toxicity as measured by survival times, mortality rates, or tests of arbitrarily-fixed short duration, and accordingly need cautious interpretation, but there are some clear examples of the effects of oxygen concentration on threshold toxicity values. Hokanson and Smith (1971) found that the lethal threshold concentration of an LAS detergent to *Lepomis macrochirus* increased from 0.4 mg l^{-1} at 2 mg DO l^{-1} to 2.2 mg l^{-1} at 7.5 mg DO l^{-1}. Thurston *et al.* (1981) reported lethal threshold concentrations of ammonia to *S.gairdneri* ranging from 0.32mg NH_3 l^{-1} at 2.6 mg DO l^{-1} to 0.81 mg NH_3 l^{-1} at 8.6 mg DO l^{-1}. Acclimation of fish to low dissolved oxygen concentrations prior to testing may influence the results of experiments. Lloyd (1960) found a small increase in the toxicity of zinc to *S. gairdneri* at low DO levels which was abolished if fish were acclimated to low DO before testing. Similar results were obtained by Adelman and Smith (1972) for *Carassius auratus* exposed to hydrogen sulphide.

Although extreme *pH values* are deleterious to aquatic organisms, the pH range 6–9 is generally considered acceptable to most species. Within this range, however, the toxicity of many poisons is influenced by pH. Particularly strongly affected are those poisons which dissociate into ionised and unionised fractions, of which one is markedly more toxic than the other. The best-known example is ammonia (Alabaster and Lloyd, 1980) which is more toxic at high pH values. The reason is that unionised ammonia has high toxicity and the ammonium ion has very low toxicity, and the proportion of unionised ammonia in solution increases rapidly with pH; thus the toxicity of ammonia is about ten times higher at pH 8 than at pH 7. Alabaster and Lloyd (1980) also cite cyanide, nickelo-cyanide complex, sodium sulphide and zinc as examples of poisons whose toxicity is substantially influenced by pH. It is reasonable to assume that pH is potentially an important determinant of toxicity for any poison which ionises in solution.

The effect of *salinity* on toxicity has received some attention since many pollutants are discharged to saline waters. Some poisons appear to be least toxic to fish at salinities corresponding to approximately 30–40% sea water, when the water is roughly isotonic with fish body fluids. Examples include zinc (Herbert and Wakeford, 1964), ammonia (Herbert and Shurben, 1965) and alkylbenzene sulphonate detergent (Eisler, 1965). The effect of salinity on zinc toxicity appears particularly large (Figure 4.12) although in this case the toxicity is expressed as 48-h LC50 and not lethal threshold concentrations. Phenol toxicity to *Salmo gairdneri* increased steadily with salinity (Brown *et al.,* 1967), 48-h LC50 values ranging from 9 mg l^{-1} in fresh water down to 5 mg l^{-1} in 60% sea water. Cadmium is reported to increase in toxicity to the estuarine fish *Fundulus heteroclitus* (Eisler, 1971) as salinity increases. There is no generally accepted hypothesis to explain salinity effects on toxicity. Herbert and Wakeford (1964) suggested that zinc was less toxic to fish in isotonic medium because of the reduced importance of osmoregulatory

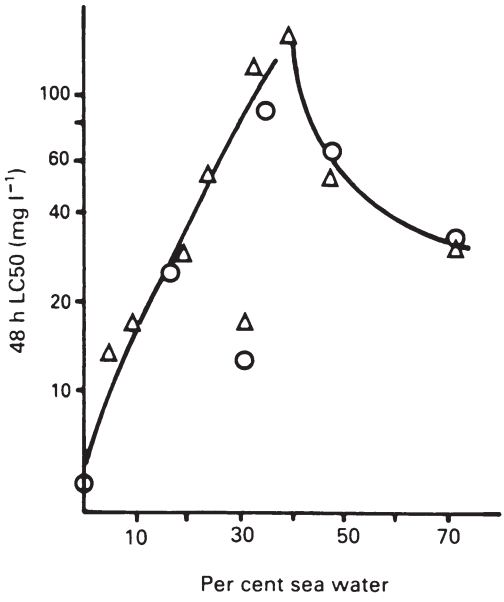

Figure 4.12 Effect of salinity on the toxicity of zinc to *Salmo gairdneri* (Herbert and Wakeford, 1964)

stress during intoxication. Skidmore (1970) showed that trout poisoned by zinc in fresh water maintained normal osmotic and ionic balance, and that death was due to asphyxiation associated with gill damage. This does not mean, however, that osmoregulatory requirements have no influence in zinc poisoning. It has been suggested (Abel and Skidmore, 1975) that the gill damage associated with poisoning by zinc and other pollutants is a consequence of a rearrangement of the gill epithelium to preserve an osmotic barrier in the face of a rapid loss of viable epithelial cells due to the action of the poison. Thus the gill damage, and consequent tissue hypoxia, may be less extensive for fish in isotonic medium, though this has never been investigated. Poisons which are more toxic in hypertonic medium may be so because teleosts drink copiously in hypertonic medium, and thus may accumulate poison more rapidly. The effect of salinity on chemical speciation of the toxicant (see above) may also be relevant.

4.2.3 Combinations of Poisons

An important environmental variable which may influence the toxicity of a poison is the presence of other poisons. Although most toxicological investigations involve the study of a single pollutant, the biota of polluted waters are usually exposed to several pollutants simultaneously.

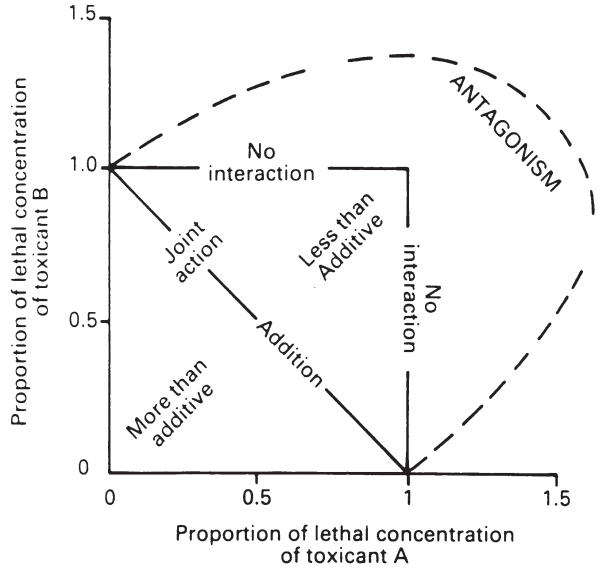

Figure 4.13 Diagram showing terms used to describe the combined effects of two pollutants (Sprague, 1970). For full explanation, see text

Sprague (1970) has pointed out that the terminology widely used to describe the behaviour of poisons acting simultaneously is confused and potentially misleading. In particular, the terms *synergism, potentiation* and *antagonism* have been defined and used in different ways by different authors, and their use is best avoided. Sprague proposed a system of nomenclature based on that of Gaddum (1948). This scheme is shown in Figure 4.13 and is the one adopted here. Sprague's description of this scheme of nomenclature is as follows.

> "The diagram represents the combination of two toxicants. The axes represent concentrations. The concentration of 1.0 toxic unit of toxicant A produces the response (death in this case) in the absence of toxicant B, and 1.0 unit of B will do the same in the absence of A. If the response is produced by combinations of the two toxicants represented by points inside the square, the toxicants are helping one another; this is called *joint action* which may be further broken down in three special cases, as follows. If the response is just produced by combinations represented by points on the diagonal straight line (e.g. 0.5A+0.5B) the effects are said to be *additive*. If the response is produced by combinations falling in the lower left triangle (e.g. 0.5A+0.2B) the effect is *more-than-additive*. If in the upper right triangle (e.g. 0.8A+0.7B) the toxicants are still working together in joint action but are *less-than-additive*. Those combinations falling exactly in the upper and right boundaries of the square show *no interaction* between the toxicants. For example, if 1.0 unit of A is required to just produce the response, no matter what concentration of B, below

1.0 unit, is present, then A is causing the response and B is neither helping nor hindering. If more than 1.0 unit of A is required to just produce the effect, because of the presence of B, this is *antagonism,* with B antagonising the effect of A. That combination of concentrations would fall on some point to the right of the square (e.g. 1.5A+0.5B). Any combination of concentrations which would fall outside the square would represent antagonism, loosely represented by the broken curved line."

A useful method for measuring the toxicity of poisons in combination was described by Brown (1969) and Sprague (1970). The concentration of a pollutant can be expressed as toxic units, that is as a proportion of its lethal threshold concentration, or of some approximation of the lethal threshold concentration such as 48-h or 96-h LC50. Thus:

$$\text{Toxic units} = \frac{\text{Concentration of poison}}{\text{Lethal threshold concentration}}$$

Thus for any poison, one toxic unit is equal to its lethal threshold concentraton. To test the toxicity of a mixture of poisons, the following procedure may be employed. Assume we wish to test the effect of two poisons acting simultaneously. Having determined the lethal threshold concentration of each poison individually, test organisms are then exposed to a mixture of the poisons which contains, say, 0.5 toxic units of each poison (i.e. half the lethal threshold concentration of each poison). If significantly more than half die, the poisons are more-than-additive. If significantly fewer than half die, the poisons are less-than-additive. Obviously it is feasible, using this technique, to investigate the effects of combinations containing more than two poisons, and of mixtures in other than equal proportions. Marking (1977) proposed a modification of this approach, whereby the toxicity of chemicals in combination may be expressed in terms of a single numerical value. Index values of zero represent additive toxicity; values significantly above, or below, zero represent more-than-additive and less-than-additive toxicity respectively. The method includes a simple test of significance for values near zero. An alternative approach (Calamari and Marchetti, 1973) is to construct complete toxicity curves for the poisons individually and in combination, converting values on the concentration axis of the graph to toxic units (Figure 4.14).

There are several examples in the literature of both additive and more-than-additive toxicity. Mixtures of copper and zinc (Lloyd, 1961; Sprague and Ramsay, 1965); copper and phenol; copper, zinc and phenol; and copper, zinc and nickel (Brown and Dalton, 1970; Marking, 1977) have all been reported to be simply additive. More-than-additive toxicity has been reported for anionic detergents with copper or mercury (Calamari and Marchetti, 1973), the piscicide rotenone with sulphoxide or piperonyl butoxide, and the organophosphate insecticides malathion and Delnav

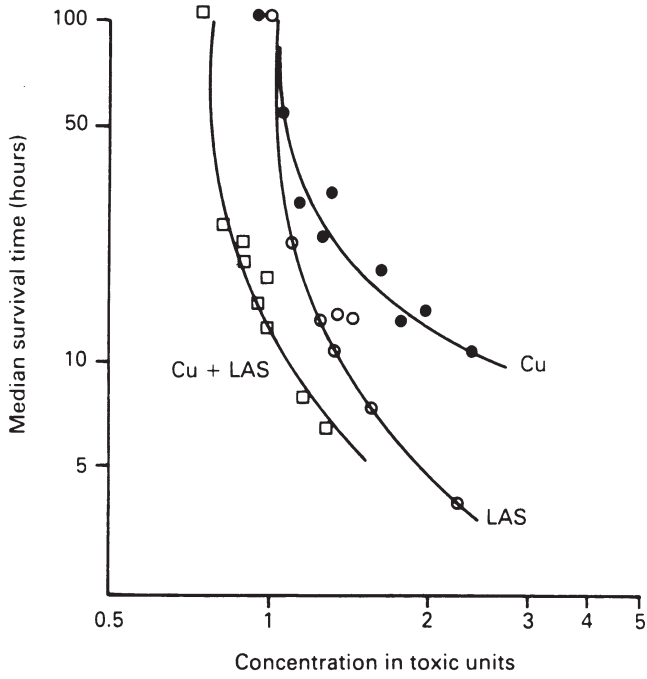

Figure 4.14 Toxicity of copper (Cu), detergent (LAS) and a copper-detergent mixture (Cu+LAS) to *Salmo gairdneri*. Poison concentrations are expressed in toxic units (Calamari and Marchetti, 1973)

(Marking, 1977). As in other types of toxicological study, it appears that tests of short duration where threshold toxicity values are not established are likely to produce misleading results. Sprague (1970) cites examples which illustrate that while threshold toxicities may be simply additive, survival times in strong mixtures may be shorter than expected on the basis of simple additive toxicity.

Examples of less-than-additive toxicity seem to be rare. Calamari and Marchetti (1973) reported that a mixture of equal proportions (in toxic units) of copper and the non-ionic detergent nonylphenol ethoxylate showed a less-than-additive toxicity to *S. gairdneri,* but this conclusion was based on a comparison of observed and expected survival times. One circumstance in which less-than-additive toxicity sometimes occurs is when a component of a mixture is present as a small fraction of the whole mixture. Thus Brown *et al.* (1969) found that the observed toxicity of a mixture of ammonia, phenol and zinc to rainbow trout was significantly less than the predicted toxicity when zinc contributed about 0.75 toxic units and the remaining 0.25 toxic units was roughly equally distributed between the phenol and the ammonia.