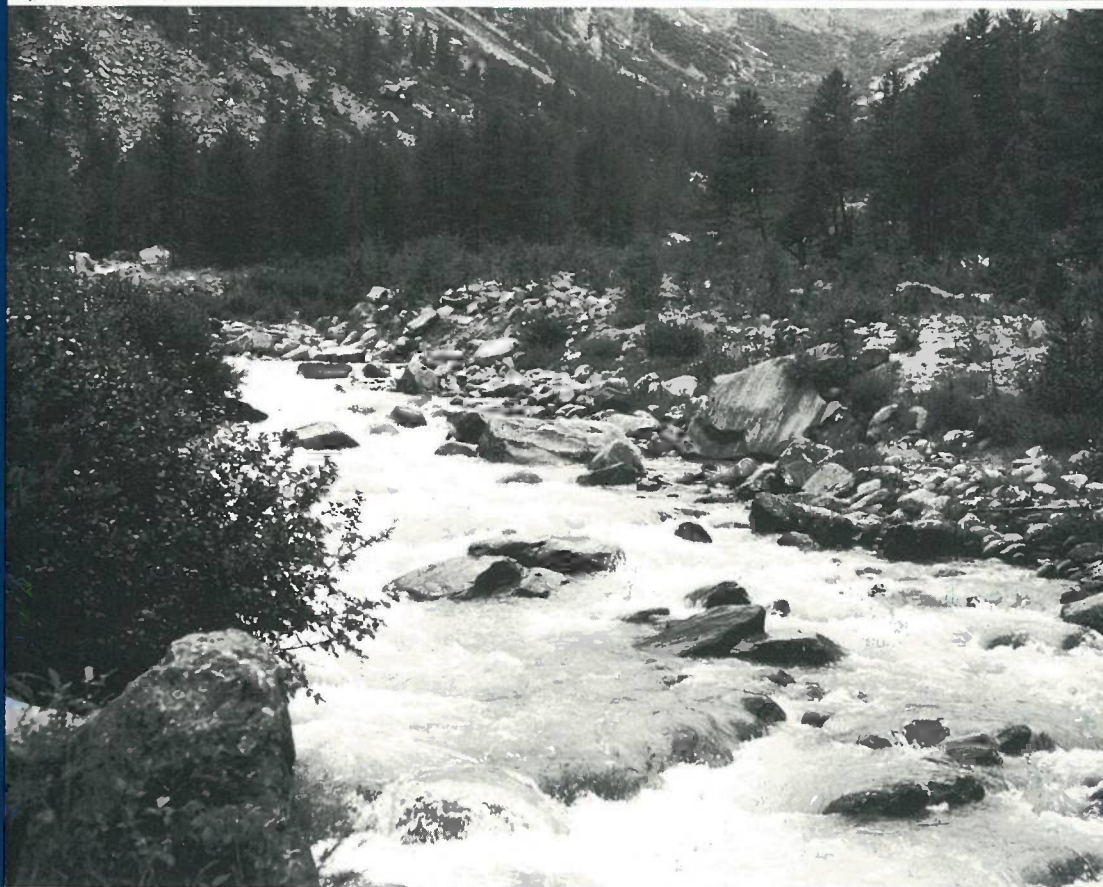# Water quality criteria for European freshwater fish

Revised report on combined effects on freshwater fish and other aquatic life of mixtures of toxicants in water

FOOD
AND
AGRICULTURE
ORGANIZATION
OF THE
UNITED NATIONS

Water quality criteria for European freshwater fish

Revised report on combined effects
on freshwater fish and other aquatic life
of mixtures of toxicants in water

Prepared by the
EIFAC Working Party on Water Quality Criteria
for European Freshwater Fish

EUROPEAN INLAND FISHERIES
ADVISORY COMMISSION
(EIFAC)

PREPARATION OF THIS DOCUMENT

The present document was prepared by the European Inland Fisheries Advisory Commission (EIFAC) Working Party on Water Quality Criteria for European Freshwater Fish and submitted to the Eleventh Session of EIFAC (Stavanger, Norway, 28 May-3 June 1980) where it was approved. It was updated in 1986 at the request of the Thirteenth Session of EIFAC (Aarhus, Denmark, 23-30 May 1984).

The report is being issued in this series where the first twelve documents of the Working Party were published: "Report on finely divided solids and inland fisheries", EIFAC Tech.Pap., (1):21 p., 1964; "Report on extreme pH values and inland fisheries", EIFAC Tech.Pap., (4):24 p., 1968; "Report on water temperature and inland fisheries based mainly on Slavonic literature", EIFAC Tech.Pap., (6):32p., 1968; "List of literature on the effect of water temperature on fish", EIFAC Tech. Pap., (8):8p., 1969; "Report on ammonia and inland fisheries", EIFAC Tech.Pap., (11): 12p., 1970; "Report on monohydric phenols and inland fisheries", EIFAC Tech. Pap., (15):19p., 1972; "Report on dissolved oxygen and inland fisheries", EIFAC Tech.Pap., (19):10 p., 1973; "Report on chlorine and freshwater fish", (20):11p., 1973; "Report on zinc and freshwater fish", EIFAC Tech.Pap., (21):22p., 1973; "Report on copper and freshwater fish", EIFAC Tech.Pap., (27):21p., 1976; "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., 1977; "Report on cadmium and freshwater fish", EIFAC Tech.Pap., (30):21 p., 1977; "Report on chromium and freshwater fish" EIFAC Tech.Pap., (43):31p., 1983; "Report on nickel and freshwater fish", EIFAC Tech.Pap., (45):20p., 1984; "Report on nitrite and freshwater fish", EIFAC Tech.Pap., (46):19p., 1984.

CONTENTS

FOREWORD

Hitherto the Working Party, in reviewing the literature on the environmental requirements of freshwater fish and fisheries, has concentrated its attention on the effect on aquatic organisms of various water quality characteristics considered separately, although the importance of the joint effect of chemicals has been emphasised and relevant data reviewed (see collected, updated reports compiled by Alabaster and Lloyd, 1980).

This report reviews the principles which have been proposed for the joint effect of toxicants and the evidence put forward in support of them. The relationships between toxicity to fish and naturally occurring water quality characteristics, such as pH, temperature, dissolved oxygen and water hardness, have been covered in the previous reviews on individual toxicants and are not considered further in this report.

For the preparation of this report the following experts were appointed to the EIFAC Working Party on Water Quality Criteria:-

Mr J.S. Alabaster, UK (Convenor)

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Dr D. Calamari, Italy
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Mr M. Grande, Norway
Mr R. Lloyd, UK (Vice-Chairman Sub-Commission III)

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Drafts which were commented upon and discussed by the Working Party were prepared by Dr Calamari (Section 1), Mr Alabaster (Sections 2, 5, and 6), Mr Lloyd (Section 3), and Dr Dethlefsen (Section 4), and the final report was produced by Mr Alabaster in consultation with Mr Lloyd.

The Working Party is grateful to those, including particularly Dr W.K. Besch, Dr S.J. Broderius, Dr G.W. Stratton, and Dr P.T.S. Wong, who provided unpublished or otherwise inaccessible data. It also wishes to acknowledge constructive comments received from Mr V.M. Brown, Dr D.I. Mount, Mr J.F.de L.G. Solbé and Dr J.B. Sprague, general editorial assistance from Mrs B.C. Alabaster, and the help of Miss I.M. Lamont in reading the text and checking the references.

Dr Alabaster updated the Report for inclusion in the 2nd edition of 'Water Quality Criteria for Freshwater Fish' (Alabaster and Lloyd, 1982). Subsequently, this Report was revised in order to include significant new advances made since 1980. The following experts were appointed to the EIFAC Working Party on Water Quality Criteria to undertake this task:-

<div style="text-align: center">

Prof D. Calamari, Italy (Convenor)
Dr H. Konemann, Netherlands
Mr R. Lloyd, UK
Mr J.F. de L.G. Solbé, UK

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Dr Konemann prepared the revised sections of the report which were discussed and finalised by the Working Party.

The typescript was produced at the Water Research Centre, Medmenham, UK.

SUMMARY

This summary is an attempt to generalise from fragmentary information, and the conclusiions reached should not be used as a basis for action without reference to the main report.

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 (eg p 96-h LC50).

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. 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.

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.

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

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 Σpt LC50s (rainbow trout) is <0.2. It is not known whether this condition is equivalent to a Σp 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 (ie fractions of the NOEC are not summed).

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.

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 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.

Meanwhile, the concentration-addition model appears to be adequate to describe the joint effect of commonly-occurring constituents of sewage and industrial wastes, and to be used to make the 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.

1. INTRODUCTION

Much of the information available on the toxicity of substances to fish and other aquatic organisms 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. In preparing tentative water quality criteria for specific pollutants, the Working Party, in its reports, has emphasised that the presence of other pollutants in the water may modify the corresponding water quality standards set for the protection of fisheries, and in reviewing the literature gave 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 in order to determine the extent to which water quality standards should be modified for those waters receiving inputs of more than one toxicant.

1.1 Fundamental concepts and approaches adopted

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 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. These reactions will not be considered explicitly in this review because they have been given attention in previous reports. However, 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 amount of toxicity 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 also my 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 (after 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 (eg 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') occurred, 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 often involved rigorous statistical test designs and treatment of data, in order to develop theoretical and empirical models; such approaches were necessarily pragmatic and did 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.

1.2 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 method 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).

The 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, and discussed in detail, mathematical models to describe the resulting dose-response curves for mixtures, and further studies have been made by Finney (1971), Ashford and Cobby (1974), Ashford (1981) and Christensen and Chen (in press). 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 $\underline{n}$ compounds ($\underline{n}$ >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 in this Report are those used in the original EIFAC Technical Paper No. 37, with the addition of those used by Konemann (1981a) and others whose work forms the new material included in this revision.

1.2.1 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) denoted 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, eg 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 Ramsey (1965) coined the term 'Toxic Unit', and their summation (M). The approach has also been used for median threshold lethal concentrations, 48-h LC50s (Table 1), for other quantal responses, such as LC10 and EC90, and for graded responses.

Table 1. Symbols used in this paper

Individual compounds

c_i	the concentration of substance i
f_i	$c_i/LC50_i$
p48hLC50	$c_i/48hLC50_i$
ptLC50	$c_i/$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 ptLC50
s_i	The standard deviation in log $LC50_i$ (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
ΣptLC50	equivalent to M, when $f_i = c_i/$threshold LC50. Similarly, Σp48h LC50 is equivalent to M when $f_i = c_i/48h$ LC50, and Toxic Units equals $\sum_{i=1}^{n} TU$
f_{max}	the largest f_i value in the mixture of n compounds
M_o	M/f_{max}
$\hat{M}$	the expected value of M in a certain model

Footnote (a) Because only a partial revision has been carried out, it has not been possible to standardise the terminology throughout this Technical Paper.

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 toxicity in terms of LC50s for the individual compounds are 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 (1984a, b, c; 1985a, b, c) and Broderius and Kahl (1985) in selecting chemicals for experiments to test models of simple similar action.

1.2.2 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 constituent toxicant; 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):

$$Q_{mix} = Q_1 \cdot Q_2 \cdot Q_3 \cdots Q_n \tag{1}$$

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 at 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 x 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_o, as defined in Table 1. For equitoxic mixtures of n chemicals $M_o = n$. 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, it may be felt, 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.

1.2.3 Classification of mixture potency

The following classification is based on that given in EIFAC Tec. Pap. No. 37 for a mixture of two substances. 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    $\underline{x} + \underline{y} < 1.0$ More than additive, or supra-additive
                                                       synergism (Ariens and Simonis, 1964) or
                                                       supra-additive interaction (Anderson and
                                                       Weber, 1975a)

For the revision of this document, new information is included on multiple mixtures of
substances with similar joint action, as well as those with dissimilar joint action, the
following model developed by Konemann (1981) is more appropriate. Also, 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_o and f_{max}	Classification of mixture potency
1 $M > M_o$, $f_{max} > 1$	Antagonism
2a $M = M_o$, $f_{max} = 1$	No addition
2b $M_o > M > 1$, $f_{max} < 1$	Partial addition
3 $M = 1$, $f_{max} < 1$	Addition
4 $M < 1$, $f_{max} < 1$	Supra addition

1.2.4 Mixture toxicity scales and indexes

Numerical scales of mixture potency have been developed by several authors. Some of
the scales which utilise Toxic Units as a base 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 (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).
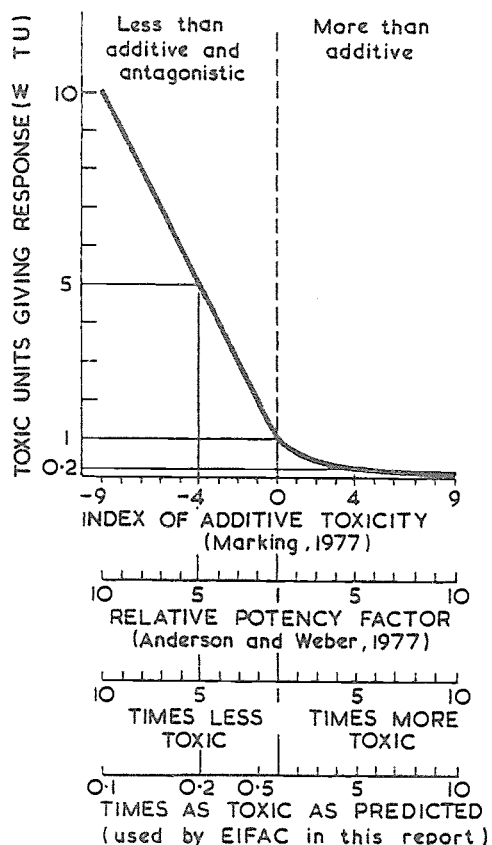
Figure 1. Relation between different terminology used
in studies of mixtures of toxicants

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

$$MTI = \frac{\log M_o - \log M}{\log M_o} = 1 - \frac{\log M}{\log M_o}$$
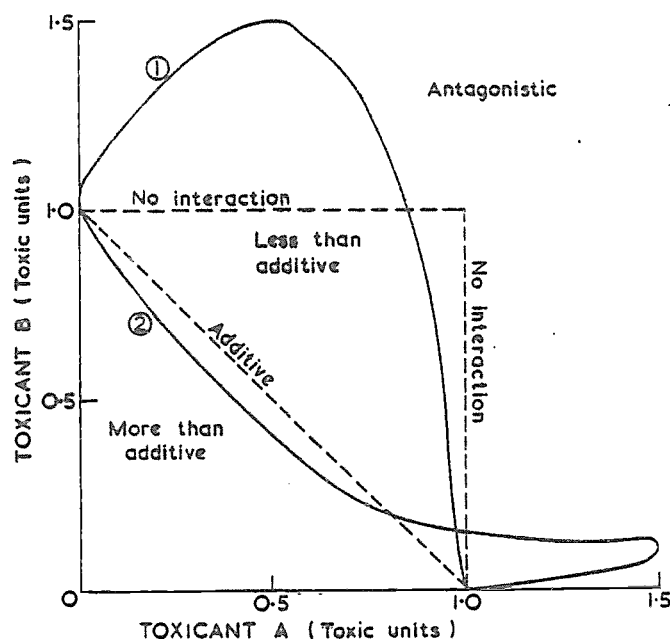
Figure 2. Types of joint action between two toxicants
in a mixture - illustrated by lines of equal
observed toxicity for two hypothetical examples

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 (ie a large f_{max}) in mixtures of unequal toxicity.

1.3 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 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. Hodson et al. (1977) give special attention to the need for replicate tests.

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.

In order to classify the toxicity of a mixture in accordance with the terminology proposed in Section 1.2.4, 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 (1981). 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_o, that is, where log f_{max} is within the 68% confidence limits of M_o.

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 $f_i.10^S$, so that for $\hat{M} = \pm 10^S \sqrt{\Sigma f_i^2}$. The value of $\sqrt{\Sigma f_i^2}$ decreases as n increases, and is minimal for equitoxic mixtures; at a certain value of n, the error in $\hat{M} = 1.0 \pm (1/\sqrt{n}).10^S$. Thus, when $n \geqslant 10$ 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 acted in simple similar or independent action with $r = +1$. It is possible that a qualitatively heterogenous mixture could produce a combined effect which coincides with one or other or 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.

1.3.1 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.

1.3.2 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 any concentration of a substance can be expressed as an equi-effective concentration of another when dealing with dose-response lines that were not parallel. Curvilinear-regression lines resulted from studying a number of mixtures and can be empirically represented 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 elipses.

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 such information becomes apparent.

1.4 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. Response-addition has been widely used in the development of pesticides where maximum lethality may be the primary aim, whereas it has been considered that for water pollution control purposes the concentration-addition model is more relevant in the context of water quality standards. For this reason the experimental data reviewed in the subsequent sections have been derived mainly from experiments based on the concentration-addition model and, in particular, to identify deviations from an additive relationship.

2. APPLICATION OF ADDITIVE INDEX TO LABORATORY STUDIES

In this part of the report the joint effect of toxicants in mixtures is described 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 (ΣTU) shows this to be less than unity. A factor (the reciprocal of ΣTU) is given to indicate by how much the effect is more than additive; conversely, when the sum is more than unity, a factor is given indicating 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 Section 1 and shown in Figs. 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.

2.1 Fish

2.1.1 The toxicity of mixtures containing constituents commonly present in sewage and industrial wastes

The constituents of sewage and industrial wastes are considered in the order ammonia, phenol, cyanide, copper, zinc, cadmium, nickel, chromium, mercury and other heavy metals, and other substances, and mixtures are cross-referenced to appropriate paragraphs. Data for non-European and marine species are included where information for European freshwater fish is scarce or absent and where such information appears to make a useful contribution to the present review. Information contained in Sections 2.1.1.1 to 2.1.1.9 is summarised in Table 2.

2.1.1.1 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 Σpt 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 ranged from 0.72 and 0.69 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 1.19 and 1.0 times more than additive for corresponding ratios of 1:0.7 and 1:0.3 respectively.

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.

(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 $^\circ$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 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 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 cupro-ammonium 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 mixture 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 and 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, and 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.

2.1.1.2 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 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 ^{O}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) Phenol, ammonia and zinc - see 2.1.1.1 (v)

(iii) Phenol, ammonia and sulphide - see 2.1.1.1 (vi)

(iv) p-cresol and cyanide

Southgate (1932) also 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.

(v) Phenol and copper

Brown and Dalton (1970) measured the 48-h 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.

(vi) 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.

(vii) 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.

(viii) Phenol and chlorine

At ambient water temperature 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).

(ix) Phenol, nickel and vanadium - see 2.1.1.7 (v)

(x) Phenol, pentachlorophenol and dinitrophenol

Verma et al. (1981) carried out static tests with phenol, pentachlorophenol and dinitrophenol, using the fish Notropterus notropterus and estimated 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.

2.1.1.3 Cyanide and other substances

(i)   Cyanide and ammonia - see 2.1.1.1 (ii)

(ii) Cyanide and p-cresol - see 2.1.1.2 (iv)

(iii) 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 marcochirus) 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 on the growth of the fish in 30 d (threshold of no effect), of a mixture in the ratio of 1:0.6 of the respective p EC, is about 0.4 times less than additive.

(iv) Cyanide and PCB

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

(v)   Cyanide, zinc and PCB

Anderson and Weber (1975a) showed that the results of Negilski and Davies (1974) 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, when compared with the reduction found with 0.2 of the 96-h LC50 of each separately, could be accounted for by response addition. A similar conclusion can be drawn from an examination of the data on production of fish biomass (from their Table 4). However, in the stream exposed to 0.1 of the 96-h LC50 of cyanide alone production of biomass was enhanced, but 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.

(vi) 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.

2.1.1.4 Copper and other substances

(i)   Copper and ammonia - see 2.1.1.1 (iii)

(ii) Copper and phenol - see 2.1.1.2 (v)

(iii) Copper and zinc

Doudoroff (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 the soft and a hard water (hardness 320 mg/l as $CaCO_3$).   Sprague and Ramsay (1965), who used these metals in water having a 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 having a hardness of 21 to 60 mg/l as $CaCO_3$.   La Roche et al. (1973) mentioned that histological examination of mummichog (Fundulus heteroclitus) exposed to mixtures of copper and zinc showed 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.   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 $CaCO_3$) using longfin dace (Agosia chrysogaster) and found that their joint effect was 1.2 times more than additive.   Anderson et al. (1979a), using zebrafish (Brachydanio rerio) reported that, whereas 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 the percentage 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 medium 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.

(iv) 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 the three was approximately additive. Marking (1977) also found that their joint action was approximately additive (unspecified LC50).

(v) 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 $^{\circ}C$ and 25 $^{\circ}C$, interaction between copper and nickel was slightly more than additive with a restricted ration, and approximately additive with an unrestricted ration.

(vi) 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 their results suggest 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.

(vii) Copper, zinc and cadmium

Eisler and Gardner (1973) measured the percentage survival of mummichog in synthetic sea water (20% salinity) in the presence of copper, zinc and cadmium and a mixture of all three, and the 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, and 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 12½ months and measured several sub-lethal effects and compared then qualitatively with that for zinc he had tested separately, and those 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, while 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).

(viii) Copper and mercury

Work has been carried out at the Istituto di Ricerca sulle Acque (Italian Institute for Water Research) (1975) (D. Calamari and R. Marchetti, pers. comm.) to measure 96-h LC50 values for rainbow trout with copper and mercury, and mixtures of the two, tested at 15 $^\circ$C in water having a hardness of 320 mg/l as $CaCO_3$; additive joint action was found between the two metals.

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.

(ix) 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.

(x) Copper and surfactant

Calamari and Marchetti (1970), measuring 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 $^\circ$C, 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, 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 $^{\circ}$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. In the latter case the joint action in terms of the Σp 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 LC5 when it was twice as additive.

(xi) 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 on bluegill of a mixture of acetic acid, acetaldehyde, acetone and copper in soft water.

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 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.

2.1.1.5 Zinc and other substances

(i) Zinc and ammonia - see 2.1.1.1 (iv)

(ii) Zinc, ammonia and phenol - see 2.1.1.1 (v)

(iii) Zinc, phenol and copper - see 2.1.1.2 (vi)

(iv) Zinc and cyanide - see 2.1.1.3 (iii)

(v) Zinc, cyanide and PCB - see 2.1.1.3 (v)

(vi) Zinc and copper - see 2.1.1.4 (iii)

(vii) Zinc, copper and nickel - see 2.1.1.4 (iv)

(viii) Zinc, copper and cadmium - see 2.1.1.4 (vii)

(ix) Zinc and cadmium

Tests with rainbow trout in hard water (J.F. de L.G. Solbe 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/l and that at lower concentrations of zinc (down to 0.5 mg/l), periods of survival were accountable by the concentration of cadmium alone. Furthermore, 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 ug/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 larvae and for 100 days from embryos, 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 they cited by R.L. Spehar with zinc alone. The authors suggested that because such fish were a sensitive indicator of toxicity it could be concluded that the chronic toxicity of mixtures of cadmium and zinc would also be largely attributable to the effect of zinc. 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 initially exposed as embryos to zinc and cadmium (Spehar et al., 1978) additive interaction was not demonstrated.

Eisler and Gardner (1973) measured the percentage survival of mummichog in synthetic sea water (20% salinity) in the presence of zinc and cadmium and mixtures of the two, and 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 appeared to differ with the 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.

(x)   Zinc and detergent

Brown, Mitrovic and Stark (1968) exposed rainbow trout to mixtures of a detergent containing 55% ABS and a fixed concentration of 0.8 mg Zn/l, and comparison of their results with those for zinc alone (Lloyd, 1960) shows that 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, and 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.

(xi) Zinc and cygon

Roales and Perlmutter (1974a) measured the 72-h LC50 of zinc and cygon (O,O-dimethyl-S-(N-methyl)carbamylmethyl phosphorodithionate) to the embryos of zebrafish and the percentage 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.

2.1.1.6 Cadmium and other substances

(i) Cadmium and copper - see 2.1.1.4 (vi)

(ii) Cadmium, copper and zinc - see 2.1.1.4 (vii)

(iii) Cadmium and zinc - see 2.1.1.5 (ix)

(iv) Cadmium, chromium and nickel

D. Calamari (pers. comm.) held large (220 g) rainbow trout in a hard water (320 mg/l as CaCO$_3$) in the presence of 0.01 mg Cd/l, 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, and subsequently he kept the fish 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 (on content of sialic acid in gills, glucide in liver and proteolytic activity in liver and red blood cells) were found in all four treatments but no evidence of effects with the mixtures more marked than those with the single metal treatments. This suggests a less than additive joint sub-lethal action of the three metals.

(v) 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 than additive at 10 days.

2.1.1.7 Nickel and other substances

(i) Nickel and copper - see 2.1.1.4 (v)

(ii) Nickel, cadmium and copper - see 2.1.1.6 (iv)

(iii) Nickel and chromium

Data provided by F.S.H. Abram (pers. comm.) 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.

(iv) 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.

(v) Nickel, vanadium and phenol

In contrast to the above results with nickel and vanadium, 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.

2.1.1.8 Chromium and other substances

(i) Chromium and cyanide - see 2.1.1.3 (vi)

(ii) Chromium and nickel - see 2.1.1.7 (iii)

(iii) 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.

2.1.1.9 Mercury and other substances

(i) Mercury and copper - see 2.1.1.4 (viii)

(ii) 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 to occur in surface waters, was markedly more than additive in toxic effect, resulting in a reduction in hatch of over 80%. The authors suggest that although both toxicants had an affinity for sulphydryl (SH) groups, they reacted 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 percentage mortality than in untreated fish when subsequently exposed for 48 h to a range of concentrations of mercury. 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, however, Heisinger et al. (1979) showed that in the presence of equimolar concentrations of selenium dioxide the 48-h LC50 of mercuric chloride 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 the selenium.

(iii) 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.

2.1.1.10 Studies on complex mixtures of organic substances

The following data have been published since 1980, and form part of the updating of this report. Because of the basic principles involved in these studies, data on Daphnia and bacteria are included to support the conclusions drawn from studies on fish. The evaluation of the data follows the format given in Section 1.2 and is summarised in Table 3.

(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 $\pm$ 0.23 and 0.82 $\pm$ 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 a 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 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 explained to a large extent the differences in toxicity between the individual chemicals, as shown by a calculated QSAR. Therefore simple similar action was 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 (1983) and Hermens (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 1-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 as used by Konemann (1981a), Hermens and co-workers have studied the effects of equitoxic mixtures on 48-h EC50 (immobilisation), 16-d LC50 and 16-d EC50 (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. Also the effects on reproduction of a mixture of five chemicals was less than additive (M = 2.0; MTI = 0.57 ± 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 phophoreum 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 ± 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 ± 0.12).

Hermens et al. (1982) determined the toxicity of a very heterogenic mixture of 24 chemicals (e.g. allylalcohol, acrylamide, dieldrin, malathion, menthol, triphenyltin chloride, decamethrin, phenol, etc.) and of five different selections of 8 chemicals out 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 combinations of 2 and 3 chemicals, each with probably 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), respectively. 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). In the same publication Hermens et al. also determined in 4-fold 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).

2.1.1.11 Summary

The results of laboratory tests with fish using mixtures of toxicants commonly found in sewage and industrial wastes, summarised in Table 2, 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 3, indicate that their joint action is either additive or slightly 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.

2.1.2 The toxicity of industrial wastes, effluents and river waters

2.1.2.1 Sewage effluents

The predictive method of estimating toxicity from ΣTU 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 $\pm$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 ΣTU differed from unity were 0.63, 0.73, 0.8, 0.81, 0.83, 0.9, 1.0, 1.06 and 1.18 (Lloyd and Jordan, 1963) and 0.48, 0.83, 0.89, 0.91, 1.04, 1.3, 1.4, 1.5 and 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, 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 unionised ammonia (0.01), zinc (0.05) and copper (0.38 to 0.08 depending upon the figure 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.

2.1.2.2 Spent gas liquor

Herbert (1962) using rainbow trout showed that, as expected, the toxicity of a spent liquor from a gas works 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.

2.1.2.3 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.

2.1.2.4 Drilling fluid

Sprague and Logan (1979) tested rainbow trout in three samples of used drilling fluid, whose 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) 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.

2.1.2.5 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

Table 2. 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)
	" "	" "	1.0:0.3 & 0.7	Additive	1.0 to 1.2	" "
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	" " "	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	
			1:7:1	More than additive	1.2	Brown, Jordan
			1:1:6	Less than additive	0.7	and Tiller (1969)
			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	
			1.0:0.02:0.1	" " "	0.6	Jenkins (1964)
			1.0:0.7:2.0	" " "	0.6	
			1.0:0.3:0.3	" " "	0.8	
Ammonia + sulphide	Fathead minnow	24-h LC50	1.0:2.2	Less than additive	0.6	
			1.0:0.3	" " "	0.6	Jenkins (1964)
			1:11	" " "	0.8	
			1.0:1.4	" " "	0.8	
Ammonia + nitrate	Guppy	72-h LC50	1.0:>0.55	Additive	<1.1	Rubin and
			1.0:<0.35	Less than additive	>0.7	Elmaraghy (1977)
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	
			1:11	" " "	0.8	
			1:3	" " "	0.7	Jenkins (1964)
			1:1	Additive	0.9	
Cyanide + zinc	Bluegill	96-h LC50		Less than additive	0.4	Cairns and Sheier (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	" " "	0.6 to 0.8	Smith et al (1979)

Table 2 (continued)

Toxicants	Species	Exposure period and response	Ratio of toxicant EC50	Joint action	Multiple of additive joint action	Reference
Copper + zinc	Rainbow trout	3-d LC50 (hard water)	1:1	Additive	1.0	Lloyd (1961)
		7-d LC50 (soft water)	1:1	"	1.0	" "
	Atlantic salmon	7-d LC50 (" ")	1:1	"	1.0	Sprague and Roussey (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)
	"	Non-specified	1:1:1	"	(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)
	"	96-h LC50	Various	More than additive	?	Eisler and Gardner (1973)
	Zebrafish	96-h LC50	?	" " "	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	" " "	1.3	Eaton (1973)
		12½ 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% " " " "	1.0:0.1:1.2	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 ABS	Goldfish	96-h LC50	1.0:0.1	More than additive	1.3	Calamari and Marchetti (1970)
			1.0:0.6	" " "	2.1	
LAS	Rainbow trout	96-h LC50	1:1	" " "	1.3	Calamari and Marchetti (1973)
+ nonyl phenyl ethoxylate	Rainbow trout	96-h LC50	1:1	Less than additive	0.8	"
Copper + paraquat	Poecilia mexicana	24-h LC50	1:0.25	More than additive	1.3	Sun and Gorman (1973)
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)
	"	10-week LC50	1:0.3	More than additive	13	"
	"	"	1:1	" " "	21	"
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)

Table 3. 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)
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)

Table 3 (cont'd)

Toxicants	Species	Response	Ratio	n	M	MTI ± sd	Reference
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 chlorphenol 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)
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 (a.o. alcohols, chloroalkanes and chloroaromatics)	Photobacterium phosphoreum	bioluminiscence inhibition EC50	equitox	21	2.0	0.77 ± 0.8	Hermens et al. (1985b)

predicted 48-h LC50 based upon the content of ammonia, phenol, cyanide, copper and zinc, was less than unity (Σp 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 Section 3. Field Studies.

2.1.3 The toxicity of pesticides

2.1.3.1 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 apparently gave 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 out 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 and Lech (1975a and b) measured the percentage 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 on 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 rather than blood flow, but 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 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 parameter 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 the author) 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.

2.1.3.2 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 the result obtained by Solon et al. (1969) 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 there was a tendency for goldfish that had been exposed to 4 µg/l of alkylbenzene sulphonate for several months to be more susceptible to subsequent exposure to 50 µg/l dieldrin in the presence of 4 µg/l ABS than fish not so exposed.

2.1.3.3 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.

2.1.3.4 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.

2.1.4 The toxicity of other substances

2.1.4.1 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 the same 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) some of which have been shown to be 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.

2.1.4.2 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 $^{\circ}$C, and 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 organism.

2.1.4.3 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.

2.1.4.4 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 only using 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 apparently found it slightly (1.3 times) more than additive in the mixture.

2.1.4.5 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 for 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).

Tsai and McKee (1980) measured the 96 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.

2.1.4.6 Summary

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

2.1.5 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.

2.1.5.1 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 2 and

illustrated in Figure 3. The more recent data summarised in Table 3 support the general conclusion for toxicants other than pesticides.
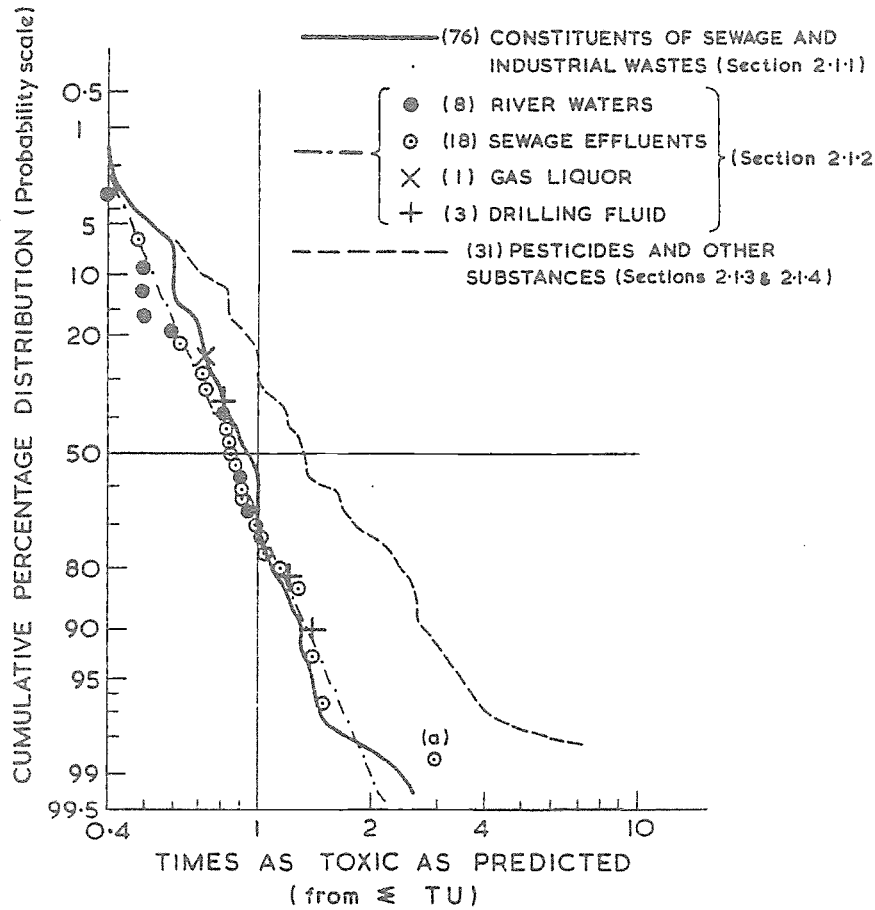
Figure 3. Summary of laboratory data on predicted/observed sum of toxic units for freshwater fish. Number of sets of data shown in parentheses. Results relate largely to short-term LC50 values to rainbow trout but include data on other species and effects on growth (see text).

'(a)' represents effluent having a high content of industrial wastes including pesticides.

2.1.5.2 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 the effect 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.

2.1.5.3 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).

2.1.5.4 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 that 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, 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. Whereas zinc and copper were additive in terms of the threshold LC50 for Atlantic salmon (Sprague and Ramsay, 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 (Section 2.1.1.10); 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.

2.1.5.5 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 that these can be profound in some cases (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, and that their data 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, as in 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 1, 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 TU's of phenol, and 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 from these experiments to be able to draw any general conclusions about the contribution of low TU values for the other toxicants present in the mixtures.
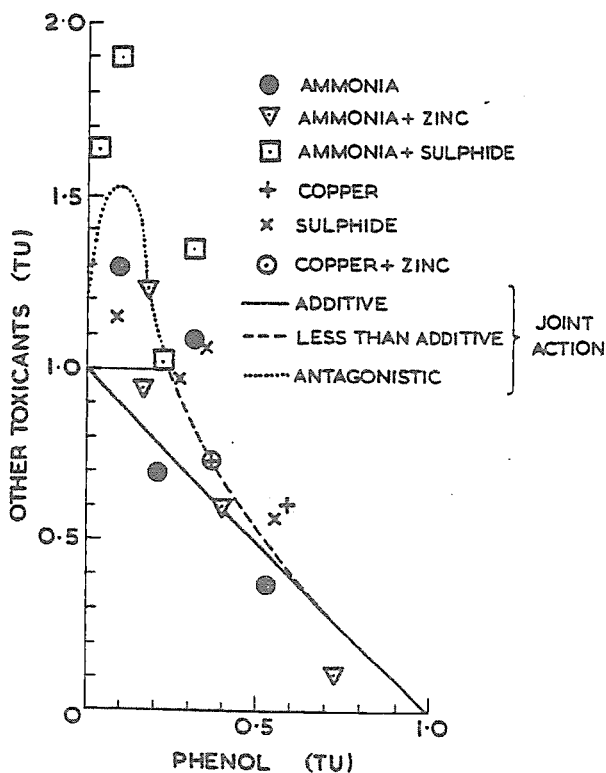

Figure 4. Toxic units of phenol and other toxicants in mixtures producing a given lethal response in fish (data calculated from those in Table 1).

2.1.5.6 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 1 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.

2.2 Invertebrates

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

2.2.1 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.

2.2.2 Copper and zinc

Buikema et al. (1977), measuring the percentage 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.

2.2.3 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 examintion 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.

2.2.4 Cadmium, malathion, methoxychlor and Arochlor 1245

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, 1976) 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.

2.2.5 Chromium and other substances

Buikema et al. (1977), measuring the percentage 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.

2.2.6 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 Section 2.1.1.10.

2.2.7 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 (x 5.3 for atrazine, x 4.5 for simazine, x 3.5 for monuron and x 3.2 for 2,4-D), while that between DDT and the herbicides was approximately additive, although the nominal concentration of DDT used 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.

2.2.8 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.

2.3 Aquatic plants

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

2.3.1 Metals

2.3.1.1 Zinc and cadmium

Hutchinson and Czyrska (1973) showed that, at a concentration of 10 µg Cd/l, the growth of Salvinia natans under a variety of conditions (except when tested alone) and of Lemna valdiviana was generally slightly inhibited, an effect that 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 (when tested alone) in the presence of 30 µg Cd/l, both alone and with either 30 or 80 µg Zn/l, and with Lemna (when 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 the presence of zinc appeared to result in a higher cadmium uptake by Lemna.

2.3.1.2 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.

2.3.1.3 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.

2.3.1.4 Cadmium and nickel

Stratton and Corke (1979) measured the percentage response of the alga Anabaena inaequalis to mixtures to 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 less than additive both for growth at 10 days (0.5-0.74 times) and for acetylene reduction (up to 0.63 times), but inconsistent for the uptake of carbon dioxide.

2.3.1.5 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.

2.3.1.6 Cadmium and mercury

Stratton and Corke (1979) measured the percentage 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.

2.3.1.7 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.

2.3.1.8 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.

2.3.2 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 just one 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.

2.3.3 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 tests, e.g. with zinc and cadmium, but even within a species such as Anabaena for which a considerable amount of data is available, results 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.

3. FIELD STUDIES

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

3.1 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 (Σp 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 Σp 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 were 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.

3.2 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 (Σpt LC50) for ammonia,

phenol, copper and zinc in the early studies (Herbert et al., 1965) and 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 Σpt LC50 was compared with the observed mortalities. Mortalities usually occurred within the 3 days' observation 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 Σpt 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 than the laboratory-derived value for copper in clean water was used by Herbert et al. (1965) to avoid over-prediction of toxicity.

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 corroborated 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 Σpt 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 Σpt 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 Σpt 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 Σpt 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, referring to the tendency of predictions based on a limited number of pollutants to under-estimate the toxicity of river waters, and to the results of long-term experiments which 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 at this stage can be added several others. The initial summation of proportions of toxic concentrations was based on threshold LC50s (Herbert, 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 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 Σp 48-h LC50 of up to a maximum of 0.4, it was not made clear that the mean values in rivers would be considerably less, and probably lower than 0.2.

It was this latter aspect that was developed by Alabaster et al. (1972), who studied the annual frequency distribution of Σp 48-h LC50 at each of a number of sampling sites in the R. Trent catchment area; these distributions tended to be log. normal. Data on the status of the fisheries at these sampling point indicated that some sort of non-salmonid fish population was present when the median Σp 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 underestimated 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 usefulness 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 in its area over a 6-year period, and 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 stickleback, 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, UK, a stream polluted mainly by ammonia and zinc, and found that it was fishless where, over a 3-year period, the median and 95-percentile values of Σp 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.

3.3 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 substance, salmonid populations were unaffected where the Σp NOEC (95 percentile) was 2.0 but were absent at 5.0. In terms of Σp 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 LC50 (rainbow trout) of these two toxicants were about 0.3 and 0.8 respectively, the biomass of the fish was reduced, and 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 up to 300 µg Zn/l and up to 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 t LC50 for salmonids respectively, giving a Σpt LC50 of 0.39 and a corresponding Σp 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 Σpt LC50 of 0.21 and a Σp 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 a considerable number of 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.

3.4 Current studies

The approach outlined above is being extended in the UK; predictive models incorporating Σp 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.

3.5 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 Σpt LC50s (rainbow trout) is <0.2. It is not known whether this condition is equivalent to a Σp 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).

4. UPTAKE OF TOXICANTS

4.1 Heavy metals and other substances

4.1.1 Copper and zinc

Wedmeyer (1968) was able to show that zinc[65] uptake of embryos of coho salmon was inhibited by dissolved copper in the range of 0 to 2.0 mg Cu/l, but facilitated at 2.0 mg/l. Although giving no further information on the interaction of these toxicants, he mentions that the differences could be related to their 'synergistic toxicity'. He also showed that Malachite green, commonly used to control fungus infections, also increased zinc permeability, presumably by reducing zinc binding on the chorion, especially when used at concentrations greater than about 1.0 mg/l.

4.1.2 Copper, cadmium and lead

Westernhagen et al. (1979) investigated the uptake of copper, cadmium and lead by embryo herring (Clupea harengus). Both copper and cadmium promoted the uptake of lead, but when concentrations of copper in the water were low, the presence of lead inhibited the uptake of cadmium while the presence of cadmium enhanced the uptake of copper. At relatively high concentrations of copper (0.075 to 0.133 mg Cu/l), the uptake of copper was promoted by the presence of lead, but inhibited by the presence of cadmium at a given concentration of cadmium; increasing concentrations of copper also tended to impede the uptake of cadmium. Lead at 0.56 and 4.44 mg/l promoted the accumulation of cadmium, but at 2.5 mg/l, inhibited it.

Hewitt and Anderson (1978) showed that a reciprocal interaction occurs whereby copper and cadmium accumulated in gills at a higher rate when each agent was present in the ambient environment concurrently, rather than singly.

4.1.3 Zinc and cadmium

Comparatively low levels of cadmium inhibited the uptake rate of zinc from water by mummichog, but inhibition of cadmium uptake by low levels of zinc was not observed (Eisler and Gardner, 1973).

Tests lasting 100 days, carried out by Spehar et al. (1978) with flagfish (Jordanella floridae) exposed to sub-lethal concentrations of mixtures of zinc and cadmium, showed that the uptake of one metal was not influenced by the presence of the other. This is consistent with their findings on the acute toxicity of the mixture, which was attributable mainly to zinc.

4.1.4 Zinc and DDT

Accumulation of Zn^{65} from sea water by the alga (Ulva rigida) was investigated by Andryushchenko and Polikarpov (1974); they found that it was 12 to 36% higher in the absence of DDT than in the presence of a concentration of 1 mg DDT/l.

4.1.5 Cadmium and methoxyclor

Nimmo and Bahner (1977) exposed shrimp (Penaeus duorarum) to cadmium and methoxychlor separately and in combination, and showed that cadmium, but not methoxychlor, was accumulated by this organism, and the methoxychlor appeared to influence the processes of accumulation and loss of cadmium from the tissues.

4.1.6 Cadmium and PCB

When PCB was present in the water together with cadmium, the accumulation of cadmium by the shrimp (Penaeus duorarum) was similar to that found when cadmium was applied alone (Nimmo and Bahner, 1977).

4.1.7 Mercury and magnesium (and azide and cyanide)

Fujita et al. (1976) studied the accumulation of mercury by the freshwater diatom Synedra ulna and found that the rate of uptake was increased by the presence of magnesium at an optimal concentration of 0.1 mM Mg^{2+} in the standard buffer solution used to grow the organism. They suggested that this was attributable to an effect of magnesium on energy-linked metabolism and showed that azide and also cyanide (both inhibitors of respiratory enzymes) at a concentration of 0.01 mM CN markedly reduced the uptake of mercury.

4.1.8 Lead and xanthate

Borg et al. (1976) found that the uptake of lead by trout fry from soft water at 10 ^{O}C was increased by increased concentrations of xanthate, and they suggested that this might account for the increased toxicity of lead, it being assumed that lead xanthate was formed as an intermediary compound in the water close to the gill, and might also increase the solubility of lead in fat. They reviewed similar effects that occur with other types of organic sulphur compounds such as dimercaptopropanol, certain dithiocarbamates, and methyl mercaptan, in combination with metals such as copper and mercury.

4.2 Pesticides

4.2.1 DDT, dieldrin and methoxychlor

Macek et al. (1970) found significant interaction between DDT and dieldrin in their uptake into the pyloric caecae of rainbow trout fed with these chemicals in their food. The presence of dieldrin increased the rate of accumulation of DDT and conversely the presence of DDT decreased the rate of accumulation of dieldrin. Dieldrin also reduced the elimination of DDT, although the converse was not true.

Mayer et al. (1970) measured the accumulation of DDT, methoxychlor and dieldrin in the fat of rainbow trout fed with these chemicals in their diet. The results were complicated. Dieldrin increased DDT storage whereas methoxychlor alone, and also interactively with dieldrin, reduced it. DDT and methoxychlor reduced dieldrin storage, depending upon the dose of dieldrin, and also reduced methoxychlor storage. Dieldrin and DDT interacted to reduce methoxychlor storage, depending on the dosage of the latter. To account for some of these results the authors suggested that DDT and possibly methoxychlor could cause selective induction of drug-metabolising enzymes in the liver and cited and discussed supporting evidence for this.

4.2.2 DDE and heptachlor epoxide

Veith et al. (1979) exposed fathead minnow to sub-lethal aqueous concentrations of DDE, heptachlor and a mixture of the two and found that concentration factors for each of the substances were similar whether or not they were present alone or together in the water.

4.2.3 Carbaryl and butylester and dichloro-nitrosalicylanilide

Statham and Lech (1976) found a more than additive lethal effect of carbaryl on 2,4-D butylester and 2',5-dichloro-4'-nitrosalicylanilide in rainbow trout. They concluded that the increased toxicity could be caused by increasing the uptake of the toxic compounds from the water, but did not carry out residue analyses to confirm this.

4.2.4 Piperonyl butoxide, aldrin, methoxychlor and trifluralin

Reinbold and Metcalf (1976) found that piperonyl butoxide greatly increased the accumulation in green sunfish (Lepomis cyanellus) of methoxychlor (15 times), aldrin (21 times and trifluralin (45 times). The effect was interpreted as a possible inhibition of mixed-function oxidases by piperonyl butoxide in preventing detoxication.

4.3 Summary

The few data available on the joint effect of toxicants on their uptake by aquatic organisms allow only tentative conclusions to be drawn.

For heavy metals, there is evidence that the uptake of zinc, lead and cadmium by fish embryos is inhibited by low concentrations of copper and enhanced by high concentrations. The uptake of cadmium is similarly affected by lead but, in one study, inhibition by low concentrations of zinc was not found. There are also observations on fish showing that the uptake of either copper or cadmium can be enhanced by the presence of the other. The failure to find any reciprocal effect on uptake between zinc and cadmium in one study involving the use of flagfish may be attributable to the particular concentrations chosen.

Substances such as DDT, methoxychlor and xanthate have been shown to increase the uptake of heavy metals in aquatic organisms but some, such as PCB, have had no apparent effect, while others, such as magnesium, azide and cyanide, have reduced uptake.

With pesticides the effect of either dieldrin, DDT or methoxychlor or combination of any two of these compounds, on the accumulation in fish through their food of any of the others, was generally inhibitory, except for the effect of DDT on the accumulation of dieldrin.

Accumulation of combinations of toxicants can be influenced by:

a)   inhibition of detoxifying enzymes which can influence the uptake or loss of xenobiotics;

b)   action upon biological surfaces, thereby affecting the permeability of membranes to other toxicants present;

c)   physiological effects, such as increasing the flow of blood through the gill, thereby increasing the uptake of toxicants; and

d)   external factors, such as forming of complexes (see also Introduction).

From the information available, no general rules for the uptake of toxicants in the presence of other toxicants can be deduced. There is no clear evidence of a correlation being found between the toxicity and the accumulation of combined toxicants, although several papers speculate on this subject.

This aspect of mixture toxicity would require much detailed research to achieve even a superficial understanding of the interaction involved. However, this should not detract from the need to carry out empirical studies on those factors (including toxicants) which affect the accumulation of substances in fish which may reach concentrations harmful to the health of consumers, including man.

5.   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 the 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.

5.1 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 that 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 (ie 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. However, unpublished work 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 appeared 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 to make this comparison 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 slight less than additive joint action of metals, but only inconsistent and unquantitative 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 water 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 Σpt LC50s (rainbow trout) is <0.2. It is not known whether this condition is equivalent to a Σp 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).

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.

5.2 Scope for further research

The research carried out so far has been directed mainly towards combinations of these 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) does 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, 1972) 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 a methodology, information has to be derived from other sources. At present, the knowledge of fish physiology and biochemistry has not reached the point where a quantitative prediction of individual toxicant effects 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 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 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, have been tested, although it must be assumed that there are no marked differences in percentage composition between samples.

5.3 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, 1982) 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 realistic 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.

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