A more detailed discussion of these points is given by Axiak and Abel (1991).

4.2 Factors Influencing Toxicity

There is a great deal of published information on the comparative toxicity of pollutants to different species under different conditions, and the present discussion is a selective review of some of the more salient features of the literature. Only influences on lethal toxicity are considered here: factors affecting sublethal toxicity have been little studied. Regrettably, much of the available literature is of limited value, or at least requires cautious interpretation, largely because of the kinds of methodological limitations discussed earlier. For example, we have seen (Figure 4.4) that comparing two LC50 values for a single time period such as 48 or 96 hours can lead to erroneous conclusions. Any biotic or abiotic influence on toxicity may affect the speed of response of the organisms as well as, or instead of, the actual quantity of poison required to kill the animals. Therefore comparisons of toxicity based on measurements of survival times, mortality rates, or fixed-time LC50 values are not adequate firmly to establish the existence or magnitude of an effect on toxicity. The importance of determining lethal threshold values is rarely more evident than when discussing comparative toxicity.

Unfortunately, much of the literature on comparative toxicity takes precisely the form of tabulations of LC50 values, or comparisons of survival times or mortality rates. Therefore the existence and magnitude of many potential influences on toxicity are not well established, despite in many cases the existence of a substantial literature. Indeed, many of the best-known influences on toxicity have been well established for many years, and much of the more recent literature fails to shed further light on the subject.

4.2.1 The Toxic Properties of the Pollutant

The toxicity of a pollutant under any given circumstances must be a function of its chemical structure or configuration, and quite small alterations in the poison molecule can produce major variations in toxicity. Synthetic anionic detergents are a group of compounds which illustrate this well. Modern anionic detergents are of the linear alkylate sulphonate type, consisting of a sulphonated benzene ring with an unbranched alkyl chain containing about 12 carbon atoms (see Figure 2.10). The degradation products of detergents are markedly less toxic to aquatic organisms than the original molecule (Kimerle and Swisher, 1977; Swisher *et al.,* 1964). Numerous studies have shown that the length of the hydrocarbon chain of an anionic detergent molecule exerts a large influence on its toxicity (Abel, 1974; Hirsch, 1963; Lindahl and Cabridenc, 1978; Maki and Bishop, 1979). These studies

are in general agreement that the toxicity of anionic detergents to fish, invertebrates and algae (expressed in terms of 48 h LC50 values) increases by up to one order of magnitude for each increase of 2 alkyl carbons in the detergent molecule, although very long hydrocarbon chains (C16 and above) tend to show reduced toxicity.

The existence of such empirical relationships between chemical structure and toxicity has since been confirmed in other groups of compounds, and has given rise to the idea of *quantitative structure-activity relationships* (QSARs). QSARs may be used to predict toxicity, even of compounds which have not yet been synthesised, and are discussed further in Section 4.6. The toxicity of both organic and inorganic poisons is greatly influenced by the physico-chemical state in which the poison is present. Heavy metal ions, for example, may exist in any of several different oxidation states; in dissolved, colloidal or particulate form; and as simple ions or as inorganic or organo-metal complexes. These various forms may have very different toxic properties. This fact explains, at least in part, the effect of some environmental conditions on toxicity.

The toxicity of copper provides a good example. Pagenkopf *et al.* (1974) recorded from published literature 96-h LC50 values for *Pimephales promelas* ranging over two to three orders of magnitude. From the chemical data provided on the environmental conditions of these experiments, they calculated the equilibrium concentrations of five possible copper species ($CuCO_3$, $Cu(CO_3)^{2-}$, , $CuOH^+$ and Cu^{2+}). The concentration of Cu^{2+} required to kill half the fish within 96 h was nearly constant, and it is apparently the major toxic species. $CuOH^+$ was found to be rather less toxic, and the other copper species apparently contributed little to the toxic action of copper. In each case examined, a substantial proportion of the total copper present was complexed with carbonate and hydroxide. It is also known that complexation of copper with dissolved organic material causes a marked reduction in its toxicity. Sewage effluent, glycine, humic substances, and suspended organic matter (Brown *et al.,* 1974) and organic chelating agents such as nitrilotriacetic acid (Shaw and Brown, 1974) markedly reduce the toxic action of copper.

The influence on toxicity of the physico-chemical state, and molecular structure or configuration of pollutants may thus have extensive implications for the conduct of toxicity tests, and for the interpretation of test results, particularly when such results are to be extrapolated in the formulation of water quality standards. Lee (1973), in his review of the topic, pointed out that with the increasing use of chronic, sublethal toxicity testing, concentrations of pollutants which are found deleterious in experimental conditions are sometimes equal to or less than the apparent 'natural' concentrations found in waters which sustain a healthy biota. An obvious possible explanation is that under laboratory conditions the poison may largely be present in a form different from that which predominates in natural waters. While it has

long been standard practice to monitor, control and record the physical environment of toxicity tests, until recently little attention has been paid to the chemistry of the poison under test conditions. Lee (1973) recommended procedures for minimising the problems which may be associated with the influence of the chemical environment on the results of toxicity tests, in particular that in the design and execution of toxicity tests, consideration should be given to the chemistry of the test substance, and to the determination of the precise form of the substance which is responsible for the observed toxic effect. It is surprising, even disappointing, that although the general principle was understood 20 years ago, toxicologists have been slow to incorporate it into routine practice, and to this extent much uncertainty remains about the accuracy of many measurements of toxicity.

4.2.2 The Effects of Environmental Conditions

Since the environmental conditions may affect both the poison and the organism under test, it is not surprising that their influence on toxicity can be large. Most of the systematic studies on the effects of environmental conditions on toxicity were carried out in the 1960s and 1970s using fish. More recent work has done little to change the general pattern which is summarised here, except to confirm that the same overall pattern appears to apply also to invertebrates.

Water hardness is among the most important environmental influences on toxicity, and its effects are particularly well known in relation to heavy metal toxicity. Lloyd (1960) found that the concentration of zinc which was lethal to rainbow trout *(S.gairdneri)* within two-and-a-half days varied by a factor of eight over the hardness range 12–320 mg l^{-1} as $CaCO_3$. Other metals are similarly influenced, including copper (Howarth and Sprague, 1978), cadmium (Calamari *et al.,* 1980) and silver (Davies *et al.,* 1978). It is now known that this effect is largely due to the effect of water hardness on the distribution of the total available metal ions between each of several inorganic complexes, which are not all equally toxic (see Section 4.2.1). However, the fact that the effect of hardness on toxicity has at least partly a biological basis can be shown by acclimating fish to hard water and exposing them to the metal in soft water. Such fish are more resistant to zinc (Lloyd, 1965) and cadmium (Calamari *et al.,* 1980) than similar fish acclimated and exposed in soft water. Hardness has also been reported to affect the toxicity of poisons other than metals, and should always be considered as a potentially significant modifier of toxicity.

The effects of *temperature* on toxicity may be expected to be complex. Temperature influences the rate of metabolic processes, including the uptake, metabolism and excretion of poisons. Increased temperature will increase the oxygen requirements of aquatic organisms, while decreasing the solubility

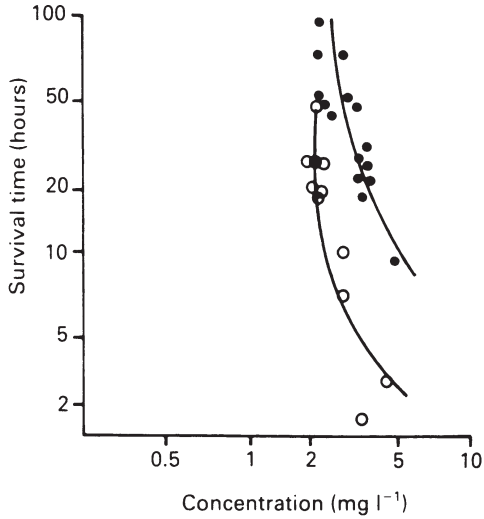

Figure 4.10 Effect of temperature on the toxicity of linear alkylate sulphonate detergent to *Lepomis macrochirus*. Open circles represent tests at 25°C, closed circles tests at 15°C (Hokanson and Smith, 1971)

of oxygen in water. The properties of the poison itself may, of course, be directly influenced by temperature; for example, through its effect on the equilibrium between molecular and ionised forms. Temperature is itself an important limiting factor to aquatic organisms. Further, as Sprague (1970) points out, it appears to be particularly important in studying temperature effects to determine lethal threshold concentrations, rather than LC50 values at arbitrarily-selected observation times. This is because temperature may influence the rate of reaction of the organism to the poison, but not the actual lethal threshold concentration. For these reasons, the large literature relating to temperature effects on toxicity affords little opportunity for reliable generalisation. It appears to be generally true that at higher temperatures, the time taken for organisms to react to a given concentration of poison is reduced. Cairns *et al.* (1975) cite numerous examples. It follows that results of short-term tests where lethal thresholds are not established, and in particular comparisons based on 48-h or 96-h LC50 values (i.e. the vast majority of reported results), are likely to be misleading and to exaggerate the magnitude of apparent temperature effects on toxicity. Reliable reports of temperature effects on lethal threshold concentrations appear to be very scarce.

These points are well illustrated by the following examples. Hokanson and Smith (1971) showed that the lethal threshold concentrations of an anionic detergent to *Lepomis macrochirus* were similar at 15°C and 25°C. However, at the higher temperature median survival times were reduced and the lethal

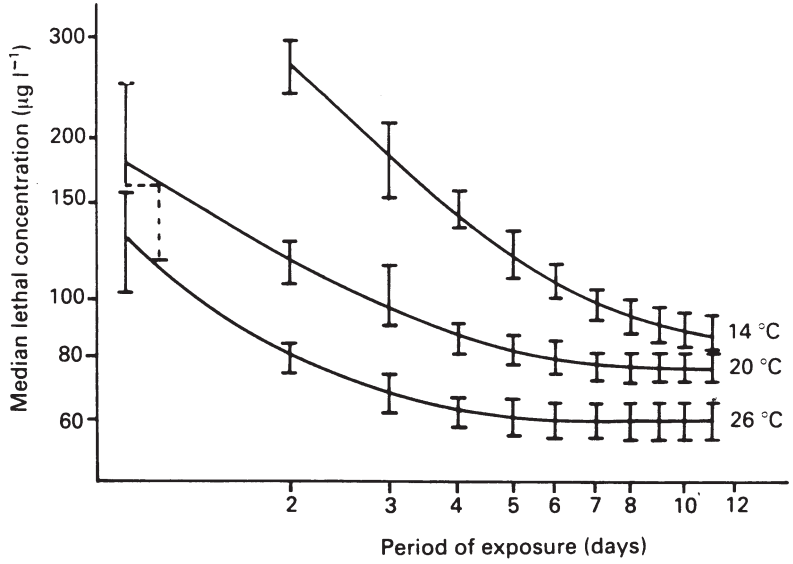

Figure 4.11 Effects of temperature on the toxicity of hydrogen sulphide to *Carassius auratus* (Adelman and Smith, 1972)

threshold time was 20–24 h at 25°C compared with 48–51 h at 15° (Figure 4.10). Adelman and Smith (1972) studied the effects of temperature on the toxicity of hydrogen sulphide to goldfish, *Carassius auratus,* in tests lasting 11 days. Their results showed that lethal threshold concentrations ranged from approximately 90 μg l^{-1} at 14°C to approximately 60 μg l^{-1} at 26°C, that is hydrogen sulphide was more toxic at the higher temperature. However, the complete toxicity curves (Figure 4.11) show clearly that the apparent increase in toxicity with temperature was much more pronounced in tests of shorter duration. An example of decreased toxicity with increasing temperature is given by Brown *et al.* (1967a, b). The 48-h LC50 of phenol to *Salmo gairdneri* was 5 mg l^{-1} at 6°C and increased to 9 mg l^{-1} at 18°C. Toxicity curves published by these authors indicate that 48-h LC50 values were in this case close to the lethal threshold concentrations. Interestingly, at all but the lowest concentrations tested the effect of increased temperature was to *reduce* median survival times, that is the toxicity curves intersect in the manner illustrated in Figure 4.7. Thus even when the effect of increased temperature is to reduce toxicity in terms of lethal threshold concentration, comparison of short-term LC50 values would lead to exactly the opposite conclusion. These examples all illustrate the danger of arbitrarily-terminated toxicity tests, and explain why, unfortunately, most of the literature relating to temperature effects on toxicity must be regarded as being of very limited value.

The effects of *dissolved oxygen* on toxicity have been less widely investigated, but in general low dissolved oxygen (DO) concentrations appear to cause an increase in the toxicity of poisons. As with temperature, the majority of reports deal with