contribution to the control and regulation of pollution, as the following examples may illustrate.

There remain instances (in the UK, about one every day of the year) of illegal, negligent or accidental discharge of polluting material to watercourses which cause observable damage to the receiving environment, frequently involving mortalities of fish. In order to investigate the causes of these, and where appropriate to take legal action against the perpetrator, it is often insufficient to rely on the circumstantial evidence that a certain substance was present at or before the time the fish died. It is obviously preferable to be able to show causation, i.e. that the substance actually caused the death of the fish. There can be circumstances where the true cause of an incident can be obscured by the fortuitous presence of an unrelated but more obvious condition; or where the nature of the suspect substance is not actually known. Therefore some measure of the toxicity of the suspect substance is often required.

In some heavily polluted rivers, the total burden of pollutants can reach, at least at times, levels which are threatening to the endogenous fauna. In order to formulate specific and cost-effective remedial measures to improve the overall water quality, a model based on a careful series of lethal toxicity tests has been found useful. This approach was described in Section 1.3.

A related application of toxicity measurements can be used to assist in improving the quality of effluents. This is particularly important for effluents which are complex and/or very variable in nature. Measuring the toxicity of a series of effluent samples at different times (see Figure 4.15), can yield useful information (Lloyd, 1991a). In this case, effluent samples 4 and 9 are markedly more toxic than the other samples. If some chemical data are available, for example regarding the composition of the effluents or the operating conditions of the plant producing them, then it may be possible to identify particular components of the effluent or particular operating conditions which are associated with increased toxicity, so that structural or process modifications can be implemented to reduce the overall impact of the effluent. In another case (Figure 4.16), an effluent contained a number of components, one of which was thought to be responsible for most of the toxicity. Comparison of the toxicity of the whole effluent with a range of concentrations of the suspect substance equal to those in the effluent samples showed that the toxicities of the two were almost identical, confirming that the suspect substance was indeed the main cause of the problem. This means, of course, that improved effluent treatment can be specifically designed to remove the chlorpyrifos.

In fact, toxicity measurements are increasingly used in the regulation of effluent discharges: permission to discharge is frequently granted or withheld at least partly on the basis of a measurement of effluent toxicity; and measurements of toxicity may be specified in discharge consents to be carried

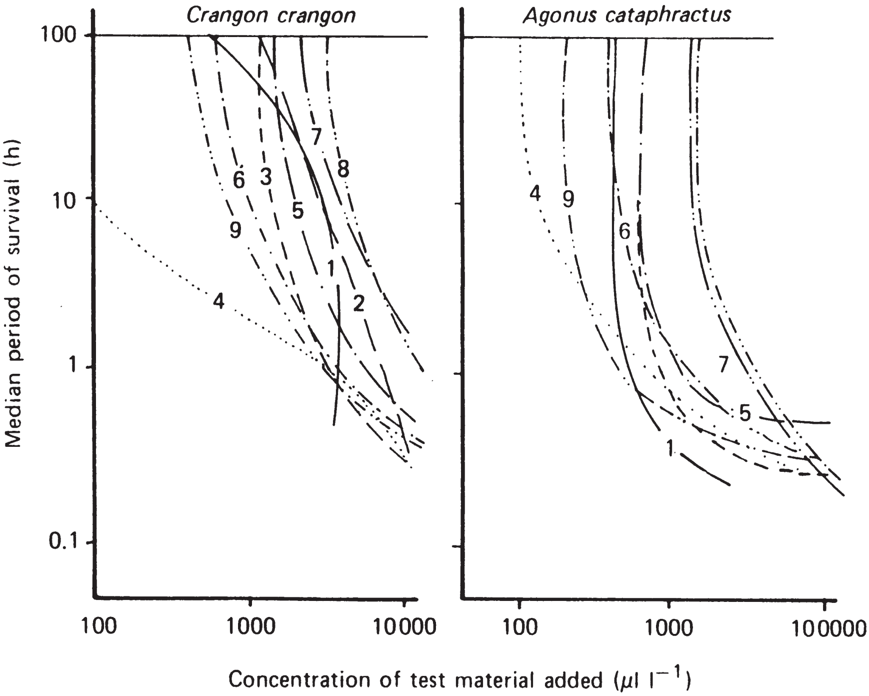

Figure 4.15 Time-series toxicity curves for a complex effluent tested against two different species under identical conditions at different times. After Lloyd (1991)

out on a regular basis. Even a simple test can influence decisions on whether or not to grant permission to discharge, or whether to carry out further research. For example, an effluent whose toxicity curve shows a distinct lethal threshold within a short time, as do most of the samples in Figure 4.15, will almost certainly be treated more leniently than one which shows little sign of such a threshold, as in Figure 4.16.

Lethal toxicity tests also form an important part of the process known as *sequential hazard assessment* (see Chapter 6). This is a codified sequence of investigations and observations on the properties of poisons or effluents, designed to allow a reasonably informed decision to be made quickly on whether or not a particular substance is environmentally acceptable. Although in an ideal world every poison and effluent should go through an exhaustive testing process, in practice it is neither feasible nor necessary to do so. In the light of all available information, and some reasonably brief toxicity measurements, it is often possible to make a rapid decision in a particular case and concentrate resources on more difficult cases. Finally, some chemicals are released to the environment under such rare and special circumstances that lethal toxicity tests provide all the information

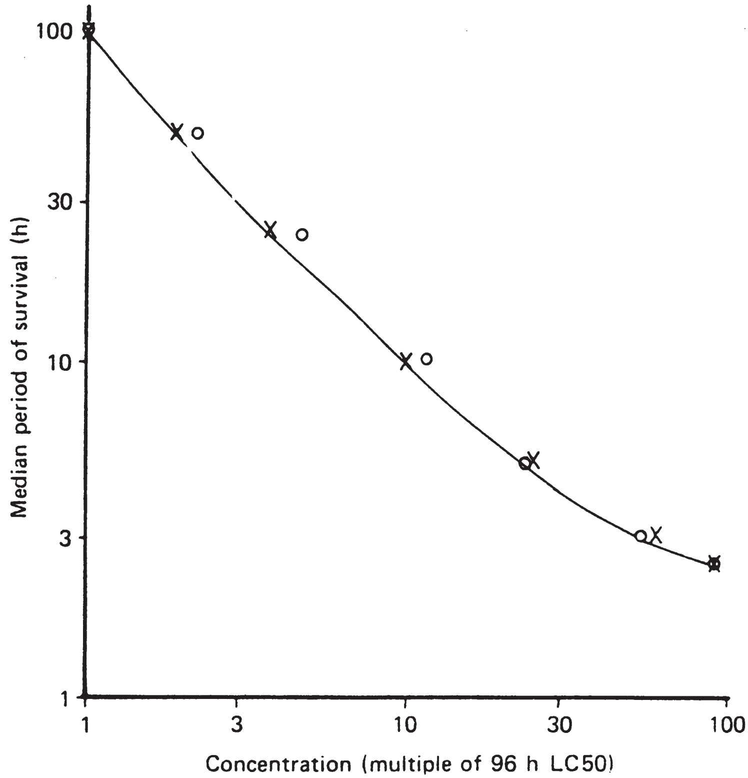

Figure 4.16 Toxicity curve for a complex effluent containing chlorpyrifos, compared with chlorpyrifos alone. Circles represent effluent containing chlorpyrifos, crosses represent chlorpyrifos alone. After Lloyd (1991)

which is likely to be required to assess their acceptability. Regulatory agencies therefore tend to license them for sale or use on the basis of lethal toxicity tests alone. Oil dispersants provide a good example. They are only used rarely, under circumstances where their effects are likely to be short-lived and localised, and only when a body of water has already been heavily contaminated with oil. However, in many countries their sale or use is forbidden unless their toxicity to living organisms is sufficiently low. By this means, manufacturers are encouraged to develop products and formulations of low toxicity, and the original, more toxic, formulations in use some 30 years ago have gradually been phased out.

4.4 Sublethal Toxicity

The fauna of polluted waters is more commonly exposed to relatively low concentrations of poison for long periods, rather than to levels of pollution which will cause rapid mortality. Therefore it is important to study the effects on aquatic organisms of exposure to sublethal levels of pollution over periods which represent at least a substantial proportion of their life cycle. The historical predominance of acute lethal toxicity studies has therefore often been criticised as being of limited relevance to real situations, but is due less to the failure of toxicologists to appreciate the point than to the many technical and conceptual difficulties involved in the measurement of sublethal toxicity.

Relatively few species can be satisfactorily maintained in the laboratory for long periods and fewer still can complete their life cycle under such conditions since their environmental requirements are complex, unknown or both. Maintenance of constant experimental conditions for periods which may exceed a year is expensive of human and physical resources, and the longer an experiment continues the greater is the chance of failure due to accident or equipment malfunction. Therefore the preferred species for research of this kind are those which can be cultured in the laboratory, are indigenous to the geographical area in which the study takes place, have reasonably short generation times and which display a more or less representative response to a wide variety of poisons; that is they should not be unusually resistant or unusually sensitive to particular poisons or categories of poison. Unfortunately in many regions few, if any, species which meet all these requirements are available.

Assuming that purely technical difficulties can be overcome, the problem remains that whereas the death of an organism is an unequivocal and easily-identifiable response to toxic action, criteria of sublethal toxic effects are less easy to define and, as will be seen, if they are recognised their biological significance is frequently difficult to assess. Nevertheless the meaningful application of data from lethal toxicity studies to many practical pollution problems is difficult, if not impossible, in the absence of some information on sublethal toxicity. Therefore a wide variety of approaches to the measurement of sublethal toxicity has been employed, and some of the more important ones are reviewed here.

4.4.1 Single-species Toxicity Tests

Because of the practical problems of maintaining animals under experimental conditions for very long periods, early studies in sublethal toxicity generally relied on the use of histological, pathological, biochemical, haematological, physiological or behavioural criteria of toxic effect in experiments lasting weeks rather than months. Sprague (1971) has reviewed some examples of these studies. More recently, the ever-increasing range of analytical and diagnostic techniques devised by biochemists and clinical chemists has been widely applied or adapted to the detection of sublethal toxic responses of fish to pollutants. Table 4.1 lists some of the techniques which have been

Variable	Variable
Cardiovascular physiology	*Enzyme assays* (various tissues)
Heart rate	Lactate dehydrogenase
Arterial pO2	Glutamic-oxaloacetic transaminase
Ventilation rate	γ-Aminolevulinate dehydrase
Cough frequency	
Opercular and buccal pressure	*Others*
Ventilation volume	Urine pyruvic acid content
Oxygen consumption	O_2 consumption of tissue homogenate
Oxygen utilisation	Electrophoretic patterns of serum proteins
Ventilation frequency	
	Body moisture content
Haematology	Body lipid content
Haematocrit	Body protein content
Haemoglobin content	Liver glycogen content
Methaemoglobin content	Liver phenylalanine content
Total blood cell counts	Urine excretion rate
Erythrocyte counts	Swimming performance
Differential leucocyte counts	Food conversion efficiency
Erythrocyte ATP concentration	Locomotor activity
Blood metabolite levels	
Lactate	
Glucose	
Sodium	
Chloride	
Cortisol	
Serum proteins	
Pyruvate	
Osmolality	
Cholesterol	
Other electrolytes	

used. The list is a representative rather than an exhaustive one, and no attempt is made here to review this aspect of pollution toxicology in detail. Rather, the discussion will focus on some conceptual and practical difficulties raised by the very diversity of criteria which have been employed.

The objective of experiments such as those represented in Table 4.1 is essentially to determine whether animals exposed to sublethal levels of pollutant are healthy or not. It might be thought that with the aid of the