

## *Special Tests in Aquatic Toxicity for Chemical Mixtures: Interactions and Modification of Response by Variation of Physicochemical Conditions*

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### ABSTRACT

There is a considerable literature on the toxicity of mixtures of chemicals to aquatic organisms, and models have been developed to quantify the types of joint action which occur. Similarly, there are abundant data on the effects of physicochemical variables such as water hardness, temperature, dissolved oxygen, pH, and complexing agents on the toxicity of single chemicals to fish, but little work has been done on the effect of these variables on the toxicity of mixtures of chemicals. The available evidence suggests that the toxicity of each chemical in a mixture will be affected by these variables to the same extent as for the single chemical. The implications of these studies for predictions of the toxicity of complex effluents after their discharge to natural waters are assessed and areas for future research are identified.

### 1 INTRODUCTION

The world literature on the effects of pollutants on aquatic organisms is concerned largely with single chemicals; the effects of mixtures have received much less attention. Furthermore, work with mixtures has been directed mainly towards combinations of common pollutants such as heavy metals, ammonia, phenol, and cyanide which are known to have caused damage to aquatic environments. Much of the available information in this field has been reviewed recently by the European Inland Fisheries Advisory Commission (EIFAC) Working Party on Water Quality Criteria (Alabaster and Lloyd, 1982); a re-evaluation of the conclusions of the Working Party in the light of recent research by K onemann (1979, 1980) and Hermens and Leeuwangh (1982) has been made by Lloyd (1985), with the following conclusions:

- (a) There is no evidence for synergism (i.e. more-than-additive action) between the common pollutants; at toxic concentrations the joint action is additive and at concentrations below those considered 'safe' there is circumstantial evidence for less-than-additive joint action. Water quality standards for individual common pollutants need not be adjusted to take into account the presence of other pollutants.
- (b) Chemicals which have a common toxic action based on a common quantitative structure-activity relationship (QSAR) are additive at all concentrations. This implies that water quality standards should be applied to each QSAR group, rather than to the individual chemicals.
- (c) Present knowledge does not allow the toxicity of a complex effluent to be ascribed to the constituent toxicants unless they all have a common QSAR or the toxicity is dominated by a single common pollutant.

However, the scattered literature on the toxicity of mixtures contains a variety of experimental methods designed to meet widely different objectives and these general conclusions can at best be regarded as tentative. The purpose of this paper is to provide an outline of the present state of the art of measuring and predicting the effects of mixtures of pure chemicals and of discharges of complex effluents on aquatic biota, and the extent to which physicochemical variables can influence the toxicity.

## 2 MODELS AND TERMINOLOGY

There are two basic models used to measure the joint action of toxicants in a mixture. The response addition model is based on the proportion of the test population which is affected by the toxicants applied singly or in mixture. This approach is applicable when an increased potency of a chemical is being sought, as with pesticides where the goal is a total mortality of the target organisms. When considering water pollution problems, however, for which control is achieved by the use of water quality standards, the concentration addition model is more appropriate. In this model, the joint action of two toxicants, A and B, can be described as follows. Concentrations of each toxicant are expressed as a proportion of their threshold (asymptotic)  $LC_{50}$  for a given species and particular test conditions. These proportions have been termed 'toxic units' (TUs) by Sprague and Ramsay (1965). Data from toxicity tests with A and B, and their mixture (A + B), are used to solve the following equation:

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

The type of joint action demonstrated depends on the values of  $x$  and  $y$  which satisfy this equation:

Values for $x$ and $y$	Type of joint action
$x$ or $y > 1.0$	Antagonistic
$x$ and $y < 1.0$ and $x + y > 1.0$	Less than additive
$x + y = 1.0$	Additive
$x + y < 1.0$	More than additive (synergism)

Because the joint action of pairs of poisons is usually additive where the individual concentrations are  $> 0.3$  TU, the expected result is  $(x + y) = 1.0$ . Therefore, the extent to which the mixture is either less or more additive than expected is given by the reciprocal of  $(x + y)$ ; if  $(x + y) = 1.5$ , the mixture is 0.67 times less toxic than expected and similarly if  $(x + y) = 0.5$  the toxicity is twice that expected.

This simple model has its drawbacks especially where the joint action is less than additive (Lloyd, 1985) and a model developed by Könemann (1979) may be more appropriate. In his model, the following terms are used (abridged from Könemann, 1979):

- $f_i$  = the concentration of a chemical in a mixture, expressed as TUs  
 $f_{\max}$  = the highest  $f_i$  of the chemicals present in a mixture  
 $M$  = the sum of the TUs in a mixture which produced the given response (in Könemann's work,  $M$  was the 14-day  $LC_{50}$  of the mixture)  
 $M_o = \frac{M}{f_{\max}}$

The type of joint action is given by the following values:

Values for $M$ , $M_o$ and $f_{\max}$	Type of joint action
$M > M_o$ , $f_{\max} > 1$	Antagonism
$M = M_o$ , $f_{\max} = 1$	No addition
$M_o > M > 1$ , $f_{\max} < 1$	Partial addition
$M = 1$ , $f_{\max} < 1$	Addition
$M < 1$ , $f_{\max} < 1$	Supra addition

To quantify the extent of the joint action, a 'mixture toxicity index' (MTI) was derived as

$$\frac{\log M_o - \log M}{\log M_o}$$

This gives a scale of 0 to 1 between 'no addition' and 'addition'. The model has several advantages over the previous one, especially where mixtures of more than two toxicants are tested. For example, it distinguishes between a mixture of five toxicants, each at a concentration of 0.2 TU and a similar mixture in which one

toxicant is at 0.9 TU and the remainder each at 0.025 TU; the potential effect of a dominant toxicant is therefore identified. This model is also capable of further refinement, if necessary, to allow a statistical treatment of the data for significance (Könemann, 1979).

### 3 TOXICITY TESTS WITH MIXTURES

It is regrettable that much of the published data on the toxicity of mixtures is given in terms of 48-hour or 96-hour  $LC_{50}$ , with no indication as to whether these can be considered as threshold or asymptotic values. The advantages of expressing experimental data for single chemicals in the form of a toxicity curve—the relationship between  $\log LT_{50}$  for a range of log concentrations, or between  $\log LC_{50}$  for a series of log exposure periods (or preferably the two combined)—have been described previously (Lloyd, 1979). The graphical representation of the idealized data from toxicity tests with toxicants A and B and mixtures of A + B which show additive joint action is shown in Figure 1. This figure shows that at high concentrations the toxicity curve for the mixtures will tend towards curve A, rather than remain in an intermediate position.

However, there are recorded instances of synergism occurring at concentrations  $> 1.0$  TU for the mixture, i.e. greater than the threshold  $LC_{50}$ . For example, both Lloyd (1961) and Sprague and Ramsay (1965) found evidence of synergism between the toxicity of copper and zinc salts to salmonids in soft water at acutely toxic concentrations, although at the threshold concentration of the mixture the joint action was additive. The toxicity curves obtained are shown diagrammatically in Figure 2.

In both experiments, the synergistic action occurred with concentrations toxic in less than one day, but it is conceivable that, with a more resistant species of fish exposed to a slow-action toxicant for which the threshold  $LC_{50}$  was reached only after a much longer period than four days, a similar synergistic action could still be operating at the 96-hour exposure period. Although it would be valid to claim that synergism occurred at these acutely toxic concentrations, it may be incorrect to predict that such synergistic joint action would occur also at the much lower toxicant concentrations which would be allowed in a watercourse by pollution control regulations.

Apart from a proper experimental design, the main requirement of toxicity tests with single chemicals and their mixtures is that the test organisms be exposed to a constant concentration of active toxicant, with other environmental variables also being held within close limits. Only with such care can accurate toxicity curves be obtained, the 95% confidence intervals of the threshold  $LC_{50}$  kept as narrow as possible and the type of joint action between the two toxicants demonstrated. This is especially important when very low concentrations of highly toxic substances are being tested.

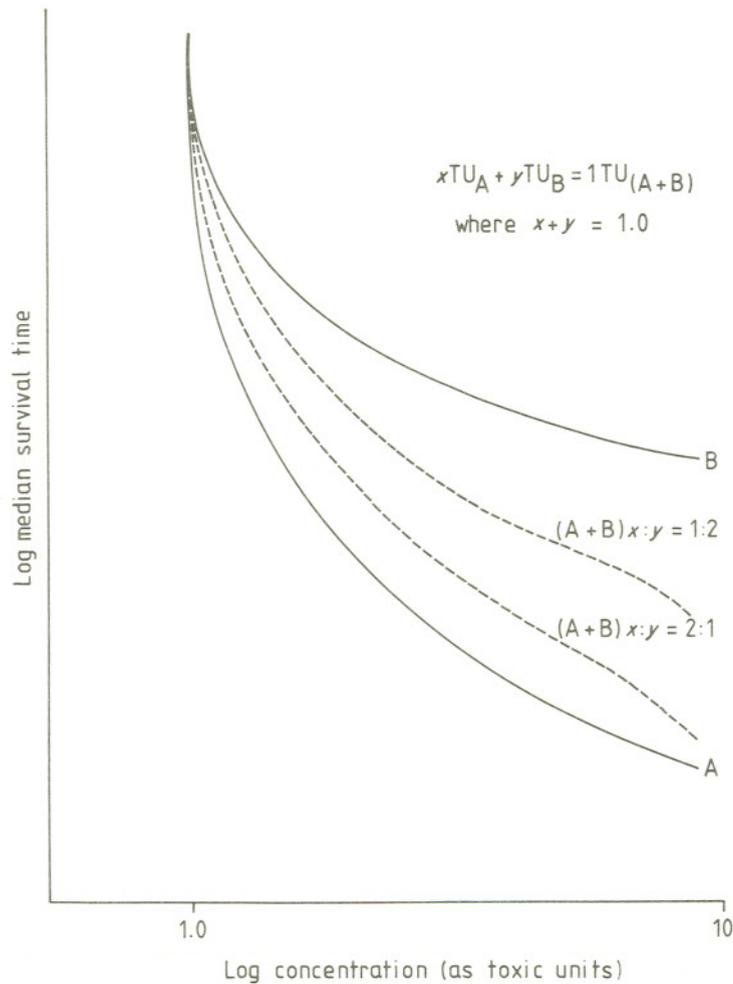


Figure 1 Idealized toxicity curves for two toxicants A and B and for mixtures of A + B, showing additive joint action

Basic toxicity curves and threshold  $LC_{50}$  were established for a number of common pollutants, both singly and as pairs or triplets, at the Water Pollution Research Laboratory, Stevenage, UK, during 1960–70. It was known at that time that the threshold  $LC_{50}$  of the individual common pollutants could be affected by one or more of a variety of natural physicochemical factors which therefore had to be taken into account when predicting the toxicity of effluents and polluted waters. These variables can be divided into two groups: those which affect the

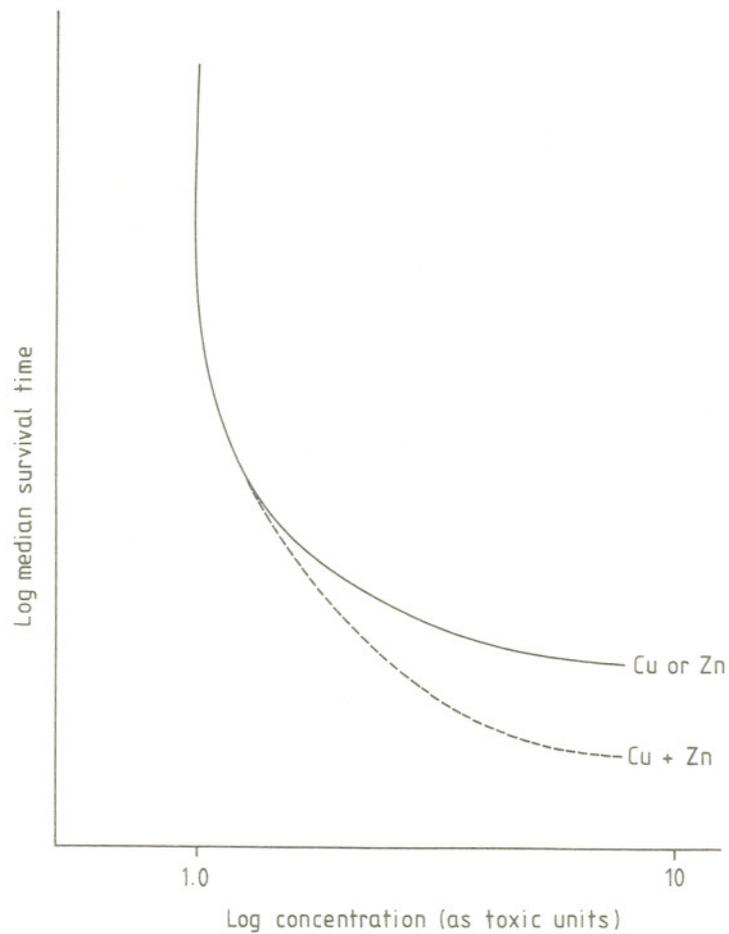


Figure 2 Idealized toxicity curves for salmonids exposed to copper and zinc separately and as a mixture, in soft water

toxic properties of the chemical and those which modify the sensitivity of the test organism. For the latter group, most of the work has been with fish, although it must be remembered that other groups of organisms may be similarly affected by these variables but to different extents. Much of the information summarized in the following two sections is derived from the EIFAC reviews of selected common pollutants (Alabaster and Lloyd, 1982).

#### 4 ENVIRONMENTAL VARIABLES AFFECTING THE TOXIC PROPERTIES OF CHEMICALS

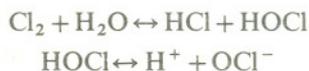
##### 4.1 pH: Ionization

There are certain weak acids and alkalies whose toxicities depend on the concentration of the non-ionized form in the solution. The best known of these is ammonia, whose toxicity increases with pH as the ionized proportion in solution becomes less:



The toxicity of ammonia increases approximately ten-fold for each unit increase in pH up to pH 8.0; at higher pH the proportional increase in non-ionized ammonia concentrations is less as its  $pK_a$  of 9.56 (at 15°C) is approached. Also, at pH greater than 8.0, the free carbon dioxide concentration in the water is very low, and the respiratory carbon dioxide excreted at the fish gills can lower the pH of the water there considerably; as a rule of thumb for water quality control purposes, the author has recommended that, at  $\text{pH} > 8.0$ , the concentrations of non-ionized ammonia should be calculated as if the pH was 8.0.

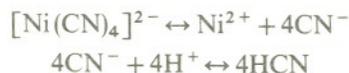
An example of a weak acid is free chlorine in water, which dissociates as follows:



It is presumed that the toxic chemical species is hypochlorous acid (HOCl) which has a  $pK_a$  of 7.5 at 25°C; a proposed water quality standard for fish of 4  $\mu\text{g}$  of hypochlorous acid per litre (Alabaster and Lloyd, 1982) corresponds to a 'free' chlorine concentration of 121  $\mu\text{g}/\text{l}$  at pH 9, of 5  $\mu\text{g}/\text{l}$  at pH 7, and of 4  $\mu\text{g}/\text{l}$  at pH 6, at which almost all the chlorine is present as hypochlorous acid. In both these cases, the maximum effect of pH is a ten-fold difference in toxicity for each unit pH change within a range considerably lower than the  $pK_a$  for weak acids.

Prediction of the toxicity of wastes containing these ionizing compounds depends on the ability to calculate or measure by analysis the concentration of the non-ionized fraction present in solution. Although the  $pK_a$  values can be influenced by temperature, in practical terms the effect is small and will not significantly change the toxicity of an effluent. Perhaps more important for compounds such as these which dissociate rapidly is the need for careful preparation of waters of different pH, and to ensure a common level of free carbon dioxide at which the changes in gill surface pH can be kept to a minimum; a concentration of 10 mg of free carbon dioxide per litre would be satisfactory, with prior acclimation of fish to this level. It is possible that failure to take this variable into account has led to a lack of precise correlation between the observed toxicity of the solution and the predicted concentration of non-ionized compound present, with subsequent attribution of the residual toxicity to the ionized form.

The effect of pH on the toxicity of metalocyanides is much more complex because of the greater number of dissociation reactions involved, and the long time required (up to ten days) to reach the dissociation equilibrium; a thorough review of the subject has been made by Leduc *et al.* (1982). A simplified example of a metalocyanide dissociation is shown for cyanonickelate (II):



It is the undissociated hydrogen cyanide (HCN) which contributes most to the toxicity; an increase in pH from 7.4 to 7.8 reduces the toxicity of cyanonickelate by ten- to 13-fold (Doudoroff, 1976). Because the ionization constants for many of the metalocyanide equilibria are not known with any accuracy, and because of the long time taken to reach equilibrium, it is difficult to measure the toxicity of the products formed; the likelihood of predicting the toxicity of a complex effluent containing metalocyanides from its chemical analysis is remote.

Clearly, toxicity tests on metalocyanides alone must be carried out with very precise pH control, but it may be necessary only to test effluents containing these compounds in a limited range of 'soft acid' to 'hard alkaline' dilution waters. This would give an approximate indication of the effect of these factors on their toxicity, and discharge controls for such effluents could then be adjusted, albeit roughly, to allow for changes in the hardness and pH of the receiving water.

#### 4.2 pH: Solubility

Solutions of metals in natural waters are at their most toxic at low pH values where the metallic ion is the dominant species. Increasing the pH will cause precipitation of the hydroxide or carbonate complexes; with aluminium, such precipitation occurs above pH 5.0, so that this metal is toxic only in markedly acid waters. Iron precipitates out of solution at pH above 6.0, but in this case the freshly precipitated metal can be highly toxic in its colloidal form, the toxicity reducing as the precipitate becomes aged (Decker and Menendez, 1974; Sykora *et al.*, 1975). Other metals, such as zinc, lead, and copper, precipitate at pH values above 7–8. There are few data on the toxicity of such precipitates to fish; almost certainly the particle size will be a determining factor and this is difficult to control and monitor experimentally in aquaria containing fish producing copious quantities of mucus.

Toxicity tests with metals at pH values close to those at which precipitation occurs should clearly define the condition of the test, the physical state of any precipitate formed, and the extent to which the test organisms are exposed to the precipitate. If particle size is thought to be important, then a carefully controlled continuous flow test is the only method of keeping the particle sizes constant for the duration of the exposure period.

### 4.3 Adsorption and Sequestration

#### 4.3.1 Suspended Solids

A variety of chemicals can become adsorbed onto inorganic or organic particles, thus restricting their availability to aquatic organisms. These include heavy metals such as cadmium and lead which can be adsorbed onto natural riverborne suspended solids (e.g. Salim, 1983) and released at a slow rate in the estuary and marine environment. Some uncertainty exists on the ability of aquatic organisms to release and absorb such bound metals in their gut but in general it would appear that only a small proportion of the total intake is derived from this route.

Another group of chemicals which can be bound by either organic or inorganic particles is pesticides; soil mobility tests show that many pesticides are strongly adsorbed to particulate matter, and field trials where pesticides are sprayed into water have shown that a rapid reduction in toxicity can occur if suspended matter is present. For example, synthetic pyrethroids are extremely toxic to fish and aquatic arthropods, but they have a much less dramatic effect than predicted from laboratory tests when sprayed onto natural turbid waters (Crossland, 1982).

Very few laboratory studies have been carried out on this aspect of toxicity modification, even though field data indicate that it may be of considerable importance; loss of bioavailability of toxic substances after discharge to natural waters is usually regarded as an additional safety factor of unknown magnitude which is not normally incorporated into hazard assessments.

#### 4.3.2 Sequestering Agents

A number of studies have shown that addition of NTA (nitrilotriacetic acid) and EDTA to heavy metal solutions reduces their toxicity considerably, because the resulting metallic complex is relatively non-toxic. Some metals, especially copper, are also bound by naturally occurring organic substances, particularly humic acids. Studies have shown that the binding of copper to humic acids becomes reduced as the calcium concentration of the water increases (Zitko *et al.*, 1973). The presence of organic substances in the water supply used for toxicity tests, as well as the production by experimental animals of sequestering substances such as mucus, should be borne in mind when planning experiments with the heavy metals.

### 4.4 Effect on the Toxicity Curve

The abiotic factors considered in the previous sections modify the bioavailability of the pollutant to the test organisms. Therefore, toxicity curves relating log median survival time to log total concentration of pollutant will be shifted horizontally towards higher concentrations (Figure 3). Changes in the shape of

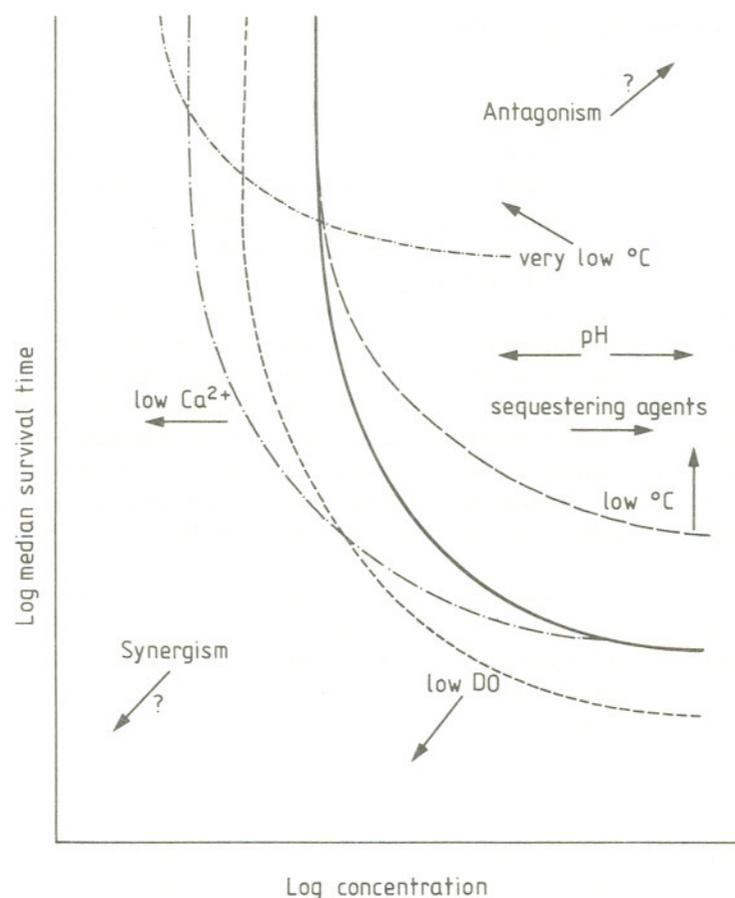


Figure 3 Effect of physicochemical variables on the position of the toxicity curve with respect to the time and concentration axes. DO = dissolved oxygen

the toxicity curve may indicate the formation of complexes of different toxicity, or, at high concentrations, the saturation of the complexing or adsorptive mechanism.

## 5 ENVIRONMENTAL VARIABLES AFFECTING THE RESPONSE OF FISH TO TOXIC SUBSTANCES

### 5.1 Water Hardness (Calcium Concentration)

It is well known that the toxicity of heavy metals is markedly increased by a reduced hardness of the water (Brown, 1968), not only because of higher

solubility at low bicarbonate alkalinities, but also because fish are more sensitive in soft water. There is some evidence that rainbow trout from hard waters have to be exposed to soft water for several days before their sensitivity to zinc is as great as that of trout reared in soft water (Lloyd, 1965). This suggests that to some extent the protective action of calcium may be intracellular and not solely a direct competition of divalent ions for sites on gill epithelial tissue. In general, the toxicity of heavy metal ions in soft water is up to ten-fold greater than that in hard water.

### **5.2 Dissolved Oxygen**

There is a considerable body of information on the increased toxicity to fish of a variety of chemicals caused by a reduction in the dissolved oxygen (DO) concentration of the water. Many of the studies have been on salmonids and there is a need for further data on other species, in particular to correlate the increased toxicity with increased respiratory flow over the gill surfaces. Problems can occur with the preparation of waters of low DO content, especially that of nitrogen supersaturation when this gas is used to strip oxygen from solution. Ideally, the experiments should be planned to give a series of toxicity curves for the different DO levels used; the effect of acclimating the fish to the experimental DO level before the test should also be investigated. However, it is unlikely that reduced DO levels within the range tolerated by natural populations of fish will increase the toxicity of pollutant by more than two-fold.

### **5.3 Temperature**

The metabolic rates of poikilotherms fall with a decrease in temperature within the tolerated range, and their survival times in toxic solutions show a corresponding increase. However, there is little change in threshold  $LC_{50}$  for those toxicants where this concentration is well defined; the toxicity curve is moved upwards with respect to the survival time axis. This general observation may not hold true for temperatures lower than 8.0°C. There is evidence, for example, that the  $LC_{50}$  of phenol to rainbow trout decreases at very low temperatures, probably because detoxification rates are slower (Alabaster and Lloyd, 1982). Again, there is a need for more work in this field, as aquatic organisms may be much more sensitive to toxicants at the low winter temperatures of temperate waters.

### **5.4 Antagonistic Effects of Other Pollutants**

There is a considerable body of evidence to suggest that aquatic organisms can increase their tolerance (at least in the short term) to those toxicants with well-defined threshold  $LC_{50}$  values, if they have been previously acclimated to

sublethal concentrations. It can be assumed that this follows the induction of the appropriate detoxification mechanisms, which in some cases may also be appropriate for other pollutants. For example, recent work has shown that previous exposure to mercury and zinc can increase the resistance of white suckers (*Catostomus commersoni*) to cadmium (Duncan and Klaverkamp, 1983); it is worth noting that these authors demonstrate the value of presenting their data as sets of toxicity curves, rather than tables of  $LC_{50}$  values.

Exposure to sublethal concentrations of toxicant can increase various physiological processes including growth rate (e.g. Dixon and Sprague, 1981). This phenomenon is known as hormesis and has been demonstrated in a range of phyla (Stebbing, 1982); it may explain the reported effect of sublethal phenol concentrations in increasing the resistance of fish to otherwise lethal concentrations of ammonia, sulphide, and copper (Alabaster and Lloyd, 1982, p. 289).

It is clear that this is a very complex field and, while it is generally recognized that the phenomenon of antagonism exists, there has been no attempt to try to quantify the effect for incorporation into water quality standards. As with the reduction in toxicant bioavailability caused by adsorption and sequestration noted above, acclimation to toxicants and antagonism between them have been treated as unutilized safety factors.

### 5.5 Effect on the Toxicity Curve

The factors considered in this section can cause changes in toxicity which result in the toxicity curve being moved either vertically or horizontally, or both. The general direction of the observed shifts is shown in Figure 3.

## 6 STUDIES ON EFFLUENTS AND POLLUTED RIVERS

Early studies on coke-oven effluents (Herbert, 1962) and sewage effluents (Lloyd and Jordan, 1963, 1964) showed that their toxicity to rainbow trout could be predicted with reasonable accuracy from an analysis of the common pollutants present. It was assumed that each pollutant was acting separately, and that the combined toxicity was the result of simple addition of their individual toxicities. Furthermore, it was assumed that the toxicity of each pollutant was modified by the appropriate physicochemical factors as outlined in the preceding sections, and that no interactions occurred. Laboratory data on the effect of these factors were used to adjust the predicted threshold  $LC_{50}$  for the individual pollutant.

However, in the majority of these effluent toxicity tests, the dilutions were prepared with the standard laboratory water supply with which the tests on the individual pollutant were also carried out, so that differences in their natural physicochemical characteristics were small. Several effluents had very low toxicities and required very little dilution, but these effluents were not dissimilar to the standard water supply in terms of water hardness and pH. Only one sewage

effluent which contained a high concentration of zinc as the main toxicant was tested in both hard and soft waters (Lloyd and Jordan, 1964). The observed toxicity of this effluent was close to that predicted from laboratory tests with zinc alone in waters of corresponding hardness. On the other hand, effluents and river waters containing appreciable amounts of copper were less toxic than predicted, and adjustments had to be made to allow for the presence of non-toxic soluble organocopper complexes.

The relationships between the '48-hour  $LC_{50}$ ' of several common pollutants and the natural physicochemical characteristics of the water, as developed from the laboratory studies on rainbow trout, were subsequently collated by Brown (1968) although some of the 48-hour  $LC_{50}$  values are in fact threshold concentrations based on longer exposure periods. This information was subsequently incorporated into a computer program to convert chemical and physical monitoring data from river sampling stations into appropriate fractions of TUs for individual common pollutants, thus opening up a new field of study. However, it is appropriate at this stage to reconsider the problems which this area of research was designed to resolve.

## **7 IDENTIFICATION OF PRINCIPAL TOXIC COMPONENTS OF EFFLUENTS**

One of the original objectives of the studies with pollutant mixtures was to enable the identification of the major toxic constituents so that the most economic or effective methods of toxicity reduction could be identified. In practice, this is likely to be feasible only if one or two of the common pollutants contribute a major proportion of the toxicity. Problems occur where several pollutants play a minor role. For example, simple addition of individual common pollutant toxicities was assumed to occur at sublethal concentrations, but this may well not be true and at  $<0.3$  TU their joint action may be less than additive. If this had been the case, predictions of effluent toxicity would have overestimated the contribution made by the minor toxic components to the total toxicity, and the residue of unexplained toxicity should have been greater. Also, the interaction between the common pollutants and other organic substances which may be present, such as aromatics, chloro-aromatics, anilines, and glycerols (Könemann, 1979, 1980) which have a common QSAR and are additive at all concentrations, is not known.

Therefore, while there is a reasonable chance that an accurate prediction could be made of the toxicity of an effluent from a base metal mining operation from a knowledge of the chemical constituents and relevant accurate toxicity test data, the prospect of ever being able to do the same for a complex effluent such as that from an oil refinery (Burks, 1982) is very remote. Even where the acute lethal toxicity can be predicted on the basis of additive joint action, there is no evidence that the proportional toxic contributions of the individual pollutants will remain

the same at concentrations below those present at the threshold  $LC_{50}$  of the effluent. Studies on interaction are comparatively simple where the single common response of mortality is measured; there is no similar common sublethal response which is appropriate for all pollutants. Furthermore, toxicity tests with aquatic organisms are usually insufficiently precise to measure, for example, whether the addition of 0.1 TU of a pollutant will produce a 10 % increase in the toxicity of a solution containing 1 TU of another pollutant.

## 8 RELATIONSHIP BETWEEN PREDICTED TOXICITY AND FISHERIES STATUS IN NATURAL WATERS

Several attempts have been made to correlate the predicted 'toxic unit content' derived from chemical analysis of natural waters with the presence or absence of fish populations; these have been reviewed by Alabaster and Lloyd (1982) and Lloyd (1985). Using the computer program referred to above, it was found that fisheries were present where the predicted sum of the individual TUs for the common pollutants (based on rainbow trout data) did not exceed 0.28 (Alabaster *et al.*, 1972). For the reasons outlined in the previous section, there is no method available to show whether the common pollutants identified were indeed contributing this amount of stress.

In addition, factors such as acclimation to the major pollutant, the antagonistic effects of small concentrations of other pollutants, and the effect of abiotic factors on the bioavailability of the pollutants (particularly the heavy metals), as described earlier, would play a considerable part in modifying the true proportional contribution of the individual pollutants. Thus, when other factors associated with an effluent discharge are also included (e.g. pulse discharges and habitat modification), the original objective of identifying principal toxic components in effluents or natural waters seems likely to be unattainable except in the simplest of situations.

## 9 CONCLUSIONS

It is clear from the foregoing analysis that our present ability to predict the toxicity of pollutant mixtures in complex effluents is inadequate for quantitative use within a regulatory context. Therefore reliance will continue to be placed on toxicity tests with whole effluents for pollution prevention control. However, a few general statements can be made on the possible qualitative effects of physicochemical variables on effluent toxicity.

(1) The toxicity of an effluent after discharge may be greater than expected for the following reasons:

- (a) the effluent contains heavy metals and the receiving water has a lower hardness than that used in the toxicity test;

- (b) the effluent contains ionizing pollutants, and the pH of the receiving water increases the toxic non-ionized fraction present;
- (c) the dissolved oxygen concentration of the receiving water is below the air saturation value used in the toxicity test;
- (d) the effluent contains pollutants which are more toxic at low, winter temperatures than at the optimum temperature for the organisms used in the toxicity test.

If (a) or (b) is thought to be potentially important for a particular effluent, then toxicity tests should be carried out with two dilution waters representing the extremes likely to be encountered in the receiving water. A lower DO level in the receiving water is unlikely to increase an effluent toxicity by more than two-fold (or less after acclimation), providing that the concentrations remain within the tolerated range. Effects of low winter temperatures are largely unknown and further research on this factor is necessary.

(2) The toxicity of an effluent after discharge may be less than expected for the following reasons:

- (a) the effluent contains heavy metals and the receiving water has a greater hardness than that used in the toxicity test;
- (b) the effluent contains ionizing pollutants and the pH of the receiving water increases the non-toxic ionized fraction present;
- (c) the effluent contains pollutants which display an additive joint action at the concentrations used within acute toxicity tests, but which are less than additive or antagonistic at the concentrations present after dilution with the receiving water;
- (d) the bioavailability of the pollutants in the effluent is reduced by precipitation, adsorption onto particles and surfaces, or sequestration;
- (e) the receiving water already contains low levels of pollutants to which the fish population is acclimated and thus have some resistance to similar pollutants in the effluent.

If (2) (a) and (b) are thought to be potentially important, then the same course of action outlined for (1) (a) and (b) above should be followed. The remaining factors are more difficult to quantify but they may have a considerable ameliorating effect; they should be borne in mind when considering the application of safety factors to effluent toxicity test data.

(3) If an effluent has an unacceptably high toxicity which is unlikely to be significantly reduced by the factors in (2) above, then the principal toxic components may be best identified by carrying out toxicity tests on the various process streams which contribute to the final effluent.

These pragmatic approaches to the problem are proposed to meet existing regulatory demands. It is clear that effluent toxicity tests may need site-specific modification in order to allow for differences in the physicochemical charac-

teristics of the receiving water. It is of even greater importance that supportive research is continued into the mechanisms of joint action, the various types of acclimation, the effect of low temperature, and the other factors described in this paper, in order to provide a deeper understanding of their potential quantitative effects, and to increase the confidence with which effluent toxicity data are used within a regulatory context. Appropriate research programmes should be formulated to meet this goal, using the guidelines outlined in this paper.

## 10 REFERENCES

- Alabaster, J. S., and Lloyd, R. (Eds.) (1982). *Water Quality Criteria for Freshwater Fish*, 2nd edition. Butterworths, London: 361 pages.
- Alabaster, J. S., Garland, J. H. N., Hart, I. C., and Solbé, J. F. de L. G. (1972). An approach to the problem of pollution and fisheries. *Symp. Zool. Soc. London*, **29**, 87–114.
- Brown, V. M. (1968). The calculation of the acute toxicity of mixtures of poisons to rainbow trout. *Water Res.*, **2**, 723–733.
- Burks, S. L. (1982). Review of pollutants in petroleum refinery wastewaters and effects upon aquatic organisms. *Environ. Int.*, **7**, 271–283.
- Crossland, N. O. (1982). Aquatic toxicology of cypermethrin. II. Fate and biological effects in pond experiments. *Aquat. Toxicol.*, **2**, 205–222.
- Decker, C., and Menendez, R. (1974). Acute toxicity of iron and aluminium to brook trout. *Proc. W. Va. Acad. Sci.*, **46**, 159–167.
- Dixon, D. G., and Sprague, J. B. (1981). Acclimation to copper by rainbow trout (*Salmo gairdneri*)—a modifying factor in toxicity. *Can. J. Fish. Aquat. Sci.*, **38**, 880–888.
- Doudoroff, P. (1976). *Toxicity to Fish of Cyanides and Related Compounds: A Review*. EPA-600/3-76-038. US Environmental Protection Agency: 154 pages.
- Duncan, D. A., and Klaverkamp, J. F. (1983). Tolerance and resistance to cadmium in white suckers (*Catostomus commersoni*) previously exposed to cadmium, mercury, zinc or selenium. *Can. J. Fish. Aquat. Sci.*, **40**, 128–138.
- Herbert, D. W. M. (1962). The toxicity to rainbow trout of spent still liquors from the distillation of coal. *Ann. Appl. Biol.*, **50**, 755–777.
- Hermens, J., and Leeuwangh, P. (1982). Joint toxicity of mixtures of 8 and 24 chemicals to the guppy (*Poecilia reticulata*). *Ecotoxicol. Environ. Saf.*, **6**, 302–310.
- Könemann, W. H. (1979). *Quantitative Structure–Activity Relationships for Kinetics and Toxicity of Aquatic Pollutants and Their Mixtures in Fish*. Ph. D. Thesis, University of Utrecht: 79 pages.
- Könemann, W. H. (1980). Structure–activity relationship and additivity in fish toxicities of environmental pollutants. *Ecotoxicol. Environ. Saf.*, **4**, 415–421.
- Leduc, G., Pierce, R. C., and McCracken, I. R. (1982). *The Effects of Cyanide on Aquatic Organisms with Emphasis upon Freshwater Fishes*. ACSCEQ Report NRCC 19246. National Research Council of Canada, Ottawa: 139 pages.
- Lloyd, R. (1961). The toxicity of mixtures of zinc and copper sulphates to rainbow trout (*Salmo gairdneri* Richardson). *Ann. Appl. Biol.*, **49**, 535–538.
- Lloyd, R. (1965). *Factors that Affect the Tolerance of Fish to Heavy Metal Poisoning. Biological Problems in Water Pollution*. 99-WP-25, pp. 181–187. US Public Health Service, Washington, DC.
- Lloyd, R. (1979). The use of the concentration–response relationship in assessing acute fish toxicity data. In Dickson, K. L., Maki, A. W., and Cairns, J. (Eds.) *Analysing the Hazard Evaluation Process*, pp. 58–61. American Fisheries Society, Washington, DC.

- Lloyd, R. (1985). The toxicity of mixtures of chemicals to fish: an overview of European laboratory and field experience. In Bergman, H. L., Kimerle, R. A., and Maki, A. W. (Eds.) *Environmental Hazard Assessment of Effluents*, pp. 42-53. Pergamon Press, New York.
- Lloyd, R., and Jordan, D. H. M. (1963). Predicted and observed toxicities of several sewage effluents to rainbow trout. *J. Proc. Inst. Sewage Purif.*, **1963**, 167-173.
- Lloyd, R., and Jordan, D. H. M. (1964). Predicted and observed toxicities of several sewage effluents to rainbow trout: a further study. *J. Proc. Inst. Sewage Purif.*, **1964**, 163-166.
- Salim, R. (1983). Adsorption of lead on the suspended particles of river water. *Water Res.*, **17**, 423-429.
- Sprague, J. B., and Ramsay, B. A. (1965). Lethal levels of mixed copper-zinc solutions for juvenile salmon. *J. Fish. Res. Board Can.*, **22**, 425-432.
- Stebbing, A. R. D. (1982). Hormesis—the stimulation of growth by low levels of inhibitors. *Sci. Total Environ.*, **22**, 213-234.
- Sykora, J. L., Smith, E. J., Synak, M., and Shapiro, M. A. (1975). Some observations on spawning of brook trout (*Salvelinus fontinalis* Mitchill) in lime-neutralised iron hydroxide suspensions. *Water Res.*, **9**, 451-458.
- Zitko, V., Carson, W. V., and Carson, W. G. (1973). Prediction of incipient lethal levels of copper to juvenile Atlantic salmon in the presence of humic acid by cupric electrode. *Bull. Environ. Contam. Toxicol.*, **10**, 265-271.

