran Dave. 1984. Toxicity of Chromate, DDT, PCP, TPBS, and Zinc to Daphnia magna Cultured in Hard and Soft Water. Bulletin of Environmental Contamination & Toxicology. 33(1):63-68.

Biesinger, Kenneth E. and Glenn E. Christensen. 1972. Effects of Various Metals on Survival, Growth, Reproduction, and Metabolism of Daphnia magna. Journal of the Fisheries Research Board of Canada. 29:1691-1700.

Biesinger, K.E., R.W. Andrew, and J.W. Arthur. 1974. Chronic Toxicity of NTA (nitrilotriacetate) and Metal-NTA Complexes to Daphnia magna. Journal of the Fisheries Research Board of Canada. 31(4):486-490.

Biesinger, K.E., G.M. Christensen, and J.T. Fiandt. 1986. Effects of Metal Salt Mixtures on Daphnia magna Reproduction. Ecotoxicology and Environmental Safety. 11(1):9-14.

Bliss, C.I. 1939. The Toxicity of Poisons Applied Jointly. Annals of Applied Biology. 26:585-615.

Borgmann, Uwe. 1983. Metal Speciation and Toxicity of Free Metal Ions to Aquatic Biota. In: Nriagu, Jerome O. (ed.). Aquatic Toxicology. John Wiley & Sons, New York: 47-72.

Borgmann, U., R. Cove, and C. Loveridge. 1980. Effects of Metals on the Biomass Production Kinetics of Freshwater Copepods. Canadian Journal of Fisheries and Aquatic Sciences. 37(4):567-575.

Broderius, Steven J. and Lloyd I. Smith, Jr. 1979. Lethal and Sublethal Effects of Binary Mixtures of Cyanide and Hexavalent Chromium, Zinc, or Ammonia to the Fathead Minnow (Pimephales promelas) and Rainbow Trout (Salmo gairdneri). Journal of the Fisheries Research Board of Canada. 36:164-172.

Brown, V.M. 1968. The Calculation of the Acute Toxicity of Mixtures of Poisons to Rainbow Trout. Water Research. 2:723-733.

Brown, V.M., D.H.M. Jordan, and B.A. Tiller. 1969. The Acute Toxicity to Rainbow Trout of Fluctuating Concentrations and Mixtures of Ammonia, Phenol and Zinc. Journal of Fish Biology. 1:1-9.

Brown, V.M. and R.A. Dalton. 1970. The Acute Lethal Toxicity to Rainbow Trout of Mixtures of Copper, Phenol, Zinc and Nickel. Journal of Fish Biology. 2:211-216.

Calamari, D. and R. Marchetti. 1970. L'analisi tossicologica nel controllo delle acque: interazioni fra inquinanti. Ig. Mod. 63:455-471.

Calamari, Davide and Roberto Marchetti. 1973. The Toxicity of Mixtures of Metals and Surfactants to Rainbow Trout (Salmo gairdneri Rich.). Water Research. 7:1453-1464.

Calamari, D. and J.S. Alabaster. 1980. An Approach to Theoretical Models in Evaluating the Effects of Mixtures of Toxicants in the Aquatic Environment. Chemosphere. 9:533-538.

Calamari, D., G.F. Gaggino, and G. Pacchetti. 1982. Toxicokinetics of Low Levels of Cd, Cr, Ni and Their Mixture in Long-term Treatment on Salmo gairdneri Rich. Chemosphere. 11(1):59-70.

Canton, H., J. Hermens, R. Wegman, and N. Steyger. 1984. Joint Effects of a Mixture of 14 Chemicals on Mortality and Inhibition of Reproduction in *Daphnia magna*. Aquatic Toxicology. 5(4):315-322.

Chapman, Gary A. 1983. Do Organisms in Laboratory Toxicity Tests Respond Like Organisms in Nature? In: Bishop, W.E., R.D. Cardwell, and B.B. Heidolph (eds.). *Aquatic Toxicology and Hazard Assessment: Sixth Symposium. ASTM Special Publication 802*. American Society for Testing and Materials, Philadelphia: 315-327.

Christensen, Erik R. 1984. Dose-Response Functions in Aquatic Toxicity Testing and the Weibull Model. Water Research. 18(2):213-221.

Christensen, E.R., J. Scherfig, and P.S. Dixon. 1979. Effects of Manganese, Copper, and Lead on *Selenastrum capricornutum* and *Chlorella stigmatophora*. Water Research. 13(1):79-92.

Connell, Des W. and Gregory J. Miller. 1984. *Chemistry and Ecotoxicology of Pollution*. John Wiley & Sons, New York. 444 p.

Doherty, Francis G. 1983. Interspecies Correlations of Acute Aquatic Median Lethal Concentration for Four Standard Testing Species. Environmental Science and Technology. 17(11):661-665.

Eaton, John G. 1973. Chronic Toxicity of a Copper, Cadmium and Zinc Mixture to the Fathead Minnow (*Pimephales promelas* Rafinesque). Water Research. 7:1723-1736.

EIFAC, European Inland Fisheries Advisory Commission Working Party. 1980. *Water Quality Criteria for European Freshwater Fish. EIFAC Technical Paper No. 37*. Food and Agriculture Organization of the United Nations, Rome. 49 p.

Flickinger, Allen Lane. 1984. Chronic Toxicity of Mixtures of Copper, Cadmium and Zinc to *Daphnia pulex*. Ph.D. Thesis. Miami University. Oxford, Ohio. 135 p.

Gotsis, O. 1982. Combined Effects of Selenium/Mercury and Selenium/Copper on the Cell Population of the Alga *Dunaliella minuta*. Marine Biology. 71:217-222.

Herbert, D.W.M. and D.S. Shurben. 1964. The Toxicity to Fish of Mixtures of Poisons. I. Salts of Ammonia and Zinc. Annals of Applied Biology. 53:33-41.

Herbert, D.W.M. and Jennifer M. Vandyke. 1964. The Toxicity to Fish of Mixtures of Poisons. II. Copper-ammonia and Zinc-phenol Mixtures. Annals of Applied Biology. 53:415-421.

Herbes, S.E. and J.J. Beauchamp. 1977. Toxic Interaction of Mixtures of Two Coal Conversion Effluent Compounds (Resorcinol and 6-Methylquinoline) to Daphnia magna. Bulletin of Environmental Contamination & Toxicology. 17(1):25-32.

Jana, Sasadhar and Monojit A. Choudhuri. 1984. Synergistic Effects of Heavy Metal Pollutants on Senescence in Submerged Aquatic Plants. Water, Air, and Soil Pollution. 21:351-357.

Könemann, H. 1981. Fish Toxicity Tests with Mixtures of More than Two Chemicals: A Proposal for a Quantitative Approach and Experimental Results. Toxicology. 19:229-238.

LeBlanc, Gerald A. 1980. Acute Toxicity of Priority Pollutants to Water Flea (Daphnia magna). Bulletin of Environmental Contamination & Toxicology. 24:684-691.

Lee, G. Fred and R. Anne Jones. 1983. Translation of Laboratory Results to Field Conditions: The Role of Aquatic Chemistry in Assessing Toxicity. In: Bishop, W.E., R.D. Cardwell, and B.B. Heidolph (eds.). Aquatic Toxicology and Hazard Assessment: Sixth Symposium. ASTM Special Publication 802. American Society for Testing and Materials, Philadelphia: 328-349.

Leeuwangh, P. 1978. Toxicity Tests with Daphnids: Its Application in the Management of Water Quality. Hydrobiologia. 59(2):145-148.

Lewis, M. 1978. Acute Toxicity of Copper, Zinc and Manganese in Single and Mixed Salt Solutions to Juvenile Longfin Dace, Agosia chrysogaster. Journal of Fish Biology. 13:695-700.

Lloyd, R. 1961. The Toxicity of Mixtures of Zinc and Copper Sulphates to Rainbow Trout (Salmo gairdnerii Richardson). Annals of Applied Biology. 49:535-538.

Lloyd, R. 1986. The Toxicity of Mixtures of Chemicals to Fish: An Overview of European Laboratory and Field Experience. In: Bergman, Harold L., Richard A. Kimerle, and Alan W. Maki (eds.). Environmental Hazard Assessment of Effluents. Pergamon Press, New York: 42-53.

Maki, A.W. 1979. Correlations between Daphnia magna and Fathead Minnow (Pimephales promelas) Chronic Toxicity Values for Several Classes of Test Substances. Journal of the Fisheries Research Board of Canada. 36:411-421.

Marking, Leif L. and Wilbur L. Mauck. 1975. Toxicity of Paired Mixtures of Candidate Forest Insecticides to Rainbow Trout. Bulletin of Environmental Contamination & Toxicology. 13(5):518-523.

Marshall, J.S. 1979. Cadmium Toxicity to Laboratory and Field Populations of Daphnia galeata mendotae. Bulletin of Environmental Contamination & Toxicology. 21:453-457.

Marshall, J.S., D.L. Mellinger, and J.I. Parker. 1981. Combined Effects of Cadmium and Zinc on a Lake Michigan Zooplankton Community. Journal of Great Lakes Research. 7(3):215-223.

Martin, Donald M. 1973. Contributions from the Department of Limnology. Number 3. Freshwater Laboratory Bioassays--A Tool in Environmental Decisions. Academy of Natural Sciences of Philadelphia, Philadelphia. 51 p.

Mason, C.F. 1981. Biology of Freshwater Pollution. Longman Group Limited, New York. 250 p.

McGinniss, Matthew J., Arthur L. Buikema, Jr., and John Cairns, Jr. 1977. Temperature Effects on the Acute Toxicity of Two Simulated Effluents to Daphnia pulex (Cladocera). Association of Southeastern Biologists Bulletin. 24(2):68-69.

Miller, Theron G., Susan L. Melancon, and Thomas W. LaPoint. 1986. Use of Effluent Toxicity Tests in Predicting the Effect of Metals on Receiving Stream Invertebrate Communities. In: Bergman, Harold L., Richard A. Kimerle, and Alan W. Maki (eds.). Environmental Hazard Assessment of Effluents. Pergamon Press, New York: 265-281.

Morris, G.M. and F.T. Buckley. 1984. The Role of the Daphnia Bioassay in the Assessment of the Quality of Effluent Discharges. Water Pollution Control. 83(4):539-546.

Moulder, Shelagh M. 1980. Combined Effect of the Chlorides of Mercury and Copper in Sea Water on the Euryhaline Amphipod Gammarus duebeni. Marine Biology. 59:193-200.

Nebeker, A.V. 1982. Evaluation of a Daphnia magna Renewal Life-Cycle Test Method with Silver and Endosulfan. Water Research. 16:739-744.

Neufeld, Ronald D. and Steven Wallach. 1984. Chemical and Toxicity Analysis of Leachates from Coal Conversion Solid Wastes. Journal of the Water Pollution Control Federation. 56(3):266-273.

Parkhurst, B.R., A.S. Bradshaw, J.L. Forte, and G.P. Wright. 1979. An Evaluation of the Acute Toxicity to Aquatic Biota of a Coal Conversion Effluent and Its Major Components. Bulletin of Environmental Contamination & Toxicology. 23:349-356.

Pelletier, Emilien. 1985. Mercury-Selenium Interactions in Aquatic Organisms: A Review. Marine Environmental Research. 18(2):111-132.

Plotkin, Stephen and Neil M. Ram. 1984. Multiple Bioassays to Assess the Toxicity of a Sanitary Landfill Leachate. Archives of Environmental Contamination and Toxicology. 13(2):197-206.

Pommery, J., M. Imbenotte, and F. Erb. 1984. Incidence de la Complexation sur la Toxicite du Cadmium et du Cuivre vis a vis de Daphnia magna. Environmental Technology Letters. 5(3):109-120.

Roush, Thomas H., Gary L. Phipps, Diane L. Spehar, Charles E. Stephan, and Quentin H. Pickering. 1985. Effects of Pollution on Freshwater Organisms. Journal of the Water Pollution Control Federation. 57(6): 667-699.

Smith, Micke J. and Alan G. Heath. 1979. Acute Toxicity of Copper, Chromate, Zinc and Cyanide to Freshwater Fish: Effect of Different Temperatures. Bulletin of Environmental Contamination and Toxicology. 22(1-2):113-119.

Spehar, Robert L. and James T. Fiandt. 1986. Acute and Chronic Effects of Water Quality Criteria-based Metal Mixtures on Three Aquatic Species. Environmental Toxicology and Chemistry. 5:917-931.

Sprague, J.B. 1969. Review Paper. Measurement of Pollutant Toxicity to Fish. I. Bioassay Methods for Acute Toxicity. Water Research. 3:793-821.

Sprague, J.B. and B. Ann Ramsay. 1965. Lethal Levels of Mixed Copper-Zinc Solutions for Juvenile Salmon. Journal of the Fisheries Research Board of Canada. 22(2):425-432.

Stephenson, R.R. and S.A. Watts. 1984. Chronic Toxicity Tests with Daphnia magna: The Effects of Different Food and Temperature Regimes on Survival, Reproduction and Growth. Environmental Pollution (Series A). 36(2):95-107.

Stokes, P.M. 1983. Responses of Freshwater Algae to Metals. In: Round, F.E. and D.J. Chapman (eds.). Progress in Phycological Research. Volume 2. Elsevier Science Publishers, New York: 87-112.

Sugatt, Richard H., Dean P. O'Grady, and Sujit Banerjee. 1984. Toxicity of Organic Mixtures Saturated in Water to Daphnia magna. Effect of Compositional Changes. Chemosphere. 13(1):11-18.

Thompson, Kenneth W., Albert C. Hendricks, and John Cairns, Jr. 1980. Acute Toxicity of Zinc and Copper Singly and in Combination to the Bluegill (Lepomis macrochirus). Bulletin of Environmental Contamination & Toxicology. 25:122-129.

Thorp, V.J. and P.S. Lake. 1974. Toxicity Bioassays of Cadmium on Selected Freshwater Invertebrates and the Interaction of Cadmium and Zinc on the Freshwater Shrimp, Paratya tasmaniensis Riek. Australian Journal of Marine and Freshwater Research. 25:97-104.

Voyer, R.A. and J.F. Heltshe. 1984. Factor Interactions and Aquatic Toxicity Testing. Water Research. 18(4):441-447.

Wang, Wuncheng. 1985. Effect of Iron and Zinc Interaction on Algal Communities. In: Cardwell, Rick D., Rich Purdy and Rita Comotto Bahner (eds.). Aquatic Toxicology and Hazard Assessment: Seventh Symposium. ASTM Special Technical Publication 854. American Society for Testing and Materials, Philadelphia: 187-201.

Winner, Robert W. 1981. A Comparison of Body Length, Brood Size and Longevity as Indices of Chronic Copper and Zinc Stresses in Daphnia magna. Environmental Pollution (Series A). 26:33-37.

Winner, Robert W. 1984. Selenium Effects on Antennal Integrity and Chronic Copper Toxicity in Daphnia pulex (deGeer). Bulletin of Environmental Contamination & Toxicology. 33(5):605-611.

Winner, Robert W. 1985. Bioaccumulation and Toxicity of Copper as Affected by Interactions Between Humic Acid and Water Hardness. Water Research. 19(4):449-455.

Yan, N.D. and R. Strus. 1980. Crustacean Zooplankton Communities of Acidic, Metal-Contaminated Lakes near Sudbury, Ontario. Canadian Journal of Fisheries and Aquatic Sciences. 37(12):2282-2293.