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The Toxicity of Pollutants to Aquatic Organisms

There are many circumstances in which the need to measure toxicity may arise. Many thousands of chemical substances are used for industrial, agricultural and domestic purposes, and their numbers increase annually. The toxicity of these chemicals and of their by-products and degradation products to aquatic animals needs to be determined, since any compound manufactured and used in substantial quantities is likely to become a contaminant of watercourses. In the case of novel compounds or formulations, toxicity testing may precede large-scale manufacture and form part of the research into the feasibility of its commercial application. Toxicity tests may be incorporated into effluent monitoring schemes. Identification of the more toxic components of complex effluents may be a prerequisite for the development and improvement of effective treatment processes. The measurement of toxicity is essential in the formulation of quality standards for receiving waters. Finally, compliance with a toxicity standard may be a legal requirement for consent to discharge an effluent. In this chapter we shall consider the basic principles of toxicology in relation to water pollutants and aquatic organisms, and the methods by which the toxicity and toxic effects of pollutants to aquatic life may be studied. The application of toxicological data in water pollution control, and their use in the formulation of water quality standards will also be considered, both in this chapter and in Chapter 6.

Toxic pollutants may exert their effects in several ways, depending upon the characteristics of the poison, of the receiving water, and of the biological community the water sustains. In extreme cases, animals may be killed by the poison. In some circumstances, poisons—insecticides, herbicides, molluscides, piscicides—are applied to water with the express purpose of killing some species and in the hope

that others will be unaffected. Lower concentrations of poison may exert sublethal toxic effects. Some poisons appear to accumulate in the tissues of organisms during their lifetime, and exert toxic effects after prolonged exposure to concentrations which are barely measurable by chemical means. It is widely suspected that some of these may pass from prey to predator organisms and achieve high concentrations in species at the top of a food web. Many poisons are known to be mutagenic, teratogenic or carcinogenic, but the study of these phenomena in aquatic organisms is in its infancy.

From a biological point of view, any toxic effect is significant if it influences, or is likely to influence, the physiology or behaviour of the organism in such a way as to alter its capacity for growth, reproduction or mortality, or its pattern of dispersal, since these are the major determinants of the distribution and abundance of species. Species which are not directly affected by a pollutant may nevertheless be indirectly influenced. For example, if a predator is deprived of its normal prey by the action of a pollutant on the prey, it may itself be numerically reduced. Alternatively it may prey upon some other species, which itself may show a numerical response. Where two competing species are unequally affected by the pollutant, both may show a change in distribution and/or abundance. Thus the effects of toxic pollutants can only be fully understood with some knowledge of trophic, competitive and other interspecific relationships.

Since pollutants can exert such a variety of toxic effects at different levels of biological organisation, an enormously wide range of investigative methods has been employed in their study. There is an enormous literature on the subject of pollutant toxicity to aquatic organisms, especially fish and invertebrates. Much of it is, unfortunately, of limited or even doubtful value, for reasons that will be made clear.

For the purposes of this discussion it is necessary at the outset clearly to define four basic terms which are widely misunderstood:

1 *Lethal toxicity:* toxic action resulting in the death of the organism.
2 *Sublethal toxicity:* toxic action resulting in adverse effects in the organism other than its death.
3 *Acute toxicity:* toxic action whose effects manifest themselves quickly (by convention, within a period of a few days).
4 *Chronic toxicity:* toxic action whose effects manifest themselves over a longer period (by convention, within periods measurable in weeks or months rather than days).

A common misunderstanding among the ill-informed is to use the terms ‘acute’ and ‘lethal’ as if they were synonymous, and to do the same with the terms ‘chronic’ and ‘sublethal’. This is clearly wrong. There are numerous examples

of sublethal toxic effects which manifest themselves quickly, and of organisms which die only after prolonged exposure to a poison. Thus it is perfectly correct to talk of acute sublethal toxicity, acute lethal toxicity, chronic sublethal toxicity or chronic lethal toxicity and in many circumstances it is important to distinguish correctly between them.

4.1 Lethal Toxicity and its Measurement

Approaches to measuring lethal toxicity vary in their complexity, in terms of the procedures employed, the apparatus required and the methods of collecting and processing the data produced. There are corresponding differences in the amount of information yielded, the degree of confidence which may be placed in the results, and the purposes for which those results may validly be used. There are many different reasons for carrying out toxicity tests, and it is important that the procedure chosen is appropriate to the purpose for which the results are required. Alabaster and Lloyd (1980) have discussed toxicity testing procedures in relation to their various applications. A useful practical guide to toxicity test methods has been published by HMSO (1983a). There are indeed several such useful manuals, not all of which are readily accessible; Abel (1991) refers to several of these. Since the results of toxicity tests may be significant in connection with pollution control legislation, national and international agencies have made some attempts to agree upon standard procedures (e.g. Alabaster and Lloyd, 1980; APHA, 1995; ASTM, 1973; Maki and Duthie, 1978). These and other sources frequently make specific recommendations on many aspects of toxicity testing methodology (see also Axiak and Abel, 1991; Reish and Oshida, 1986; Sprague, 1969, 1970, 1973; UNEP/FAO/IAEA, 1989; Ward and Parrish, 1982). In some circumstances, special procedures are required in order to obtain meaningful results. This can arise where, for example, the properties of the organism under test, or of the poison to be tested, are unusual in some way; or where the pattern of occurrence of the pollutant in water does not follow the usual pattern. Some examples of modified test procedures are described by Axiak and Abel (1991); see also Section 4.1.5. Some of the simpler methods are widely used but provide very limited information, and their results need to be interpreted with more caution than is sometimes exercised. The reasons for this will become clear later in this chapter, but it is first necessary to understand the principles of more rigorous procedures.

4.1.1 The Experimental Conditions

The basic requirement is for groups of animals to be exposed to each of a series of concentrations of poison in suitable containers. A toxicity test does not differ from other types of experiment in that due consideration should be given to matters such as sample size, acclimation of animals to the experimental conditions, and

maintenance of a constant environment. Normally samples of at least ten are used, and the appropriate range of poison concentrations may be estimated on the basis of preliminary experiments so as to cause most of the animals to die over a period ranging from a few hours to a few days. However, it is often necessary to investigate the effects of lower concentrations such that the experiment may last for several weeks or more. The main practical difficulties encountered are in ensuring constant environmental conditions, maintaining the poison concentrations at their nominal levels, and minimising stress to the animals.

The toxicity of many poisons is greatly influenced by environmental conditions such as pH, temperature, water hardness and dissolved oxygen concentration. Clearly such variables should ideally be measured and controlled during any test, particularly since the presence of the animals themselves is likely to cause a gradual deterioration in the initial experimental conditions. This will arise due to utilisation of dissolved oxygen and excretion of carbon dioxide and other toxic metabolites such as ammonia. Poison concentrations may vary during the experiment due to absorption and metabolism by the animals, chemical and microbiological breakdown, by evaporation, or through adsorption onto the sides of the test vessels. These considerations suggest that the test containers should be fairly large and that the test solutions should be replaced regularly. It is generally considered that a minimum test volume of two to three litres per gram of animal tissue, to be renewed at least once daily, is normally required. The preferred arrangement, however, is to construct a 'continuous flow' apparatus in which the test solutions are automatically replenished, usually achieving a total replacement of solution every six to eight hours. The replacement rate may be greater or less depending upon the size of the test containers, the size, activity level and metabolic rate of the animals, and the volatility or degradability of the poison under test. In practice, most investigators use apparatus of their own design and construction, and of varying degrees of complexity.

4.1.2 Data Collection and Analysis

The raw data from a toxicity test take the form of a record of increasing mortality in each test container as time passes. The survival time of each individual in the experiment should be recorded accurately. In practice the series of observation times recommended by Sprague (1973) are usually adequate, namely at 0.25, 0.5, 0.75, 1, 2, 4, 8, 14±2, 24, and 33±3, hours, and thereafter at daily intervals.

The next step is to estimate the median survival time (LT50) of each group of animals, that is the time required for half the animals to die. The most widely-used technique is the rapid graphical method of Litchfield (1949), which is derived from a procedure developed by Bliss (1935, 1937). For each group of animals, a graph is plotted of cumulative percentage mortality

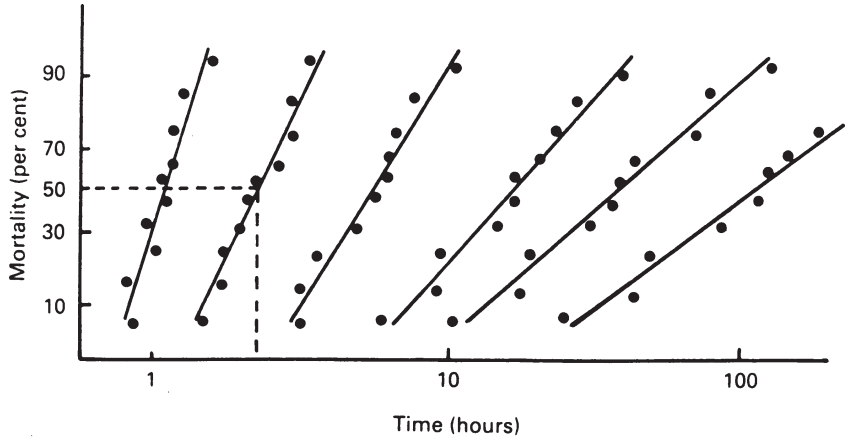

Figure 4.1 Probit lines resulting from plots of cumulative percentage mortality in each test tank against time, using logarithmic–probability graph paper. The dotted lines show how median survival times are read from each line. Lines on the left are for the higher poison concentrations

against elapsed time. For reasons which will be discussed later, the mortality data are transformed to probits (or plotted on a probability scale) and the time values are transformed to log times. The straight line of best fit is then drawn through each set of points. This line may be computed, but in practice lines fitted by eye are often satisfactory. The result is a set of 'probit lines' or time-mortality curves as shown in Figure 4.1. Values of LT50 can now be read off each line as shown. The time for each percentage response, for example LT10, LT90, can also be read off if required. Normally values for LT16, LT50 and LT84 are required. To estimate confidence limits, the *slope function, S,* for each probit line is determined by

$$S = \frac{\dfrac{\text{LT } 84}{\text{LT } 50} + \dfrac{\text{LT } 50}{\text{LT } 16}}{2} .$$

Using the values of S and N (where N = the number of animals in the test tank), the factor f is computed:

$$f = \text{antilog}\left(\frac{1.96 \log S}{\sqrt{N}}\right) = S^{1.96/\sqrt{N}}.$$

Litchfield (1949) provides a nomograph for the computation of f from values of S and N. The procedure is slightly more complex if not all the animals die during the experiment, or if a 'split' probit line (see below) is obtained. The upper and lower confidence limits of the LT50 are given by LT50×f and LT50/f respectively. LT50 values and their confidence limits for each group of animals can be plotted against