In the case of ammonia, Lloyd and Orr (1969) demonstrated that concentrations below 0.12 toxic units exerted no effect on the water permeability of the fish. If toxic levels of ammonia exert their effect at least partly by increasing water permeability of the fish, as is apparently the case, this finding suggests an explanation for the non-contribution of low concentrations of ammonia to the toxicity of mixtures. For most poisons, however, no such detailed knowledge of their mode of actions exists, and their behaviour in mixtures can be predicted only on the basis of empirical findings without any explanation of the underlying mechanism. Nevertheless a series of attempts by British workers to predict the toxicity of mixtures, and to test such predictions against field observations of the fishery status of polluted rivers, have been reasonably successful. Sprague (1971) reviews some early attempts and more recent examples include those of Alabaster *et al.* (1972), Solbé (1973) and Garland and Rolley (1977). In British rivers the most common toxic pollutants are ammonia, phenols, cyanide, zinc and copper. For those poisons, summation of the fractional toxicities expressed in toxic units as described above gives generally good agreement between predicted and observed toxicities in laboratory experiments. The application of this technique in field conditions was discussed in Chapter 1.

4.2.4 Fluctuating Concentrations

The concentrations of pollutants in receiving waters are rarely constant; they fluctuate, often quite widely and with rapid periodicity. Consequently the effects of fluctuations of poison concentration are of some interest. Additionally, even in the best-designed toxicity tests some fluctuation in the poison concentration is occasionally unavoidable, and information on the response of test animals to fluctuating concentrations would also be of value in this context. Surprisingly, there have been relatively few investigations of the point. Brown *et al.* (1969) determined the 48-h LC50 of ammonia, zinc, and an ammonia-zinc mixture to *Salmo gairdneri*. They then exposed replicate groups of fish to a constant 48-h LC50 of each poison, and to fluctuating concentrations such that the mean concentration was a 48-h LC50 but the actual concentration varied between 0.5 and 1.5 times the 48-h LC50 at intervals of one to four hours. In the majority of cases there was no significant difference between the survival times of fish exposed to constant concentrations and those exposed to fluctuating concentrations, suggesting that fluctuations of ±50% of the nominal concentration do not affect the response of the fish. However, in the case of ammonia, where the periodicity of the fluctuations was two hours rather than one, the median survival time was significantly reduced. Thurston *et al.* (1981) reported similar findings, also using trout exposed to ammonia. In these experiments fish were alternately exposed to ammonia and clean water, the periodicity of the fluctuations being six or 12 hours. On the basis of 96-h LC50 values for stable and fluctuating concentrations, the

fish were more sensitive to the fluctuating concentrations, than to a constant concentration equivalent to the mean value. One explanation of this (Brown *et al.,* 1969) is that when the periodicity of fluctuation is long, fish will suffer irreversible damage during the exposure to the peak concentration which cannot be compensated for during exposure to the lower concentration. An extreme case of fluctuating concentrations is the test designed to determine the effects of a relatively short exposure to poisons, followed by an extended period of recovery in clean water (Abel, 1980b; Abel and Garner, 1986). In this case, tests based on continuous exposure to lethal concentrations were found severely to underestimate the impact of short, high-level exposures as might occur during an accidental discharge.

4.2.5 Biotic Factors Influencing Toxicity

Animals may show both interspecific and intraspecific variation in susceptibility to pollutants. It is beyond the scope of the present discussion to review in detail all the relevant literature. However, it is possible to draw attention to some of the more salient general findings regarding the way in which the biological characteristics of the test organisms influence their response to toxic pollutants.

Among fishes, interspecific variation in susceptibility is probably smaller than was once thought, and may be less important than variation due to environmental conditions. Because of the large influence of environmental conditions on toxicity, it is arguably unwise to place too much reliance on comparisons between the results of different investigators who have measured toxicity under different environmental conditions. In many studies, environmental variables which are known to have a large influence on toxicity have not been measured or specified; and there have been relatively few studies in which several species have been tested under similar conditions.

Thatcher (1966) found that 96-h LC50s of an alkylbenzene sulphonate detergent to 11 species ranged from 7.7 mg l^{-1} to 22 mg l^{-1}. For a linear alkylate sulphonate detergent tested against five species, 96-h LC50s varied from 3.3 mg l^{-1} to 6.4 mg l^{-1} (Thatcher and Santner, 1967). Lethal threshold concentrations of the fungicide captan to three fish species ranged from 29 to 64 μg l^{-1} (Hermanutz *et al.,* 1973). The 96-h LC50 of hydrogen cyanide to five species varied from 57 to 191 μg l^{-1} (Smith *et al.,* 1978). Interspecific variations in short-term LC50 values of about threefold to fourfold appear to be typical for many types of poison (Sprague, 1970). Larger differences have occasionally been reported, particularly for pesticides. Eisler (1970) determined the 96-h LC50 of 12 insecticides for seven estuarinespecies. Interspecific variations for endrin were 0.05–3.1 μg l^{-1}; for dieldrin, 0.9–34 μg l^{-1}; for heptachlor, 0.8–194 μg l^{-1}; and for malathion, 27–3250 μg l^{-1}.

The importance of determining lethal thresholds, rather than relying on short-term LC50 values, is again evident from Ball's (1967a) studies on the relative susceptibility of fish species to ammonia. Although Salmonid fishes are widely

considered to be more sensitive to pollutants than coarse fish, he showed that the lethal threshold concentration of ammonia for *Salmo gairdneri* was the same as that for three Cyprinid species *(Abramis brama, Rutilus rutilus* and *Scardinius erythrophthalmus).* However, the trout reacted far more rapidly to the ammonia than did the cyprinids, so that in tests of short duration they would appear to be more sensitive. Ball (1967c) also measured the toxicity of zinc to trout and four coarse fish species. In this case the trout were markedly more sensitive: the threshold LC50 value for trout was 4.6 mg l^{-1}, significantly lower than the corresponding values for *Rutilus rutilus, Abramis brama* and *Perca fluviatilis,* which lay between 14.3 and 17.3 mg l^{-1}. The gudgeon, *Gobio gobio,* had a seven-day LC50 (not a threshold value) of 8.4 mg l^{-1}. As seen earlier, the toxicity of both zinc and ammonia is greatly influenced by environmental conditions, and it is likely that variation in susceptibility between trout under different environmental conditions is actually greater than that between trout and other fish species. The results of Smith *et al.* (1978) indicate that environmentally-induced variability in the toxicity of cyanide to fishes is of a similar magnitude to interspecific variability.

A further point arising from Ball's (1967a) study has been referred to earlier but is also relevant here. It appears that some species may show much greater individual variation in susceptibility to a poison than others. Thus in one of Ball's experiments, the five-day LC50 values of undissociated ammonia to roach *(Rutilus rutilus)* and rudd *(Scardinius erythrophthalmus)* were identical, but the slopes of the probit lines differed, indicating that roach were more variable in response than rudd. The practical implications relate to the application of toxicity data to field observations, and to the formulation of water quality standards. In an example given by the author, two-thirds of the five-day LC50 of ammonia would kill only 1% of a rudd population, but 16% of a roach population. This example again illustrates the advantages of a full analysis of toxicity test data, and the limitations of comparative studies based on simple determinations of short-term LC50 values.

Several authors have undertaken comparative studies of the susceptibility of invertebrate species to pollutants. In most of these, however, authors have chosen representative species from each of several orders, classes or phyla. Not surprisingly, the susceptibility of such 'representative' species shows some very wide variations, often of two or three orders of magnitude in terms of short-term LC50 values, in comparison with the phylogenetically more uniform fishes (e.g. Bell, 1971; Gaufin *et al.,* 1965; Rye and King, 1976). However, there have been some studies of the comparative toxicity of poisons to fairly closely-related species and it is useful to consider some examples in the present context. For five genera of oligochaetes exposed to four poisons, variation between 96-h LC50 values was generally less than one order of magnitude, although the two marine genera tested appeared highly resistant to the lethal action of cadmium (Chapman *et al.,* 1982). There were four species of *Daphnia* (Winner and Farrell, 1976). Sanders (1970) measured

only small differences between the 72-h LC50 values of copper tested against the toxicity of several herbicides to six crustaceans representing four orders. In general, variations in 48-h LC50 values between species were within one order of magnitude or less, but some species displayed considerable resistance to some or all of the herbicides, and some herbicides elicited interspecific variations in 48-h LC50 values spanning three orders of magnitude. These and similar examples from the literature indicate that it is not easy to draw firm conclusions about the relative susceptibility of invertebrate species to many poisons; there remains a need for continuous review in an accessible form of the data which is available.

4.2.6 Intraspecific Variation

The effects of size and age of fish on their susceptibility to poisons has been the subject of surprisingly few systematic investigations, although the point is important both for the practical application of toxicological data and because laboratories which routinely carry out toxicity tests cannot always obtain animals of standard size and/or age. In most reports, fish of different sizes have also been of different ages, so the separate influences of these two variables remains largely unknown. Nevertheless it is common practice to standardise as far as possible on the age and size of animals, and to restrict the size/age range of specimens in a test as narrowly as is practicable.

Adelman *et al.* (1976) measured the toxicity of pentachlorophenol to goldfish *(Carassius auratus)* and fathead minnows *(Pimephales promelas)* of different sizes and ages. Differences in threshold LC50 values were small and probably of no practical significance. However, the range of sizes and ages tested was small, for example, from four to 14 weeks of age and 13–30 mm in length for the fathead minnow. Kumaguru and Beamish (1981) found a more marked effect of size on the toxicity of the pesticide permethrin to *Salmo gairdneri*. The 96-h LC50 values ranged from 3 μg l^{-1} for fish weighing 1 g, to 287 μg l^{-1} for 50-g fish. In contrast Stendahl and Sprague (1972) reported that small rainbow trout (1.5g) were more resistant than larger (12 g) fish to vanadium, although lethal threshold concentrations were not established for the smaller fish and the difference in susceptibility is probably small.

The young of most species show a clear division of their life cycle into distinct stages, and there have been several investigations of the relative susceptibility to poisons of the different life stages of fish. (Toxicity tests spanning whole life cycles are discussed later.) Skidmore (1965) found that zebrafish *(Brachydanio rerio)* were most susceptible to zinc between 4 and 13 days after hatching. Eggs were considerably more resistant than newly-hatched fry, and after 13 days resistance increased rapidly. This seems to be a general pattern, similar results having been reported for species exposed to detergents (Marchetti, 1965), hydrogen sulphide (Smith and Oseid, 1972) and heavy metals (Chapman, 1978).

An important potential source of intraspecific variation in susceptibility to poisons is genetic variation between different strains or populations of a species. Many waters have been subjected to pollution for a period equivalent to many generations of the organisms living there, and it may be expected that natural selection would lead to an increase in the tolerance of populations living in polluted waters. Thus natural populations may in fact be more tolerant of pollutants than those used in laboratory experiments, which are usually inbred strains or acquired from unpolluted habitats. It is important, however, to distinguish increased resistance due to genetic adaptation, from that due to acclimation effects (i.e. long-term exposure to low levels conferring increased resistance to a subsequent high-level exposure) which are not genetically determined and of which a few examples exist in the literature (Sprague, 1970). Also, since such differences in tolerance are likely to be relatively small, short-term LC50 values or median survival times at fairly high concentrations are not really adequate measures of toxicity. Thus examples of genetically-acquired resistance to poisons are few. Brown (1976) reported that Isopod crustaceans *Asellus meridianus* from metal-polluted streams were more resistant to copper and lead than animals from clean streams, and that the resistance persisted to the F$_2$ generation. Rahel (1982) reported that perch *(Perca flavescens)* from acid lakes were more tolerant to low pH than those from alkaline lakes. He did not show that the difference was genetically based, but acclimation experiments failed to produce increased tolerance in fish from alkaline lakes. Swarts *et al.* (1978) measured the resistance of several strains of brook trout *(Salvelinus fontinalis)* to low pH and investigated the effects of genetic and environmental influences on resistance. They found that fish could not be acclimated to low pH, that strain differences in resistance to low pH were detectable, but that an attempt to produce a resistant strain by breeding from fish which had survived exposure to low pH was unsuccessful.

4.3 Applications of Lethal Toxicity Measurements

Until fairly recently, the literature on the toxicity of pollutants was dominated by measurements of lethal toxicity, and the relevance of these measurements was widely questioned. There are, however, genuine technical and conceptual difficulties with the measurement of sublethal toxicity (see Section 4.4), and as these difficulties have been resolved there has been much more emphasis on measurements of sublethal toxicity at poison concentrations which more realistically simulate the levels at which organisms are more usually exposed in the field. However, it would be a mistake to think that lethal toxicity testing is of no value, or that it has been altogether superseded by more recently-developed sublethal test methods. On the contrary, lethal toxicity testing continues to make a valuable and very practical