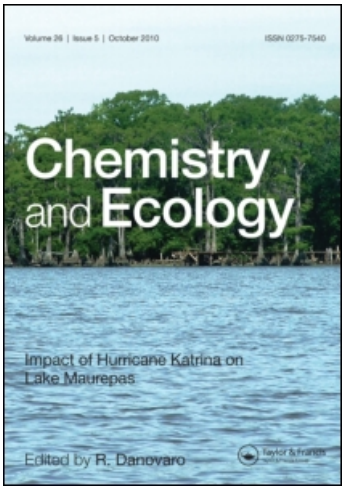

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

Water Quality Criteria for European Freshwater Fish

J. S. Alabaster[a]; D. Calamari[a]; V. Dethlefsen[a]; H. Konemann[a]; R. Lloyd[a]; J. F. Solbé[a]
[a] EIFAC Working Party on Water Quality Criteria for European Freshwater Fish,

To cite this Article Alabaster, J. S. , Calamari, D. , Dethlefsen, V. , Konemann, H. , Lloyd, R. and Solbé, J. F.(1988) 'Water Quality Criteria for European Freshwater Fish', Chemistry and Ecology, 3: 3, 165 — 253
To link to this Article: DOI: 10.1080/02757548808070844
URL: http://dx.doi.org/10.1080/02757548808070844

Chemistry and Ecology, 1988, Vol. 3, pp. 165–253
Reprints available directly from the publisher.
Photocopying permitted by license only

Printed in the United Kingdom

Water Quality Criteria for European Freshwater Fish†

J. S. ALABASTER, D. CALAMARI, V. DETHLEFSEN, H. KONEMANN, R. LLOYD and J. F. SOLBÉ

EIFAC Working Party on Water Quality Criteria for European Freshwater Fish

(Received July 1, 1987)

1. For water pollution control purposes, the concentration-addition model for describing the joint effects of mixtures of toxicants on aquatic organisms is appropriate; in this model the contribution of each component in the mixture is expressed as a proportion of the aqueous concentration producing a given response in a given time (e.g. *p* 96-h LC50).

2. Examination of available data using this model shows that for mixtures of toxicants found in sewage and industrial effluents, the joint acutely-lethal toxicity to fish and other aquatic organisms is close to that predicted, assuming simple addition of the proportional contribution from each toxicant. The observed median value for the joint effect of these toxicants on fish is 0.95 of that predicted, and the corresponding collective value for sewage effluents, river waters, and a few industrial wastes, based on the toxicity of their constituents, is 0.85, while that for pesticides is 1.3.

3. The less-than-predicted effect of commonly-occurring toxicants in some mixtures may be partly attributable to small fractions of their respective LC50 values having a less-than-additional effect. However, recent research has shown that for some organic chemicals which have a common quantitative structure-activity relationship (QSAR), their joint action as determined by acute toxicity is additive at all concentrations.

4. The few (unpublished) data available for the long-term lethal joint effect on fish of toxicants in mixtures suggest that they may be markedly more than additive, a phenomenon that needs confirmation and further investigation.

5. In the few studies on the sub-lethal effects on fish (eg growth), the joint effect of toxicants has been consistently less-than-additive which suggests that as concentrations of toxicants are reduced towards the levels of no effect, their potential for

† This review was prepared as a working party report for the European Inland Fisheries Advisory Commission (EIFAC), and issued as EIFAC Technical Paper 37 Rev. 1; it is reprinted here with some minor modifications with permission from Food and Agriculture Organisation, Rome.

addition is also reduced. There appear to be no marked and consistent differences between the response of different species to mixtures of toxicants.

6. Field studies have shown that reasonably accurate toxicity predictions based on chemical analysis can be made if the waters which are polluted are acutely lethal to fish, and that a fish population of some kind can exist where the median $\sum pt$ LC50s (rainbow trout) is <0.2. It is not known whether this condition is equivalent to a $\sum p$ NOEC of <1.0 (ie the sum of the individual fractions of the NOEC for the species present), or to a NOEC of <1.0 for each individual toxicant (i.e. fractions of the NOEC are not summed).

7. In general, the joint effect of the common toxicants on lethal and sub-lethal responses of fish is not explained by variations in the uptake of the individual toxicants concerned; this may not apply for those chemicals with a common QSAR, although there is little experimental evidence in this field.

8. There is an immediate need for more empirical studies on the joint effect of mixtures of toxic units of individual components, and the relation between long- and short-term lethal and non-lethal joint effects. This applies to mixtures of commonly-occurring toxicants as well as to mixtures of organic chemicals with a common QSAR. The data obtained should be reinforced by studies on the mechanisms of interaction of toxicants. More field studies which relate water quality to the structure and productivity of fish populations are also required, involving direct measurements of fractional toxicity of the river water wherever possible.

9. The concentration-addition model appears to be adequate to describe the joint effect of commonly-occurring constituents of sewage and industrial wastes, and for tentative predictions of the joint effect on fish populations of toxicants present at concentrations higher than the EIFAC recommended values. However, concentrations lower than the EIFAC recommended values may make an increasingly lesser contribution to the toxicity of mixtures of toxicants and there may be a need to adjust the tentative water quality criteria downwards where two or more toxicants are present at concentrations close to these values. For toxicants with a common QSAR, their additive joint action may necessitate the setting of water quality criteria for this group as a whole and not on the basis of individual compounds. However, too little is known of their precise joint action where the combined concentration produces a sub-lethal response.

KEY WORDS Pollution; Mixtures; Fish; Aquatic life; Water quality standards.

INTRODUCTION

Information available on the toxicity of substances to fish and other aquatic organisms often relates to materials tested singly under laboratory conditions, or considered separately in field studies, yet it is uncommon to find a river or lake which is polluted by a single toxicant, and usually several harmful substances are present together in significant quantities in a polluted water. The presence of other pollutants in the water may modify the corresponding water quality so that standards can be set for the protection of fisheries,

by giving attention to data on mixtures with other toxicants. The main purpose of the present review is to collate the information available to us on the effect of such mixtures on fish.

When a potentially toxic substance is present in water, several processes may be involved before an aquatic organism shows a response. In the aquatic phase, the substance may interact with other constituents or conditions in the water; for example, pH will affect the dissociation of acids and alkalis, and humic acids will form complexes with some heavy metals, particularly copper. Where such processes are inadequately understood or even unknown, and more than one potentially toxic substance is present, they may have an overriding effect on the subsequent response of the organism and lead to erroneous conclusions about the type of joint action of the substances present. It is essential, therefore, that the biological availability of the toxicant concentrations in a mixture is known before firm conclusions are reached on their combined action.

Secondly, within the organism, physiological processes, including absorption (mainly through the gills, gut and skin), transport and distribution by the circulatory system, metabolic transformation, accumulation at various sites, and excretion, may all influence the degree of toxicity of a substance and its metabolites present in the body fluids, tissues and organs and thus the quantity available at the site or sites of action. These processes may also be affected by other water quality characteristics of the ambient environment of the organism that are not necessarily harmful in themselves.

Thirdly, where an organism is exposed to two or more potentially toxic substances, interaction between different physiological processes within the organism may occur, including (see Sjoquist and Alexanderson, 1972) those affecting the chemicals' absorption, binding to plasma proteins, distribution, transport and release from tissues, action on receptor sites, metabolism and elimination, all of which may contribute to the response of the whole organism, such as death, growth, avoidance behaviour and accumulation of chemicals in tissues and organs.

Attention to interactions at receptor sites within tissues has centred on the assumption that occupation of these sites by chemicals is regulated by the law of mass action and depends on the concentrations there. This concept has been elaborated by considering several possibilities, including the effect of two biologically

active substances, of a compound active if applied singly, and another inactive alone but modifying the response to the first, and of two inactive compounds acting only in combination. Further elaboration arises from considering such concepts as common receptors and common sites of effects, different sites of action and a common target system, and different receptors and common sites of effects. These approaches have been developed mainly in the fields of pharmacology and pesticide research and as yet there has been little attempt to apply such fundamental concepts to the action of mixtures of common pollutants on fish and other aquatic life.

Nevertheless, the problem of mixtures of poison was recognised by a few early workers who carried out appropriate tests with fish and found it possible to account for the harmful effect of a mixture of chemically similar poisons in water by a summation of their individual toxic fractions (e.g. Southgate, 1932; Bucksteeg *et al.*, 1955; Cherkinsky, 1957; Friedland and Rubleva, 1958); on the other hand, some experiments indicated that more-than-additive effects (or 'synergism') could occur, as with mixtures of some heavy metals (Bandt, 1946; Doudoroff, 1952). This latter finding has been commonly quoted to support the setting of water quality standards for pollutants considerably more stringent than those indicated as reasonable on the basis of tests and observations made on the effects of single substances. In the last 25 years, however, a considerable amount of more detailed and longer-term work has been carried out on mixtures of toxicants on fish and to a lesser extent on other aquatic organisms. These have often involved rigorous statistical test designs and treatment of data, in order to develop theoretical and empirical models; such approaches are necessarily pragmatic and do not invoke a fundamental consideration of the problem at the biochemical level as outlined above. This material is critically reviewed here to examine the extent to which the effect of mixtures of toxicants on fish and other aquatic organisms can be described, modelled and predicted, and to identify areas where future research should be concentrated.

MODELS AND TERMINOLOGY

In dealing with the joint effects on organisms of two or more toxicants, it is necessary in the context of water pollution control to

determine the extent to which the measured response is the result of additive action, and also the range and limits of concentrations and proportions of the toxicants in a mixture which produce the measured effect. Various authors have proposed methods of modelling and data analysis to describe the types of combined effect that occur. As a result, the terminology has become confused, despite several revisions and reviews (for example, Ariens, 1972; Fedeli *et al.*, 1972).

A fundamental approach to the terminology and classification was made by Bliss (1939), and this was elaborated in a number of publications by Plackett and Hewlett (1948, 1952, 1963, 1967) and Hewlett and Plackett (1950, 1959, 1964), who defined four types of joint action with respect to quantal responses (Plackett and Hewlett, 1952).

	Similar joint action	Dissimilar joint action
Interaction absent	*Simple similar action*	*Independent action*
Interaction present	*Complex similar action*	*Dependent action*

A joint action is defined as similar or dissimilar depending on whether the sites of primary action of two chemicals are the same or different, and as interactive or non-interactive depending on whether one chemical does or does not influence the biological action of the other. In their publications, Hewlett and Plackett developed mathematical models to describe the resulting dose-response curves for mixtures; further studies have been made by Finney (1971), Ashford and Cobby (1974), Ashford (1981) and Christensen and Chen (1985). In aquatic toxicity research the approach developed by Plackett and Hewlett has been followed by Anderson and Weber (1976) and Muska and Weber (1977).

When applying this classification to mixtures of more than two chemicals, problems can arise because the different pairings can fall into different classes of joint action, and other joint actions are possible between different pairs. Therefore, a mathematical description of the joint toxicity of a mixture of n compounds ($n > 2$) is possible only in a few cases, for which the absence of interaction seems to be a prerequisite. The models discussed here apply to the classes of simple similar action and independent action, and can be

applied to mixtures of two or more chemicals. The symbols used are listed in Table I with the addition of those used by Konemann (1981a) and others.

Simple similar action or concentration-addition model (Anderson and Weber, 1975a)

In theory, the simplest explanation of an additive joint effect of toxicants in a mixture is that the mode of action of each toxicant is qualitatively identical, even though a common effect is produced by a different concentration of each. Bliss (1939) described this mode of physiological interaction as a similar joint action; his method for examining this in studies of whole organisms using quantal response regressions has been applied to the results of several mixtures and their constituents, e.g. by Anderson and Weber (1975a). They applied the term 'concentration-addition' to denote this empirical model.

If the effective concentration of each toxicant is taken as unity, then the effective concentration of a mixture will be obtained when the sum of each toxicant concentration, expressed as the fractions of the effective concentration of each toxicant, equals unity. This model of additive joint action has been used to evaluate the joint effects of mixtures using, for example, the proportions of the median lethal concentration (f_i) for which Sprague and Ramsay (1965) coined the term 'Toxic Unit', and their summation (M). This approach has also been used for median threshold lethal concentrations, 48-h LC50s (Table I), for other quantal responses, such as LC10 and EC90, and for graded responses.

In mathematical terms the occurrence of concentration addition is characterised by $M = 1$. For a mixture of n chemicals with identical f_i values (hereafter called equitoxic mixture) giving 50% mortality, the concentrations of the separate chemicals will be $LC50_i/n$. Knowledge of the concentration response curves is not strictly necessary, although several authors consider this as a prerequisite for determining whether the mixture displays simple similar action.

Konemann (1981a) has developed the use of quantitative structure-activity relationships (QSARs) to support evidence of simple similar action. Such QSARs can be determined, for example, within a homologous series of organic chemicals where the

TABLE I
Symbols used in this paper

Individual compounds	
c_i	the concentration of substance i
f_i	$c_i/\text{LC50}_i$
p 48-h LC50	$c_i/48\text{-h LC50}_i$
pt LC50	$c_i/\text{threshold LC50}_i$; the threshold concentration is lethal within an extended exposure period to 50 percent of the organisms tested (used particularly alongside data for short exposure periods)
TU	Toxic Unit; equivalent to f_i and more particularly to pt LC50
s_i	the standard deviation in $\log \text{LC50}_i$ (a measure of reproducibility)
Q_i	the probability of survival of an organism exposed to f_i
Mixtures	
n	the number of compounds in the mixture
M	$\sum_{i=1}^{n} f_i$, when the standard response is produced
$\sum pt$ LC50	equivalent to M, when $f_i = c_i/\text{threshold LC50}$. Similarly, $\sum p$ 48-h LC50 is equivalent to M when $f_i = c_i/48\text{-h LC50}$, and Toxic Units equals $\sum_{i=1}^{n} \text{TU}$
f_{max}	the largest f_i value in the mixture of n compounds
M_0	M/f_{max}
$\hat{M}$	the expected value of M in a certain model

Note: both the notations of Sprague (1970) and Konemann (1981a) have been used.

toxicity in terms of LC50s for the individual compounds is positively correlated with physico-chemical properties such as the octanol:water partition coefficient. A detailed examination of the value and limitations of this approach can be found in Kaiser (1984).

If a group of chemicals is shown to possess a strong QSAR, it is likely that they would have a simple similar action in a mixture. This assumption has been used successfully by Konemann (1981a), Hermens *et al.* (1984a, b, c; 1985a, b, c) and Broderius and Kahl (1985) in selecting chemicals for experiments to test models of simple similar action.

Response-addition (Anderson and Weber, 1975a) *or independent-joint-action* (Bliss, 1939) *model*

In theory, another and more complicated joint effect of toxicants in a mixture is found when each constituent acts on a different physiological or biochemical system but contributes to a common

response; several authors, for example, Plackett and Hewlett (1948, 1952) and Finney (1971), have approached this problem quantitatively. The expected proportion of individuals responding to a mixture depends upon the correlation between the susceptibilities of the individual organisms to each toxic constituent; the lowest response occurs where susceptibilities are positively correlated and the highest where they are negatively correlated, i.e. acting independently. The assumption is that, since the response is log-normally distributed for each component, it can be expected to be a log-normal bivariate for the joint action of the two (Plackett and Hewlett, 1948). If an organism is exposed simultaneously to two poisons A and B, which act independently, it can be killed by a lethal dose of either A or B, or by a combination of both. But in order to predict the proportion of organisms expected to be killed by the mixture, it is necessary to know to what extent the tolerance of the organism to A is correlated with its tolerance to B. If there is complete negative correlation, all the organisms susceptible to A are tolerant to B and *vice versa*; the mixture would then be more effective than if A and B were completely or partially positively correlated. The model has been applied to the results with several mixtures, for example, by Anderson and Weber (1975a; 1976) and Broderius and Smith (1979).

When a third chemical is added, the correlation with the tolerances for the first 2 chemicals cannot be negative in both cases if the tolerances for the first 2 are wholly negatively correlated. Therefore, the concept of negative correlation has no application for mixtures of many chemicals. For these mixtures the correlation coefficient r can vary from 0 to +1. We will discuss here the 2 extreme cases.

Independent action, $r = 0$

When the tolerances are not correlated ($r = 0$) the probability of survival at exposure to a mixture Q_{mix} can be obtained by multiplying the Q_i values for each of the separate compounds of the mixture (Finney, 1971):

$$Q_{mix} = Q_1 \cdot Q_2 \cdot Q_3 \cdots Q_n \qquad (1)$$

For a mixture of 10 compounds, with identical Q_i-values, the Q_i-value to produce the standard response, $Q_{mix} = 0.5$, is 0.93, and

for 100 compounds, 0.993 (this agrees with 0.7% mortality on exposure to the separate chemicals). Assuming the log-probit plot of the separate chemicals to be linear, and supposing a slope of all log-probit plots (as defined by Litchfield and Wilcoxon, 1949) of 1.2, for a mixture of 100 chemicals, $0.6 \times LC50$ of each of the separate chemicals would be necessary to produce 50% mortality of organisms exposed to the mixture. At this level, however, concentration-response curves are usually not very reliable. Therefore, response addition with $r = 0$ cannot be used to predict the toxicity of large mixtures with a reasonable accuracy.

Independent action, $r = +1$

When tolerances are correlated completely positively ($r = +1$), Q_{mix} is equal to the smallest Q_i-value, so $Q_{mix} = 0.5$ when $f_{max} = 1$. The total strength of a mixture at that condition, M, is M_0, as defined in Table I. For equitoxic mixtures of n chemicals $M_0 = n$. This model will be called 'no addition' and can be used without knowledge of the concentration-response curves.

One comment must be made about the concept of independent action. In the words of Plackett and Hewlett (1967): "... thus dependent action ... is more likely to occur in reality than independent action, because an organism is at least largely a coordinated whole. Nevertheless, independent action, if approximated only occasionally in reality, is important theoretically, for it leads to a limiting mathematical model that appears to be an essential step towards the construction of others more commonly realistic".

Anderson and Weber (1975a) point out that parallelism between response curves for different compounds in a mixture may be a prerequisite for concentration-addition, but cannot be relied upon to distinguish it from response-addition.

Anderson and d'Apollonia (1978) proposed that the common response to a response-additive mixture is never greater than that predicted on the basis of the potency for threshold or above-threshold concentrations of each constituent in the mixture. Therefore, in theory, water quality criteria which set 'safe' (sub-threshold) levels for individual response-additive substances should protect organisms against their combined effects in mixtures.

Classification of mixture potency

The following classification is based on that for a mixture of two substances (EIFAC Tec. Pap. No. 37). Data from toxicity tests with substances A and B, and their mixture (A + B) are used to solve the following equation.

$$x\,TU_A + y\,TU_B = 1\,TU_{(A+B)}.$$

	Values found for x and y	*Classification of mixture potency*
1	x or $y > 1.0$	Antagonism
2	x and $y < 1.0$ and $x + y > 1.0$	Less than additive, or infra-additive interaction (Warren, 1971; Anderson and Weber, 1975a)
3	$x + y = 1.0$	Additive
4	$x + y < 1.0$	More than additive, or supra-additive synergism or supra-additive interaction (Anderson and Weber, 1975a)

New information on multiple mixtures of substances with similar joint action, as well as those with dissimilar joint action, has been included in the following model developed by Konemann (1981b). It has advantages for mixtures in which the substances have a wide range of f_i values because a dominant toxicant in a non-equitoxic mixture is identified.

	Values found for M, M_0 and f_{max}	*Classification of mixture potency*
1	$M > M_0, f_{max} > 1$	Antagonism
2a	$M = M_0, f_{max} = 1$	No addition
2b	$M_0 > M > 1, f_{max} < 1$	Partial addition
3	$M = 1, f_{max} < 1$	Addition
4	$M < 1, f_{max} < 1$	Supra addition

Mixture toxicity scales and indexes

Numerical scales of mixture potency have been developed by

several authors. Some of the scales using Toxic Units are shown in Figure 1.

For two-component mixtures the scale of units corresponds to that on the ordinate in Figure 1. Such deviations from unity are not necessarily the same for all combinations of a particular two-component mixture of toxicants, as found, for example, by Bianucci and Legnani (1973), Herbes and Beauchamp (1977) and Sprague and Logan (1979), and illustrated in Figure 2 by isopleths of equal observed toxicity (Loewe, 1953 and Gaddum, 1953) for two hypothetical examples of a mixture of two toxicants. The area above and to the right of such a curve represents the conditions in which the expected mortality would be greater than 50%, whereas the area below and to the left represents that in which it would be less than 50%. In example 1, joint action is always less than additive, but in a mixture containing 0.5 toxic units of A and 1.5 toxic units of B (the sum of observed/predicted toxicity being 2) the particular mixture is half as toxic as expected from strictly additive joint action indicating a marked antagonism. In example 2, joint action between the toxicants is slightly more than additive at most proportions of their respective toxic units, the greatest effect

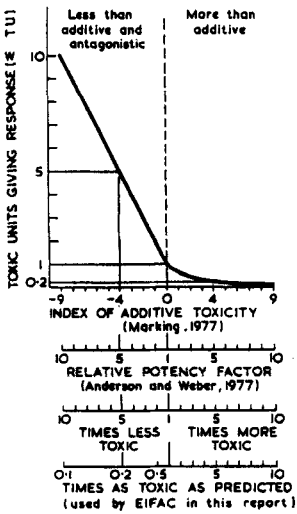

FIGURE 1 Relation between different terminology used in studies of mixtures of toxicants.

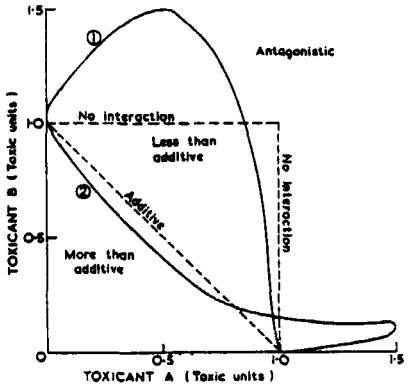

FIGURE 2 Types of joint action between two toxicants in a mixture—illustrated by lines of equal observed toxicity for two hypothetical examples.

(1.11 times more toxic than expected) being in a mixture of 0.4 toxic units of toxicant A and 0.5 toxic units of toxicant B; however the mixture containing 1.5 toxic units of A and 0.1 units of B shows marked antagonistic joint action between toxicants.

Although toxicants may be additive in terms of a threshold median lethal concentration when present in a mixture, the shorter periods of survival at high, rapidly lethal, concentrations may suggest a greater than additive effect. This has sometimes been described as 'synergism', and has been found, for example, in the case of mixtures of copper and zinc (Lloyd, 1961; Sprague and Ramsay, 1965) and copper and detergent (Calamari and Marchetti, 1970).

Ideally, a scale for the toxicity of a mixture should meet the following requirements (Konemann, 1981):

(a) it should provide constant values for the two reference points—'no addition' and 'addition'—independently of the number of compounds in the mixture and the ratio between the concentrations in terms of the f_i or TU;
(b) it should have a logarithmic form because of the log-normal distribution of toxic concentrations with reference to the toxic response.

None of the above scales meet these two requirements; Kone-

mann (1981b) proposed the following Mixture Toxicity Index (MTI) which overcomes some of the problems of devising a widely applicable scale.

$$\text{MTI} = \frac{\log M_0 - \log M}{\log M_0} = 1 - \frac{\log M}{\log M_0}.$$

This cannot be displayed graphically because of the incorporation of the variable f_{max} into the Index. However, the scale associated with the various possibilities of joint action is as follows:

Antagonism	*No addition*	*Partial addition*	*Addition*	*Supra addition*
Negative value	0	>0 to <1	1	>1

It should be noted that this Index indirectly takes into account the number of substances in the mixture, especially when these are present at low, equitoxic, concentrations. In such cases, for example, the scale of M values representing the range from simple addition to no addition is from 1 to 20 for an equitoxic mixture of 20 substances, whereas it is from 1 to 2 for an equitoxic mixture of two substances. Thus an equitoxic twenty component mixture having a M value of 1.5 ($f_{max} = 0.075$) has an MTI of 0.86, whereas an equitoxic two component mixture having a similar M value has an MTI of 0.41. This Index also takes into account the effect of a dominant toxic substance (i.e. a large f_{max}) in mixtures of unequal toxicity.

EXPERIMENTAL DESIGN AND DATA ANALYSIS

Difficulty in interpreting the results reported in this literature survey has often been due to lack of consistency in experimental design and lack of proper statistical analysis. Brown (1978) has discussed standard requirements for dose-effect studies in both these areas. For example, two particularly important aspects of data analysis, not always dealt with adequately by experimentalists, are the choice of appropriate transformations for dose and response, and the testing of statistical significance of differences between observed and predicted results.

Various transformations have been suggested for the units of dose and response, but Finney (1964) recommends using those that appear to fit the observed data, without ignoring additional relevant information about the mechanisms of action. Brown (1978) compared the dose-response relations of three models (normal probability, logistic (sic) and sine) and while there was little difference between them over nearly three orders of magnitude of dose, extrapolations to doses three order of magnitude lower than the median produced wide divergences.

There is also a need for appropriate statistical tests for determining significant deviations from unity in a concentration-additive index. For example, one method includes in its computations the standard deviation at the median response (e.g. LC50), without consideration of the possible differences in slope of the dose-response lines of the individual constituents (Abt *et al.*, 1972). Thus, any conclusion about joint action at the median level may not apply to the full response range unless there is parallelism between the dose-response relationships. Hodson *et al.* (1977) give special attention to the need for replicate tests.

In order to classify the toxicity of a mixture in accordance with the terminology proposed above, it is necessary to test the statistical difference between the measured toxic response of organisms exposed to a mixture of substances and the predicted toxic response based on concentration addition or response addition. An approach to this statistical analysis has been proposed by Konemann (1981a). In making a comparison between observed and predicted responses, two sources of error have to be taken into account: the error in the experimental data obtained for the mixture (M) and the error in the expected response ($\hat{M}$) derived from errors in the toxicity values (f_i) obtained for the single chemicals.

Deviation from the two reference points of the MTI (no addition and addition) can be tested statistically when the standard deviation, s_i (which is used to indicate the 68% confidence limits of log LC50), is known. The standard deviation, s_i, which is important in this context, cannot be deduced from a single LC50 measurement, but only from the reproducibility of the LC50 (Finney, 1971). In the following sections, the value of s_i is assumed to be constant for all LC50s in order to simplify the discussion, but obviously different values may have to be used in practice.

No addition (the expected result for independent action with $r = +1$):
This is possible when $\hat{M}$ does not differ significantly from M_0, that is, where $\log f_{max}$ is within the 68% confidence limits of M_0.

Concentration addition (the expected result for simple similar action):
In theory, $\hat{M}$ cannot be calculated by simply adding f_i values because the distribution of f_i is log normal. The value of $\hat{M}$ is therefore not unity but $10^{1.15s^2}$ (Diem and Lentner, 1968). However, when s is in the region of 0.1 (a usual value for determinations made within one laboratory), this deviation from unity can be neglected, and the error in $\hat{M}$ can be treated as if the distribution was normal. The error in f_i approximates to $f_i \cdot 10^s$, so that for $\hat{M} = \pm 10^s \sqrt{\sum f_i^2}$. The value of $\sqrt{\sum f_i^2}$ decreases as n increases, and is minimal for equitoxic mixtures; at a certain value of n, the error in $\hat{M} = 1.0 \pm (1/\sqrt{n}) \cdot 10^s$. Thus, when $n \geq 10$ in an equitoxic mixture, the error in $\hat{M}$ is negligible compared to the error in M.

When the number of compounds in a mixture is small, or when some occur in relatively high concentrations, it is possible for the confidence limits of these two models to overlap. However, even when an experiment provides data which fit one or other of the models, it cannot be assumed without further information that the chemicals in the mixture act in simple similar or independent action with $r = +1$. It is possible that a qualitatively heterogeneous mixture could produce a combined effect which coincides with one or other of these models. Therefore, there is a need to approach the toxicity of mixtures of a large number of chemicals in a more quantitative, rather than a qualitative, manner.

These aspects of mixture toxicity reinforce the need to incorporate statistical analysis of data into the design of mixture toxicity experiments, although the results from equitoxic mixtures are usually more readily interpreted than those from mixtures in other ratios. Furthermore, quantitative interpretation becomes easier when more chemicals are included in mixture, and especially for series of chemicals with strongly correlated QSAR.

Modifying factors

Several factors may affect the results of experiments designed to test the additivity of the effect of toxicants in a mixture. These include the type of response (i.e. whether long-term or short-term and whether lethal or sub-lethal), the magnitude of response, the type of and proportion between chemical constituents, and biological (e.g. life stage, size, acclimation, history, diet) and environmental (e.g. water hardness, pH, etc.) variables.

Multi-factorial studies

One approach to assessing the role of modifying factors on the toxicity of mixtures of substances is through multi-factorial studies. In theory, this approach allows for the measurement of all possible joint interactions without the necessity of examining all possible combinations. The complexities inherent in such studies have been discussed by Street *et al.* (1970). Suitable experimental designs have been described by Kempthorne (1952) and Cochran and Cox (1957) for general statistical problems, and by Finney (1964) and Das and Kulkarni (1966) for toxicity tests; these enable the size of such studies to be reduced by assuming that certain interactions between the dose and the response are negligible, although this increases the complexity of the statistical analysis. There have, however, been few attempts, for example, Marubini and Bonanomi (1970), Benijts–Claus and Benijts (1975) and Gray and Ventilla (1973) to apply purely factorial experimental design to quantitative responses. Alderdice and Forrester (1968) used a multi-factorial, response-surface approach to demonstrate the effects of concurring environmental modifying factors on the toxicity of pollutants to fish during development. However, these experiments were limited to a short exposure period.

Another approach which combines aspects of the multi-factorial approach with the quantal response approach for binary mixtures of Anderson and Weber (1975a), is that of Fedeli *et al.* (1977). They assumed that when dealing with dose-response lines that were not parallel, any concentration of a substance can be expressed as an equi-effective concentration of another. Curvilinear-regression lines resulted from studying a number of mixtures and can be represented empirically by an exponential parabolic regression. The

maximum attainable joint effect may be predicted at a particular ratio of the concentrations of the two components. Finally, the authors arrived at an equation for a paraboloid whose equi-effective horizontal sections are shown to correspond to a series of co-axial ellipses.

In practical terms, the use of a multi-factorial model can lead to difficulties, especially where some combinations of factors may lead to long response times, and maintaining the non-variable parameters constant becomes difficult. It is sometimes simpler, and more instructive, to begin with bifactorial experiments and proceed to more complicated models when a need for more complexity becomes apparent.

Conclusions

The choice of the most suitable model to study the joint effects of mixtures of poisons on fish and other organisms depends on the type of information required and the objective. Response-addition has been widely used in the development of pesticides where recognizing maximum lethality may be the primary aim, whereas for water pollution control the concentration-addition model may be more relevant. For this reason the experimental data reviewed have been derived mainly from experiments based on the concentration-addition model, in particular, to identify deviations from an additive relationship.

APPLICATION OF ADDITIVE INDEX TO LABORATORY STUDIES

The joint effect of toxicants in mixtures is described here as being 'more than additive' (see Figure 1) when tests are carried out to measure the toxic units of individual toxicants, if, when tested together, their sum ($\sum$ TU) shows this to be less than unity. A factor (the reciprocal of $\sum$ TU) is used to indicate by how much the effect is more than additive; conversely, when the sum is more than unity, a factor indicates by how much the effect is less than additive. Information is also provided on whether there is evidence for no

physiological interaction and antagonism, as defined in the previous section and shown in Figures 1 and 2. Where possible, the approximate ratio of the TU's of the constituent toxicants of mixtures is also given. Additionally, especially if TU's cannot be quoted or calculated, the interaction is sometimes described in terms of response-addition of short-term period of survival and percentage response.

FISH

Constituents commonly present in sewage and industrial wastes (see Table II):

Ammonia and other substances

(i) *Ammonia and phenols*

Herbert (1962) carried out tests with rainbow trout (*Salmo gairdneri*) using made-up gas liquor phenols and ammonia, and a mixture of both at a concentration of dissolved oxygen close to the air-saturation value (ASV). Results were rather variable, perhaps because the ratio of *pt* LC50 un-ionised ammonia to that of phenols varied during the tests from 1:1 to 1:2, but the joint effect of the two was approximately additive (between 0.7 and 1.0 times that expected from $\sum$ *pt* LC50); similar results were also obtained at 33 and 66% ASV.

Jenkins (1964) carried out static tests with fathead minnow (*Pimephales promelas*) using ammonium chloride and phenol separately and together in two different mixtures; from the data for 24-h LC50 values (in his Table VI), joint action was not consistent and was about 0.7 times less than additive for a ratio of *p* 24-h LC50 for these two toxicants of 1:0.1 and 1:0.3 at pH 8 and 8.5 respectively, to 1.2 and 1.0 times more than additive for corresponding ratios of 1:0.7 and 1:0.3.

Lloyd and Swift (1976) considered the interaction between ammonia and phenol and concluded that the rate of uptake and concentration of phenol in fish muscle was not caused by high ambient concentrations of ammonia, and that no physiological basis could be found to account for the observed additive toxic action of these two toxicants.

TABLE II
Summary of laboratory data on the joint action of mixtures of toxicants on fish

Toxicants	Species	Exposure period and response	Ratio of toxicant EC50	Joint action	Multiple of additive joint action	Reference
Ammonia + phenol	Rainbow trout	Threshold LC50	1:1 to 1:2	Additive	0.7 to 1.0	Herbert (1962)
	Fathead minnow	24-h LC50	1.0:0.1 & 0.3	Less than additive	0.7	Jenkins (1964)
	Fathead minnow	24-h LC50	1.0:0.3 & 0.7	Additive	1.0 to 1.2	Jenkins (1964)
Ammonia + cyanide	Rainbow trout	96-h LC50	1:1	Additive	1.2	Broderius and Smith (1979)
		30-d EC50 (growth)	1.0:0.7	Less than additive	0.6	Smith *et al.* (1979)
Ammonia + copper	Rainbow trout	48-h LC50	1:1	Additive	1.0	Herbert and Vandyke (1964)
		LC25	1:1	More than additive	1.2	
		LC10	1:1	More than additive	1.4	
Ammonia + zinc	Rainbow trout	Threshold LC50	1.0:0.5	Additive	1.0	Herbert and Shurben (1964)
		Hard water	1:2	Additive	1.0	
		Soft water	1:1	Less than additive	0.8	
Ammonia + phenol + zinc	Rainbow trout	48-h LC50	1.0:1.0:0.5	Additive	1.0	Brown, Jordan and Tiller (1969)
			1:7:1	More than additive	1.2	
			1:1:6	Less than additive	0.7	
			1.0:0.2:0.1	Additive	0.9	
Ammonia + phenol + sulphide	Fathead minnow	24-h LC50	1.0:0.1:1.1	Less than additive	0.5	Jenkins (1964)
			1.0:0.02:0.1	Less than additive	0.6	
			1.0:0.7:2.0	Less than additive	0.6	
			1.0:0.3:0.3	Less than additive	0.8	
Ammonia + sulphide	Fathead minnow	24-h LC50	1.0:2.2	Less than additive	0.6	Jenkins (1964)
			1.0:0.3	Less than additive	0.6	
			1:11	Less than additive	0.8	
			1.0:1.4	Less than additive	0.8	
Ammonia + nitrate	Guppy	72-h LC50	1.0:>0.55	Additive	<1.1	Rubin and Elmaraghy (1977)
			1.0:<0.35	Less than additive	>0.7	

TABLE II
(*Contd.*)

Toxicants	Species	Exposure period and response	Ratio of toxicant EC50	Joint action	Multiple of additive joint action	Reference
Phenol + copper	Rainbow trout	48-h LC50	1:1	Less than additive	0.85	Brown and Dalton (1970)
Phenol + copper + zinc	Rainbow trout	48-h LC50	1:1:1	Less than additive	0.9	Brown and Dalton (1970)
Phenol + sulphide	Fathead minnow	24-h LC50	1:3.5	Less than additive	0.8	Jenkins (1964)
			1:11	Less than additive	0.8	
			1:3	Less than additive	0.7	
			1:1	Additive	0.9	
Cyanide + zinc	Bluegill	96-h LC50		Less than additive	0.4	Cairns and Scheier (1968)
	Fathead minnow	96-h LC50	1:1	More than additive	1.4	Broderius and Smith (1979)
		30-d EC50 (growth)	1.0:0.6	Less than additive	0.4	Smith *et al.* (1979)
Cyanide + chromium	Fathead minnow	96-h LC50	1:1	Less than additive	0.8	Broderius and Smith (1979)
		30-d EC50 (growth)	1.0:0.8 1.0:0.1	Less than additive	0.6 to 0.8	Smith *et al.* (1979)
Copper + zinc	Rainbow trout	3-d LC50 (hard water)	1:1	Additive	1.0	Lloyd (1961)
		7-d LC50 (soft water)	1:1	Additive	1.0	Lloyd (1961)
	Atlantic salmon	7-d LC50 (soft water)	1:1	Additive	1.0	Sprague and Ramsay (1965)
	Longfin dace	96-h LC50	1:0.75	More than additive	1.2	Lewis (1978)
Copper + zinc + nickel	Rainbow trout	48-h LC50	1:1:1	Additive	0.7	Brown and Dalton (1970)
	Rainbow trout	Non-specified	1:1:1	Additive	(0.4 to 1.2)	Marking (1977)

Copper + nickel	Guppy	7-d EC50 (growth, restricted ration)	?	Slightly more than additive	?	Muska and Weber (1977)
		7-d EC50 (growth, unrestricted ration)		Additive		
Copper + cadmium	Mummichog (salinity 20%)	Effect on lateral line	?	Less than additive	?	La Roche *et al.* (1973)
	Mummichog (salinity 20%)	96-h LC50	Various	More than additive	?	Eisler and Gardner (1973)
	Zebrafish	96-h LC50	?	More than additive	2.0	Hewitt and Anderson (1979)
Copper + zinc + cadmium	Mummichog (salinity 20%)	96-h LC50	Various	More than additive	?	Eisler and Gardner (1973)
	Fathead minnow	96-h LC50 (Cu) Threshold LC50 (Cd and Zn)	1.0:0.1:1.2	More than additive	1.3	Eaton (1973)
		$12\frac{1}{2}$ months EC50	1.0:0.1:1.2	Varied, depending on response measured		Eaton (1973)
		90% red. in no eggs	1.0:0.1:1.2	More than additive	>1.6	
		50% red. in no eggs		Less than additive	<0.9	
Copper + mercury	Rainbow trout	96-h LC50	?	Additive	?	D. Calamari and R. Marchetti (pers. comm.)
mercury (methyl)	Blue gourami	96-h LC50	1.0:0.25 to 1.0:1.15	Less than additive	?	Roales and Perlmutter (1977)
Copper + manganese	Longfin dace	96-h LC50		Less than additive	0.67	Lewis (1978)
Copper + surfactant	Goldfish	96-h LC50	1.0:0.1	More than additive	1.3	Calamari and Marchetti (1970)
ABS			1.0:0.6	More than additive	2.1	
LAS	Rainbow trout	96-h LC50	1:1	More than additive	1.3	Calamari and Marchetti (1973)
+ nonyl phenyl ethoxylate	Rainbow trout	96-h LC50	1:1	Less than additive	0.8	Calamari and Marchetti (1973)
Copper + paraquat	*Poecilia mexicana*	24-h LC50	1:0.25	More than additive	1.3	Sun and Gorman (1973)

TABLE II
(*Contd.*)

Toxicants	Species	Exposure period and response	Ratio of toxicant EC50	Joint action	Multiple of additive joint action	Reference
Zinc + cadmium (see text)				Various		
Zinc + detergent (55% ABS) See text for additional tests	Rainbow trout	72-h LC50	1:3.8	Additive	0.9	Brown, Mitrovic and Stark (1968)
Cadmium + chromium + nickel	Rainbow trout	3-month physiological	?	Less than additive	?	D. Calamari (pers. comm.)
Cadmium + mercury	?	96-h LC50	?	Additive	1.0	Hewitt and Anderson (pers. comm.)
		10-d LC50	?	More than additive	2.0	
Mercury + surfactant (LAS)	Rainbow trout	96-h LC50	Various	Additive	1.1	Calamari and Marchetti (1973)
Chromium + nickel	Rainbow trout	96-h LC50	1:0.3	Additive	0.9	F. S. H. Abram (pers. comm.)
	Rainbow trout	10-week LC50	1:0.3	More than additive	13	F. S. H. Abram (pers. comm.)
	Rainbow trout	10-week LC50	1:1	More than additive	21	F. S. H. Abram (pers. comm.)
Chromium + surfactant	Alborella	96-h LC50	1:17 to 1.0:0.8	Less than additive	0.6 to 0.7	Bianucci and Legnani (1973)

(ii) *Ammonia and cyanide*

Experiments with solutions containing both ammonia and cyanide showed that the combination was more toxic than either substance alone (Wuhrmann and Woker, 1948). More recently, the acutely lethal effect on rainbow trout of un-ionised ammonia and hydrogen cyanide in mixtures, in the ratio of 1:1 of the respective *p* 96-h LC50 values, has been tested in a hard water (220 mg/l as $CaCO_3$) at 10°C and shown to be slightly (1.16 times) more than additive (Broderius and Smith, 1979). Similar results have been obtained using smolts of salmon (*Salmo salar*) in fresh water at ASV and with values between 0.61 and 1.25 at lower percentage ASV and higher salinity (J. S. Alabaster, D. G. Shurben and M. J. Mallett, pers. comm.). Examination of Broderius and Smith's Figure 1 and data in Smith *et al.* (1979) on growth in 30-d (for both the threshold of no effect and for 50% reduction, compared with controls) suggests that the effect of these toxicants in a mixture in the ratio of 1:0.7 of respective p EC (effective concentration) values of ammonia and cyanide is about 0.6 times less than additive.

(iii) *Ammonia and copper*

Vamos and Tasnadi (1967) used copper sulphate to reduce the toxicity of ammonia in carp ponds, and suggested that the cuproammonium compounds formed are not toxic. However, Herbert and Vandyke (1964) measured the 48-h LC50 of un-ionised ammonia and copper and mixtures of the two, in the ratio 1:1 *p* 48-h LC50, using rainbow trout in a hard water (320 mg/l as $CaCO_3$); the joint effect of the two was not significantly different from additive but, on the basis of LC25 and LC10, it was 1.2 and 1.4 times respectively more than additive. The authors pointed out that 75% of the copper and 0.3% of the ammonia was calculated to be present as cuprammonium ions, with $Cu(NH_3)_3^{2+}$ being predominant and suggested, therefore, that the toxicity of copper and cuprammonium ions was similar, possibly because a large proportion of the latter would dissociate to copper ions and ammonia at the gill surface.

(iv) *Ammonia and zinc*

Herbert and Shurben (1964) measured the *t* LC50 of un-ionised ammonia and zinc and mixtures of the two, in the ratio of 1.0:0.5

and 1:2 of their respective *pt* LC50 values, using rainbow trout in a hard water (320 mg/l as $CaCO_3$) at ASV; they found that the joint effect of the two was 0.96 and 0.97 times less than additive. At 40% ASV and with a ratio of 1:1 of the respective *pt* LC50 values, the effect was 0.85 times less than additive; at ASV and the same ratio in a soft water (44 mg/l as $CaCO_3$), it was 0.8 times less than additive.

(v) *Ammonia, phenol and zinc*

Brown, Jordan and Tiller (1969) measured the 48-h LC50 to rainbow trout of ammonia, phenol and zinc and mixtures of all three, using different ratios of the respective *p* 48-h LC50 values. The joint effect was additive (where the ratio of the respective *p* 48-h LC50 values was 1.0:1.0:0.5); 1.2 times more than additive (with a ratio of 1:7:1), 0.7 times less than additive (ratio 1:1:6, in two cases); or 0.9 times less than additive (ratio 1:0.2:0.1). The authors point out that slightly different results are obtained using 48-h LC50 values for the three toxicants from other published sources, although the joint action was still 1.4 times more than additive in the mixture in the ratio 1:7:1, and was still 0.7 times less than additive in the mixture in the ratio 1:1:6.

(vi) *Ammonia, phenol and sulphide*

Jenkins (1964) carried out static tests with fathead minnow using ammonium chloride, phenol and sodium sulphide separately and together in two different mixtures; from the data for 24-h LC50 values (in his Table VI), joint action between the toxicants ranged from 0.47 and 0.61 times less than additive for a ratio of *p* 24-h LC50 of 1:0.1:1.1 and 1:0.02:0.1 for these three toxicants at pH 8 and 8.5 respectively, to 0.6 and 0.83 times less than additive for corresponding ratios of 1:0.7:2 and 1:0.3:0.3.

(vii) *Ammonia and sulphide*

Jenkins (1964) also carried out 24-h tests using ammonium chloride and sodium sulphide separately and together in two different mixtures; his data for LC50 values show that the joint action of the toxicants was 0.56 and 0.64 times less than additive for a ratio of

p 24-h LC50 of 1:2.2 and 1:0.3 for these two toxicants at pH 8 and 8.5 respectively and 0.78 and 0.81 times more than additive for corresponding ratios of 1:11 and 1:1.4.

(viii) *Ammonia and nitrate*

Rubin and Elmaraghy (1977) used the fry of guppy (*Poecilia reticulatus*) to test the individual and joint effect of potassium nitrate and un-ionised ammonia. The results indicate no more than 1.13 times more than additive effect except in mixtures in which the *p* 72-h LC50 of ammonia is below or at 0.35 when joint action was at a minimum of 0.7 times less than additive; in the latter case there appears to be no interaction between the two, i.e. toxicity is entirely attributable to potassium nitrate in the mixture, with ammonia making no contribution.

Monohydric phenols and other substances

(i) *Monohydric phenols*

Southgate (1932) measured the survival period of rainbow trout in tests lasting a few hours in the presence of *p*-cresol and phenol and mixtures of the two. He noticed that the behaviour of the fish was similar in the two toxicants and found that the effect of phenol and *p*-cresol on the very short-term (11–12½ min) survival of rainbow trout was additive. He also carried out similar tests with *p*-cresol and 2,6-xylenol and mixtures of the two. In this case the behaviour of the fish in the two toxicants was different, and he found that the toxicity of *p*-cresol was increased in the presence of xylenol, but that the converse was not true. Calculation of the 1-h LC50 values shows that the joint action of *p*-cresol and 2,6-xylenol in the ratio 1:1.5 of their respective 1-h LC50 values was 1.5 times more than additive. Herbert (1962) compared the survival of rainbow trout at 9°C in simulated spent still gas liquors from a gas works and a coke oven, made up with a variety of monohydric phenols (including 13% *p*-cresol and 0.5% 2,6-xylenol), with the survival of fish in pure phenols; in one experiment the toxicity (in terms of *p* 24-h LC50) was respectively about 1.3 and 1.1 times that of phenol alone, and in another it was not significantly greater.

(ii) *p-cresol and cyanide*

Southgate (1932) measured the short-term survival of rainbow trout in the presence of potassium cyanide and *p*-cresol and mixtures of the two. The toxicity of *p*-cresol was increased in the presence of cyanide but the converse was not true. Calculation of the 26-min LC50 values shows that the joint action of *p*-cresol and cyanide in the ratio of about 1:1.2 of their respective 26-min LC50 values was 1.7 times more than additive.

(iii) *Phenol and copper*

Brown and Dalton (1970) measured the 48-hr LC50 to rainbow trout of phenol and copper and mixtures of the two in a hard water (240 mg/l as $CaCO_3$) in the ratio of 1:1 of their respective *p* 48-h LC50 values and found that their joint action was 0.85 times less than additive.

(iv) *Phenol, copper and zinc*

Brown and Dalton (1970) measured the 48-h LC50 to rainbow trout of phenol, copper and zinc and mixtures of the three in a hard water (240 mg/l as $CaCO_3$) in the ratio of 1:1:1 of their respective *p* 48-h LC50 values and found that their joint action was 0.9 times less than additive.

(v) *Phenol and sulphide*

Jenkins (1964) carried out static tests with fathead minnow using phenol and sodium sulphide separately and together in two different mixtures; from the data for 24-h LC50 values (in his Table VI) interaction between the toxicants ranged from 0.82 and 0.81 times less than additive for a ratio of *p* 24-h LC50 of 1:3.5 and 1:11 for these two toxicants at pH 8 and 8.5 respectively, to 0.71 and 0.85 times more than additive for corresponding ratios of 1:3 and 1:1.

(vi) *Phenol and chlorine*

At environmental water temperatures phenol reacts with chlorine to form chlorinated phenols which are more toxic than phenol; for example, the 96-h LC50 of pentachlorophenol is 0.21 mg/l for

goldfish (*Carassius auratus*) and fathead minnow (Adelman and Smith, 1976), and for mosquitofish (*Gambusia affinis*) the 6-d LC50 is about a third of that of phenol (Katz and Cohen, 1976).

(vii) *Phenol, pentachlorophenol and dinitrophenol*

Verma *et al.* (1981) carried out static tests with phenol, pentachlorophenol and dinitrophenol, using the fish *Notropterus notropterus* and estimating the 96-h LC50 values. They also tested all mixtures of two and mixtures of three at several different ratios of the respective 96-h LC50 values. Examination of their data shows that on average the mixtures were 0.78 less than additive, the range being 0.34 to 1.47 (as detailed in their Table 1). In interpreting these results it should be noted that the confidence limits for individual tests differed by a factor of 1.5 on average, ranging from close to unity to 1.8.

(For mixtures of phenol, ammonia and zinc or sulphide, and phenol, nickel and vanadium, see preceding sections.)

Cyanide and other substances

(i) *Cyanide and zinc*

The toxicity to fathead minnow of solutions containing sodium cyanide and zinc sulphate was related mainly to the level of molecular cyanide present (Doudoroff *et al.*, 1966).

Cairns and Scheier (1968) measured the 96-h LC50 of cyanide and zinc and a mixture of the two using bluegill (*Lepomis macrochirus*) and found that the joint action was 0.42 times less than additive. On the other hand, Broderius and Smith (1979) made similar measurements, in duplicate, with fathead minnow in a hard water (220 mg/l as $CaCO_3$) at 25°C, using mixtures in the ratio of 1:1 of the respective *p* 96-h LC50 values, and showed that the joint action was 1.4 times more than additive. However, examination of their Figure 1 and raw data in Smith *et al.* (1979) suggests that the effect of a mixture in the ratio of 1:0.6 of the respective *p* EC, on the growth of the fish in 30 d (threshold of no effect), is about 0.4 times less than additive.

(ii) *Cyanide and PCB*

Anderson and Weber (1975a) found that in the majority of cases interaction between cyanide and PCB was response-additive.

(iii) *Cyanide, zinc and PCB*

Negilski and Davies (1974) found that for reduction in the biomass of juvenile chinook salmon (*Oncorhynchus tshawytscha*) maintained in artificial streams and exposed to a mixture comprising 0.2 of each of the 96-h LC50 values of cyanide, zinc and PCB, and was greater than that found with 0.2 of the 96-h LC50 of each separately. Anderson and Weber 1975a) showed that these results (see their Table 4) could be accounted for by response addition. However, in the stream exposed to 0.1 of the 96-h LC50 of cyanide alone production of biomass was enhanced; if this is equated to no reduction in production, the reduction found in the stream containing all three toxicants can be shown to be about 0.6 of that expected from strict addition.

(iv) *Cyanide and chromium*

The acutely lethal effect on fathead minnow of chromium and cyanide in mixtures in the ratio of 1:1 of the respective 96-h LC50 values tested in a hard water (220 mg/l as $CaCO_3$) at 25°C has been shown to be slightly (0.76 times) less than additive (Broderius and Smith, 1979). Their Figure 1 and raw data (Smith *et al.*, 1979) suggest that the effect on growth in 30 d (threshold of no effect) of mixtures in the ratio 1:0.8 and 1:0.1 is also slightly (0.6 to 0.8 times) less than additive.

(For mixtures of cyanide and ammonia, cyanide and *p*-cresol, see preceding sections.)

Copper and other substances

(i) *Copper and zinc*

Doudroff (1952) found that whereas fathead minnow usually survived an 8-h exposure to solutions containing either 8 mg Zn/l or 0.2 mg Cu/l in soft water, most succumbed within this period in a mixture of copper and zinc at one-eighth of these concentrations respectively. Lloyd (1961) obtained somewhat similar results with rainbow trout in soft water (hardness 14 mg/l as $CaCO_3$), but found that at relatively low concentrations, corresponding to less than the 72-h and 7-d LC50 of copper and zinc respectively, the effect of the mixtures was simply additive in both soft and hard water (hardness 320 mg/l as $CaCO_3$). Sprague and Ramsay (1965), who used these

metals in water with a similar hardness of 14 mg/l as $CaCO_3$, also found that the toxicity of mixtures to juvenile Atlantic salmon (*Salmo salar*) could be accounted for by the simple addition of the corresponding *p* 7-d LC50 of the separate metals. Recently, Thompson *et al.* (1980) reached the same conclusion using bluegill in a water with a hardness of 21 to 60 mg/l as $CaCO_3$. La Roche *et al.* (1973) found by histological examination of mummichog (*Fundulus heteroclitus*) exposed to mixtures of copper and zinc that the effects of copper on the lateral line were not aggravated by the presence of zinc.

Somewhat different results have been found for other species. Using longfin dace (*Agosia chrysogaster*) Lewis (1978) measured the 96-h LC50 of copper and zinc, and a mixture of the two in a hard water (218 mg/l as $CaCO_3$) and found that their joint effect was 1.2 times more than additive. Using zebrafish (*Brachydanio rerio*) Anderson *et al.* (1979a) reported that, whereras the toxicity expressed as 96-h LC50 of both zinc and copper was reduced with increase in calcium and magnesium ions over a range of 20 to 300 mg/l as $CaCO_3$, the toxicity of a mixture of the two remained virtually the same over this range; thus the effect of copper and zinc in the mixture was strictly additive in soft water but progressively more than additive with increase in hardness. In these experiments the pH and alkalinity were held constant, whereas these would normally increase with hardness.

Eisler and Gardner (1973) measured survival of mummichog in synthetic sea water (20% salinity) in the presence of copper and zinc and mixtures of the two. Although tests were carried out under static conditions and concentrations of dissolved copper were known to have fallen considerably during the tests, the results, though not entirely consistent, suggest that the interaction between copper and zinc was several-fold more than additive.

Anderson and Weber (1975a) carried out tests using mature male guppy and found that the toxicity of a mixture of copper and zinc was 2.65 times more than additive and later (Anderson and Weber, 1976) suggested that this might be attributable to the two metals having a similar mode of toxic action, correlated perhaps with a similar affinity for sulphur-containing ligands present at high concentrations in the gill. Their tests were carried out in a water of moderate hardness (126 mg/l as $CaCO_3$), and they pointed out that

the results of Sprague (1964) and Lloyd (1961) with these two metals in a soft water (about 20 mg/l as $CaCO_3$) also showed greater than additive effects in the short term whereas in a much harder water (about 320 mg/l as $CaCO_3$) (Lloyd, 1961, and Brown and Dalton, 1970) the effects were simply additive.

(ii) *Copper, zinc and nickel*

Brown and Dalton (1970) measured the 48-h LC50 to rainbow trout of copper, zinc and nickel, and mixtures of the three, in a hard water (240 mg/l as $CaCO_3$) in the ratio of 1:1:1 of their respective *p* 48-h LC50 values and found that the joint effect of three was approximately additive. Marking (1977) also found that their joint action was approximately additive (unspecified LC50).

(iii) *Copper and nickel*

Weinstein and Anderson (1978), using zebrafish, showed that copper and nickel were more than additive at both the lethal (more than two-fold) and sub-lethal levels, and also reported (Anderson *et al.*, 1979a) that this was markedly affected by the relative proportion of each in the mixture, the higher the proportion of nickel, the lower the percentage mortality. However, Muska and Weber (1977) report briefly that, in 7-d tests on the effect of copper and nickel and their mixture on the growth and food consumption of juvenile guppy at 7°C and 25°C, interaction between copper and nickel was slightly more than additive with an restricted ration, and approximately additive with a unrestricted ration.

(iv) *Copper and cadmium*

Although La Roche *et al.* (1973) mentioned that histological examination of mummichog exposed to mixtures of copper and cadmium showed that the effects of copper on the lateral line were not aggravated by the presence of cadmium, Eisler and Gardner (1973) measured the percentage survival of mummichog in synthetic sea water (20% salinity) in the presence of copper and cadmium and mixtures of the two, and suggested that interaction between the two toxicants was several-fold more than additive on both a response and concentration basis.

Hewitt and Anderson (1979), and P. D. Anderson (pers. comm.) using zebrafish in 96-h tests, found the lethal effect of copper and cadmium in mixtures to be approximately 2-fold more than additive.

(v) *Copper, zinc and cadmium*

Eisler and Gardner (1973) measured survival of mummichog in synthetic sea water (20% salinity) in the presence of copper, zinc and cadmium and a mixture of all three; results suggest that interaction between them was several-fold more than additive on a response and concentration basis, except possibly in one case where the ratio of *p* 96-h LC30 for the three was 1:1:0.1, where the interaction seemed to be approximately response additive.

Eaton (1973) tested the toxicity to the fathead minnow of a mixture of copper, zinc and cadmium in the ratio 1:0.1:1.2 of the 96-h LC50 of copper and the lethal threshold values of cadmium and zinc respectively; the data show that the joint action at 96 h was 1.3 times more than additive. The author also used the same proportions of these three metals in tests lasting $12\frac{1}{2}$ months and measured several sub-lethal effects; these were compared qualitatively with effects for zinc alone, and for copper and cadmium tested separately by other workers. The evidence presented (in his Figure 1) suggests that, for a reduction of 90% in the number of eggs produced per female, joint action may be more than additive, but for a reduction of 50% it may be less than additive, with zinc playing the predominant adverse role. In addition, reduction in the size of the fish, and inhibition of sexual development, was found at a concentration of copper in the mixture of about 0.3 of that of copper alone, suggesting a greater than additive effect. On the other hand, hatchability was actually better in the mixture than in the presence of an almost identical concentration of cadmium alone, suggesting a less than additive joint action (perhaps with zinc).

(vi) *Copper and mercury*

Some studies have been made to measure 96-h LC50 values for rainbow trout with copper and mercury, and mixtures of the two, tested at 15°C in water having a hardness of 320 mg/l as $CaCO_3$;

additive joint action was found between the two metals (D. Calamari and R. Marchetti, pers. comm.).

Roales and Perlmutter (1974b) measured the 96-h LC50 of copper and methyl mercury to blue gourami (*Trichogaster tricopterus*) and the percentage mortality at 96 h in mixtures of the two in tap water in which the pH was adjusted to 7.4 by the addition of sodium bicarbonate; a less than additive interaction was found where the ratio of *p* 96-h LC50 of copper to that of methyl mercury was between 1:0.25 and 1:1.5. These authors later showed (Roales and Perlmutter, 1977) that exposure of this species for one week to 0.1 of the 96-h LC50 of copper, or methyl mercury or a mixture of the two, produced a marked decline in immune response to antigens of infectious pancreatic necrosis virus and *Proteus vulgaris*; the response was probably too large to distinguish the type of interaction between copper and methyl mercury, although there was no evidence that it was not simply additive.

(vii) *Copper and manganese*

Lewis (1978) measured the 96-h LC50 of copper and manganese, and a mixture of the two in a hard water (218 mg/l as $CaCO_3$) using the longfin dace and found that joint action was 0.67 times less than additive.

(viii) *Copper and surfactant*

Calamari and Marchetti (1970), measured the 96-h LC50 to goldfish in a hard water (190 to 240 mg/l as $CaCO_3$) at a temperature of 14.7 to 15.9°C, and found that the joint action of copper and sodium alkyl benzene sulphonate (ABS) was more than additive, the effect increasing with increase in the ratio of the respective *p* 96-h LC50 values of copper and ABS (alkylbenzene sulphonate), from 1.3-fold, for a ratio of 1:0.1, to 2.1-fold for a ratio of 1:0.6. The same authors (1973) working with rainbow trout in a hard water (290 to 310 mg/l as $CaCO_3$) and 15 to 15.6°C also reported marked reductions in periods of survival compared with those predicted assuming additive interaction, with both mixtures of copper and ABS and of copper and LAS (linear alkylbenzene sulphonate). In the latter case the joint action in terms of the $\sum p$ 96-h LC50 was 1.25 times more than additive. However, with mixtures of copper

and nonyl phenol ethoxylate, joint action was slightly (probably 0.8 times) less than additive.

Tsai and McKee (1980) using goldfish found that the toxicity of mixtures of copper and LAS was additive except in the ratios 1:0.5 of the 96-h LC50, when it was twice as additive.

(ix) *Copper and other substances*

Tsai and McKee (1980) also found that, depending upon the proportion of the toxicants present, mixtures of copper and chloramines, and mixtures of copper, chloramines and LAS were between 1.3 and 1.7 more than additive.

Cairns and Scheier (1968) report an additive effect in soft water on bluegill of a mixture of acetic acid, acetaldehyde, acetone and copper.

Sun and Gorman (1973) tested the 24-h LC50 of copper sulphate and paraquat and a mixture of the two in the ratio of 1:0.25 to *Poecilia mexicana* in a soft water (hardness 32 mg/l as $CaCO_3$); the joint action between copper sulphate and paraquat was 1.3 times more than additive. On the other hand, Fitzgerald (1963) found that the 24-h LC50 of copper sulphate to the bluntnose minnow (*Hyborhynchus notatus*) and several sunfish species was 0.5 mg/l whereas a concentration of 500 mg/l in the presence of 1000 mg/l of citric acid at either pH 6 or 8.5 did not kill any fish within 24 h.

(For mixtures of copper and ammonia, copper and phenol, see preceding sections.)

Zinc and other substances

(i) *Zinc and cadmium*

Tests with rainbow trout in hard water (J. F. de L. G. Solbé and V. A. Cooper, pers. comm.) have shown that the 48-h LC50 for zinc was about 3.8 mg/l in the presence of cadmium at concentrations of up to 2 mg/l Cd and that at lower concentrations of zinc (down to 0.5 mg/l), periods of survival were accountable by the concentration of cadmium alone. In another experiment in which rainbow trout yearlings were exposed for four weeks to mixtures of either 30 or 40 μg Cd/l and zinc at concentrations of up to 500 μg/l in a hard water (245 mg/l as $CaCO_3$) at pH values of about 7.8, there was a

tendency for mortality to be least at intermediate concentrations of zinc, suggesting less than additive interaction between the two metals (Cooper and Solbé, 1978). Moreover, pre-exposure of the fish for 5 days to 109 μg Zn/l, followed by intermittent exposure up to 35 μg Cd/l (six 2-h periods of cadmium exposure within 72 h), protected all fish from the lethal effects of 35 μg Cd/l in continuous exposure for 44 days. During this period there was a 50 percent mortality of a batch of fish exposed to 35 μg Cd/l, but not pretreated as above.

Spehar *et al.* (1978) exposed flagfish (*Jordanella floridae*), for 2 weeks from the day-old stage and for 100 days from the embryo stage, to zinc and cadmium and mixtures of the two. Survival of larvae (not exposed as eggs) was reduced in the presence of mixtures of zinc and cadmium to an extent similar to that found in previous work cited with zinc alone. The authors suggested that because such larval fish are sensitive indicators of toxicity the chronic toxicity of mixtures of cadmium and zinc could also be largely attributable to the effect of zinc alone. This would agree with the findings of Eaton (1973) for the effect on the production of embryos in mixtures of zinc, copper and cadmium which was largely attributable to the effect of zinc. With larvae of flagfish exposed initially as embryos to zinc and cadmium (Spehar *et al.*, 1978) additive interaction was not demonstrated.

Eisler and Gardner (1973) measured survival of mummichog in synthetic sea water (20% salinity) in the presence of zinc and cadmium and mixtures of the two; the results suggest that interaction was several-fold more than additive on both a response and concentration basis.

Anderson *et al.* (1979a) report that the combined toxicity of cadmium and zinc appears to differ with stage in the life cycle.

Gallimore and Anderson (1979) and P. D. Anderson (pers. comm.) found that zinc was more toxic than cadmium to eggs of zebrafish, but that the converse was true of the larval, juvenile and adult stages. However, at each stage in the life cycle, zinc and cadmium were consistently concentration-additive in their joint lethal toxicity. The decreases in susceptibility of zebrafish from larvae through to adult states exposed to zinc, cadmium and their mixtures were correlated with a decrease in weight-specific metabolic rate.

(ii) *Zinc and detergent*

Brown, Mitrovic and Stark (1968) exposed rainbow trout to mixtures of a detergent containing 55% ABS and fixed concentration of 0.8 mg Zn/l, and compared results with those for zinc alone (Lloyd, 1960); joint action (based upon *p* 72-h LC50 values in the ratio 1:3.8) was 0.9 times less than additive. They also carried out similar experiments with fish that had been acclimated for 100 days beforehand to 0.8 mg Zn/l. Comparison of their results with those of Edwards and Brown (1966) for zinc alone for fish acclimated for 60 days suggests that, in this case, the joint action between zinc and detergent was 1.5 times more than additive.

(iii) *Zinc and cygon*

Roales and Perlmutter (1974a) measured the 72-h LC50 of zinc and cygon (0,0-dimethyl-S-(N-methyl)carbamylmethyl phosphorodithionate) to the embryos of zebrafish and mortality at 72 h in mixtures of the two in distilled water; the results indicate additive joint action with a ratio of *p* 72-h LC50 of zinc to that of cygon of 1:0.7 but less than additive action with a ratio of 1:4.

(For mixtures of zinc with ammonia, with ammonia and phenol, with phenol and copper, with cyanide, with cyanide and PCB, with copper, with copper and nickel, see preceding sections.)

Cadmium and other substances

(i) *Cadmium, chromium and nickel*

Arillo *et al.* (1982) held large (220 g) rainbow trout in a hard water (320 mg/l as $CaCO_3$) in the presence of 0.01 mg Cd/1, 0.2 mg Cr/l and 1.0 mg Ni/l, separately and together, for a period of six months after an initial period of one month in clean water; subsequently the fish were kept for a three-month recovery period in clean water. There was no significant mortality among the fish but preliminary results indicate that adverse physiological effects were found (on content of sialic acid in gills, glucide in liver and proteolytic activity in liver and red blood cells) in all four treatments; no evidence was found of more marked effects with the mixtures than with the single metal treatments. This suggests a less than additive joint sub-lethal action of the three metals.

(ii) *Cadmium and mercury*

Anderson *et al.* (1979a) suggested that apparent safe levels of poorly accumulated toxicants, like cadmium, could enhance the acute toxicity of cumulative toxicants such as mercury. L. A. Hewitt and P. D. Anderson (pers. comm.) showed that while the joint lethal action of these two metals was additive at 96 h, it was two-fold more than additive at 10 days.

(For mixtures of cadmium with copper, with copper and zinc, and with zinc, see preceding sections.)

Nickel and other substances

(i) *Nickel and chromium*

Data for the survival of rainbow trout in mixtures of nickel and chromium in a hard water (hardness 270 mg/l as $CaCO_3$) at 15°C show that, for the 96-h LC50 in the ratio of 3:1 of the *p* LC50 values for chromium and nickel, there is additive joint action but for the 10-week LC50 in the same ratio, and also in the ratio 1:1, joint action was apparently 13- and 21-times more than additive respectively (F. S. H. Abram, pers. comm.)

(ii) *Nickel and vanadium*

Anderson *et al.* (1979b) found that the lethal effects of mixtures of nickel and vanadium became progressively less-than-additive as the proportion of vanadium increased.

(iii) *Nickel, vanadium and phenol*

In contrast, Anderson *et al.* (1979b) found that when the concentrations of these two metals increased in proportion to that of phenol in tertiary mixtures, the lethal effect of the mixture became progressively (2-fold) more than additive.

(For mixtures of nickel with copper, and with copper and cadmium, see preceding sections.)

Chromium and other substances

Chromium and surfactant

Bianucci and Legnani (1973) using alborella (*Alburnus albidus*) to test the toxicity of mixtures of potassium dichromate and sodium

benzene sulphonate in a hard water (hardness 200 mg/l as $CaCO_3$) at 20°C, showed that, over a wide range of ratios of the respective fractions of the 96-h LC50 (1:17 to 1:0.8), their combined effect on the LC50 was between 0.6 and 0.7 times less than additive; similar results were obtained for 24- and 48-h LC50 values.

(For mixtures of chromium with cyanide, and with nickel, see preceding sections.)

(i) *Mercury and selenium*

Huckabee and Griffith (1974) showed that although the presence of 1 mg/l of both selenium (added as SeO_2) and mercury (added as $HgCl_2$) resulted in a reduction in the hatch of eggs of carp (*Cyprinus carpio*) of only 0.4% and 0.6% respectively, a mixture of the two, each at a concentration of 1 mg/l (the lowest concentration tested, but unrealistically high in relation to those reported for surface waters), was markedly more toxic than additive, resulting in a reduction in hatch of over 80%. The authors suggest that although both toxicants have affinity for sulphydryl (SH) groups, they react together, when metabolised, to form less reactive complexes or compounds and might therefore be expected to interact less than additively; however their results suggest that reaction with SH groups might occur directly in the outer membrane of the egg.

Kim *et al.* (1977) however, showed that pretreatment of northern creek chub (*Semotilus atromaculatus*) for 48 h with 3 mg Se/l (0.4 of the lethal concentration) resulted in a lower mortality than in untreated fish when subsequently exposed for 48 h to a range of mercury concentrations. Insufficient information is available to determine whether or not the protection afforded by this treatment resulted from the effect of selenium on the accumulation of mercury in the fish.

More recently, Heisinger *et al.* (1979) showed that the 48-h LC50 of mercuric chloride in the presence of equimolar concentrations of selenium dioxide to goldfish was 0.8 times less than the corresponding value in its absence, and 0.7 times less when exposure to mercury and selenium followed a 24-h pretreatment with selenium.

(ii) *Mercury and surfactant*

Calamari and Marchetti (1973) measured the 96-h LC50 of mercury and LAS and reported that joint action was more than additive, but

their data show that the effect was not large (1.1-fold), and probably not statistically significant.

(For mixtures of mercury and copper, see preceding sections.)

Studies on complex mixtures of organic substances

The following data have been published since 1980; data on *Daphnia* and bacteria are included to support the conclusions drawn from studies on fish. The data are summarised in Table III.

(i) *Chlorobenzenes*

Konemann (1981a) measured the 14-d LC50 of equitoxic mixtures of 3 and 10 chlorobenzenes (diCB–pentaCB) to guppies. The toxicity of these mixtures was slightly less than additive, M being 1.4 and 1.5 respectively (MTI = 0.68 ± 0.23 and 0.82 ± 0.10). Chlorobenzenes were expected to have simple similar modes of action based on a QSAR analysis.

(ii) *Chlorophenols*

The toxicity of an equitoxic mixture of phenol and 10 chlorophenols (14-d LC50) to guppies appeared to be completely concentration additive (Konemann, 1981a). This result was expected since simple similar action was likely on the basis of QSAR analysis.

(iii) *Chloro- and alkylanilines*

Hermens *et al.* (1984a) determined the 14-d LC50s of mixtures of substituted anilines to guppies. The toxicity of a mixture of equitoxic concentrations of six alkylanilines was concentration additive ($M = 1.1$; MTI = 0.95 ± 0.13), as was the case for a mixture of aniline and ten chloroanilines ($M = 1.1$; MTI = 0.96 ± 0.10). Also the toxicity of the mixture of the equitoxic concentration of all 17 of these anilines was completely concentration additive (M again 1.1; MTI = 0.96 ± 0.08). QSAR analysis had indicated the likelihood of simple similar action.

(iv) *Reactive organic halides*

Hermens *et al.* (1985a) showed that the joint toxicity (14-d LC50) to the guppy of 9 reactive organic halogen compounds (such as

TABLE III
Summary of laboratory data on the joint action of complex mixtures of organic substances to fish

Toxicants	Species	Response	Ratio	*n*	*M*	MTI ± *sd*	Reference
1,4-dichlorobenzene, 1,2,3-tri-chlorobenzene, 1,2,3,4-tetrachlorobenzene	guppy	14-d LC50	equitox	3	1.4	0.69 ± 0.23	Konemann (1981a)
all di-, tri-, tetra- and pentachlorobenzenes	guppy	14-d LC50	equitox	10	1.5	0.82 ± 0.10	Konemann (1981a)
3 chlorobenzenes, 2 chlorotoluenes, 3 chloroalkanes, diethylether, acetone	guppy	14-d LC50	equitox	10	1.1	0.96 ± 0.10	Konemann (1981a)
50 chloroaromatics, chloroalkanes, alcohols and ethers	guppy	7-d and 14-d LC50	equitox	50	0.9	1.02 ● 0.06	Konemann (1981a)
phenol and 10 chlorophenols	guppy	14-d LC50	equitox	11	1.8	1.0 ± 0.1	Konemann (1981a)
10 chlorobenzenes and toluenes	*Daphnia magna*	immobilisation 48-h IC50	equitox	10	1.0	1.0 ± 0.1	Hermens *et al.* (1984b)
benzene derivatives and aliphatic compounds	*Daphnia magna*	immobilisation 48-h IC50	equitox	10	0.9	1.05 ± 0.1	Hermens *et al.* (1984b)
4 chlorobenzenes and a chlorotoluene	*Daphnia magna*	reproduction 16-d EC50	equitox	5	2.0	0.57 ± 0.17	Hermens *et al.* (1984b)
4 chlorobenzenes and a chlorotoluene	*Daphnia magna*	16-d EC50	equitox	5	1.0	1.00 ± 0.14	Hermens *et al.* (1984b)
10 non-reactive hydrocarbons (mostly alcohols and chlorobenzenes)	*Daphnia magna*	growth 16-d no observed effect concentration	equitox	10	1.8	0.74 ± 0.11	Hermens *et al.* (1985c)
6 alkylanilines	guppy	14-d LC50	equitox	6	1.1	0.95 ± 0.13	Hermens *et al.* (1984a)
aniline and 10 chloroanilines	guppy	14-d LC50	equitox	11	1.1	0.96 ± 0.10	Hermens *et al.* (1984a)
aniline, 6 alkylanilines and 10 chloroanilines	guppy	14-d LC50	equitox	17	1.1	0.96 ± 0.08	Hermens *et al.* (1984a)

TABLE III (*continued*)

Toxicants	Species	Response	Ratio	*n*	*M*	MTI ± *sd*	Reference
9 reactive organic halides	guppy	14-d LC50	equitox	9	1.0	1.0	Hermens *et al.* (1985a)
1-pentanol and 1-hexanol	fathead minnow	96-h LC50	equitox	2	0.87	1.20 ± 0.33	Broderius and Kahl (1985)
idem and 1-heptanol	fathead minnow	96-h LC50	equitox	3	0.89	1.11 ± 0.21	Broderius and Kahl (1985)
idem and 1-octanol	fathead minnow	96-h LC50	equitox	4	0.88	1.09 ± 0.17	Broderius and Kahl (1985)
idem and 1-nonanol	fathead minnow	96-h LC50	equitox	5	0.97	1.02 ± 0.14	Broderius and Kahl (1985)
3 alcohols and 1 ketone	fathead minnow	96-h LC50	equitox	4	1.07	0.95 ± 0.17	Broderius and Kahl (1985)
1 alcohol and 1 ketone	fathead minnow	96-h LC50	equitox	2	1.04	0.94 ± 0.33	Broderius and Kahl (1985)
idem and 1 ether	fathead minnow	96-h LC50	equitox	3	0.96	1.04 ± 0.21	Broderius and Kahl (1985)
idem and 1 nitrile	fathead minnow	96-h LC50	equitox	4	0.89	1.08 ± 0.17	Broderius and Kahl (1985)
1 alcohol, ketone, ether, nitrile and 1 tertiary aromatic amine	fathead minnow	96-h LC50	equitox	5	1.04	1.98 ± 0.14	Broderius and Kahl (1985)
idem and 1 alkylhalide	fathead minnow	96-h LC50	equitox	6	0.87	1.08 ± 0.13	Broderius and Kahl (1985)
idem and 1 chlorobenzene	fathead minnow	96-h LC50	equitox	7	0.89	1.06 ± 0.12	Broderius and Kahl (1985)
14 alcohols, ketones, ethers, alkylhalides, benzenes, nitriles, tertiary aromatic amines	fathead minnow	96-h LC50	equitox	14	1.15	0.95 ± 0.09	Broderius and Kahl (1985)
21 of the above compounds	fathead minnow	96-h LC50	equitox	21	1.23	0.93 ± 0.08	Broderius and Kahl (1985)

substituted benzene, phenol, aniline, xylene, alkene, 2 pesticides, 2 metals	guppy	14-d LC50	equitox	9	2.5	0.58 ± 0.12	Konemann (1981a)
14 heterogenic organic and inorganic chemicals	*Daphnia magna*	48-h LC50	approx equitox	14	1.2	0.92 ± 0.11	Hermens *et al.* (1984c)
idem	*Daphnia magna*	16-d EC50	approx equitox	14	2.6	0.43 ± 0.18	Hermens *et al.* (1984c)
5 selections of 8 chemicals out of 24 heterogenic organic chemicals	guppy	14-d LC50	equitox	8	1.1–2.7	0.74 – 0.95	Hermens and Leeuwangh (1982)
24 heterogenic organic chemicals	guppy	14-d LC50	equitox	24	2.3	0.74 ± 0.08	Hermens and Leeuwangh (1982)
4 different combinations of a non-reactive hydrocarbon with a chloroaniline	guppy	14-d LC50	equitox	2	0.7–1.3	0.62 – 1.51	Hermens *et al.* (1985a)
11 non-reactive hydrocarbons and 11 chloroanilines (in 4-fold)	guppy	14-d LC50	equitox	22	1.2–1.5	0.87 – 0.94	Hermens *et al.* (1985a)
4 different combinations of a non-reactive hydrocarbon with a chlorophenol	guppy	14-d LC50	equitox	2	1.2–1.5	0.42 – 0.74	Hermens *et al.* (1985a)
11 non-reactive hydrocarbons and 11 chlorophenols (in 4-fold)	guppy	14-d LC50	equitox	22	1.2–1.5	0.87 – 0.94	Hermens *et al.* (1985a)
4 different combinations of a chlorophenol with a chloroaniline	guppy	14-d LC50	equitox	2	1.4–1.5	0.42 – 0.51	Hermens *et al.* (1985a)
11 chlorophenols and 11 chloroanilines (in 4-fold)	guppy	14-d LC50	equitox	22	1.0–1.2	0.94 – 1.00	Hermens *et al.* (1985a)
4 different combinations of a chlorophenol with a chloroaniline	guppy	14-d LC50	equitox	2	1.4–1.5	0.42 – 0.51	Hermens *et al.* (1985a)
11 chlorophenols and 11 chloroanilines (in 4-fold)	guppy	14-d LC50	equitox	22	1.0–1.2	0.94 – 1.00	Hermens *et al.* (1985a)
4 different combinations of a non-reactive hydrocarbon with a reactive organic halide	guppy	14-d LC50	equitox	2	0.8–1.8	0.15 – 1.15	Hermens *et al.* (1985a)

TABLE III (*continued*)

Toxicants	Species	Response	Ratio	*n*	*M*	MTI ± *sd*	Reference
9 non-reactive hydrocarbons and 9 reactive organic halides (in 4-fold)	guppy	14-d LC50	equitox	18	1.1–1.4	0.88 – 0.97	Hermens *et al.* (1985a)
4 different combinations of a non-reactive hydrocarbon with a chlorophenol and a chloroaniline	guppy	14-d LC50	equitox	3	1.4–1.6	0.57 – 0.69	Hermens *et al.* (1985a)
11 non-reactive hydrocarbons and 11 chlorophenols and 11 chloroanilines (in 4-fold)	guppy	14-d LC50	equitox	33	1.2–1.5	0.88 – 0.95	Hermens *et al.* (1985a)
21 non-reactive hydrocarbons (e.g. alcohols, chloroalkanes and chloroaromatics)	*Photobacterium phosphoreum*	bioluminescence inhibition EC50	equitox	21	2.0	0.77 ± 0.8	Hermens *et al.* (1985b)

1,4-dichloro-2-butene, chloroacetone and 1-chloro-2,4-dinitrobenzene) in equitoxic concentrations was strictly concentration additive ($M = 1.0$). The chemicals were selected on the basis of their reactivity towards 4-nitrobenzypyridine (a nucleophilic substitution reaction). This reactivity explains to a large extent the differences in toxicity between the individual chemicals, as shown by a calculated QSAR; simple similar action was thus expected *a priori.*

(v) *Chemicals of different structures but with similar modes of actions*

Konemann (1981b) showed that the toxicity of many non-dissociating, non-reactive organic chemicals was not very structure specific and could be predicted by a simple QSAR. It is likely that these chemicals have a common mode of action, based on membrane perturbation, which can result in anaesthesia. This effect constitutes the minimum toxicity, since it is likely that all organic chemicals can exert this effect in principle, although it is often masked by more specific modes of action which are exerted at lower concentrations. The validity and wide applicability of this approach has been confirmed by others, including Veith *et al.* (1983), Lipnick and Dunn (1983) and Hermens *et al.* (1984b). For a mixture of 50 of these non-reactive chemicals (chloroaromatics, chloroalkanes, alcohols and ethers) in equitoxic concentrations, the toxicity was strictly concentration additive ($M = 1.0$). Thus concentrations as low as 0.02 LC50 appeared to contribute to the lethality of the mixture.

Broderius and Kahl (1985) obtained similar results for the 4-d LC50s of equitoxic mixtures of 2 to 21 chemicals (alcohols, ketones, ethers, alkylhalides, benzenes, nitriles and tertiary aromatic amines) to fathead minnows, M varying from 0.9 to 1.2 (MTI from 0.93 ± 0.08 to 1.2 ± 0.3). Broderius and Kahl verified the similarity of the modes of action of these chemicals by comparing the slopes of their concentration response curves, which proved to be similar. These authors also tested 8 binary mixtures (each consisting of l-octanol and one other chemical from the group mentioned above), each in 7 different ratios of concentrations. The M values of all these mixtures were largely within the range 0.8 to 1.3.

For similar chemicals to those used by Konemann (1981a), Hermens and co-workers studied the effects of equitoxic mixtures on 48-h EC50 (on immobilisation), 16-d LC50 and 16-d EC50 (on reproduction) for *Daphnia magna* (Hermens *et al.*, 1984b) and on the growth of this species (16-d no observed effect concentrations). For mixtures of 10 chemicals the effects were again strictly concentration additive for immobilisation and mortality, but significantly less than additive for growth reduction ($M = 1.8$; MTI $-$ 0.74 ± 0.11; Hermens *et al.*, 1985d). In the latter case some dissimilarity in modes of action was indicated by a relatively large standard deviation of the QSAR-equation. Further, the effects on reproduction of a mixture of five chemicals was less than additive ($M = 2.0$; MTI $= 0.57 \pm 0.17$).

Hermens *et al.* (1985c) also determined the EC50 (inhibition of bioluminescence) of an equitoxic mixture of 21 of the same category of chemicals as described above, to *Photobacterium phosphoreum* using the Microtox test (developed by Beckman Instruments Inc.). The toxicity of the mixture was less than concentration additive ($M = 2.0$; MTI $= 0.77 \pm 0.08$), suggesting a certain dissimilarity in mode of action between these chemicals. This dissimilarity was also confirmed to some extent by the relatively large standard deviation of the calculated QSAR equation.

(vi) *Chemicals with different modes of action*

Konemann (1981a) determined the toxicity of a mixture of equitoxic concentrations of 9 chemicals, selected to have widely varying modes of action (2 metals, 2 non-chlorinated pesticides and some chlorinated hydrocarbons with different functional groups); the toxicity of this mixture was less than concentration additive ($M =$ 2.5; MTI $= 0.58 \pm 0.12$).

Hermens and Leeuwangh (1982) determined the toxicity of a very heterogeneous mixture of 24 chemicals (e.g. allylalcohol, acrylamide, dieldrin, malathion, menthol, triphenyltin chloride, decamethrin, phenol, etc.) and of five different selections of 8 chemicals of these 24, in equitoxic concentrations. The toxicity of four of the mixtures of 8 chemicals differed little from concentration additive, M varying from 1.1 to 1.3 and MTI from 0.87 to 0.85 (± 0.11). The toxicity of the other mixture of 8 chemicals ($M = 1.7$;

MTI = 0.74 ± 0.11) and of the mixture of 24 chemicals ($M = 2.3$; MTI = 0.74 ± 0.08) were less than additive.

For a different set of 14 chemicals with probably dissimilar modes of action, Hermens *et al.* (1984c) determined the toxicity to *Daphnia magna*. For the 48-h LC50, the equitoxic mixture was concentration additive ($M = 1.2$; MTI = 0.92 ± 0.11). The effect on reproduction (EC50) was less than additive ($M = 2.6$; MTI = 0.43 ± 0.18).

Hermens *et al.* (1985b) determined the toxicity to *Photobacterium phosphoreum* of combinations of 2 and 3 chemicals, each probably with a different mode of action. The chemicals belonged to 4 different groups; within each group similarity of mode of action was assumed, on the basis of calculated QSARs. The four groups consisted of 11 chloroanilines (A), 11 chlorophenols (B), 11 non-reactive hydrocarbons (C) and 9 reactive organohalides (D). Four different combinations of single chemicals from groups A and B were less than additive (M from 1.4 to 1.5, MTI from 0.42 to 0.51). Similar results were obtained for combinations from B and C and from A, B and C; the toxicity of mixtures of chemicals from A and C varied more widely (M from 0.7 to 1.3; MTI from 0.15 to 1.15). Hermens *et al.* also determined the toxicity of all members of Groups A and B, of B and C, of C and D and of A, B and C. The toxicity of all these combinations was concentration additive or slightly less than additive (M from 1.0 to 1.5; MTI from 0.87 to 1.0).

Summary

The results of laboratory tests with fish using mixtures of toxicants commonly found in sewage and industrial wastes, summarised in Table II, show that joint action for acutely lethal concentrations range from 0.4 to 2.6 times those predicted from simple addition of the individual p LC50 values, the median value being about 0.9. More recent data on complex organic mixtures, shown in Table III, indicate that their joint action is either additive or slighly less-than-additive. Thus there is no evidence of marked antagonistic or more-than-additive ('synergistic') effects. Although the results of two unpublished experiments suggested that marked more-than-additive joint action might occur with prolonged exposure to some

heavy metals, there has been no subsequent support for these findings.

THE TOXICITY OF INDUSTRIAL WASTES, EFFLUENTS AND RIVER WATERS

(i) *Sewage effluents*

The predictive method of estimating toxicity from Σ TU has been used to estimate the relative importance of the commonly occurring poisons (ammonia, monohydric phenols, zinc, copper and free cyanide) in sewage effluents in industrial areas in the United Kingdom, samples being taken from a variety of disposal works covering a range of treatment processes and receiving various types of industrial wastes (Lloyd and Jordan, 1963 and 1964); the toxicity of the effluents to rainbow trout was measured under controlled conditions and predicted on the assumption that the toxicity of the mixture of all five poisons could be calculated from chemical analyses in a similar manner to that used in laboratory studies. In 13 out of 18 toxic effluents the predicted toxicities were within ±30 percent of the observed values and 6 effluents were correctly predicted to be non-toxic; only 2 effluents were more toxic than predicted, probably because of the presence of unidentified poisons. The factors by which the observed Σ TU differed from unity were 0.63 to 1.18 (Lloyd and Jordan, 1963) and 0.48 to 3.0 (Lloyd and Jordan, 1964).

Esvelt *et al.* (1973) measured the 96-h LC50 of municipal wastes discharged to San Francisco Bay in 43 tests using golden shiner (*Notemigonus crysoleucas*) and found an average value of 2.2 TU of which only 0.74 TU was not attributable, from statistical correlation, to ammonia or MBAS (methylene blue active substances), i.e. these two toxicants accounted for 0.66 of the observed toxicity; too few data on other water quality characteristics were available to assess their possible contributions.

Servizi *et al.* (1978) measured the 96-h LC50 of effluent from a primary sewage treatment plant using sockeye salmon (*Onchorhynchus nerka*). They could attribute about 0.4 of the toxicity to anionic surfactants and cyanide, but further examination

of their data suggests that the figure would rise to between 0.54 and 0.84 if contributions from un-ionised ammonia (0.01), zinc (0.05) and copper (0.38 to 0.08 depending upon the value assumed for the toxicity of copper in the presence of organic matter), were also taken into account.

Thus what little evidence is available for sewage effluents containing industrial wastes shows that a large proportion of the observed acute lethal toxicity can be accounted for in terms of Σp LC50 of commonly occurring toxicants.

(ii) *Spent gas liquor*

Herbert (1962) showed that, as expected, the toxicity of a spent liquor from a gas works to rainbow trout was largely attributable to its content of ammonia and monohydric phenols, it being very similar to that expected from the additive effect of ammonia and phenol.

(iii) *Pulp and paper effluent*

Compounds isolated from two acidic fractions of effluents from kraft mills were thought, by Leach and Thakore (1975), on the basis of chemical analysis and bioassays, to account for most of the toxicity of other acidic fractions of such effluents.

Later, Leach and Thakore (1977) gave examples showing that there was no clear evidence for more than additive or less than additive toxicity of constituents identified in 11 effluents from debarking, kraft pulping, caustic extraction and mechanical pulping. Measured toxicity was on average 1.03 times that predicted from chemical analysis, the range being 0.81 to 1.54 and often within the range of experimental error of the toxicity tests and chemical analyses.

(iv) *Drilling fluid*

Sprague and Logan (1979) tested rainbow trout in three samples of used drilling fluid, for which composition was estimated rather than measured, and found that the toxicity (based upon 96-h LC50) was 1.4, 1.2 and 0.8 times as toxic as predicted from the sum of estimated p 96-h LC50 values, although the differences from unity

did not appear to be statistically different. However, they tested a simulated drilling fluid containing barium sulphate, potassium chloride, ferrochrome lignosulphonate, bentonite, an industrial xanthate gum, paraformaldehyde and potassium chromic sulphate, and found it (significantly) only 0.6 times as toxic as predicted. Significant differences from predicted toxicity were also found with this fluid when 7 other components were added singly to it. With each added component three mixtures were tested, the ratio of component to fluid being 1:0.43, 1:1 and 1:2.3, and in all cases antagonism was found with one of the three mixtures, the least toxic of which was 0.3 times that expected on the basis of simple addition. Interaction appeared to be less than additive in eight of the remaining twelve mixtures and more than additive in the other four, but none of these appeared to be significantly different from additive interaction. The authors suggested that the adsorptive capacity of the bentonite might have accounted for some of the reduction in toxicity found.

(v) *River waters*

J. F. de L. G. Solbé and F. H. Davies (pers. comm.) tested the toxicity to rainbow trout of samples of water from the R. Churnet, UK, taken on five separate occasions when the predicted 48-h LC50 based upon the content of ammonia, phenol, cyanide, copper and zinc, was less than unity ($\sum p$ 48-h LC50, 0.08 on average). After adding either zinc (on all occasions), nickel (4 occasions), copper or ammonia (one occasion each), to increase the predicted value above unity, the measured 48-h LC50 values were, on average, only 0.66 of those expected.

For data from on-site tests with river waters see Field Studies, below.

THE TOXICITY OF PESTICIDES

(i) *Mixtures of pesticides*

Macek (1975) measured the percentage mortality within 1 to 4 days of bluegill exposed to 29 different mixtures of pairs of pesticides at individual concentrations that were expected to produce less than

40% mortality in 72 h. The results were expressed as the proportion of the total mortality in all concentrations of both chemicals tested singly, to the total mortality for all corresponding concentrations of chemicals tested together, and do not allow conventional analysis. The proportions ranged from 0.28 to 11.7 and are distributed approximately lognormally, with a median value of 1.25 and lower and upper limits of the standard deviation of 0.66 and 2.3 respectively. Six of the mixtures when also tested with rainbow trout gave apparently similar results. This could imply that on average response-addition was slightly more than additive.

Marking and Mauck (1975) measured the toxicity to rainbow trout of 20 of 21 possible paired mixtures of 7 insecticides in the ratio of 1:1 of their respective *p* 96-h LC50 (of unspecified time). For 9 pairs, joint toxicity was between 0.5 and 0.7 times less than additive, for nine others it was not significantly different from additive, and for the remaining two it was 1.4 and 1.7 times more than additive.

Statham (1975a and b) measured the survival of rainbow trout exposed for 4 h to either 2,4-D butyl ester, dieldrin, rotenone or pentachlorophenol, each in the presence of carbaryl, a choline esterase inhibitor, at a concentration of 0.11 of the 96-h LC50, to which the fish had been exposed for 2 h immediately beforehand. More than additive interaction was found in all cases, and in the case of 2,4-D, toxicity (expressed as 4.5 h LC50) was increased 2.7-fold. The more than additive effect of carbaryl with dieldrin and 2,4-D was shown to be reduced considerably in the presence of 10 mg/l atropine, and the additive effect of arecoline on the toxicity of 2,4-D and PCB was also reduced by atropine. The authors (Statham and Lech, 1975a and b) suggested that the effect of carbaryl was related to its muscarine action, and later (Statham and Lech, 1976) showed that carbaryl at a concentration which increased the acutely lethal toxicity of 2,4-D and also of Bayer 73 (2.3-fold in terms of 4.5-h LC50) also significantly increased the concentration of these substances in the blood and whole body of the fish, possibly by affecting the permeability of the gill, but it did not decrease their rates of elimination. Atropine, alone of several blocking agents, also inhibited the carbaryl-induced increase in concentration of 2,4-D and Bayer 73 in the blood.

Anderson and Weber (1975a) found that the effects of PCB and

HEOD (dieldrin) were response-additive, while Marking and Dawson (1975) measured the 96-h LC50 to bluegill of malathion and delnav and found that joint action was markedly (8.2-fold) more than additive.

The 24-h LC50 of 2,4-D(tributyl ester) and trifluralin for insecticide-resistant strains of mosquitofish were about twice as high as the corresponding values for insecticide-susceptible fish (Fabacher and Chambers, 1974) suggesting possible pesticide-induced physiological changes in these organisms.

Fabacher *et al.* (1976) showed that the mortality of mosquitofish in a mixture of methyl parathion and the defoliant tributyl phosphorotrithioate in the ratio of 1:2 of fractions of their respective 48- and 24-h LC50 values was several-fold more than additive.

Dethlefsen (1977) concluded, from extensive studies of the development of eggs and the mortality of larvae of cod (*Gadus morhua*) made in the presence of DDT and DDE, that the adverse effects of the two together was generally slightly more than additive, depending on the response measured.

Koenig (1977) fed adults of the saltmarsh fish (*Adinia xenica*) with a diet containing either pp DDT or mirex, or both, and observed the mortality among the progeny embryos and larvae (expressed as LD50 of the dose administered to the parental females). Interaction between the toxicants was about 1.6 times more than additive. This is consistent with the independent action of these two toxicants in that mortality attributable to DDT occurred sooner after fertilisation than that attributable to mirex, and that the symptoms of poisoning for the two toxicants (reviewed by Koenig) are quite different.

The mortality of rainbow trout dosed *via* their food with DDT, or methoxychlor, in the presence of dieldrin, was less than that resulting from dosing with DDT alone (Mayer *et al.*, 1972).

Krieger and Lee (1973) showed that simultaneous treatment of mosquitofish with diquat and an insecticide did not affect the toxicity of DDT, aldrin or parathion, but markedly enhanced that of carbaryl and inhibited aldrin epoxidation.

Ludke *et al.* (1972) found, with several species of fish, a less than additive toxic effect with mixtures of parathion and aldrin, and similar results were obtained by Ferguson and Bringham (1966)

with mosquitofish exposed to all possible paired combinations of endrin, DDR, toxaphene and methyl parathion.

Bender (1969) reported tests with fathead minnow which showed more than additive interaction between malathion and each of its two main products of hydrolysis.

(ii) *Pesticides and surfactants*

Solon *et al.* (1969) measured the 96-h LC50 to fathead minnow of parathion, DDT and endrin alone, and each in the presence of 1 mg/l (0.003 96-h LC50) LAS. The joint action between LAS and the insecticides was about 1.9, 1.2 and 0.9 times that expected of simple addition.

Solon and Nair (1970) carried out further tests on phosphate pesticides and confirmed this result for parathion, and also found that joint action of LAS and the pesticide was more than additive for methyl parathion (2.1 times), ronnel (1.2 times), trithion (1.7 times) and trichloronat (1.7 times), additive for guthion, and less than additive for EPN (0.85 times) and dicapton (0.85 times), all based upon *p* 48-h LC50. They commented that there was no correlation between the type of interaction found and chemical structure, and speculated that, because of the low aqueous solubility of all the toxicants tested, the additive joint action of LAS might be attributable to its increasing their solubility at the gill or skin membrane, although an effect of LAS on enzymes involved in oxidising and detoxifying them was also seen as a possibility.

Dugan (1967) showed that goldfish exposed to 4 μg/l of alkylbenzene sulphonate for several months became more susceptible to subsequent exposure to 50 μg/l dieldrin in the presence of 4 μg/l ABS than fish not so exposed.

(iii) *Pesticides and miscellaneous substances*

Howland (1969) reported additive joint action between *p* 96-h LC50 values for rotenone and antimycin but his results actually suggest that interaction is, if anything (0.72 times) less than additive.

Marking (1977) reported that there was greater than additive joint action between rotenone and piperonyl butoxide (2.4 times) and rotenone and sulphoxide (between 1.4 and 3.1 times depending

upon the ratio of the two used) when the toxicity (unspecified) was tested using rainbow trout.

A number of other examples, in which the lethal toxicity of mixtures of pesticides and other miscellaneous substances is greater than that predicted from the *p* LC50 of the constituents, are also cited by Alabaster (1969) for formulated products.

Summary

While many data on the acute lethal toxicity of mixtures of pesticides and other substances to fish show that joint action is close to additive, a relatively high proportion, compared with toxicants commonly found in sewage and industrial wastes, show that it is several-fold more than additive.

THE TOXICITY OF OTHER SUBSTANCES

(i) *Chlorine and other substances*

Schaut (1939) found that, following chlorination of solutions containing potassium thiocyanide at a concentration of 6 mg/l, 'minnows' were affected to the same extent as by 3.6 mg/l of sodium cyanide; he considered this to be a consequence of hydrogen cyanide production, although Allen, Blezard and Wheatland (1948) studied the same phenomenon and concluded that the poison was more likely to be cyanogen chloride. Chlorine can combine with a wide variety of organic substances to form stable organo-chlorine compounds (Jolley, 1973) harmful to fish at concentrations as low as 0.001 mg/l (e.g. Gehrs *et al.*, 1974). There is no information on the effect of chlorine in the presence of other poisons with which it does not react chemically.

(ii) *Trifluoromethyl nitrophenol and Bayer 73*

Bills and Marking (1976) measured the toxicity of a mixture of 3-trifluoromethyl-4-nitrophenol (TFM) and 2,5-dichloro-4-nitrosalicylanilide (Bayer 73) in the ratio 49:1, using fingerling brown trout (*Salmo trutta*), rainbow trout, lake trout (*Salvelinus namaycush*), brook trout (*Salvelinus fontinalis*), channel catfish (*Ictalurus*

punctatus), bluegill and yellow perch (*Perca flavescens*) in soft water at 12°C, also rainbow trout in hard water, and eggs and fry of coho salmon (*Oncorhynchus kisutch*). In all cases joint action between the components was additive, except for rainbow trout tested at pH 6.5 and 8.5, when it was (0.7 and 0.6 times, respectively) less than additive. Although Howell *et al.* (1964) reported a mixture of these substances killing ammocoetes of sea lamprey (*Lampetra marinus*) at concentrations which were not toxic when applied singly, Dawson *et al.* (1977) found their effect additive or less than additive to this species.

(iii) *Antimycin and other substances*

Berger (1971), quoted in Marking and Dawson (1975), reported that mixtures of antimycin and TFM were 'synergistically toxic' to black bullhead (*Ictalurus melas*), largemouth bass (*Micropterus salmoides*), and yellow perch, but Marking and Dawson (1975) found that the *p* 96-h LC50 values of antimycin and TFM were additive when tested with bluegill, suggesting that there may be differences between species.

Marking and Dawson (1975) also found additive joint action between antimycin and dibrom, when tested with rainbow trout, but a marked antagonistic reaction between antimycin and potassium permanganate (0.01 times as toxic as expected from additive joint action).

Marking (1969) showed that the interaction between the *p* 96-h LC50 values of antimycin and rhodamine B was additive.

(iv) *Quinaldine sulphate and MS-222*

Dawson and Marking (1973) measured the 24- and 96-h of LC50 mixtures of quinaldine sulphate and MS 222, in the ratio of 1:2 and 1:4 *p* 96-h LC50 using rainbow trout, brown trout, brook trout and bluegill, and in the ratio 1:4 using only lake trout, and found that the joint action between the two, based upon their earlier work with the substances tested separately, was on average 1.3-fold more than additive (range 1.11 to 1.41), with no marked or significant differences between different ratios or species used. Berger (1969), quoted in Gilderhus *et al.* (1973), tested the toxic effect of MS 222

and quinaldine sulphate and a mixture of the two, and found it apparently slightly (1.3 times) more than additive in the mixture.

(v) *Others*

Leteux and Mayer (1972) reported that the efficacy of malachite green and formaldehyde as therapeutic agents was increased (about 1.8 times) when present together in a mixture. Devlaminck (1960) showed that it was possible to sum the toxic fractions of individual poisons in mixtures of 2, 3 and 4 dissimilar substances over a 6 h exposure period.

Abram and Wilson (1979) studied the acute toxicity of o-chlorobenzaldehyde and malononitrile; their data show more than additive joint action between the two (1.4 to 2.7 times) in mixtures of median lethal concentrations between 12 and 96 hours and ratios of the LC50 values of the two substances from 1.3:1 to 8.7:1 (time was confounded with ratios in these tests).

Tsai and McKee (1980) measured the 96-h LC50 of LAS and chloramines and their mixtures in the ratio of 1:0.5 to 1:2 *p* 96-h LC50, using goldfish, and found that toxicity was slightly (1.2 to 1.4 times) more than additive.

Summary

The data on the toxicity of mixtures of pesticides show that joint action may be often more than additive.

CONCLUSIONS ON THE ADDITIVE EFFECT OF TOXICANTS ON FISH

The extent to which the joint effect of toxicants on freshwater fish deviates from additive, may depend upon several factors, including the type of response (for example, whether long-term or short-term and whether lethal or non-lethal), the magnitude (percentage) of response, the type of toxicant and the proportion in which it is present in a mixture, water quality characteristics (such as hardness), species, stage in the life cycle of the fish and prior acclimation to toxicant, and size of the ration.

(i) *Additivity of acute lethal toxicity*

Examination of acutely lethal concentrations to freshwater fish of mixtures of toxicants from 76 sets of data, of which 62 relate to pairs of toxicants, and the remainder to mixtures of three, all referring to commonly-occurring constitutents of sewage and industrial wastes (namely ammonia, phenol, cyanide, copper, zinc, cadmium, nickel, chromium, mercury and other metals and other substances), has shown that these are between 0.4 and 2.6 times those predicted from the sum of the proportions of the respective toxic units of the constituents, with a median value of 0.95; 87% of the results were within 0.5 and 1.5 of the predicted values. Somewhat similar results have been found for 18 sewage effluents based upon the toxicity of toxicants known to be present and for 8 samples of river water, the toxicity of which has been increased experimentally by the addition of known amounts of ammonia, phenol, cyanide, copper and zinc, and also for several drilling fluids and a gas liquor; the median value for all of these is 0.85 of the predicted value. With pesticides and other substances, however, the mixtures tend to be somewhat more toxic than predicted, with a median value of 1.3. These results are summarised in Table II and illustrated in Figure 3. The more recent data summarised in Table III support the general conclusion for toxicants other than pesticides.

(ii) *Magnitude of response*

Most of the data reported on lethal concentration relate to median values, but in one study with ammonia and copper (Herbert and Vandyke, 1964) the joint action, while additive in terms of the median, was 1.2 and 1.4 more than additive for the LC25 and LC10 values respectively. Also, in the case of mixtures of copper, zinc and cadmium on the production of eggs by fathead minnow, interaction was approximately additive for a reduction of 90% but less than additive for a reduction of 50%. However, it is possible that such a result is an artifact resulting from progressive differences in proportional bio-availability of the three chemicals with increase in total concentration.

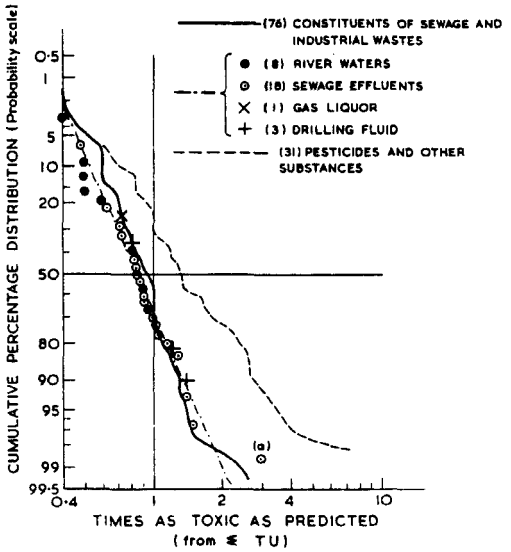

FIGURE 3 Summary of laboratory data on predicted/observed sum of toxic units for freshwater fish. Number of sets of data shown in parentheses. Results relate largely to short-term LC50 values to rainbow trout but include data on other species and effects on growth (see text). '(a)' represents an effluent having a high content of industrial wastes including pesticides.

(iii) *Time of response*

Few data are available on the relation between short- and long-term joint toxic action of constituents in mixtures. However, it has been shown for mixtures of cadmium and mercury (L. A. Hewitt and P. D. Anderson, pers. comm.) and for mixtures of chromium and nickel (F. S. H. Abram, pers. comm.) that, while joint action may be additive in the short term (4-d LC50) it may be more than additive in the longer term (two-fold at 10 days for cadmium and mercury and up to 21-fold at 10 weeks for chromium and nickel).

(iv) *Type of response*

Broderius and Smith (1979), examining mixtures of ammonia and cyanide, cyanide and zinc, and cyanide and chromium, found that the type of joint toxicity observed for fish exposed to lethal concentrations of these mixtures was not found when they were

subject to sub-lethal levels of the same toxicants; for example, whereas the joint acutely lethal action of ammonia and cyanide on rainbow trout was 1.16 times more than additive, the joint effect on growth over 30 d was 0.6 times as additive. The corresponding figures for fathead minnow exposed to zinc and cyanide were 1.4 and 0.4 respectively although for this species exposed to chromium and cyanide the figures were 0.8 and between 0.6 and 0.8 respectively. There are few other data on the effect of mixtures on growth; the range of factors by which the observed effect differs from the predicted is 0.4 to 1.0, and for one set of data on fish production (without comparable data on acutely lethal effects) the figure is 0.6 (Neglinski and Davis, 1974) and for another on growth in the presence of HCN and arsenic (G. Leduc, pers. comm.) it is 0.6 and 0.8.

It is possible, therefore, that as concentrations of toxicants are reduced towards the level of no effect (NOEC), their potential for addition is also reduced. However, this may not apply to sub-lethal physiological effects on the whole organism; for example whereas zinc and copper were additive in terms of the threshold LC50 for Atlantic salmon (Sprague and Ramsey, 1965), their joint effect on avoidance behaviour was slightly (about 1.3 times) more than additive (Sprague, Elson and Saunders, 1965). Nor does it apply to the acute toxicity of mixtures containing many toxicants with a common QSAR (see above); however, the toxic contribution to a heterogenous mixture of such a combination whose combined toxicity is considerably lower than the acute concentration is not known.

(v) *Relative proportion of toxicant*

Comparatively little attention has been given to the effect on joint toxicity of the relative proportions of the toxicants present, although it has been amply demonstrated in some cases that these can be profound (see, for example, Sprague and Logan, 1979). It has been shown that one constituent at concentrations below a certain *p* LC50 may not contribute any toxic effect, for example with mixtures of ammonia and nitrate (Rubin and Elmaraghy, 1977), and with mixtures of copper and surfactant (Calamari and Marchetti, 1972). This reinforces the proposition made in the previous section.

Sprague (1970) pointed out that the toxicity of mixtures of ammonia, phenol and zinc was over-estimated by Brown *et al.* (1969) in three of four cases when two of the toxicants were present at 0.1 to 0.14 TU whereas when they were present at 0.2 TU or more, interaction was additive. The data of Brown *et al.* also suggest that the level of no effect for acute toxicity is about 0.2 TU for metals. If this were a general phenomenon, one would expect the results when plotted (Figure 2), to lie to the right and parallel to the line marked 'additive', but within the triangle marked 'less than additive'. Examination of all the data summarised in Table II, however, shows only a slight tendency in this direction.

The most clear-cut picture relates to mixtures of phenol and other substances as plotted in Figure 4; in this case a relatively large number of tests has been carried out at low TUs of phenol. While the figure can be interpreted as indicating less-than-additive joint action when the TU of phenol is less than about 0.3, it also shows that at a TU of phenol of about 0.1, there is an apparent marked antagonism between phenol and other toxicants; however, there are too few data even from these experiments to be able to draw any general conclusions abot the contribution of low TU values for the other toxicants present in the mixtures.

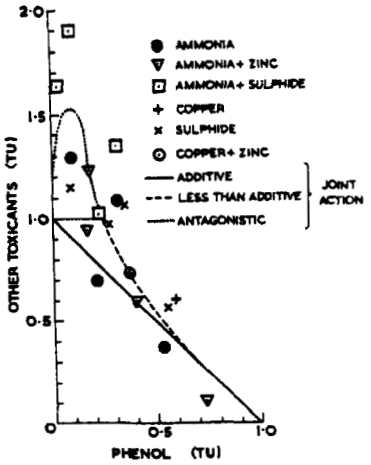

FIGURE 4 Toxic units of phenol and other toxicants in mixtures producting a given lethal response in fish (data calculated from those in Table II).

(vi) *Species of fish*

There are few data available to compare the additive effect of a given mixture on toxicity to two or more species of fish, and none are available to make this comparison for identical experimental conditions. Yet, from Table II it can be seen that for mixtures of ammonia and phenol with rainbow trout and fathead minnow, and for mixtures of copper and zinc with rainbow trout, Atlantic salmon and longfin dace, similar results are obtained. However, for mixtures of cyanide and zinc, the joint action is apparently 0.4 times as additive for bluegill and 1.4 times as additive for fathead minnow, while the results for copper, zinc and cadmium are equivocal. In any event, there is not strong evidence for marked consistent differences between species in their response to the joint effect of mixtures of toxicants.

INVERTEBRATES

Fewer data are available on the effect of mixtures of substances on aquatic invertebrates than on fish.

(i) *Phenols*

Herbes and Beauchamp (1977), in tests with *Daphnia magna,* demonstrated less than additive, and also antagonistic, joint action between methylquinoline and resorcinol, depending upon the relative *p* 48-h LC50 of each present in the mixture, the lowest LC50 of the mixture being about 0.6 of that predicted from additive joint action.

(ii) *Copper and zinc*

Buikema *et al.* (1977), measuring the response (immobility) of the rotifer *Philodina acuticornis* to mixtures of copper and zinc, obtained rather variable results, but found no evidence that joint action was not additive. Biesinger *et al.* (1974) give an example of chemical reduction in toxicity of copper and zinc by chelation with NTA using *Daphnia* as the test organism.

(iii) *Zinc and other substances*

Gray and Ventilla (1973) measured the depression in growth rates of a sediment-living marine protozoan, *Cristigera* sp., in the presence of zinc, lead and mercury singly, and in all their combinations, and found both more and less than additive interaction depending on the concentrations tested.

Maksimov (1979) measured the numbers of rotifers present in a natural plankton community at the end of a 17-d test period in the presence of mixtures of zinc and chromium. An examination of his data suggests that the joint effect of the toxicants in reducing numbers by one order of magnitude is about 2.4-fold more than additive.

(iv) *Cadmium, malathion, methoxychlor and Arochlor* 1254

Bahner and Nimmo (1975) using the estuarine shrimp *Penaeus duorarum*, concluded that in mixtures combining cadmium with malathion, or methoxychlor, or both methoxychlor and Arochlor 1254 (PCB), as well as in a complex industrial waste in which cadmium was present, interaction between the toxicants was additive. They (Nimmo and Bahner, 1977) also measured the survival of the shrimp for at least 25 days in the presence of cadmium and methoxychlor at several concentrations and in a mixture of the two. Examination of the results on the basis of the estimated 26-d LC50 values suggests that interaction between these two toxicants was more than additive. Further 10-d tests with different proportions of the two did not show no more than additive interaction.

(v) *Chromium and other substances*

Buikema *et al.* (1977), measuring the response (immobility) of the rotifer *Philodina acuticornis* to mixtures of two toxicants, obtained rather variable results, but found more than additive interaction with chromium and chlorine and no marked departure from additive interaction with mixtures of chromium and fluoride and of chromium and copper.

(vi) *Other substances*

Nitrosalicylanilide and 4-nitro-3-trifluoromethyl phenol were tested individually and in mixtures of the two for toxicity to the ostracod *Cypretta kawatai* (Kawatski, 1973), and examination of the 96-h LC50 values shows that joint action is about 1.4 times more than additive for mixtures in which the toxicants are in the ratio of 1:1 and 49:1 of their respective *p* 96-h LC50s values.

Additive, less than additive, and non-interactive toxicity to *Daphnia magna* of mixtures of 2 and 3 chemicals was observed by Freeman and Fowler (1953) using inorganic substances or very similar organic compounds.

Additional data for organic chemicals are given in a previous section and Table III.

(vii) *Pesticides*

Lichenstein *et al.* (1973) measured the percentage mortality of 3rd instar larvae of the mosquito (*Aedes aegypti*) held for 24 h in static solutions containing initial concentrations of either 0.016 mg/l parathion or 0.18 mg/l DDT alone (in which the percentage mortality was 15 and 10 respectively), and also in the presence of 10 mg/l of one of four herbicides. The interaction between parathion and the herbicides was more than additive (×5.3 for atrazine, ×4.5 for simazine, ×3.5 for monuron and ×3.2 for 2,4-D), while that between DDT and the herbicides was approximately additive, although the nominal concentration of DDT was probably not maintained because it was higher than the solubility value.

Lichenstein *et al.* (1974) showed that an aqueous extract of the plant, dill (*Anethum graveolus*), and a constituent of the plant, d-carvone, increased the percentage mortality of 3rd instar larvae of the mosquito (*Aedes aegypti*) over 24 h in the presence of carbaryl, carbofuran, parathion and DDT.

Summary

In general, the few data available for the joint effect of toxicants on aquatic invertebrates show, as in the case of fish, that for constituents commonly present in sewage and industrial wastes, where

the concentration-addition model can be tested, it is approximately additive. Also, in the case of those pesticides tested, the joint effects are generally markedly more than additive.

AQUATIC PLANTS

Fewer data are available on the combined effects of toxicants on aquatic plants than on other organisms.

Metals

(i) *Zinc and cadmium*

Hutchinson and Czyrska (1973) showed that, at a concentration of 10 μg Cd/l, the growth of *Salvinia natans* and of *Lemna valdiviana* under a variety of conditions was generally slightly inhibited. This effect was increased in the presence of either 50 or 80 μg Zn/l (except when *Lemna* was tested when in competition with *Salvinia*), even though those concentrations of zinc alone were stimulatory. Similar results were obtained, however, with *Salvinia* (tested alone) in the presence of 30 μg Cd/l, both alone and with either 30 or 80 μg Zn/l, and with *Lemna* (tested in competition with *Salvinia*) when tested in the presence of 30 μg Cd/l and 80 μg Zn/l.

On the other hand, the data of Say (1977) showed that there was between 1.4 and 1.7 times more than additive interaction between cadmium and zinc in their combined effect on the inhibition of growth of the alga *Homidium rivalare,* a threshold response being obtained, for example at *pt* EC values of cadmium and zinc in the ratios 1:0.4 and 1:5.

Laborey and Lavollay (1967) measured the percentage reduction in the growth of *Aspergillus niger* in the presence of cadmium and zinc separately, and together in a number of mixtures, and examination of their data shows that the joint action was 0.4 and 0.5 times less than additive in the presence of 70 and 25 mg Mg/l respectively.

Thus contradictory results have been obtained with mixtures of zinc and cadmium, depending upon species, but they are within the range 0.4 to 1.7 times as toxic as predicted.

Hutchinson and Czyrska (1973) found that zinc appeared to result in a higher cadmium uptake by *Lemna.*

(ii) *Zinc and sulphide*

An example of a less than additive effect, attributable to chemical interaction in the water, is given by Hendricks (1978) for the effect of zinc and sulphide on oxygen production by the alga *Selenastrum capricornutum.*

(iii) *Copper and nickel*

Hutchinson and Czyrska (1973) found that when nickel and copper were present at the same time the uptake rates of both metals by *Lemna* were increased.

(iv) *Cadmium and nickel*

Stratton and Corke (1979) measured the response of the alga *Anabaena inaequalis* to mixtures to cadmium and nickel; examination of their data, including those for the toxicants tested singly (from their other cited papers) to calculate concentrations corresponding to 50% reduction in response compared with controls, suggests that joint action is less than additive both for growth at 10 days (0.5–0.74 times) and for acetylene reduction (up to 0.63 times). For the uptake of carbon dioxide joint action for different mixtures was inconsistent.

(v) *Cadmium, nickel and mercury*

Stratton and Corke (1979) measured the percentage response of *Anabaena inaequalis to* mixtures of cadmium, nickel and mercury, and an examination of their data, including those for the toxicants tested singly (from their other cited papers) to calculate concentrations corresponding to 50% reduction in response compared with controls, suggests that joint action is (0.4 times) less than additive (with the three toxicants in the ratio 1:0.9:1.1 of their respective *p* EC50 values). Joint action is, however, inconsistent for reduction of acetylene being more than additive (1.2-fold) with the three toxicants in the ratio 1:3.6:0.4 of their respective *p* EC50 values and less than additive (0.6 times) with a corresponding ratio of

1:3.5:2; it is also inconsistent for uptake of carbon dioxide, being additive with a ratio of EC50 values of 1:1.2 and less than additive (0.5 times) with a corresponding ratio of 1:1:1.

(vi) *Cadmium and mercury*

Stratton and Corke (1979) measured the response of *Anabaena inaequalis* to mixtures of cadmium and mercury, and an examination of their data, including those for the toxicants tested singly (from their other cited papers) to calculate concentrations corresponding to 50% reduction in response compared with controls, suggests that joint action is less than additive for growth at 10 days (0.46 times) and for uptake of carbon dioxide (up to 0.7 times), and more than additive for acetylene reduction (1.1 to 2.3 times). The results were, however, different when exposure to the two metals was in sequence rather than simultaneous, and depended also upon the concentrations of each used.

(vii) *Cadmium and lead*

Pietiläinen (1975) using natural phytoplankton communities tested in the laboratory, measured the reduction in primary production within a 24-h period in the presence of cadmium, lead and mixtures of the two. EC50 values calculated from these data showed that joint action was 0.7 times less than additive with cadmium and lead present in the ratio 1:0.1 of their respective EC50 values and about 0.2 times with a corresponding ratio of 1:11.

(viii) *Nickel and mercury*

Stratton and Corke (1979) measured the percentage response of *Anabaena inaequalis* to mixtures of cadmium and nickel, and an examination of their data, including those for the toxicants tested singly (from their other cited papers) to calculate concentrations corresponding to 50% reduction in response compared with controls, suggests that joint action is more than additive for growth at 10 days (1.2 times), unless exposure is not simultaneous, when it can be additive if exposure is to mercury followed by nickel and less than additive if exposure is first to nickel. Joint action appears to be

less than additive for acetylene reduction (up to 0.8-fold) and inconsistent for carbon dioxide production.

Pesticides and other substances

Mosser *et al.* (1974) observed substantial growth inhibition when a strain of the marine diatom *Thalassiosira pseudonana* was treated simultaneously with 10 μg/l PCB and 100 μg/l DDE, while growth of the diatom was only slightly reduced when treated with either of these substances at the same concentrations, suggesting a more than additive joint action. In contrast, the joint action of PCB and DDT was less than additive; when the diatom was treated with 50 μg/l PCB, growth was almost stopped, but the simultaneous addition of 500 μg/l DDT restored growth to about two-thirds of that in control cultures. The addition of DDT-12 to 24 h after inoculation with PCB, also reversed the inhibition caused by the latter. The mechanism appeared to be an intracellular interaction rather than a physical process, such as co-precipitation, because it was reversible on removal of the pesticide. Additive joint action was also demonstrated between DDT and DDE.

Walsh *et al.* (unpublished, but quoted in Walsh, 1978) found that the herbicide 2,4-D inhibited the toxicity to algae of nickel and aluminium.

Tsay *et al.* (1976) demonstrated that copper ions inhibited toxicity of 2 mg/l paraquat (1,1-dimethyl-4,4′-bipyridinium ion) to *Chlorella pyrenoidosa*, whereas cyanide ion enhanced it.

Recent data for the green algae (*Chlamydomonas*) tested in mixtures of arsenic and PCB (Christensen and Zielski, 1980) show that toxicity (concentrations producing 50% reduction in growth) is about 0.8 times as great as expected from purely additive effects of the components.

Summary

Very few data are available on the joint effect of toxicants on plants compared with those for fish and for other aquatic organisms. For heavy metals, results vary according to the species tested, e.g. even within a species such as *Anabaena* for which a considerable amount

of data is available, results with zinc and cadmium vary inconsistently according to the toxicant and proportion of toxicant used, and the response measured. Nevertheless, all the data fall in the range 0.2 to 2.3 times as toxic as predicted for the concentration-addition model, with a median value of about 0.7.

For pesticides and other substances there are too few data on which to draw general quantitative conclusions but joint action is more than additive in some cases and less than additive in others.

FIELD STUDIES

This section describes a number of field studies which serve to confirm conclusions on the additivity of toxicants derived from laboratory experiments on the toxicity of mixtures of poisons to fish.

Stream receiving gas liquor

The recognition that the toxicity to fish of gas liquors from coke oven plants could be predicted in the laboratory by summing the proportions of 24-h LC50s ($\sum p$ 24-h LC50) for ammonia and gas liquor phenols (Herbert, 1962) was followed by investigations in a stream polluted by gas liquors. Batches of rainbow trout were held in cages in the stream below the discharge and water samples were taken every two hours for subsequent analysis. Values for $\sum p$ 24-h LC50 for gas liquor and un-ionised ammonia were compared with the observed fish mortality; a correct prediction was assumed if half a batch of fish died when the sum exceeded 1.0 for any period during the 24-hour exposure period or when less than half the batch died and the sum did not exceed 1.0. Although the results of 40 individual days' observations gave an 80 percent correct prediction on this basis, the predictive model had considerable deficiencies. The concentration of pollutants showed rapid fluctuations and on several days was very low. Inspection of the data shows that the 24-h LC50s for both ammonia and gas liquor phenols could be reduced by up to five-fold without affecting the accuracy of the predictions. These field experiments failed to confirm the laboratory findings.

Rivers receiving sewage effluents containing industrial wastes

Following laboratory experiments on the predicted and observed toxicity to fish of sewage effluents containing industrial wastes (Lloyd and Jordan, 1963, 1964) a series of field experiments was carried out in which rainbow trout were exposed to polluted river waters either in cages, or, if the dissolved oxygen content of the water was too low, in aerated aquaria on the river bank. Again, water samples were taken every two hours and analyses of labile water quality characteristics were made immediately. Predictions of toxicity were made by summing the proportions of the threshold LC50 ($\sum p$ LC50) for ammonia, phenol, copper and zinc in the early studies (Herbert *et al.*, 1965); cyanide was included later (Brown *et al.*, 1970). Other metals were analysed for, but the concentrations found were thought to be of insignificant importance for acute toxicity. The time series of $\sum pt$ LC50 was compared with the observed mortalities. Mortalities usually occurred within a 3 day obervation period and since, in the hard river waters investigated, the threshold LC50s were close to the 48-h values, it was assumed that 50 percent of the fish should die within 48 hours if the $\sum pt$ LC50 exceeded unity during that period. Generally, it was found that the calculated toxicity tended to under-predict the observed mortalities, and, for some heavily polluted rivers, only 60 percent of the observed toxicity was accounted for by the predictive method (Brown *et al.*, 1970). Where a river contained elevated levels of copper, which might have been complexed with soluble organic matter, a 48-h LC50 higher (up to three fold) than the laboratory-derived value for copper in clean water was used by Herbert *et al.* (1965).

These experiments showed that for those river waters which were acutely toxic to rainbow trout, the predicted toxicity was close to that observed, even though the concentrations of any one poison would have been insufficient alone to kill fish. Under-predictions could be explained by the probable presence of unmeasured pollutants. To this extent, the field investigations generally support the laboratory experiments.

However, parallel attempts were made to derive from the laboratory and field data on acute toxicity a prediction of the maximum values of $\sum pt$ LC50 or 48-h LC50 which would allow

natural fish populations to survive, as opposed to causing mortality among captive rainbow trout. For example, Herbert *et al.* (1965) thought that a fishery of some kind could be maintained in waters where the toxicity was kept below 0.2 of the threshold LC50, provided that the dissolved oxygen was maintained at a satisfactory level. A new approach to this aspect of the problem was begun by Edwards and Brown (1966) who attempted to find the limiting $\sum pt$ LC50 above which a fishery would not exist. Pollutant concentrations in single water samples taken from 100 stations in the R. Trent, UK, were measured and the values of $\sum pt$ LC50 for the individual poisons present were correlated with the status of the fish populations there. They concluded that non-salmonid fish populations could generally exist where the $\sum pt$ LC50 for rainbow trout did not exceed 0.3 to 0.4, provided that the dissolved oxygen concentration was greater than 50 percent of the air saturation value and the suspended solids no greater than 60 to 100 mg/l. This value of 0.3 to 0.4 was similar to that obtained from a recalculation of data given by Allan *et al.* (1958) for the survival of a mixed non-salmonid fishery in a sewage effluent channel (Herbert *et al.*, 1965).

Later, Brown *et al.* (1970) criticised the adoption of these values on several grounds, including the tendency of predictions based on a limited number of pollutants to under-estimate the toxicity of river waters. Long-term experiments showed that substantial mortalities occurred among rainbow trout exposed to concentrations as low as 0.2 of the 48-h LC50, particularly with chromium and cadmium. Also, the fish populations observed at field sampling stations may have been able to move in and out of the polluted stretch and were not necessarily continuously exposed to the toxic conditions.

To this list of potential sources of error several others can be added. The initial summation of proportions of toxic concentrations was based on threshold LC50s (Herbert *et al.*, 1965) which, for the significant poisons and environmental conditions encountered in the field experiments, were close to the 48-h LC50s (Herbert *et al.*, 1965). Subsequently, 48-h LC50s were quoted in the basic method for calculating the toxicity of mixtures of toxicants (Brown, 1968) even though some of the values given (for example, copper and zinc in soft water) were for far longer exposure periods. These data were

still based on laboratory experiments with rainbow trout, and the relevance of the predictive model to field situations where a number of other, less sensitive, species of fish existed was not seriously questioned, mainly because of the lack of basic data for these species (Brown, 1968). Finally, in proposing limiting values for $\sum p$ 48-h LC50s of up to a maximum of 0.4, it was not made clear that the mean values in rivers would be considerably less, probably lower than 0.2.

This aspect was developed by Alabaster *et al.* (1972), who studied the annual frequency distribution of $\sum p$ 48-h LC50s at each of a number of sampling sites in the R. Trent catchment area; these distributions tended to be lognormal. Data on the status of the fisheries at these sampling points indicated that some sort of non-salmonid fish population was present when the median $\sum p$ 48-h LC50 (rainbow trout) was below 0.28, with a corresponding 95 percentile value of about 0.60. This upper limit was higher than those previously proposed; one possible cause was that, at the sites where the fish population was marginal, a significant proportion of the toxicity was caused by copper and although it was recognised from earlier work that the presence of organo-copper complexes would reduce the toxicity, an adjustment similar to that used by Herbert *et al.* (1965) was not made. However, it was recognised that the use of 48-h LC50s underestimates the toxic contribution made by cadmium and nickel in the long-term and that values for *pt* LC50 (rainbow trout) would be considerably higher for those sites where marginal fisheries existed. The scientific validity of using rainbow trout data in a model to predict the status of non-salmonid fisheries was questioned, but the general utility of the approach was defended on pragmatic grounds.

The same approach was also applied to data made available by Yorkshire Water, UK, on the quality of river water at 14 points within its area over a 6-year period; results were similar to those found for the River Trent catchment. There were too few data to define the exact boundary distribution of calculated toxicity between fishless and fish supporting waters, but the limiting p 48-h LC50 (rainbow trout) found for the Trent discriminated clearly between 12 stations, there being one supporting only minnows and sticklebacks, on the fishless side of the boundary, and another marginal case where the river had for unknown periods been both

fish-supporting and fishless (J. S. Alabaster and I. C. Hart, pers. comm.).

Solbé (1973) analysed chemical and fisheries data from the Willow Brook, Northamptonshire, UK, a stream polluted mainly by ammonia and zinc, and found that it was fishless over a 3-year period, where the median and 95-percentile values of $\sum p$ 48-h LC50 (rainbow trout) were 0.45 and 1.45 respectively. Good non-salmonid fish populations existed where the corresponding values were less than 0.25 and less than 0.9, and introduced brown and rainbow trout survived where values of 0.17 and 0.62 were found.

Lake and river systems receiving heavy metals

A similar exercise, but with monthly sampling, was reported by EIFAC (1977) in which the status of salmonid fish populations in a Norwegian lake and river system was correlated with the concentrations of copper and zinc present. In this study, the heavy metal concentrations (as 95 percentiles) were expressed as proportions of the corresponding EIFAC proposed water quality standards (synonomous with the no observed effect concentration, NOEC) for brown trout. A number of factors prevented this study from showing whether a satisfactory water quality for fisheries exists where the NOECs for individual pollutants are not exceeded, or whether the summed p NOECs should not exceed unity. Making allowances for the possible complexing of copper ions with soluble organic material, salmonid populations were unaffected where the $\sum p$ NOEC (95 percentile) was 2.0 but were absent where the value was 5.0. In terms of $\sum p$ 48-h LC50 (from EIFAC 1976, 1977), the corresponding 95 percentile values are 0.28 and 0.79.

Copper and zinc were also found to be the principal toxicants present in trout streams (hardness about 20 mg/l as $CaCO_3$) in the catchment of the R. Mawddach in Wales, UK; where the annual median and 95 percentile p 48-h LC50s (rainbow trout) of these two toxicants were about 0.3 and 0.8 respectively, the biomass of the fish was reduced; where the corresponding values were about 0.5 and 1.0 respectively, fish were absent (Cremer and Warner, pers. comm.). The ratio of p 48-h LC50 of copper to that of zinc varied

spatially and temporally, but annual median values ranged from 1:3 to 1:2 for different stations.

In a similar study of Canadian lakes in the Flin Flon area, Van Loon and Beamish (1977) found a slightly reduced population of fish in Hamell Lake (total hardness based on Ca of 40 mg/l as $CaCO_3$) where the average concentrations in 3-monthly samples were 300 μg Zn/l and 15 μg Cu/l. There were only small seasonal differences in heavy metals content of the water. Based on EIFAC data, the zinc and copper present represented 0.22 and 0.17 of the *pt* LC50 for salmonids respectively, giving a $\sum pt$ LC50 of 0.39 and a corresponding $\sum p$ NOEC (95 percentile) of 3.1. Cliff Lake, with similar hardness, contained zinc concentrations of up to 120 μg/l and copper up to 11 μg/l, representing a $\sum pt$ LC50 of 0.21 and a $\sum p$ NOEC (95 percentile) of 1.4; this lake contained an abundance of fish, including coregonids. These values are in agreement with those obtained in the Norwegian study.

It is possible, therefore, that concentrations of poisons of less than the individual NOECs do not have an additive action in mixtures; alternatively, since the pollution in these three study areas had been present for many years, some acclimation by the fish populations, or genetic selection, may have occurred. There was certainly no evidence of more than additive joint action for these two metals.

Current studies

The approach outlined above is being extended in the UK; predictive models incorporating $\sum p$ 48-h LC50s and *p* NOEC for different fish species groups are being used to correlate water quality at contaminated river sites with the status of fisheries there (J. S. Alabaster, R. Lloyd and J. F. de L. G. Solbé, pers. comm.). One factor which has emerged is the importance of dissolved oxygen, which has had to be incorporated separately into the model.

Summary

In summary, it has been shown that toxicity predictions based on chemical analysis can be made if the waters which are polluted are

acutely lethal to fish, and that a fish population of some kind can exist where the median $\sum pt$ LC50s (rainbow trout) is <0.2. It is not known whether this condition is equivalent to a $\sum p$ NOEC of <1.0 (i.e. the sum of the individual fractions of the NOEC for the species present) or to a NOEC of <1.0 for each individual toxicant (i.e. where the fractions of the NOEC are not summed).

UPTAKE OF TOXICANTS

The EIFAC Technical paper 37 Rev. 1 contains a review of information on the uptake by aquatic organisms of chemicals present as mixtures. It was concluded that there are comparatively few data available on the joint effect of toxicants on their uptake in aquatic organisms. However, it is clear that, in the case of metals, the uptake of one may be increased or decreased according to the concentration of the other metal present; in the presence of other substances it may be either increased or decreased or not affected at all, depending on the substances present. With pesticides, feeding trials have shown the interaction between dieldrin, DDT and methoxychlor to be complicated but generally mutually inhibitory. In general, the joint effect of toxicants on the lethal and sub-lethal responses of fish are not explained by changes in the uptake of the substances concerned.

SUMMARY AND CONCLUSIONS

The choice of the most suitable model to study the joint effects of mixtures of poisons on fish and other organisms depends on the type of information required. For the production of mixtures with maximum lethality, e.g. in the development of pesticides, response-addition has been widely used, whereas for the protection of freshwater organisms by the application of water quality standards, the concentration-addition model is more relevant. For this reason the experimental data reviewed in this report have been derived mainly from experiments based on the latter model and particular attention has been given to identifying the extent of deviations from a strictly additive relationship. Where possible, raw data presented

within a response-addition model have been re-examined on the basis of the concentration-addition model.

The conclusions drawn from the data are discussed in relation to the formulation of water quality criteria for freshwater fish regarding mixtures of toxicants. Attention is drawn to research needs.

Summary of data

Most of the laboratory data reviewed relate to freshwater fish. For those species, the extent to which the joint action of toxicants deviate from addition may depend upon several factors, including: the type of toxicant and its proportional contribution to the toxicity of the mixture; water quality characteristics (such as hardness) and its effects on toxicant speciation; the test species, the stage in the life cycle and prior acclimation to the toxicant, and the size of the ration in growth studies; the measured response (for example, whether long-term or short-term, or whether lethal or non-lethal) and the magnitude (percentage) of the responses. Some research has been conducted which indicates the relative importance of these variables in the joint action of toxicant mixtures but more is required.

For mixtures of commonly occurring constituents of sewage and industrial wastes (for example, ammonia, phenol, cyanide, copper, zinc, cadmium, nickel, chromium and mercury) tested as pure chemicals in the laboratory, it has been shown that the acute lethal toxicity is between 0.4 and 2.6 times that predicted from the sum of the proportions of the respective toxic units (f_i) of the constituents, with a median value of 0.95; 87% of the results lie within the range 0.5 to 1.5 of the predicted values. More recent laboratory data on the toxicity of complex mixtures of organic substances also indicate that their joint action is either additive or slightly less than additive. Somewhat similar results have been found for (a) sewage effluents, based on the predicted contribution of toxicants known to be present, (b) samples of river water, the toxicity of which has been increased experimentally by the addition of known amounts of ammonia, phenol, cyanide, copper and zinc, and (c) several drilling fluids and a gas liquor; the median value for all of these is 0.85 of the predicted value.

For some mixtures of pesticides and other substances, however,

the acute lethal toxicity tends to be somewhat greater than predicted; however, the median value was about 1.3 and other, more recent, data tend to confirm an additive joint action.

Most of the data reported derived from a lethal response relate to median lethal values (LC50s), but one study with rainbow trout using ammonia and copper showed that the joint action, while additive in terms of the LC50, was 1.4 times more than additive for the LC10 value. Whether this effect is of practical significance is a matter for conjecture and possibly further research.

Comparatively little attention has been given to the effect on joint toxicity of the relative toxic proportions of the chemicals present, although it has been amply demonstrated tha these can be profound. In particular, it has been shown that one constituent at concentrations below a certain *pt* LC50 may not contribute any toxic effect, for example with mixtures of ammonia and nitrate, and of ammonia, phenol and zinc. Some data suggest that the critical *pt* LC50 is between 0.1 and 0.2 but the bulk of the data reviewed in this report have not been derived from experiments designed specifically to examine this question. In the case of phenol, however, there is fairly consistent evidence that, at *p* LC50 values above about 0.3, its joint effect with other toxicants is additive, while at *p* LC50 values lower than this, it is less-than-additive, and lower than 0.1, antagonistic.

To some extent, the lack of a suitable model and statistical techniques has contributed to the tentative nature of the conclusions drawn from such experiments with mixed substances of unequal toxicity; the use of the Mixture Toxicity Index may be of value in future work in this field. More recent studies based on this model have confirmed that the joint action of pairs of reactive toxicants vary from less than additive to more than additive, but with an increase in the number of equitoxic substance in the mixture (i.e. lower values of f_i), the results become more consistent and generally display a slightly less than additive joint action. Under these conditions, no 'safe' value of f_i has been established; individual chemicals at concentrations of 0.1 LC50 still contribute to some extent to the toxicity of the mixture.

The conclusions drawn so far apply to mixtures of common substances which are reactive in their joint action. There is also a wide range of organic substances which are largely unreactive in

their toxic action, and which fall into groups with very similar modes of toxic action; this commonality can be demonstrated by QSARs based on physico-chemical properties of the molecule. It has been shown conclusively that mixtures of the substances exhibit a simple similar action, and that the concentration addition model is applicable to their mixtures. However, in contrast to the tentative conclusions drawn for reactive chemicals, such chemicals with a common QSAR and showing simple similar action are additive in their joint action even at concentrations as low as 0.02 LC50.

Few data are available on the relation between the short- and long-term joint toxic action of constituents in mixtures. Unpublished work, however, has shown that for mixtures of cadmium and mercury, and of chromium and nickel, the lethal toxicity may be additive in the short-term, but markedly more than additive in the long-term. These results are not supported by more recent data. Such investigations need to be extended to other mixtures, particularly those which can exert a lethal effect after a long exposure period. Apart from this uncertainty, there is no evidence that mixtures of the common reactive substances, or of non-reactive substances, have markedly more than a concentration additive lethal joint toxic action, and for equitoxic mixtures of more than five substances the joint action is usually slightly less than additive. For all practical purposes, therefore, the possibility of supra-addition or synergism can be discounted for complex mixtures.

In contrast, the few studies with mixtures of toxicants on sub-lethal responses of fish indicate that their combined effect on growth was consistently less additive than the corresponding effect on survival; furthermore, one study showed that the joint effect of toxicants both on fish growth and production was less than additive. It is possible, therefore, that as concentrations of toxicants are reduced towards the level of no effect (NOEC), their potential for addition is also reduced. However, although recent evidence suggests that substances at such concentrations may still contribute towards the toxicity of lethal mixtures, it is unlikely that water quality standards for common toxicants would need to be reduced by more than 50% in order to allow for possible interaction with other toxicants present. In the case of avoidance behaviour of salmonids, the joint effect of sub-lethal concentrations of zinc and copper appear to be slightly more than additive.

There are few data available to compare the joint effect of a given mixture of toxicants to two or more species of fish, and none are available for identical experimental conditions. Nevertheless, there is no strong evidence for inter-specific differences in response to the joint effect of mixtures of toxicants, and the greatest difference found relates to mixtures of cyanide and zinc, for which the joint action was apparently 0.4 times as additive for bluegill and 1.4 times as additive for fathead minnow.

The data available for aquatic invertebrates, like those for fish, show a generally additive joint action for constituents commonly present in sewage and industrial wastes; similarly, data for complex organic mixtures also show a similarity of response for invertebrates and fish. However, as with fish, a more than additive joint action has been demonstrated for some pesticides. There are data for plants which also show a generally slightly less than additive joint action of metals, but only inconsistent and non-quantitative data are available for the joint action of pesticides.

Field studies have shown that predictions of acute toxicity can be based on chemical analysis for those waters which contain sufficient common toxicants to be acutely lethal to fish; also that a fish population of some kind can exist in less polluted waters where the median $\sum pt$ LC50 (rainbow trout) is <0.2. It is not known whether this condition is equivalent to a $\sum p$ NOEC of <1.0 (i.e. the sum of the individual fractions of the NOEC for the species present), or to a NOEC of <1.0 for each individual toxicant (i.e. fractions of the NOEC are not summed).

Scope for further research

The research carried out so far has been directed mainly towards combinations of those toxicants commonly present in water and for which valid water quality standards are required for the protection of aquatic life. Evaluation of the data suggests that, with a very few possible exceptions, markedly more than additive effects (synergism) do not occur even when lethal responses are tested, and where normally the joint effects are within the range of 0.5 to two-fold times as additive. Furthermore, the general inference of some of the data reviewed here is that toxicant concentrations lower than the NOEC do not contribute to the toxicity of a mixture containing a dominant toxicant; in other words, they are 'levels of no effect' both

singly and in mixtures. A more conservative approach was proposed in Water Quality Criteria (US Environmental Protection Agency, 1973) suggesting that concentrations lower than 0.2 NOEC do not add to the toxicity of a mixture; this fraction was derived from a general feeling of what might be the case, rather than from the existence of explicit experimental evidence. Recent research has shown that very low concentrations of toxicants in complex equitoxic mixtures can contribute to the combined lethality, and for classes of organic substances with a common and well defined QSAR, there is apparently no lower concentration at which individual toxicants do not contribute their full toxic potential.

In the context of regulatory activities, the evidence reviewed in this report may form a sufficient basis for a working hypothesis; if not, then further research will be required in the following areas:

(a) effects of toxicants singly and in mixtures on biochemical and physiological processes and their interaction, including interaction at receptor sites;
(b) field studies on fish populations at sites where mixtures of toxicants occur at concentrations close to the NOEC;
(c) further studies on the wide range of organic substances which can occur in rivers and which may, in combination, substantially add to the toxicity even where the individual concentrations are very small. The joint action of different classes of such compounds may be an important component of such research, and the identification and use of QSARs may assist in the interpretation of the data obtained;
(d) identification of toxicant concentrations which do not add to the toxicity of other substances present either in equitoxic mixtures or in mixtures containing a dominant toxicant.

All these areas of research pose considerable problems. One of the main difficulties of extending the existing concentration-additive experimental techniques to mixtures containing toxicants at less than the NOEC is that there is no response common to all toxicants which can be measured with sufficient precision for additive effects to be detected at the low concentrations that have to be used.

In the absence of such methodology, information has to be derived from other sources. At present, knowledge of fish physiology and biochemistry has not reached the point where a quantitative prediction of individual toxicant response is possible. Even with

the knowledge of how a substance, by itself, affects each of a variety of biochemical and physiological processes, it may not be possible to predict the total effect on a particular function. With mixtures of toxicants the problem is even greater, even when the relationship between dose and response for the individual components is well described (e.g. Veldstra, 1956). For example, Lloyd and Swift (1976) in discussing various physiological and biochemical responses of rainbow trout to ammonia and phenol, singly and together, considered that knowledge was not yet sufficient to explain their joint effect on survival, or on the accumulation of phenol.

Studies of mechanisms of physiological interactions are expensive and time-consuming and represent an area where inspired research is required if new insights are to emerge.

The evaluation of the effect of toxicant mixtures on natural or captive fish populations in field situations poses a different set of problems. One of the major difficulties is the large number of chemical analyses required to quantify all the variables which might affect fish health. A detailed examination of contaminated lakes might be rewarding since the concentrations of toxicants there are less likely than in flowing waters to fluctuate rapidly and thus sampling frequency can be reduced. When relating water quality to the presence or absence of fish, there is a need to measure toxicity directly, rather than to rely on predictions alone; even when undiluted river water is not demonstrably toxic it may be possible to make some estimate of the fractional LC50 of the water by making several separate laboratory estimations based on the addition of different toxicants in sufficient quantity to make the water demonstrably toxic. Also, Esvelt *et al.* (1973) have demonstrated how, for individual samples (of effluent) in which there is less than 50% kill, the toxicity expressed as a fraction of the LC50 can be estimated by extrapolation provided a sufficient number of samples from the same source, but having a higher toxicity, has been tested, although it must be assumed that there are no marked differences in percentage composition between samples.

Tentative water quality criteria

The question arises as to whether the tentative water quality criteria for individual toxicants proposed by EIFAC (see Alabaster and

Lloyd, 1980) are appropriate or not to mixtures of the toxicants concerned. The original report on mixtures concluded that "The information reviewed . . . on the effect of mixtures of toxicants on fish would support the conclusions that the tentative water quality criteria promulgated by EIFAC would be applicable to situations where more than one of the toxicants considered were present. Therefore, there would appear to be little justification to set more stringent standards in such situations". This conclusion was based on the fact that the water quality criteria were small proportions of the long-term LC50 of these toxicants and thus considered unlikely to exhibit additive joint action when several toxicants at these low concentrations were present as a mixture.

Further experience with the toxicity of chemical mixtures to aquatic life confirms that cases of supra-addition or synergism between chemicals likely to be found in rivers are very rare and indeed may not exist. However, the recent evidence tends to support the conclusion that low, sub-lethal concentrations of organic chemicals may still exert a harmful effect when present in a mixture. Therefore, it is a safe but conservative assumption that the toxicity of any mixture of such chemicals in the aquatic environment approximates to additive joint action. For other chemicals such as those reviewed individually by EIFAC, the case for no additive joint action at low concentrations (that is, at or below the tentative water quality criteria) is now less certain and it would be prudent to assume that partial addition exists, pending further research in this area.

REFERENCES

Abram, F. S. H. and P. Wilson (1979). The acute toxicity of CS to rainbow trout. *Water Res.*, 13: 631–5.

Abt, K., J. Grauwiler and H. Schön (1972). Acute toxicity of drug combinations. A method for evaluating the interaction of the active components. *Proc. Eur. Soc. Stud. Drug Toxicity*, 13: 192–5.

Adelman, I. R. and L. L. Smith, Jr. (1976). Fathead minnows (*Pimephales promelas*) and goldfish (*Carassius auratus*) as standard fish in bioassays and their reaction to potential reference toxicants. *J. Fish. Res. Board Can.*, 33(2): 209–14.

Alabaster, J. S. (1969). Survival of fish in 164 herbicides, insecticides, fungicides, wetting agents and miscellaneous substances. *Inst. Pest. Control.*, 12 (March/April issue): 29–35.

Alabaster, J. S., J. H. N. Garland, I. C. Hart and J. F. de L. G. Solbé (1972). An approach to the problem of pollution and fisheries. *Symp. Zool. Soc. Lond.*, 1972: 87–114.

Alabaster, J. S. and R. Lloyd (eds) (1980). Water quality criteria for freshwater fish. London, Butterworths, 297 p.

Alderdice, D. F. and C. R. Forrester (1968). Some effects of salinity and temperature on early development and survival of the English sole (*Parophys vetulus*). *J. Fish. Res. Board Can.*, 25(3): 495–521.

Allan, I. R. H., D. W. M. Herbert and J. S. Alabaster (1958). A field and laboratory study of fish in a sewage effluent. *Fish. Invest. Minist, Agric. Fish. Food G.B. (I. Salm. Freshwat. Fish.)*, 6(2): 76 p.

Allen, L. A., N. Blezard and A. B. Wheatland (1948). Formation of cyanogen chloride during chlorination of certain liquids; toxicity of such liquids to fish. *J. Hyg.*, 46: 184–93.

Anderson, P. D. and S. d'Apollonia (1978). Aquatic animals. *In* Principles of ecotoxicology, edited by G. C. Butler, London, John Wiley and Sons, Section 3, Chapter 9 (SCOPE Report, 12).

Anderson, P. D., H. Horovitch and N. L. Weinstein (1979a). Pollutant mixtures in the aquatic environment: A complex problem in toxic hazard assessment. *Tech. Rep. Fish. Mar. Serv. Can.*, (862): 100–14.

Anderson, P. D. and L. J. Weber (1975a). The toxicity to aquatic populations of mixtures containing certain heavy metals. *In* Proceedings of the International Conference on heavy metals in the envornment, Canada, October 27–31. Toronto, University of Toronto, Institute of Environmental Studies, pp. 933–53.

Anderson, P. D. and L. J. Weber (1975b). Toxic responses as a quantitative function of body size. *Toxicol. Appl. Pharmacol.*, 33: 471–83.

Anderson, P. D. and L. J. Weber (1976). The multiple toxicity of certain heavy metals: additive actions and interactions. *In* Proceedings of the Workshop on toxicity to biota of metal forms in natural waters, edited by R. W. Andrew, P. V. Hodson and D. E. Konasewich. Windsor, Canada, International Joint Commission Research Advisory Board, pp. 263–82.

Anderson, P. D. *et al.* (1979b). The multiple toxicity of vanadium, nickel and phenol to fish. Prepared for the Alberta Oil Sands Environmental Research Program by Department of Biology, Concordia University, AOSERP Report 79: 109 p.

Ariens, E. J. (1972). Adverse drug interactions. Interactions of drugs on the pharmaco-dynamic level. *Proc. Bur. Soc. Stud. Drug Toxicity*, 13: 137–63.

Arillo, A., C. Margiocco, F. Melodia, P. Mensi (1982). Biochemical effects of longterm exposure to Cr, Cd, Ni on rainbow trout (*Salmo gairdnerii* Rich): influence of sex and season. Chemosphere 11: 47–57.

Ashford, J. R. (1981). General models for the joint action of mixtures of drugs. *Biometrics*, 37: 457–74.

Ashford, J. R. and J. M. Cobby (1974). A system of models for the action of drugs applied singly or jointly to biological organisms. *Biometrics*, 30: 11–31.

Ashford, J. R. and C. S. Smith (1964). General models for quantal response to the joint action of a mixture of drugs. *Biometrika*, 51: 413–28.

Ashford, J. R. and C. S. Smith (1965). An alternative system for the classification of mathematical models for quantal responses to mixtures of drugs in biological assay. *Biometrics*, 21: 181–8.

Bahner, L. H. and D. R. Nimmo (1975). Methods to assess effects of combinations of toxicants, salinity and temperatures on estuarine animals. *In* Trace substances in environmental health, edited by D. D. Hemphill. Columbia, Missouri, University of Missouri, pp. 169–77.

Bandt, H. J. (1946). Über Versteärkte Schadwirkungen auf Fische, insbesondere uber erhönte Giftwirkung durch Kombination von Abwassergiften. *Beitr. Wasser Chem.*, 1: 15.

Bender, M. B. (1969). The toxicity of the hydrolysis and breakdown products of malathion to the fathead minnow (*Pimephales promelas,* Rafinesque). *Water Res.*, 3: 571–82.

Benijts-Claus, C. and F. Benijts (1976). The effect of low lead and zinc concentrations on the larval development of the mud-crab (*Rhithropanopeus harrisii* Gould). *In* Sub-lethal effects of toxic chemicals on aquatic animals, edited by J. H. Koeman. New York, Elsevier, pp. 43–52.

Bianucci, F. and P. Legnagni (1973). Toxicity to *Alburnus alburnus* var. *alborella* of mixtures of hexavalent chromium and anionic detergent. *Ig. Mod.*, 60: 531–7.

Biesinger, K. E., R. W. Andrew and J. W. Arthur (1974). Chronic toxicity of NTA (nitrilotri-acetate) and metal-NTA complexes to *Daphnia magna. J. Fish. Res. Board Can.*, 31(4): 486–90.

Bills, T. D. and L. L. Marking (1976). Toxicity of 3-trifluoromethyl-4-nitrophenol (TFM), 2′,5-dichloro-4′-nitrosalicylanilide (Bayer 73), and a 98:2 mixture to fingerlings of seven fish species and to eggs and fry of coho salmon. *Invest. Fish Control USFWS,* (69): 9 p.

Bliss, C. I. (1939). The toxicity of poisons applied jointly. *Ann. Appl. Biol.*, 26: 585–615.

Broderius, S. and M. Kahl (1985). Acute toxicity of organic chemical mixtures to the fathead minnow. *Aquat. Toxicol.*, 6: 307–322.

Broderius, S. J. and L. L. Smith (1979). Lethal and sub-lethal effects of binary mixtures of cyanide and hexavalent chromium, zinc or ammonia to the fathead minnow (*Pimephales promelas*) and rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Board Can.*, 36(2): 164–72.

Brown, C. C. (1978). The statistical analysis of dose-effect relationships. *In* Principles of ecotoxicology, edited by G. C. Butler. London, John Wiley and Sons, Chapter 6 (SCOPE Report, 12).

Brown, V. M. (1968). The calculation of the acute toxicity of mixtures of poisons to rainbow trout. *Water. Res.*, 2: 723–33.

Brown, V. M. and R. A. Dalton (1970). The acute lethal toxicity to rainbow trout of mixtures of copper, phenol, zinc and nickel. *J. Fish Biol.*, 2: 211–6.

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. *J. Fish Biol.*, 1: 1–9.

Brown, V. M., V. V. Mitrovic and G. T. C. Stark (1968). Effects of chronic exposure to zinc on toxicity of a mixture of detergent and zinc. *Water Res.*, 2: 255–63.

Brown, V. M., D. G. Shurben and D. Shaw (1970). Studies on water quality and the absence of fish from some polluted English streams. *Water Res.*, 4: 363–82.

Bucksteeg, W., H. Thiele and K. Stoltzel (1955). Die Beeinflussund von Fischen durch Giftstoffe aus Abwassern. *Vom Wasser.*, 22: 194–211.

Buikema, A. L., Jr., C. L. See and J. Cairns, Jr. (1977). Rotifer sensitivity to combinations of inorganic water pollutants. OWRT Project A-071-VA VPI-VWRRC-BULL 92, December.

Cairns, J. and A. Scheier (1968). A comparison of the toxicity of some common industrial waste components tested individually and combined. *Progr. Fish-Cult.*, 30: 3–8.

Calamari, D. and R. Marchetti (1970). L'analisi tossicologica nel controllo delle acque: internazioni fra inquinanti. *Ig. Mod.*, 63: 455–71.

Calamari, D. and R. Marchetti (1972). Applicazione di un modello per lo studio delle interazioni tra due sostanze in tossicologica ittica. *N. Ann. Ig. Microbiol.*, 23: 419–25.

Calamari, D. and R. Marchetti (1973). The toxicity of mixtures of metals and surfactants to rainbow trout (*Salmo gairdneri* Rich.). *Water Res.*, 7: 1453–64.

Cherkinsky, S. N. (1957). The theoretical basis for hygienic standardization of simultaneous pollution of watercourses with several harmful substances. *Gig. Sanit.*, 22(8): 3–9 (in Russian, English summary).

Christensen, E. R. and C.-Y. Chen (1985). A general non-interactive multiple toxicity model including probit, logit and Weibull transformations. *Biometrics*, 41: 711–725.

Christensen, E. R. and A. A. Zielski (1980). Toxicity of arsenic and PCB to a green alga (*Chlamydomonas*). *Bull. Environ. Contam. Toxicol.*, 25: 43–8.

Cochran, W. G. and G. M. Cox (1957). Experimental designs. New York, Wiley and Sons, 2nd ed.

Cooper, V. A. and J. F. de L. G. Solbé (1978). Reducing the toxicity of cadmium sulphate to rainbow trout (*Salmo gairdneri*) by preliminary exposure of fish to zinc sulphate, with and without intermittent exposure to cadmium. A progress report. *Water Res. Cent. ILR*, (750): 8 p.

Das, M. N. and G. A. Kulkarni (1966). Incomplete block designs for bioassays. *Biometrics*, 22: 706–29.

Dawson, V. K. and L. L. Marking (1973). Toxicity of mixtures of quinaldine sulfate and MS-222 to fish. *Invest. Fish Control USFWS*, (53): 11 p.

Dawson, V. K., K. B. Cumming and P. A. Gilderhus (1977). Efficacy of 3-trifluoromethyl-4-nitrophenol (TFM), 2′,5-dichloro-4′-nitrosalicylanilide (Bayer 73), and a 98:2 mixture as lampricides in laboratory studies. *Invest. Fish Controls USFWS*, (77): 11 p.

Dethlefsen, V. (1977). The influence of DDT and DDE on the embryogenesis and the mortality of larvae of cod (*Gadus morhua* L.). *Meereforschung*, 25: 115–48.

Devlaminck, F. (1960). Etude de mélanges de toxiques, appliquée a l'évaluation de la nocivité, a l'égard du poisson, des effluents de cokeries. Liege, Centre Belge pour l'Etude et Documentation des Eaux.

Diem, K. and C. Lentner (eds) (1968). Wissenschaftliche Tabellen, 7th edition. Basel, CIBA-Geigy.

Doudoroff, P. (1952). Some recent developments in the study of toxic industrial wastes. *Proc. Pac. N.W. Ind. Waste Conf.*, 4: 21–5.

Doudoroff, P., G. Leduc and C. R. Schneider (1966). Acute toxicity to fish of solutions containing metal cyanides, in relation to concentrations of molecular hydrocyanic acid. *Trans. Am. Fish. Soc.*, 95: 6–22.

Dugan, P. R. (1967). Influence of chronic exposure to anionic detergents on toxicity of pesticides to goldfish. *J. Water Pollut. Control Fed.*, 39: 63–71.

Eaton, J. G. (1973). Chronic toxicity of a copper, cadmium and zinc mixture to the fathead minnow (*Pimephales promelas* Rafinesque). *Water Res.*, 7: 1723–36.

Edwards, R. W. and V. M. Brown (1966). Pollution and fisheries: a progress report. *Water Pollut. Control*, 66: 63–78.

EIFAC Working Party on Toxicity Testing Procedures (1975). Report on fish toxicity testing procedures. *EIFAC Tech. Pap.*, (24): 25 p.

EIFAC Working Party Quality Criteria for European Freshwater Fish with the Cooperation of the United Nations Environment Programme (UNEP) (1976). Report on copper and freshwater fish. *EIFAC Tech. Pap.*, (27): 21 p.

EIFAC Working Party on Biological Monitoring (1977). Report on the effect of zinc and copper pollution on the salmonid fisheries in a river and lake system in central Norway. *EIFAC Tech. Pap.*, (29): 34 p.

Eisler, R. and G. R. Gardner (1973). Acute toxicology to an estuarine teleost of mixtures of cadmium, copper and zinc salts. *J. Fish Biol.*, 5: 131–142.

Esvelt, L. A., W. J. Kaufman and R. E. Selleck (1973). Toxicity assessment of treated municipal wastewaters. *J. Water Pollut. Control Fed.*, 45: 1558–72.

Fabacher, D. L. and H. Chambers (1974). Resistance to herbicides in insecticide-resistant mosquitofish (*Gambusia affinis*). *Environ. Lett.*, 7: 15–20.

Fabacher, D. L., J. D. Davis and D. A. Fabacher (1976). Apparent potentiation of cotton defoliant DEF by methyl parathion in mosquitofish. *Bull. Environ. Contam. Toxicol.*, 16: 716–8.

Fedeli, L. *et al.* (1972). Quantitative evaluation of joint drug action. *Proc. Eur. Soc. Stud. Drug Toxicity*, 13: 231–45.

Ferguson, D. E. and C. R. Bringham (1966). The effects of combinations of insecticides on susceptible and resistant mosquitofish. *Bull. Environ. Contam. Toxicol.*, 1: 97–103.

Finney, D. J. (1964). Statistical methods in biological assays. London, Griffin, 2nd ed.

Finney, D. J. (1971). Probit analysis. Cambridge University Press, 318 p. 3rd ed.

Fitzgerald, G. P. (1963). Factors affecting the toxicity of copper to algae and fish. Paper presented to the Division of Water and Waste Chemistry, American Chemical Society, ACS National Meeting, September 8–13.

Freeman, L. and I. Fowler (1953). Toxicity of combinations of certain inorganic compounds to *Daphnia magna* Straus. *Ind. Wastes*, 25: 1191–5.

Friedland, S. A. and M. N. Rubleva (1958). The problem of hygienic standards for waters simultaneously polluted with several harmful substances. *Gig. Sanit.*, 23: 12–6.

Gaddum, J. M. (1953). Bioassays and mathematics. *Pharmacol. Rev.*, 5: 87–134.

Gallimore, B. and P. D. Anderson (1979). Life cycle patterns in lethal tolerance of fish exposed cadmium, zinc and their mixtures. *Pharmacology*, 21(3): 250 (abstr.).

Gehrs, C. W. *et al.* (1974). Effects of stable chlorine-containing organics on aquatic environments. *Nature, Lond.*, 249: 675–6.

Gray, J. S. and R. J. Ventilla (1973). Growth rates of sediment-living marine protozoan as a toxicity indicator for heavy metals. *Ambio*, 2: 118–21.

Heisinger, J. F., C. D. Hansen and J. H. Kim (1979). Effect of selenium dioxide on the accumulation and acute toxicity of mercuric chloride in goldfish. *Arch. Environ. Contam. Toxicol.*, 8: 279–83.

Hendricks, A. C. (1978). Response of *Selenastrum capricornutum* to zinc sulfides. *J. Water Pollut. Control Fed.*, 50: 163–8.

Herbert, D. W. M. (1962). The toxicity to rainbow trout of spent still liquors from the distillation of coal. *Ann. Appl. Biol.*, 50: 755–77.

Herbert, D. W. M., D. H. M. Jordan and R. Lloyd (1965). A study of some fishless rivers in the industrial Midlands. *J. Inst. Sewage Purif.*, 1965: 569–82.

Herbert, D. W. M. and D. G. Shurben (1964). The toxicity to fish of mixtures of poisons. 1. Salts of ammonia and zinc. *Ann. Appl. Biol.*, 53: 33–41.

Herbert, D. W. M. and J. M. Vandyke (1964). The toxicity to fish of mixtures of poisons. 2. Copper–ammonia and zinc–phenol mixtures. *Ann. Appl. Biol.*, 53: 415–21.

Herbes, S. E. and J. J. Beauchamp (1977). Toxic interaction of mixtures of two coal

conversion effluent components (resorcinol and 6-methyl-quinoline) to *Daphnia magna*. *Bull. Environ. Contam. Toxicol.*, 17: 25–32.

Hermens, J., E. Broekhuyzen, H. Canton and R. Wegman (1985c). Quantitative structure-activity relationships and mixture toxicity studies of alcohols and chlorohydrocarbons: effects on growth of *Daphnia magna*. *Aquat. Toxicol.*, 6: 209–17.

Hermens, J., E. Broekhuyzen, H. Canton, and R. Wegman (1985a). Quantitative structure-activity relationships and mixture toxicity studies of alcohols and chlorohydrocarbons: Effects on growth of *Daphnia magna*. *Aquat. Toxicol.*, 6: 209–217.

Hermens, J., F. Buser, P. Leeuwangh and A. Musch (1985b). Quantitative structure-activity relationships and mixture toxicity of organic chemicals to *Photobacterium phosphoreum*: the Microtox test. *Ecotoxicol. Environ. Safety*, 9: 17–25.

Hermens, J., H. Canton, P. Janssen and R. de Jong (1984b). Quantitative structure-activity relationships and toxicity studies of mixtures of chemicals with anaesthetic potency: acute lethal and sublethal toxicity to *Daphnia magna*. *Aquat. Toxicol.*, 5: 143–54.

Hermens, J., H. Canton, N. Steyger and R. Wegman (1984c). Joint effects of a mixture of 14 chemicals on mortality and inhibition of reproduction of *Daphnia magna*. *Aquat. Toxicol.*, 5: 315–22.

Hermens, J. and P. Leeuwangh (1982). Joint toxicity of mixtures of 8 and 24 chemicals to the guppy (*Poecilia reticulata*). *Ecotoxicol. Environ. Safety*, 6: 302–10.

Hermens, J., P. Leeuwangh and A. Musch (1984a). Quantitative structure-activity relationships and mixture toxicity studies of chloro- and alkylanilines at an acute lethal toxicity level to the guppy (*Poecila reticulata*). *Ecotoxicol. Environ. Safety*, 8: 388–94.

Hermens, J. P. Leeuwangh and A. Musch (1985a). Joint toxicity of groups of organic aquatic pollutants to the guppy (*Poecila reticulata*). *Ecotoxicol. Environ. Safety*, 9: 321–26.

Hewitt, L. A. and P. D. Anderson (1979). Aspects of supra-additive interactions between cadmium and copper in fish exposed to lethal mixtures. *Pharmacology*, 21(3): 210 (abstr.).

Hewlett, P. S. and R. L. Plackett (1950). Statistical aspects of the independent joint action of poisons, particularly insecticides. II. Examination of data for agreement with the hypothesis. *Ann. Appl. Biol.*, 38: 527.

Hewlett, P. S. and R. L. Plackett (1959). A unified theory for quantal responses to mixtures of drugs: non-interactive action. *Biometrics*, 15: 591–610.

Hewlett, P. S. and R. L. Plackett (1964). A unified theory for quantal responses to mixtures of drugs: competitive action. *Biometrics*, 20: 566.

Hewlett, P. S. and R. L. Plackett (1979). An introduction to the interpretation of quantal responses in biology. London, Edward Arnold, 82 p.

Hodson, P. C. *et al.* (1977). Statistical considerations in planning aquatic bioassays. *Environ. Prot. Serv. Tech. Rep. Can.*, (EPS-5-AR-77-1): 15–31.

Howell, J. H. *et al.* (1964). Synergism of 5,2'-dichloro-4'-nitrosalicylanilide and 3-trifluoromethyl-4-nitrophenol in a selective lampricide. *Tech. Rep. Great Lakes Fish. Comm.*, No. (8): 21 p.

Howland, R. M. (1969). Interaction of antimycin A and rotenone in fish bioassays. *Prog. Fish-Cult.*, 31(1): 33–4.

Huckabee, J. W. and N. A. Griffith (1974). Toxicity of mercury and selenium to the eggs of carp (*Cyprinus carpio*). *Trans. Am. Fish. Soc.*, 103(4): 822–5.

Hutchinson, T. C. (1973). Comparative studies of the toxicity of heavy metals to phytoplankton and their synergistic interactions. *Water Pollut. Res. Can.*, 8: 68–90.

Hutchinson, T. C. and H. Czyrska (1973). Cadmium and zinc toxicity and synergism to aquatic plants. *Water Pollut. Res. Can.*, 7: 59–65.

Jenkins, C. R. (1964). A study of some toxic components in oil refinery effluents. Ph.D. Thesis submitted to Oklahoma State University. Stillwater, Oklahoma, USA, 73 p.

Jolly, R. L. (1973). Chlorination effects on organic constituents in effluents from domestic sanitary sewage treatment plants. *Publ. Oak Ridge Natl. Lab.*, (ORNL-RM-4290).

Kaiser, K. L. E. (ed.) (1984). *QSAR in Environmental Toxicology*. D. Reidel Publishing Co., Dordrecht, Holland, 406 p.

Katz, B. M. and G. M. Cohen (1976). Toxicities of 'excessively' chlorinated organic compounds. *Bull. Environ. Contam. Toxicol.*, 15(6): 611–7.

Kawatski, J. A. (1973). Acute toxicities of antimycin A, Bayer 73 and TRM to the ostracod *Cyptetta kawatai. Trans. Am. Fish. Soc.*, 102: 829–31.

Kempthorne, O. (1952). The design and analysis of experiments. New York, J. Wiley and Sons.

Kim, J. H., B. Birks and J. F. Heisinger (1977). Protective action of selenium against mercury in northern creek chubs. *Bull. Environ. Contam. Toxicol.*, 17: 132–6.

Koenig, C. C. (1977). The effects of DDT and Mirex alone and in combinations on the reproduction of a salt marsh cyprinodont fish (*Adinia xenica*). *In* Physiological responses of marine biota to pollutants, edited by F. J. Vernberg *et al.* New York, Academic Press, pp. 357–76.

Konemann, H. (1981a). Fish toxicity tests with mixtures of more than two chemicals: a proposal for a quantitative approach and experimental results. *Toxicol.*, 19: 229–38.

Konemann, H. (1981b). Quantitative structure-activity relationships in fish toxicity studies. Part 1: relationship for 50 industrial pollutants. *Toxicol.*, 19: 209–21.

Krieger, R. I. and P. W. Lee (1973). Inhibition of *in vivo* and *in vitro* epoxidation of aldrin and potentiation of toxicity of various insecticide chemicals by diquat in two species of fish. *Arch. Environ. Contam. Toxicol.*, 1: 112.

Laborey, F. and J. Lavollay (1967). Sur la toxicité exercée par Zn^{++} et Cd^{++} dans la croissance *d'Aspergillus niger*, l'antagonisme de ces ions et l'interaction Mg^{++}, Zn^{++}, Cd^{++}. *C.R. Hebd. Seances Acad. Sci. Paris (Ser. D. Sci. Nat.)*, 24: 2937–40.

LaRoche, G. *et al.* (1973). Analysis of toxic response in marine poikilotherms. *In* Bioassay techniques and environmental chemistry, edited by G. E. Glass. Ann Arbor, Mich., Ann Arbor Science Publishers Inc., pp. 199–216.

Leach, J. M. and A. N. Thakore (1975). Isolation and identification of constituents toxic to juvenile rainbow trout (*Salmo gairdneri*) in caustic extraction effluents from kraft pulp mill bleach plants. *J. Fish. Res. Board Can.*, 32(8): 1249–57.

Leach, J. M. and A. N. Thakore (1977). Compounds toxic to fish in pulp mill waste streams. *Prog. Wat. Tech.*, 2: 787–98.

Leteux, F. and F. P. Mayer (1972). Mixtures of malachite green and formalin for controlling *Ichthyophthirius* and other protozoan parasites of fish. *Prog. Fish-Cult.*, 34(1): 21–6.

Lewis, M. (1978). Acute toxicity of copper, zinc and manganese in single and mixed salt solutions to juvenile longfin dace, *Agosia chrysogaster. J. Fish Biol.*, 13: 695–700.

Lichtenstein, E. P. *et al.* (1974). Insecticidal and synergistic components isolated from dill plants. *J. Agric. Food Chem.*, 22: 658–64.

Lichtenstein, E. P., T. T. Liang and B. N. Anderegg (1973). Synergism of insecticides by herbicides. *Science, Wash.*, 181: 847–9.

Lipnick, R. L. and W. J. Dunn (1983). A MLAB study of aquatic structure-toxicity relationships. *In* Quantitative Approaches to Drug Design, edited by J. C. Dearden. Proceedings of the Fourth European Symposium on Chemical Structure-biological Activity: Quantitative Approaches, Bath (U.K.), September 6–9, 1982. Amsterdam, Elsevier, pp. 265–6.

Litchfield, J. F. and F. Wilcoxon (1949). A simplified method of evaluating dose-effect experiments. *J. Pharm. Exp. Therap.*, 96: 99.

Lloyd, R. (1960). Toxicity of zinc sulphate to rainbow trout. *Ann. Appl. Biol.*, 48(1): 84–94.

Lloyd, R. (1961). The toxicity of mixtures of zinc and copper sulphates to rainbow trout (*Salmo gairdneri* Richardson). *Ann. Appl. Biol.*, 49: 535–8.

Lloyd, R. and D. H. M. Jordan (1963). Predicted and observed toxicities of several sewage effluents to rainbow trout. *J. Proc. Inst. Sewage Purif.*, 1963: 167–73.

Lloyd, R. and D. H. M. Jordan (1964). Predicted and observed toxicities of several sewage effluents to rainbow trout: a further study. *J. Proc. Inst. Sewage Purif.*, 1964: 163–6.

Lloyd, R. and D. J. Swift (1976). Some physiological responses by freshwater fish to low dissolved oxygen, high carbon dioxide, ammonia and phenol with special reference to water balance. *In* Effects of pollutants on aquatic organisms, edited by A. P. M. Lockwood. Cambridge, Cambridge Union Press, pp. 47–69.

Loewe, S. (1953). The problem of synergism and antagonism of combined drugs. *Arzeimitt.-Forsch.*, 3: 285–90.

Ludke, J. L., J. R. Gibson and G. Lusk (1972). Mixed-function oxidase activity in freshwater fishes. Aldrin epoxidation and parathion activation. *Toxicol. Appl. Pharmacol.*, 21.

Macek, K. J. (1975). Acute toxicity of pesticide mixtures to bluegills. *Bull. Environ. Contam. Toxicol.*, 14: 648–52.

Marking, L. L. (1969). Toxicity of rhodamine B and fluorescein sodium to fish and their compatibility with antimycin A. *Prog. Fish-Cult.*, 31(3): 139–42.

Marking, L. L. (1977). Method for assessing additive toxicity of chemical mixtures. *In* Aquatic toxicology and hazard evaluation, edited by F. W. Mayer and Hamelink. Philadelphia, American Society for Testing and Materials, STP634.

Marking, L. L. and V. K. Dawson (1975). Method for assessment of toxicity or efficacy of mixtures of chemicals. *Invest. Fish. Control*, USFWS, (67): 1–8.

Marking, L. L. and W. L. Mauck (1975). Toxicity of paired mixtures of candidate forest insecticides to rainbow trout. *Bull. Environ. Contam. Toxicol.*, 13: 518–23.

Marubini, E. and L. Bonanomi (1970). Quantitative evaluation of toxicity tests carried out on mixtures of drugs. *Proc. Eur. Soc. Stud. Drug Toxicity*, 11: 113–21.

Mayer, F. L., J. C. Street and J. M. Neuhold (1972). DDT intoxication in rainbow trout as affected by dieldrin. *Toxicol. Appl. Pharmacol.*, 22: 347–54.

Morse, P. M. (1978). Some comments on the assessment of joint action in herbicide mixtures. *Weed Sci.*, 26: 58–71.

Mosser, J. L. *et al.* (1974). Interactions of PCBs, DDT and DDE in a marine diatom. *Bull. Environ. Contam. Toxicol.*, 12: 665–8.

Muska, C. C. and L. J. Weber (1977). An approach for studying the effects of mixtures of toxicants. *Proc. West. Pharmacol. Soc.*, 20: 427–30.

Negilski, D. S. and G. E. Davis (1974). Individual and combined effects of cyanide, pentachlorophenol and zinc on juvenile chinook salmon (*Oncorhynchus tshawytscha*) (*Walbaum*) and invertebrates in model stream communities. *Tech. Pap. Ore. Agric. Exp. Stn.*, (4238): 21 p.

Nimmo, W. R. and L. W. Bahner (1977). Metals, pesticides and PCBs: Toxicities to shrimp singly and in combination. *In* Estuarine proceedings Vol. 1. Uses, stresses and adaptation to the estuary, edited by M. Wiley. New York, Academic Press, pp. 523–32.

Pietiläinen, K. (1975). Synergistic and antagonistic effects of lead and cadmium on aquatic primary production. *In* Proceedings of the International Conference on heavy metals in the environment, Canada, October 27–31. Toronto, University of Toronto, Institute of Environmental Studies, pp. 861–74.

Plackett, R. L. and P. S. Hewlett (1948). Statistical aspects of the independent joint action of poisons, particularly insecticides. 1. The toxicity of a mixture of poisons. *Ann. Appl. Biol.*, 35: 347–58.

Plackett, R. L. and P. S. Hewlett (1952). Quantal responses to mixtures of poisons. *J. R. Statist. Soc.*, 14: 141–63.

Plackett, R. L. and P. S. Hewlett (1963). A unified theory for quantal responses to mixtures of drugs: the fitting to data of certain models for two non-interactive drugs with complete positive correlation of tolerances. *Biometrics*, 19: 517.

Plackett, R. L. and P. S. Hewlett (1967). A comparison of two approaches to the construction of models for quantal responses to mixtures of drugs. *Biometrics*, 23: 27.

Puri, P. S. and J. Senturia (1972). On the mathematical theory of quantal responses assay. *Proc. Berkeley Symp. Math. Stat. Probab.*, 6(4): 231–47.

Roales, R. R. and A. Perlmutter (1974a). Toxicity of zinc and cygon, applied singly and jointly to zebrafish embryos. *Bull. Environ. Contam. Toxicol.*, 12: 475–80.

Roales, R. R. and A. Perlmutter (1974b). Toxicity of methylmercury and copper, applied singly and jointly to the blue gourami (*Trichogaster trichopterus*). *Bull. Environ. Contam. Toxicol.*, 12: 633–9.

Roales, R. R. and A. Perlmutter (1977). Effects of sub-lethal doses of methylmercury and copper, applied singly and jointly, on immune response of blue gourami (*Trichogaster trichopterus*) to viral and bacterial antigens. *Arch. Environ. Contam. Toxicol.*, 5: 325–31.

Rubin, A. J. and G. A. Elmaragy (1977). Studies on the toxicity of ammonia, nitrate and their mixtures to guppy fry. *Water Res.*, 11: 927–35.

Say, P. J. (1977). Microbial ecology of high zinc level streams. Ph.D. Thesis University of Durham, 295 p.

Schaut, G. G. (1939). Fish catastrophes during droughts. *J. Am. Water Works Assoc.*, 31: 771–822.

Servizi, J. A., D. W. Martens and R. W. Gordon (1978). Acute toxicity at Annacis Island Primary sewage treatment plant. *Prog. Rep. Int. Pac. Salmon Fish. Comm.*, (38): 12 p.

Sjöquist, F. and B. Alexanderson (1972). Drug interaction. A critical look at their documentation and clinical importance. *Proc. Eur. Soc. Stud. Drug Toxicity*, 13: 167–79.

Smith, L. L. *et al.* (1979). Acute and chronic toxicity of HCN to fish and invertebrates. Washington, D.C., U.S. Environmental Protection Agency, (EPA-600/3-79-009): 115 p.

Solbé, J. F. de L. G. (1973). The relation between water quality and the status of fish populations in Willow Brook. *Water Treat. Exam.*, 22: 41–61.

Solon, J. M. and J. M. Nair (1970). The effect of a sub-lethal concentration of LAS

on the acute toxicity of various phosphate pesticides to fathead minnow (*Pimephales promelas*) Rafinesque). *Bull. Environ. Contam. Toxicol.*, 5: 408–13.

Solon, J. M., J. L. Lincer and J. M. Nair (1969). The effect of sub-lethal concentration of LAS on the acute toxicity of various insecticides to fathead minnow. *Water Res.*, 3: 767–75.

Southgate, B. A. (1932). The toxicity of mixtures of poisons. *Q. J. Pharmacol.*, 5: 639–48.

Spehar, R. L., E. N. Leonard and D. L. DeFoe (1978). Chronic effects of cadmium and zinc mixtures on flagfish (*Jordanella floridae*). *Trans. Am. Fish. Soc.*, 107(2): 354–60.

Sprague, J. B. (1964). Lethal concentrations of copper and zinc for young Atlantic salmon. *J. Fish. Res. Board Can.*, 21(1): 17–26.

Sprague, J. B. (1970). Measurement of pollutant toxicity to fish. 2. Utilizing and applying bioassay results. *Water Res.*, 4: 3–22.

Sprague, J. B. and W. J. Logan (1979). Separate and joint toxicity to rainbow trout of substances used in drilling fluids for oil exploration. *Environ. Pollut.*, 19(4): 269–82.

Sprague, J. B. and B. A. Ramsay (1965). Lethal levels of mixed copper–zinc solutions for juvenile salmon. *J. Fish. Res. Board Can.*, 22(2): 425–32.

Sprague, J. B., P. F. Elson and R. L. Saunders (1965). Sub-lethal copper–zinc pollution in a salmon river—a field and laboratory study. *Int. J. Air Water Poolt.*, 9: 531–43.

Statham, C. N. (1975a). Synergism of the acute toxic effects of 2,4-D butyl ester, dieldrin, rotenone and pentachlorophenol in a rainbow trout by carbaryl. *Toxicol. Appl. Pharmacol.*, 33: 188.

Statham, C. N. (1975b). Potentiation of the acute toxicity of several pesticides and herbicides in trout by carbaryl. *Toxicol. Appl. Pharmacol.*, 34: 83–77.

Statham, C. N. and J. J. Lech (1976). Studies on the mechanism of potentiation of the acute toxicity of 2,4-D N-butyl ester and 2′,5-dichloro-4′-nitrosalicylanicide in rainbow trout by carbaryl. *Toxicol. App. Pharmacol.*, 36: 281–96.

Stratton, G. W. and C. T. Corke (1979). The effect of mercuric, cadmium and nickel ion combinations on a blue–green alga. *Chemosphere*, 8(10): 731–40.

Street, J. C., F. L. Mayer and D. J. Wagstaff (1970). Ecological significance of pesticide interactions. *In* Pesticides Symposia, edited by W. B. Deichman. Halos, Miami.

Sun, L. T. and M. L. Gorman (1973). The toxicity to fish of herbicides recommended for weed control in the Rewa. *Fiji Agric. J.*, 35: 31–3.

Thompson, K. W., A. C. Hendricks and J. Cairns (1980). Acute toxicity of zinc and copper singly and in combination to the bluegill (*Lepomis macrochirus*). *Bull. Environ. Contam. Toxicol.*, 25: 122–9.

Tsai, C. and J. A. McKee (1980). Acute toxicity to goldfish of mixtures of chloramine, copper and linear alkylate sulfonate. *Trans. Am. Fish. Soc.*, 109: 132–41.

Tsay, S.-F., J.-M. Lee and J. Q. Lynd (1976). The interactions of Cu^{++} and Cn^{-} with paraquat phytotoxicity to a *Chlorella*. *Weed Sci.*, 18: 596–8.

US Environmental Protection Agency (1973). Water quality criteria, 1972. Washington, D.C., Environmental Protection Agency, (EPA.R3.73.003), 594 p.

Vamos, R. and R. Tasnadi (1967). Ammonia poisoning in carp. 3. The oxygen content as a factor in influencing the toxic limit of ammonia. *Acta Biol. Szeged*, 13: 99–105.

Van Loon, J. C. and R. J. Beamish (1977). Heavy-metal contamination by atmospheric fallout of several Flin Flon area lakes and the relation to fish populations. *J. Fish. Res. Board Can.*, 34(7): 899–906.

Veith, G. D., D. J. Call and L. T. Brooke (1983). Structure-toxicity relationships for the fathead minnow, *Pimephales promelas*: Narcotic industrial chemicals. *Can. J. Fish. Aquat. Sci.*, 40: 743–8.

Verma, S. R., S. Rani and R. C. Dalela (1981). Synergism, antagonism and additivity of phenol, pentachlorophenol and dinitrophenol to a fish (*Notropterus notropterus*). *Arch. Environ. Contam. Toxicol.*, 10: 365–70.

Walsh, G. E. (1978). Toxic effects of pollutants on plankton. *In* Principles of ecotoxicology, SCOPE 12, edited by G. C. Butler. London, John Wiley and Sons, Chapter 12 (SCOPE Report 12).

Warren, C. E. (1971). Biology and water pollution control. Philadelphia, W. B. Saunders Company, 434 p.

Weinstein, N. L. and P. D. Anderson (1978). Lethal and sub-lethal toxicities of copper–nickel mixtures to the zebrafish (*Brachydanio rerio*). *Tech. Rep. Fish. Mar. Serv. Can.*, 818.

Wuhrman, K. and Woker, H. (1948). Experimentelle Untersuchungen Über die Ammoniak—und Blansäuruergiftung. *Schweiz Z. Hydrol.*, 77: 210–244.