

## CHAPTER 9

### *Aquatic Animals*

P. D. ANDERSON AND S. D'APOLLONIA

*Department of Biological Sciences, Concordia University, 1455 de Maisonneuve Blvd.  
W. Montreal, P.Q. H3G 1M3*

9.1 INTRODUCTION . . . . .	187
9.2 SUBLETHAL TOXICITY . . . . .	188
9.3 PHYSIOLOGICAL SYSTEMS FOR SUBLETHAL TESTS OF POLLUTANTS . . . . .	191
(i) Osmoregulation . . . . .	191
(ii) Respiration . . . . .	192
(iii) Blood . . . . .	194
(iv) Locomotion . . . . .	195
(v) Growth . . . . .	198
(vi) Reproduction . . . . .	198
9.4 TERATOGENESIS . . . . .	199
9.5 MUTAGENESIS . . . . .	201
9.6 CARCINOGENESIS . . . . .	202
9.7 MULTIPLE TOXICITY . . . . .	204
(i) Presumptive Mechanisms of Interaction . . . . .	206
(ii) Multiple Toxicity Models – based on Interactions at the Dynamic Level . . . . .	207
(iii) Multiple Toxicity Models – based on Interactions at the Kinetic Level . . . . .	212
(iv) Application of Multiple Toxicity Models . . . . .	213
(v) Cancer . . . . .	213
(vi) Water Quality Management . . . . .	213
9.8 REFERENCES . . . . .	214

#### 9.1. INTRODUCTION

Natural waters are the ultimate recipients of much of the chemical wastes generated by man's industrial, agricultural, and domestic activities and released into the geosphere. Wastes enter the aquatic environment directly, by point source dumping, or indirectly, by rain, snow, or fall-out and by surface water run-off and ground-water leachings. Aquatic ecosystems, to varying degrees, are adaptable; they have a variety of physical, chemical, and biological mechanisms by which wastes may be assimilated without serious implications to endemic biota. When chemical contaminants reach levels in excess of the assimilative capacity of receiving waters they affect the survival, reproduction, growth, and movement of organisms. As a result the distribution and abundance of populations change with a possible alteration in the energy budget of the ecosystem. The end effect is observed as perturbations in community structure and successional patterns. Man's economic,

recreational, and agricultural interests in aquatic ecosystems may be jeopardized by pollution. Furthermore, man's health may be harmed by toxic contaminants upon their uptake from drinking water and from the consumed flesh of aquatic organisms.

Chemical wastes can impair the natural state of an ecosystem through either nutrient enrichment or toxic processes. Enrichment by man-mobilized organics and minerals may promote excessive growth rates in autotrophs whose abundance then alters environmental modifying factors such as oxygen concentration and temperature. The resulting conditions may be unfavourable to other organisms which either migrate or perish often to be replaced by less desirable species. A change in the nutritional balance of an ecosystem can increase the susceptibility of certain organisms to the toxic action of chemical wastes. In these instances, nutrient enrichment may be regarded as an accessory factor in toxicity (Fry, 1947). Moreover, the incomplete catabolism of excess nutrients by decomposers can, in itself, be toxicogenic. The technology is, however, available to regulate effectively the releases of biostimulatory wastes at their major sources, e.g. in municipal and industrial effluents. This technology coupled with recent methods of monitoring eutrophication (Vollenweider and Dillon, 1974) offers a promising solution to the problems created in aquatic ecosystems by biostimulatory wastes.

The management of toxic wastes entering natural waters would appear more difficult if only because poisonous contaminants often arise from multiple or diffuse sources. The seriousness of this form of pollution is compounded by the facts that toxicants may be highly potent and selective as lethal and sublethal agents, resistant to denaturation by environmental and biological agents, altered by environmental and biological agents to more toxic forms, transported widely by environmental and biological vectors and accumulated by organisms whereby the levels of a toxicant undergo magnification within the food chain.

The following account provides approaches deemed to be useful in assessing sublethal toxicity with particular reference to teratogenesis, mutagenesis, and carcinogenesis. Furthermore, a rationale is presented for the investigation of the potentially harmful additive and synergistic interactions of pollutant mixtures to aquatic fauna.

It will be noticed that the tests of sublethal effects described in the sequel apply to individual organisms or to small groups studied in the laboratory. At present these are the principal means of assessing the effects of environmental pollutants in spite of the fact that the methodology for applying laboratory results to field situations is in serious need of development. Some discussion of this is to be found in Section IV.

## 9.2. SUBLETHAL TOXICITY

The deleterious effects of chemical pollutants on aquatic ecosystems may result not only from toxicity that directly causes the death of an organism, but also from



a variety of sublethal effects. Sublethal impairment of an animal's development or its capacity to perform and adapt can reduce the chances for survival and the potential for growth and reproduction. These constraints, though possibly slight for individual members of a population, may be highly detrimental to that population's long-term ecological success as measured by its abundance and distribution. The consequences may be as severe as extinction. Ultimately, such changes in populations lead to perturbations of community structural and successional patterns.

This subtle yet insidious pattern of sublethal toxicity makes the assessment of levels below which the respective chemical contaminants do not pose a toxic hazard to an ecosystem, an immensely complex problem. Adequate monitoring of natural ecosystems to measure ecologically significant levels (i.e. levels that are detrimental under field conditions) usually results in severe disruption of the community under study. The only practical recourse in water quality management is to devise laboratory screening tests from which are extrapolated pollutant levels that are deemed 'safe' for aquatic ecosystems. The task in this latter approach is to decide what level of impairment observed under laboratory conditions would constitute a detrimental effect under field conditions.

The popular use of 'application factors' (5–10% or the 48-hour  $LC_{50}$  for non-persistent pollutants and 1–10% of the 48-hour  $LC_{50}$  for persistent pollutants, Sprague, 1971) as a means of predicting 'safe' levels derived from measurements of lethality has been criticized by many pollution biologists. They argue that the assumptions in the application factor approach are not supported empirically and advocate as an alternative the use of chronic, sublethal tests. However, as of 1972 fewer than 40% of the water quality criteria for toxicants set by the Environmental Protection Agency of the United States were based on sublethal tests (Sprague, 1976).

Sublethal studies conducted in the laboratory are amenable to predicting 'safe' or ecologically insignificant levels of toxicants if responses are quantified and dose–response relationships are established. By converting these numerical data to a quantal (all or none) form, the effective concentration (EC) for a given sublethal response can be computed for various percentages of the test organisms (Figure 9.1). Sprague (1971) has proposed that the EC's be used to set water quality criteria for safeguarding aquatic organisms. Also recommended as a measure for predicting 'safe' levels is the incipient threshold concentration computed by plotting median effective concentrations ( $EC_{50}$ ) against time (Figure 9.2).

With a few notable exceptions, such analyses of sublethal toxicity have not been applied. This scarcity of quantitation and the lack of predictive models that can be used to correlate sublethal impairment and population hazard are perhaps the major deficiencies in the field of sublethal toxicity and its application to environmental assessment.

Another important problem in the design of sublethal bioassays is the choice of test organisms. Test species, representative of the ecosystem to which the eventual

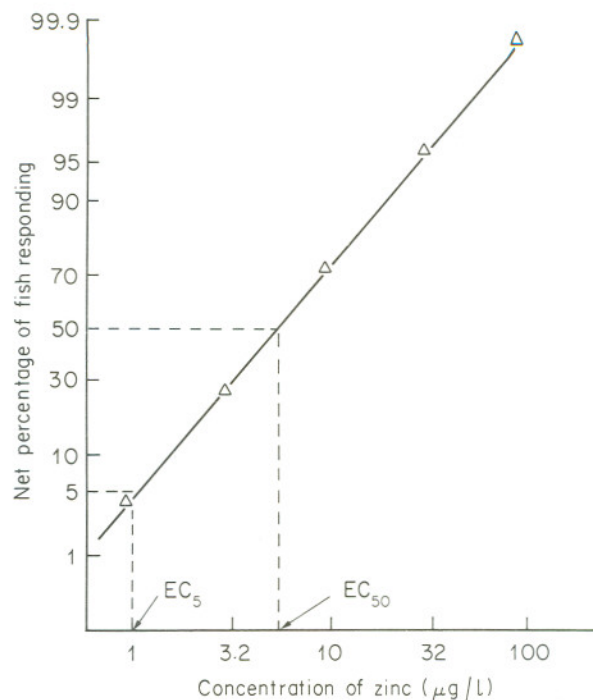


Figure 9.1 Example of probit analysis applied to sublethal studies of avoidance behaviour in trout. The technique allows the computation of the median effective concentration ( $\text{EC}_{50}$ ) and the effective concentration for 5 per cent of the test population ( $\text{EC}_5$ ). (Reprinted with permission from *Water Res.*, 2, 367. Sprague, J. B. Avoidance reactions of rainbow trout to zinc sulphate solutions. © 1968, Pergamon Press, Ltd.)

criteria are to be applied, should be selected for their sensitivity, availability, and position in the food chain. For marine environments, Eisler (1972) listed teleosts, crustaceans, and molluscs in order of increasing tolerance to lethal levels of several organochlorine and organophosphate insecticides. It cannot be assumed that this order of sensitivity would be exemplified at sublethal levels; pollutants may have modes of toxicity that are highly selective and therefore particularly potent to only certain organisms. In consideration of the extreme variety in organisms and toxicants in aquatic ecosystems, neither one screening test nor one test species should be relied upon for the establishment of criteria to protect against sublethal impairment. It is suggested that knowledge of modes of action of toxicants may prove useful in predicting sensitive organisms in an aquatic ecosystem. This proposal is based on the assumption that cells having similar functions and similar metabolic pathways in various organisms are similarly affected by a given chemical entity (Loomis, 1974).



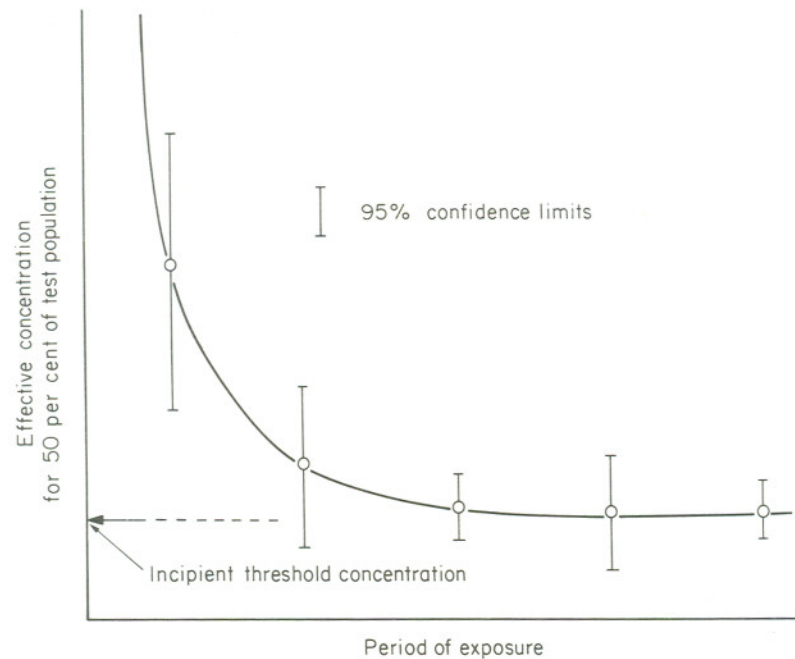


Figure 9.2 Illustration of a sublethal toxicity curve derived by plotting  $EC_{50}$  values against the period of exposure. The incipient threshold concentration represents the point at which the curve becomes asymptotic to the time scale. (Reproduced by permission of Ann Arbor Science Publishers, Inc., from Brown, 1973)

Organisms have evolved intricate regulatory mechanisms vital to survival, growth, and reproduction within a hostile environment. Toxic damage to these physiological systems, although not necessarily lethal under laboratory conditions, often produces impairment under natural conditions. The following section offers a brief survey of physiological systems deemed to have ecological significance for aquatic organisms and some functional laboratory tests used to evaluate the sublethal toxicity of discrete chemical toxicants as they apply to fish. The principles may be applied to other organisms. It is not a complete review of the topic; further information may be found in recent reviews (Sprague, 1971; Mitrovic, 1972; Rosenthal and Alderdice, 1976).

### 9.3. PHYSIOLOGICAL SYSTEMS FOR SUBLETHAL TESTS OF POLLUTANTS

#### (i) Osmoregulation

Aquatic organisms often expend considerable energy regulating the concentrations of osmotically active constituents in body fluids. For example, freshwater fish

are hyper-osmoregulators and must counteract the tendency to lose ions and gain water. Thus, ions are selectively taken up at the gill epithelium and retained by the urinary bladder epithelium. Ionic regulation across a concentration gradient imposes the largest energy expenditure on standard metabolism in freshwater fish (Renfro *et al.*, 1973). Gills, a primary site of osmoregulation, as well as respiration, may be highly vulnerable to lesions because they are in immediate contact with aquatic toxicants. These considerations suggest that inquiries into the sublethal effects of pollutants should include tests for osmoregulatory damage.

There is considerable evidence that certain heavy metals (e.g. mercury, zinc, and copper) cause structural gill damage (Skidmore, 1970; Mitrovic, 1972) and interfere with ion transport across gill epithelium (Lewis and Lewis, 1971; Renfro *et al.*, 1973). Toxicant-induced disruptions in osmoregulation are reflected in alterations in the ion content of blood (Lewis and Lewis, 1971; O'Conner and Fromm, 1975) or in changes in the rate of urine flow (Lloyd and Orr, 1969; Swift and Lloyd, 1974).

Renfro *et al.* (1973) have shown that both mercuric chloride and methylmercury bind to gill tissue where they inhibit sodium uptake in killifish. The rates of mercury clearance from gill tissue correlate with the disappearance of this sodium uptake inhibition. Furthermore, there was a dose-related decrease in sodium—potassium-activated ATPase activity in bladder homogenates isolated from fish exposed to varying concentrations of methylmercury.

This enzyme is found in high concentrations in all epithelial membranes involved in active ion exchange. In anadromous fish adapting to sea water, the activity of sodium—potassium-activated ATPase increases in proportion to sodium excretion (Giles and Vanstone, 1976). Consequently, this period of adjustment may be particularly sensitive to mercurial contaminants and other osmoregulatory antagonists which inhibit sodium—potassium-activated ATPase (Davis and Wedemeyer, 1971; Janicki and Kinter, 1971).

Osmoregulatory variables, such as plasma ion concentrations, rates of urine flow, and activities of ion transport enzymes, would appear to offer sensitive systems for the quantitative determination of effective sublethal concentrations of many toxicants.

## (ii) Respiration

Fish have evolved ventilatory, circulatory, and haematological systems for the uptake and transport of oxygen to cellular oxidation sites. These systems are integrated to deliver an oxygen supply to tissues sufficient for oxidative phosphorylation and other energy requirements of the cells. Homeostatic mechanisms are present to regulate oxygen tension at cellular respiratory sites independent (within set limits) of varying respiratory stress. These mechanisms may be either short-term responses (e.g. increased heart rate, increased blood pH) or long-term adaptations (e.g. increased haematocrit). All these require an additional expenditure of energy which, under certain conditions (e.g. food shortage, low

ambient oxygen concentration) or during times of increased energy demand, the organism cannot supply. Although respiratory distress imposed by a pollutant may have a detrimental effect on fish there are very few studies in which a sublethal effect has been proved to cause population perturbations. The effects of pollutants on ventilation and gas transport have been investigated.

#### (a) Ventilation

The muscular expansion and contraction of the buccal and opercular cavities maintains a flow of water over gill surfaces in teleost fishes. These respiratory movements are under physiological control of the respiratory centre and are modified by both internal (oxygen concentration, carbon dioxide concentration, pH) and external (temperature, oxygen depletion) factors (Shelton, 1971). Under normal circumstances the ventilatory pump is coupled to the cardiac pump, so that there is an adequate residence time of blood in the gills for oxygen exchange. Generally, an increase in ventilation acts to increase the oxygen supply at the gills at a low additional energy cost (Cech and Wohlschlag, 1973). Large increases in respiratory movements however may result in a lower oxygen-carrying capacity of the blood with resulting tissue anoxia (Cech *et al.*, 1976).

A number of techniques have been used to monitor respiratory movements (Heath, 1972). Implanted cannulae, appropriately positioned pressure transducers, and the physical separation of inspired and expired water allow ventilatory volume, ventilatory frequency, and opercular and buccal pressures to be monitored simultaneously under conditions of least stress (Davis and Randall, 1973). In this way alterations in respiratory movements have been recorded in response to sublethal concentrations of a variety of toxicants (Cairns and Sparks, 1971; Davis, 1973; Lunn *et al.*, 1976). Although alterations in respiratory movements are relatively easy to monitor, modifying conditions are difficult to control. Activity, time of day, internal and external oxygen and carbon dioxide concentration, and temperature modify ventilation rate. Furthermore the required anaesthetic and surgery are stressors that are manifested by alterations in ventilatory responses (Houston *et al.*, 1971). Perhaps for these reasons quantitative studies of the effect of toxicants on respiratory activity and the possible detrimental effect of this sublethal response on fish, have not been made.

A more promising respiratory activity for sublethal studies is 'coughing' (the reversal of water flow over gills, presumably to clear them). Schaumberg *et al.* (1967) have suggested that the cough reflex be used as an indicator of sublethal toxicity. An increased cough reflex has been correlated with low levels of pesticides (Lunn *et al.*, 1976), copper (Sellers *et al.*, 1975), and kraft mill effluent (Davis, 1973). An elevated cough frequency interferes with oxygen uptake at the gills and reduces the efficiency of oxygen uptake (Davis, 1973) and if continued might cause tissue anoxia and respiratory collapse. Although alterations in cough reflex are relatively easy to monitor using either implanted cannulae and pressure transducers,



or surface electrodes, fish quickly accommodate to the toxicant and the cough rate returns to normal. Thus the relevance of this sublethal response to populations' impairment can be questioned.

#### (b) Gas Transport

The amount of oxygen reaching tissue sites is a function of not only the oxygen-carrying capacity of the blood but also the blood flow. The rate of blood flow is modulated by peripheral vascular resistance to flow (measured by blood pressure) and cardiac output (heart rate  $\times$  stroke volume). Although both arterial and venous blood pressures are easily monitored the effects of a toxicant on blood pressure, to our knowledge, have not been measured. Heart rate and amplitude of contraction are under nervous control and can be measured by either electrocardiography or by the methods used to monitor respiratory movements and oxygen uptake. A number of cardiovascular functions can be measured directly (e.g. blood pressure, heart rate, oxygen consumption); cardiac output and therefore stroke volume can also be determined from the Fick relationship (Cech and Wohlschlag, 1973; Davis and Randall, 1973). Although studies have shown that heart rate is altered by a number of toxicants (Pfuderer and Francis, 1973; Lunn *et al.*, 1976), the response is transitory and thus can only be used for short-term experiments; furthermore, the possible long-term detrimental effects of a decreased heart rate have not been established.

#### (iii) Blood

Blood forms a unique compartment between the external and internal tissue compartments in animals: all substances that enter or leave tissues, be they nutrients, toxicants, excretory products, or gases, pass through blood. Most toxic substances alter blood composition either directly (e.g. erythrocyte destruction) or indirectly by altering osmotic and ion regulation, gas uptake, bilirubin formation, and catabolism. Because of the crucial role of blood in oxygen uptake many pollutants cause anaemia and tissue hypoxia. These sublethal effects can not be considered a successful acclimation to an environmental change because if they persist they result in reduction in the survival potential of the fish (Sprague, 1971).

Haematological analysis is an established procedure for the diagnosis of disease and poisonings in medicine. Blood is relatively easy to obtain, store, analyse, and quantitate. Analyses for haematocrit, haemoglobin content, iron content, ion composition, or serum protein analysis offer quick screening methods for assessing the health of fish (Wedemeyer and Chatterton, 1970; Blaxhall and Daisley, 1973).

Haematological analysis can also be used to determine the incipient lethal concentration of a toxicant (McLeay, 1973). For example, Buckley *et al* (1976) assayed haemoglobin content and the percentage of mature erythrocytes in coho salmon exposed to residual chlorine for twelve weeks. A linear dose-response

relation was established between both these variables and total residual chlorine. McKim *et al.* (1970) demonstrated statistically significant alterations in a number of blood variables in brook trout to short-term (6-day) copper exposure. The copper concentration, however, resulted in the death of 40% of the fish, scarcely a sublethal effect.

#### (iv) Locomotion

Most non-sedentary aquatic animals depend on swimming to maintain their position against a current, to obtain food, to escape predators, and to reach spawning beds. Thus a toxicant that interferes with swimming is likely to have far-reaching effects. Swimming represents the integrated result of many physiological processes including behaviour, sensory perception, and muscular activity. These three aspects of locomotion are discussed below.

##### (a) Behaviour

Fish have a number of behavioural patterns which enable them to select environments favourable to survival and reproduction. These behavioural patterns provide useful measures of sublethal toxicity because they represent the integrated result of many biochemical and physiological processes. Furthermore, even under laboratory conditions, behavioural patterns remain consistent with those in the field and behavioural measurements often require less physical manipulation of the test organism than physiological measurements.

Two approaches used to monitor the effect of a toxicant on behaviour are avoidance studies in a 'free-choice' situation by unconditioned fish and impairment of a conditioned response in trained fish.

In avoidance studies, fish are introduced into an experimental tank in which there is either a continuous gradient from water to toxicant (Ishio, 1965) or a differential partition of a toxicant and water (Sprague, 1968). The distribution of fish in the tank or the time spent in the uncontaminated water are the indices of the avoidance reaction.

Fish have been shown to avoid sublethal concentrations of detergents (Abel, 1974), heavy metals (Sprague, 1968; Ishio, 1965), and insecticides (Hansen *et al.*, 1972) at sublethal concentrations; goldfish avoid fenitrothion at a concentration two orders of magnitude below the incipient lethal level (Scherer, 1975). Westlake and Kleerekoper (1974) have shown that the extent of avoidance and, conversely, attraction to copper is dependent on the slope of the copper ion gradient rather than the absolute copper concentration. Thus a steep gradient may elicit avoidance while a shallow gradient may elicit attraction.

In this latter experiment the bimodal response to the rate of change in the concentration of a toxicant presents a problem in applying the data to quantal analysis, but Sprague (1968) was able to employ quantal analysis in estimating the median effective concentration of zinc that elicits an avoidance reaction in fish



(Figure 9.1). Avoidance by salmon of polluted segments of their migratory routes to spawning areas did have far-reaching effects on their population (Saunders and Sprague, 1967).

A phenomenon related to avoidance reaction is temperature preference. There is evidence that pollutants like DDT alter temperature preference in fish (Anderson, 1971).

The effect of a pollutant on the ability of fish to be conditioned (or learn) can also be used as a measure of sublethal toxicity. Fish can be trained either by reward for performance (e.g. feeding) or by punishment for failure to perform (e.g. electric shock, Warner *et al.*, 1966). This experimental approach has shown that a number of insecticides at sublethal concentrations reduce both learning and retention of a conditioned response in fish (Warner *et al.*, 1966; Hatfield and Johansen, 1972; Davy *et al.*, 1973). It is highly probable that learning impairment has a detrimental effect on such complex behaviour patterns as migration and reproduction. However the ecological significance of alterations in learning in fish has not been studied. Preliminary evidence would suggest that salmon completely recovered their learning ability within 1 week following exposure to 1000 ppm fenitrothion (Hatfield and Johansen, 1972).

#### (b) Swimming Performance

Brett (1967) and Sprague (1971) have suggested that swimming performance be used as a criterion in determining sublethal impairment. The critical swimming speed (the fastest swimming speed reached) or the maximum sustained swimming speed (the maximum swimming speed maintained for an hour) can be determined by forcing fish to swim against an applied water current in order to maintain a stationary position. The water current is increased at a fixed rate and the swimming speeds (fish lengths/sec) determined from the water current. The design of the swimming chamber is critical as turbulence, drag, and temperature fluctuations must be minimized. The food ration and respiratory ability of the fish also influence swimming performance (Howard, 1975). Despite the difficulties due to fish variability and equipment design, cyanide (Neil, 1957), fenitrothion (Peterson, 1974), hydrogen sulphide (Oseid and Smith, 1972), and bleached kraft pulpmill effluent (Howard, 1975) have been shown to impair swimming performance.

A more promising bioassay for a sublethal effect related to swimming performance is the determination of the critical rate of rotation of a rotating water mass at which fish cannot compensate for torque (Lindahl *et al.*, 1977). Using this technique, Bengtsson (1974) showed that exposure to zinc at levels as low as 0.06 ppm in the water for 190 days significantly reduced the ability of minnows to compensate for torque. This sublethal effect occurs at a concentration of zinc lower than that for any other sublethal impairment reported.

A similar effect of methylmercury has been reported by Lindahl and



Schwanbom (1971a,b). With roach, the 'critical rate of rotation', which measures the ability of the fish to compensate for torque, was inversely proportional to the methylmercury content of the fish muscles.

(c) Perception

Sense perception is thought to play a crucial role in many activities vital to the survival of fish. For example, the homing abilities of migrating fish are mediated through their olfactory, visual, and auditory senses (Hoar, 1975). Impairment of these sensory functions by chemical toxicants would have adverse consequences for fish populations. Furthermore, because many sensory receptors are in immediate contact with the ambient environment they are prime target sites for uptake of toxicant and for subsequent impairment. Considering the importance of olfaction in fish biology, Hara *et al.* (1976) and Bardach *et al.* (1965) studied the effects of a number of pollutants on olfactory response. The alterations in response to a standard stimulus, serine, caused by a pollutant can be detected by recording the electrical responses of the olfactory bulb. After exposure to sublethal concentrations of copper or mercury the electrical activity generated by the olfactory bulbs of anaesthetized immobilized rainbow trout in response to serine was depressed (Figure 9.3). Such depression in olfactory response can be quantified and is dose-dependent.

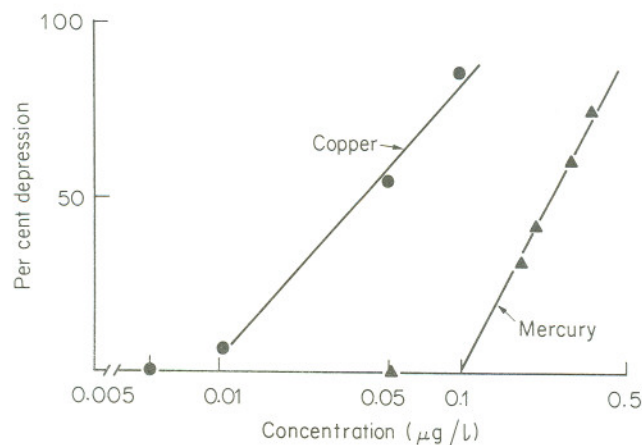


Figure 9.3 Relationship between per cent depression of olfactory response and the ambient concentration of discrete solutions of mercury and copper at 4 hours exposure. (Reproduced by permission of Minister of Supply and Services, Canada, from Hara *et al.*, 1976)

**(v) Growth**

All organisms must continually repair and replace bodily structures to survive. To increase in size, organisms must obtain energy and materials from the environment in excess of minimum requirements. Growth is dependent on the metabolic state of the organism, thus on its age, on its physiological state, and on environmental controlling and limiting factors (Fry, 1947). Both natural and pollutant stressors may affect food conversion in animals. Pollutants can be an indirect stress on growth by altering the quality and quantity of available food and a direct stress by increasing the metabolic cost of other life functions (Warren, 1971).

Growth rate was initially suggested as a good stress indicator (Sprague, 1971), but proper execution of growth studies, particularly over long periods of time, requires careful control of ration level and locomotory activity. Furthermore, the ration level must be such that the growth rate does not become asymptotic during the experimental period (Warren, 1971). For these reasons, certain results of studies on the influence of toxicants on growth are contradictory (Mount and Stephen, 1969; McKim and Benoit, 1971; Lett *et al.*, 1976). A complicating factor is that many pollutants, especially heavy metals, initially reduce appetite so that less food is eaten (Lett *et al.*, 1976). These latter authors found that after prolonged exposure to toxicants fish exhibit an increased appetite. Consequently, in the long-term studies there is no growth retardation in test fish.

Recently, the effectiveness of growth studies for the establishment of 'safe' levels has been challenged (Sprague, 1976). Nevertheless, Webb and Brett (1973) showed measurements of growth rate and conversion efficiency to be more sensitive indicators of the sublethal effects of sodium pentachlorophenate than swimming performance.

Warren and Davis (1971), through the use of artificial streams, have added a promising new dimension for investigating effects of pollutants on intertrophic relationships that govern growth and production of fish. Such experimental approaches may provide an effective intermediary in extrapolations from laboratory to field conditions.

**(vi) Reproduction**

The biogenic potential of a population may be adversely affected by a pollutant in a number of ways:

- (1) physiological stresses may reduce gametogenesis and therefore egg and sperm production and fertilization success;
- (2) behavioural alterations may reduce spawning or parental care of eggs and thus reduce the number of fertilized eggs and their rate of hatching;

- (3) egg or sperm viability may be reduced;
- (4) teratological effects on the developing embryo may reduce hatching success and larval survival.

Regardless of the mode of action, pollutants affecting reproduction reduce the number of viable offspring.

The reproduction process appears to be highly sensitive to the adverse effects of certain pollutants (Brungs, 1969). Laboratory test systems are now available for the quantitative evaluation of not only survival and growth but also reproduction of fish during chronic exposure to pollutants (Benoit, 1975).

These latter experiments, although informative, are time requiring and expensive. Lesniak (1977) applied light- and electron microscopy as tools for the quantitative measurements of oocyte retardation in rainbow trout, exposed to cyanide during short-term (two weeks) experiments. This approach may offer a satisfactory alternative to the *in vivo* reproductive test.

Two special aspects of reproductive impairment, teratogenesis and mutagenesis, are considered separately in the following two sections.

#### 9.4. TERATOGENESIS

All chemical contaminants are probably toxic to embryos and larvae at some level, but only certain substances impair the ontogenic processes of sequential differentiation, growth and metamorphosis at levels that are normally tolerated by later stages of the life cycle. The adverse effects of such selectively acting compounds — herein designated as embryotoxicants — can range from a simple retardation in developmental rate through a variety of distortions in form (teratogenesis) or function in embryos (Rosenthal and Alderdice, 1976). Whether the action of these toxicants is lethal by contributing directly to the death of an organism during ontogeny or sublethal by reducing indirectly the survival potential of an organism at some subsequent stage in its life, the consequence of embryotoxicity can be a serious reduction in the distribution and abundance of animal populations. It follows that a check list in the toxicity screening of environmental contaminants should include, in conjunction with reproduction surveys, tests identifying and quantifying teratogenic and other forms of selective embryotoxicity.

Whereas certain species appear to serve as sensitive indicators of the potentially adverse effects of pollutants on juvenile and adult organisms inhabiting different trophic levels (Reish, 1972), evidence would suggest that species genotype is also a significant determinant of susceptibility in embryotoxicity (FDA, 1970). This specificity is credited to the tendency of teratogens to act on, through or in conjunction with, unstable genetic loci found only in certain species. As a result there may be limited predictive value in using indicator organisms to estimate the



relative hazard of teratogens and other embryotoxicants to a broad spectrum of animal groups. Rosenthal and Alderdice (1976), who tabulated the responses of eggs and larvae of marine fish to a wide selection of stressors including pollutants, suggested that embryotoxicity often involves a limited number of similar physiological and biochemical mechanisms common to many species. By our definition the pollutants that these authors selected to illustrate their rationale were not 'true' teratogens or embryotoxicants because deleterious effects were caused at concentrations equal to or greater than levels affecting adult and juvenile organisms (McKee and Wolf, 1963). The problem of whether embryotoxicants can be characterized by studies involving limited kinds of test organisms would appear unresolved.

Egg capsules, jelly-like coatings and extraembryonic membranes are effective screening devices which often impede the access of pollutants to the developing organism. The high resistance of eggs and embryos to the toxic effects of many pollutants may be explained in part by the protection provided by these coatings. Developing organisms may however be more vulnerable to contaminants absorbed from yolk deposits which occurred during oogenesis; this possibility should be considered in the design of experiments in teratology (Davis, 1972). Studies of teratogenesis should also include complete life-cycle examinations because deleterious effects may be initiated well in advance of physiological or structural manifestations of toxicity (FDA, 1970).

Certain events in the time course of development are particularly vulnerable to the action of teratogens. These 'sensitive' periods include gastrulation, early organogenesis, hatching, and metamorphosis (Rosenthal and Alderdice, 1976). Sufficient periods of toxicant exposure prior to or during these sensitive periods may be necessary for effects to be manifested (e.g. Anderson and Battle, 1967). The cell proliferation stage, i.e. cleavage, that immediately follows fertilization in the indeterminate type of vertebrate development is known to be relatively insensitive to toxic insult. Embryonic development of crustaceans and molluscs exhibits determinant cleavage where, unlike the former omnipotent pattern, each daughter cell or blastomere is the sole primordium of major organ systems in the adult organism (Balinsky, 1975). The implications of this pronounced difference in the pattern of ontogeny between major groups of aquatic organisms in reference to their respective susceptibility to toxicants require investigation.

Amongst the various agents reported by FDA (1970) as possible teratogens are classes of chemicals known to pollute aquatic ecosystems. They include alkylating agents, azo dyes, salicylates, and analogues and antimetabolites of nucleic acid metabolism. Rosenthal and Alderdice (1976) described the embryotoxicity of certain heavy metals (particularly cadmium), DDT, dinitrophenol, cyanide, oil dispersants and extracts, benzene and vinyl chlorides, amongst others, but failed to demonstrate, as suggested previously, that embryonic development was more sensitive to these latter toxicants than physiological, structural, or behavioural properties of adult or juvenile organisms.

As observed by Davis (1972), few studies have explored the teratogenic effects of interacting pollutants in aquatic organisms even though the importance of such investigations has been enunciated in mammalian research (Wilson, 1964).

### 9.5. MUTAGENESIS

The list of aquatic pollutants suspected of being mutagens has increased dramatically in the last three decades. Amongst the more prominent contaminants attributed with mutagenic capabilities are nitrites, certain pesticides, alkylating agents, commercial solvents (Sanders, 1969). Somatic mutations may contribute to premature senescence and may initiate neoplastic growths within an organism's life span. Gametic mutations may be inherited and thereby cause at some point during subsequent generations either death or a reduced potential for survival (Durham and Williams, 1972). The implication that mutagens are adversely affecting the health of contemporary and future generations of aquatic organisms can not be dismissed. Mutagenicity testing should become an integral part of screening programmes for the assessment of the toxic hazard that chemical contaminants represent to aquatic biota.

Definitive mutagenic tests such as specific locus, backcross, and genetic loading studies are tedious, time consuming, and expensive (FDA, 1971). The only reasonable alternative in accomplishing the task of surveying potential mutagens is to employ rapid 'indicator' tests, the significance of which is then extrapolated to field conditions.

The kinds and numbers of indicator tests required for deducing mutagenic activity are still in a stage of research and development. Nevertheless most investigators advocate a multi-component or tier approach which screens for evidence of DNA damage, chromosomal aberrations, metabolite mutagenicity, and mutation transmissibility by gametes to subsequent generations (Flamm, 1974).

For a study of DNA damage, bacterial cultures are often employed on the assumption that genetic material is similar in all organisms and that a chemical which is mutagenic in one species is likely to be mutagenic in others (Ames *et al.*, 1973). Either cell cultures or *in vivo* cytogenetic examination appear effective for chromosomal aberration studies. These investigations are complicated by the fact that chromosomal breakage and repair occurs spontaneously in organisms, the rate of which can be altered by hormones and background radiations (Chu, 1971; Schmid, 1973). Host-mediated assays, in which an organism is administered both a candidate mutagen and then, by another route, indicator bacteria, are carried out to detect the mutagenic action of metabolites while at the same time providing the advantages of a bacterial assay system (Legator and Malling, 1971). Heritability of mutations caused by pollutants can be investigated through dominant lethal tests with the recommendation that studies span several generations (Epstein, 1973). Extrapolations from these tests on microorganisms to whole animals must be made



with caution because false negatives and false positives have occurred in all these tests.

The use of bacteria in tests for environmental mutagens and in screening of environmental carcinogens is mentioned in Chapter 13.

A different approach has been developed by Osterman-Golkar (1975) who treated *E. coli* with several alkylating agents and found a good correlation between the degree of alkylation and mutation rate. The amount of *in vivo* alkylation of haemoglobin in mice was used as a method for monitoring the dose of alkylating agent received by the animals (Osterman-Golkar *et al.*, 1976).

## 9.6. CARCINOGENESIS

The epidemiological studies presently available are not sufficient to allow an assessment of the hazard that chemical pollutants as oncogenic agents represent to the welfare of aquatic organisms. However, given that carcinogens initiate lesions by reacting with DNA (Kotin, 1976) and considering the similarity of genetic material in all organisms, some impression of the impact of carcinogens on aquatic populations may be gained from the extensive data on man. The World Health Organization (WHO, 1964) has estimated that up to 85% of all human tumours are caused by factors related to man's environment. Ninety per cent of these oncogenic factors are considered to be chemicals (Boyland, 1969). The National Institute for Occupational Safety and Health in the U.S.A., as of 1974, implicated more than 1,300 chemical pollutants as candidate carcinogens for man. Their threat to the health of man is immense in view of the fact that twenty five per cent of all deaths in the United States are accredited to cancer (Lassiter, 1976). Because many of the above candidate carcinogens are common contaminants of natural waters, one can only infer that aquatic biota are being jeopardized by pollutants causing cancer (Stich *et al.*, 1975).

There is some evidence to suggest that tumours occur with a higher incidence in aquatic organisms inhabiting polluted, rather than pristine, waters. Brown *et al.* (1973) after an extensive five-year survey found a 4.4% incidence of neoplasms in fish taken from a polluted watershed. This frequency of tissue lesions was significantly different from the 1.03% occurrence of tumours identified in similar fish species captured from uncontaminated waters. In a polluted habitat, catfish, *Ictalurus nebulosus*, which are bottom dwelling and therefore likely to contact high concentrations of chemical contaminants, had a high frequency of hepatomas (12.2%). Harshbarger (1974) also observed the highest incidence of neoplasms (epidermal papillomas and carcinomas) in benthic fish, e.g. catfish, from polluted rivers. Although neoplasms are extremely rare in aquatic invertebrates, particularly molluscs (Wolfe, 1974), sarcomas, with an average incidence of 12%, were detected in oysters, *Ostrea lurida* and mussels, *Mytilus edulis* residing in an estuary receiving pulp mill effluent (Farley, 1974). Carcinomas were fatal to 80% of the afflicted detritus-feeding clams, *Macoma balthica*, found in a watershed receiving agricultural wastes (e.g. pesticides, herbicides, and ammonia).



In all the epidemiological studies listed above, the epizootic occurrence of tumours varied seasonally and the possibility that viruses were acting as oncogenic agents can not be ruled out. Viruses have been associated with the occurrence of fish tumours for years (Nigrelli, 1952; Mawdesley-Thomas, 1971). Mulcahy (1974) suggested a viral cause in malignancies (12.5%) afflicting certain genetic stocks of northern pike, *Esox lucius*, caught in natural waters of Ireland. Nevertheless, chemical carcinogenesis has been demonstrated empirically in fish. For example, salmonoids, more than any other group of animals, are susceptible to the hepatoma-inducing action of aflatoxins (Wales and Sinnhuber, 1972). Khudoley (1972) produced liver tumours in guppies, *Poecilia reticulata*, through exposure to aminoazobenzene derivatives.

One reasonable explanation for these epidemiological results is that the higher tumour incidences were promoted by the combined action of pollutant chemicals and viruses (see section on multiple toxicity). Kirschbaum *et al.* (1940) demonstrated enhanced incidences and growth rates of tumours caused by viruses in the presence of chemicals. Furthermore, Kotin and Wisely (1963) suggested that interactions between viruses that are not considered oncogenic and chemical carcinogens may be important in the occurrence of neoplasms. In any case, future definitive tests for carcinogens must consider the viral background and the natural ecological settings of aquatic organisms.

Ames *et al.* (1973) have devised a rapid, sensitive, and inexpensive test for screening candidate carcinogens on the assumption that most chemical carcinogens

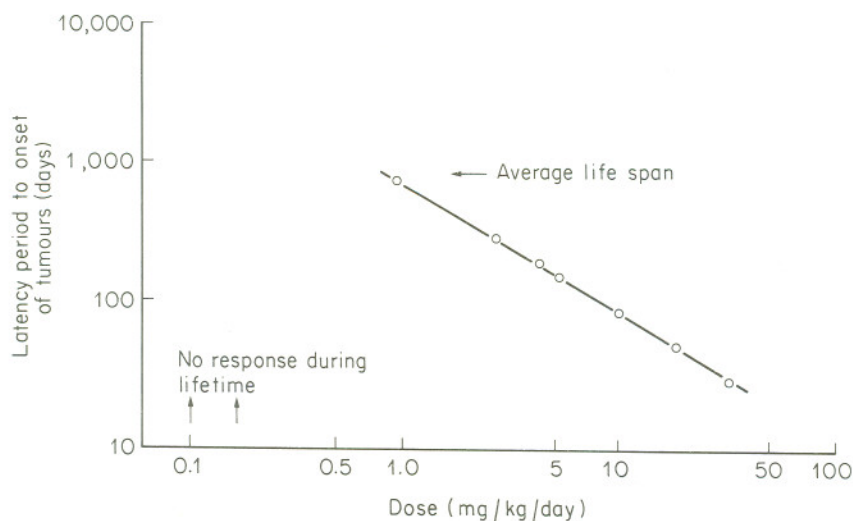


Figure 9.4 Toxicity curve showing the time to occurrence of hepatomas as a function of dose, in rats administered *p*-dimethylaminoazobenzene. (Reproduced with permission from National Academy of Sciences/National Research Council, 1959)

cause tumours by somatic mutation. The test uses a special set of bacterial strains combined with liver homogenates for carcinogen activation. Whether adaptations of this test system would be effective for identifying oncogenic agents in aquatic organisms — given that many tumours may be the consequence of interactions between viruses and chemicals — is a question requiring investigation.

With a knowledge of the dose—response relation for a carcinogen, definitive tests should be designed to employ high dosages with at least one likely to yield a maximum incidence of tumours thereby reducing latency periods to within manageable time periods (Durham and Williams, 1972). Other considerations in the design of definitive tests are the choice of short-lived species, the inclusion of both sexes, the route of administration, the presence of viruses, bacteria and parasites, and the possibilities of cocarcinogens (FDA, 1971).

In accordance with the relation between dose and response depicted in Figure 9.4, Evans (1966) showed that the time of appearance of radium-produced bone cancers in humans was inversely related to dose. When the dose of radium was small enough the latent period was so long that it exceeded the life expectancy. This resulted in an apparent zero incidence of bone cancer; a phenomenon that he called 'practical threshold'.

### 9.7. MULTIPLE TOXICITY

In receiving waters multicontaminant pollution by the biota appears to be the rule rather than the exception. *In situ* chemical monitoring of aquatic ecosystems has confirmed the virtual ubiquity of pollutant mixtures in both the ambient environment and tissues of organisms (FAO, 1972; Kerr and Vass, 1973). The facts gathered to date show that unique forms of toxicity can be ascribed to the concurrent and sequential exposure of organisms to two or more pollutants (Sprague, 1970).

Types of multiple toxicity particularly hazardous to aquatic fauna are characterized by:

- (1) an effect greater than that predicted on the basis of the potency of each component of a mixture;
- (2) an effect different from and therefore more toxic or insidious than that predicted by a knowledge of the toxicity of individual constituents of a mixture.

Organisms are not safeguarded against either of these effects by water quality standards which set permissible levels based on single toxicants. Because the movement of pollutants from various diffuse or point sources gives rise to 'coincidental' mixtures in the ecosystem, multiple toxicity risks can not be completely avoided by the application of water quality standards based on bioassay

criteria for complex effluents monitored 'at the pipe'. There is a need to understand the mechanisms of multiple toxicity and to derive approaches which quantify the effects. Such knowledge would provide the authorities, who are charged with the responsibility of determining valid water quality criteria and standards, with a rationale that adequately estimates the adverse effects of toxicant mixtures.

Multiple toxicity is the consequence of interaction between constituents of chemical mixtures. This interaction can be either chemical or physiological and can occur at one or more of three phases of pollutant movement and activity in the ecosystem as illustrated in Figure 9.5.

Chemical interaction involves the mutual influence between pollutants that results in, for example, new compounds, complexes, chelates, or modifications in valency. One would expect this form of interaction to occur primarily in the environmental phase, although incidences of chemical combinations are known to occur within the organism (Gaddum, 1957). Physiological interactions can occur in the dynamic phase by altering the sequence of events commencing with, and following from, the binding of a toxicant to the target tissue. Within this sequence,

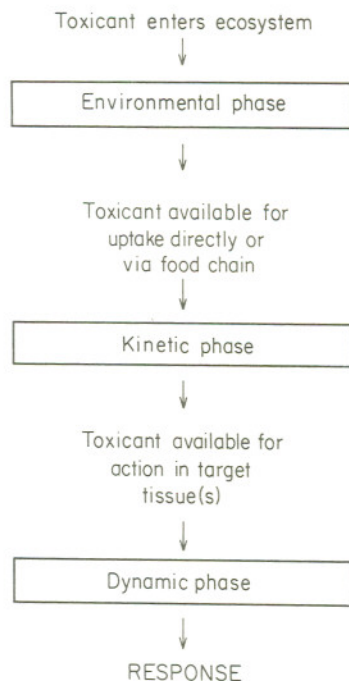


Figure 9.5 Phases of interactions between chemical constituents of pollutant mixtures (modified from Ariens, 1972)



it is useful to distinguish between the processes of affinity (i.e. events in binding with tissue receptors) and intrinsic activity or efficacy (i.e. events initiated by binding). Physiological interactions also occur in the kinetic phase by altering mechanisms of toxicant uptake, distribution, deposition, degradation, and excretion. Kinetic processes determine the concentration of a contaminant or its metabolite(s) available in body compartments, e.g. blood, tissue and excreta, as a function of time and dosage.

The following discussion is limited to multiple toxicity caused by physiological interactions\*. A rationale is presented in an attempt to stimulate and guide further research in the field of multiple toxicity.

#### **(i) Presumptive Mechanisms of Interaction**

Possible modes of physiological interaction which create hazardous forms of multiple toxicity are proposed to occur as follows:

In the dynamic phase between,

- (a) pollutant constituents which act at the same site(s) in target tissue(s);
- (b) pollutant constituents which act at different sites possibly in different target tissues but which contribute to a common adverse response;
- (c) pollutant constituents where one is normally inactive as a toxic agent but in combination changes the response of an organism to one or more of the other toxic constituents;
- (d) pollutant constituents that mutually produce a toxic response different from the response induced by each toxicant alone.

In the kinetic phase between,

- (e) pollutant constituents which alter toxicant availability to the target tissue;
- (f) pollutant constituents which enhance or induce (e.g. by the mixed oxidase system in liver) the production of metabolites more toxic than the original pollutants.

\*An understanding of the problems of chemical interaction may be achieved through precise analytical knowledge of pollutant kinds, forms, and quantities that result from reciprocal actions between constituents, either in receiving waters or in target organisms. It is obviously essential to have this information before proceeding with a quantitative and qualitative assessment of physiological interactions.

## (ii) Multiple Toxicity Models – based on Interactions at the Dynamic Level

## (a) Strict Addition

The simplest form of pollutant interaction occurs when constituents of a pollutant mixture have qualitatively similar toxic effects and these effects combine additively, although the concentration or amount of one or another required to produce a given effect may be quite different. Thus if  $C_{s1}$ ,  $C_{s2}$ , etc., are concentrations of pollutants 1, 2, etc., which produce identical effects, i.e. if

$$E(C_{s1}) = E(C_{s2})$$

where  $E(C_{s1})$  = the biological effect of pollutant  $P_1$  at concentration  $C_{s1}$  then it is also true that

$$E(\frac{1}{2}C_{s1} + \frac{1}{2}C_{s2}) = E(C_{s1}) = E(C_{s2}) \quad (9.1)$$

and more generally

$$E\left(\frac{1}{n} \sum_{j=1}^n C_{sj}\right) = E(C_{s1}) = \dots = E(C_{sn}) \quad (9.2)$$

The concept of 'Toxic Units' (Sprague, 1970) concerns the calculation of a quantity

$$q = \sum_{j=1}^n \frac{C_j}{C_{sj}} \quad (9.3)$$

where  $C_j$  is the actual concentration of pollutant  $j$ , and the comparison of this quantity to unity. If  $q = 1$ , we would predict that such a combination of toxic substances would lead to the specified effect, even though the concentration of any one may be below toxic levels. This concept has been used to compare multiple-pollutant effects in a variety of systems (Anderson and Weber, 1975). Simply stated, equations (9.1) to (9.3) mean that a similarly acting constituent contributes to its mixtures in proportion to its relative potency. Expressed in another way, the contribution of any constituent to the toxic effect of a mixture may be calculated by multiplying its concentration by its relative potency. Empirically the respective contributions in dosage of each toxicant are added to achieve a quantitative measure of the potency of the mixture. This criterion of dose summation inherent in equations (9.1) to (9.3) has come to be known as strict addition.

Bliss (1939) discussed a way of examining strict additivity in the case where one of the pollutants (in fact, by assumption, all of them) gives a linear graph when probit response was plotted against the logarithm of the dose. In that case one pollutant would have a dose-response curve of the form,

$$Y = a + b \log X_1 \quad (9.4)$$

where

$Y$  = probit of response

$X_1$  = concentration of pollutant no. 1

If additivity is expected, then a second pollutant could be combined with the first in a proportion  $\pi$ , so that

$$Y = a + b \log [\pi X_1 + (1 - \pi) \rho_{21} X_1] \quad (9.5)$$

that is, an amount  $\pi$  of a solution of pollutant  $X_1$  and an amount  $(1 - \pi)$  of a solution of pollutant no. 2 of concentration  $X_2 = \rho_{21} X_1$ , would have the same effect. If now  $\pi = 0$ , the equation becomes

$$Y = a + b \log \rho_{21} + b \log X_1 \quad (9.6)$$

i.e. an equation which has the same slope but is laterally shifted. Figure 9.6 illustrates this methodology applied to the multiple toxicity of nickel and copper.

Strict addition may not occur between toxicants which have a similar action. If the response is larger than that expected for the algebraic summation of dosages (i.e.  $q > 1$  in equation 9.3), then the effect is termed supra-additive — a form of synergism; if the response is less than predicted for additivity (i.e.  $q < 1$  in equation 9.3), then the effect is termed infra-additive — a form of antagonism\*. The degree of this displacement from strict addition is often illustrated as isobols, curves for equal biological response, as seen in Figure 9.7. A relative potency factor ( $q$  observed/ $q$  expected) has been suggested as a measure of the supra- and infra-additive effects (Anderson and Weber, 1975).

#### (b) Response Addition

Several authors (Bliss, 1939; Plackett and Hewlett, 1952; Finney, 1971) have advanced a quantitative approach for the assessment of such an interaction between pollutant constituents which act at different sites but contribute to a common response. In accordance with their model, toxic constituents, although acting on different systems, may contribute to a common response only if their respective concentrations are equal to or greater than the threshold computed from quantal response curves for single toxicants. This mechanism of physiological interaction is called independent action (Bliss, 1939).

\*Synergism and antagonism were terms originally synonymous with supra- and infra-additive effects as defined above (Gaddum, 1953). However, through general usage the definitions of these terms have been broadened to mean respectively *any* effects of a mixture greater and lesser than the toxicity or potency estimated on the basis of the effects of its constituents taken individually. To avoid confusion, this chapter will follow the suggestion of Ariens and Simonis (1964) and qualify the type of synergistic and antagonistic effects, e.g. supra-additive synergism.



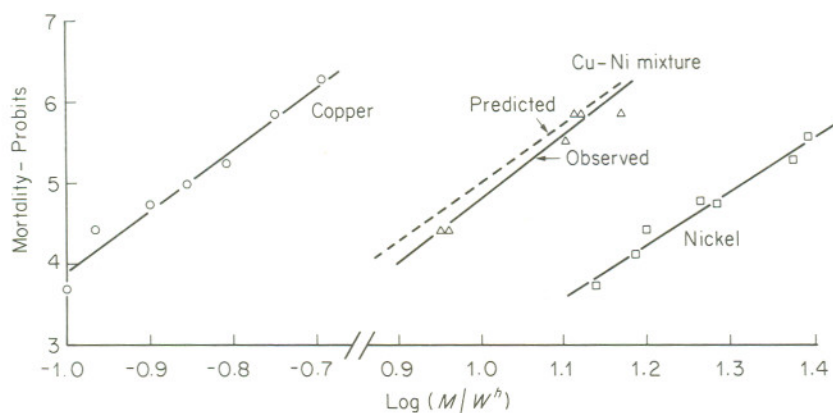


Figure 9.6 Linear regressions for discrete solutions and for mixtures of copper and nickel. The observed toxicity curve for the mixtures is not significantly different from that predicted in accordance with the Bliss (1939) model of similar action. (Reproduced by permission of International Heavy Metals Conference Committee, from Anderson and Weber, 1975)

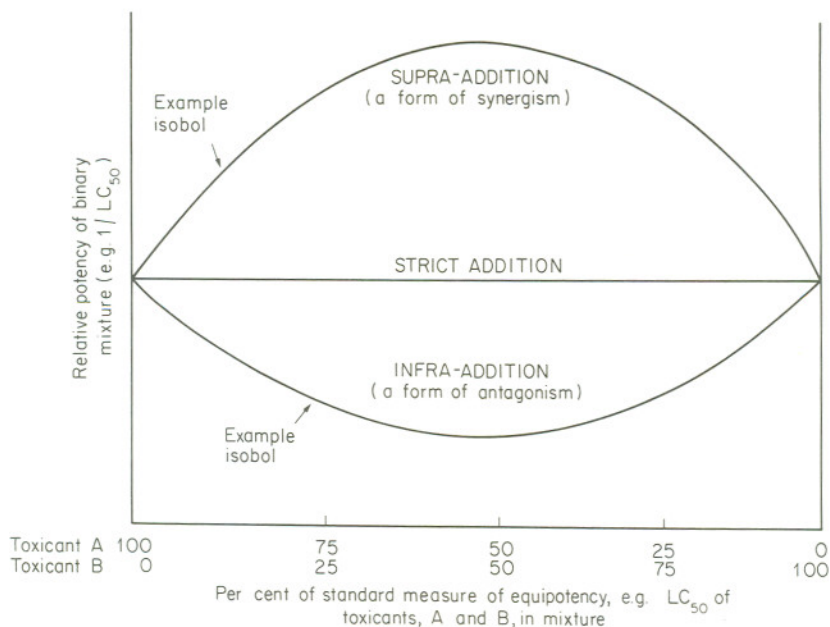


Figure 9.7 Possible types of responses which can occur between two hypothetical toxicants, A and B, which have similar actions. (Reproduced by permission of W. B. Saunders Co., Philadelphia, Pa., from Warren, 1971)

At the outset, it is generally not known whether tolerances ( $LD_{50}$ s) to different toxicants are correlated. Therefore, it is not meaningful to attach great significance to parameters, such as regression coefficients, that arise from the study of dose-response curves for one pollutant at a time. One can, however, compare the response to a combination of doses with what would be expected if they acted independently. For a quantal response to two toxicants, this is calculated as follows ( $P_r$  = probability):

$$\begin{aligned} P(\text{response}) &= 1 - P_0 \text{ (no response)} \\ &= 1 - (1 - P_1)(1 - P_2) \\ &= P_1 + P_2 - P_1P_2 \end{aligned}$$

where  $P_1$  and  $P_2$  are the probabilities of exhibiting a response to pollutant 1 or 2 at concentration  $X_1$  or  $X_2$  respectively.

For several toxicants, the expanded form is,

$$P_m = 1 - (1 - P_1)(1 - P_2) \dots (1 - P_n) \quad (9.7)$$

where

$$\begin{aligned} P_m &= \text{proportion of individuals responding to a mixture} \\ P_1, P_2 \dots P_n &= \text{proportion of individuals responding to pure solutions of} \\ &\quad \text{each constituent at concentrations } X_1, X_2 \dots X_n \text{ respectively} \\ &\quad \text{(Finney, 1971)} \end{aligned}$$

To find an example of the application of this method to aquatic organisms, refer to Anderson and Weber (1975). These authors have suggested that the criterion of independent action be called response addition\*.

This model makes possible the prediction of effects of mixtures that, by our original definition, are not hazardous. The common effect in each is never greater than that predicted on the basis of the potency of each component of a mixture. In this case water quality standards which establish safeguards against individual, independently acting toxicants also protect organisms against their combined effect in mixtures. Nevertheless equation (9.7) provides a useful quantitative approach for screening combinations of toxicants for those constituents which would not create, through interaction, hazardous forms of multiple toxicity. Further research may identify patterns of independent action by which a mixture's effect is greater than that predicted by equation (9.7).

Another formulation for calculating the total effect of several pollutants in several effluents has been given by Esvelt *et al.* (1973).

\*This item is introduced to maintain coherence of thought whereby strict and response addition represent the empirical aspects of the mechanisms of similar and independent action respectively. This terminology avoids inference to a knowledge of mode of action which may be unknown or unattainable.



*(c) Sensitization and Potentiation*

Theoretically, synergistic interaction can occur as a consequence of a non-toxic pollutant promoting either the binding or the toxic action of another toxic pollutant. Antagonistic interactions occur when either of these is inhibited.

It may be possible to separate, empirically, affinity from intrinsic interactions on the assumption that in the former the non-toxic component acts prior to or concurrently with the toxic agent whereas in the latter the non-toxic agent may act after the binding of the toxicant to its site of action. This temporal distinction is deemed useful for the development of quantitative methods for the assessment of this type of interaction. Ariens (1972) identified synergism due to the former affinity-related interactions as sensitization, and those due to the latter type of interaction as potentiation.

A relative measure of the joint potency of constituents interacting in this manner can be obtained through the use of isobols as demonstrated in Figure 9.8 (Hewlett, 1969).

Over a certain range an increase in the dose of B, the potentiating or sensitizing agent, would result in a corresponding decrease in the dose ( $M$ ) of toxicant A required to induce a particular standard of effect, e.g.  $LC_{50}$ . Eventually, a level of B is reached beyond which no further synergism occurs. As seen in Figure 9.8, this

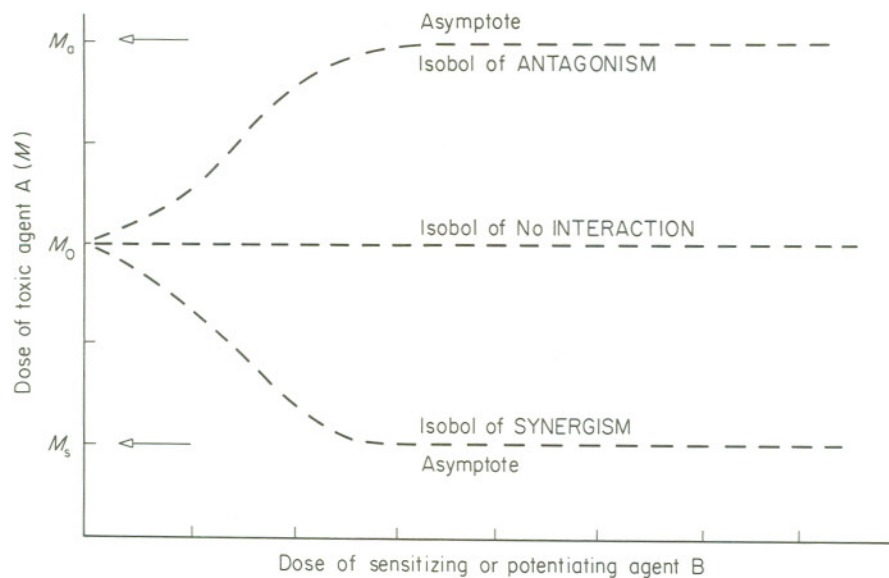


Figure 9.8 Isobols for a mixture consisting of a pollutant A, an active toxicant if applied singly, and a pollutant B, a non-toxicant but which antagonizes or synergizes the response to pollutant A. (Reproduced by permission of The Biometric Society, from Hewlett, 1969)

asymptote allows the computation of a minimum effective dose  $M_s$  of toxicant A. The ratio  $M_0/M_s$  is a relative measure of the maximum potency of B as a synergistic agent in a mixture containing A. The ratio  $M_0/M_a$  measures agent B's potency as an antagonist.

(d) *Permissive Synergism*

There is recent evidence that pollutants can interact to produce an effect different from those of the individual toxicants. For example, Crocker *et al.* (1974) suggested that Reye's syndrome was an expression of ammonia toxicity generated by the simultaneous or sequential interaction of general toxic agents. The same symptoms of toxicity were not evident in studies of the poisonous constituents taken individually. The proposal was that the ammonia problem arose in the presence of the mixture due to one poison's attack on the liver which was then unable to detoxify at an adequate rate the increased levels of ammonia generated as a by-product of another toxic agent's action elsewhere in the body. We were unable to find evidence of aquatic studies to represent this model.

(iii) **Multiple Toxicity Models – based on Interactions at the Kinetic Level**

(e) *Uptake Interactions*

These interactions can create enhanced, and consequently hazardous, effects through an increased availability of one or more toxicants at their respective target sites. For example, Bingham and Falk (1969) showed a 1,000-fold increase in the potency of the carcinogen, benzo(a)pyrene, when administered in solution in *n*-dodecane rather than in toluene. Similarly, oil solvents enhanced the toxicity of DDT, BHC, toxaphene, and chlordane to aquatic organisms (Cope, 1971). In both instances, the accelerated rate of uptake of the toxicant promoted by the second pollutant augmented the magnitude of response beyond that predicted.

(f) *Interactions at Sites of Detoxification*

Evidence exists that synergism can occur due to interactions between toxicants at sites of detoxification. Piperonyl butoxide suppresses allethrin denaturation by inhibiting mixed oxidase systems in liver, thus enhancing the toxicity of allethrin (Menzie, 1972). On the other hand, certain combinations of non-carcinogenic and carcinogenic hydrocarbons resulted in a reduced incidence of tumours (Falk *et al.*, 1968). A similar mechanism of antagonism was shown to occur between two carcinogens (Gelboin, 1967). A measure of protection against the lethal toxicity of chlordane and parathion was gained by organisms exposed to mixtures including chlorinated hydrocarbons (Triolo and Coon, 1966). Thus both synergistic and antagonistic interactions can occur at the kinetic level.



#### (iv) Application of Multiple Toxicity Models

Often, the suggestion that a pollution-related disease is linked to the action of more than one chemical contaminant comes only from extensive epidemiological studies (Koeman and van Genderen, 1972; Brown *et al.*, 1973). This limitation may explain the dearth of knowledge and quantitative methodology relative to most of the models proposed. However this paucity of information should not negate the probable occurrence and hazard of these interactions in the aquatic environment. Below are two examples of the applicability of this rationale to cancer and to water quality management.

#### (v) Cancer

Kotin (1976) proposed that most, if not all, forms of chemical carcinogenesis are potentially triggered by a contaminant which binds covalently to the genetic material (DNA) of the cell. Subsequent development of neoplastic tissue, however, depends upon a number of intrinsic factors, such as DNA repair mechanisms and abnormal protein production and activity in the cell, as well as extrinsic factors, such as the competence of the immunological response and the nutritional and hormonal state of the organism. Kotin (1976) termed the events involving carcinogen-DNA binding, the initiation phase, and those which followed, the promotion phase. Since the classical study of Berenblum and Shubik (1947), it has been shown that the promotion phase of certain forms of cancer is enhanced by a synergizing agent either administered concurrently with or subsequently to the carcinogen (Weisburger *et al.*, 1965; Bingham *et al.*, 1976). These synergizing agents are collectively called cocarcinogens and are believed not to be carcinogenic in themselves. It appears that certain carcinogen-cocarcinogen relations fit the model of sensitization and potentiation.

If the assumption is correct, then standard measures of the relative potency of cocarcinogens as synergizing agents could be obtained through the use of Hewlett's isobol method. It may also be useful to explore the nature of cocarcinogens as either potentiating or sensitizing agents in accordance with the model. Of interest in the pursuit of this distinction in cocarcinogenic action is knowledge of the degree of specificity which each form displays in this collaboration with carcinogens. The threat of cocarcinogens to the health of the ecosystem is deemed to increase with decreasing specificity of their synergistic action in carcinogenesis.

#### (vi) Water Quality Management

Of the multiple toxicity models proposed, strict addition and various synergistic forms of interaction meet the original criteria of what constitutes a hazard in the aquatic environment.

The standard based on the toxic unit principle is calculated as follows, (Esvelt *et al.*, 1973)

$$T_{cr} = \frac{T_{c1}Q_1 + T_{c2}Q_2 + \dots + T_{cn}Q_n}{Q_t} = 0.05TU \quad (9.8)$$

where

$T_{cr}$  = total allowable relative concentration in receiving waters

$T_{c1} - T_{cn} = \frac{C_{actual}}{LC_{50}}$  for each contaminant respectively

0.05 = application factor

$Q_t$  = total effluent flow = sum of  $Q_1, Q_2 \dots Q_n$

TU = toxic unit = 1 (Seba, 1975)

and may prove to be satisfactory as a measure of protection for the health of aquatic biota in receiving waters of strictly additive toxicants. However, the eventual applicability of this standard requires knowledge supporting its inference that toxic constituents which obey the strictly additive criterion at the lethal level interact by the same mechanism at the sublethal level.

Some authors (Sprague, 1970; Seba, 1975) have proposed that strict addition methodology quantifies adequately the multiple toxicity of most pollutant mixtures. However, even a cursory survey of toxicology literature reveals a diversity of effects — and presumably modes of action — between poisonous pollutants studied singly. One might reasonably expect that this complexity would be compounded for the toxicity of mixtures and that the adverse effects of combinations of toxicants could not be simply characterized by the mechanism of similar action (i.e. strict addition). In fact, recent investigations of the effects of pollutant mixtures on aquatic biota report a variety of lethal and sublethal response patterns indicating not only strict addition but also response addition, synergisms, and antagonisms, (Sprague, 1970; LaRoche *et al.*, 1973; Halter and Johnson, 1974; Roales and Perlmutter, 1974; Anderson and Weber, 1975, 1976; Hutchinson and Czyska, 1975; Macek, 1975; Sellers *et al.*, 1975; Reinbold and Metcalf, 1976; Statham and Lech, 1976; Herbes and Beauchamp, 1977). These latter reports do not support reliance on a single water quality standard for the safeguarding of organisms against toxic mixtures. Additional research is required, particularly in the area of synergistic interactions.

## 9.8. REFERENCES

- Abel, P. D., 1974. Toxicity of synthetic detergents to fish and aquatic invertebrates. *J. Fish Biol.*, **6**, 279–98.
- Ames, B. N., Lee, F. D., and Durston, W. E., 1973. An improved bacterial test system for the detection and classification of mutagens and carcinogens. *Proc. U.S. Natl. Acad. Sci.*, **70**, 782–6.



- Anderson, J. M., 1971. Assessment of the effects of pollutants on physiology and behaviour. *Proc. Roy. Soc. London*, B177, 307-20.
- Anderson, P. D. and Battle, H. I., 1967. Effects of chloramphenicol on the development of the zebrafish, *Brachydanio rerio*. *Can. J. Zool.*, 45, 191-204.
- Anderson, P. D. and Weber, L. J., 1975. The toxicity to aquatic populations of mixtures containing certain heavy metals. *Proc. International Conference on Heavy Metals in the Environment*, 2, 933-53. Institute for Environ. Studies, Univ. of Toronto, Toronto.
- Anderson, P. D. and Weber, L. J., 1976. The multiple toxicity of certain heavy metals: additive actions and interactions. In *Proc. of Workshop on Toxicity to Biota of Metal Forms in Natural Water* (Eds. R. W. Andrew, P. V. Hodson, and D. E. Konasewich), Publ. Int. Joint. Comm. Res. Ad. Bd. Windsor, Canada, pp. 263-82.
- Ariens, E. J., 1972. Adverse drug interactions. *Proc. Eur. Soc. Study Drug Tox.*, 13, 137-63.
- Ariens, E. J. and Simonis, A. M., 1964. A molecular basis for drug action. *J. Pharm. Pharmacol.*, 16, 137-57.
- Balinsky, B. I., 1975. *An Introduction to Embryology*, 4th ed., W. B. Saunders Co., Philadelphia, U.S.A., 648 pp.
- Bardach, J. E., Fujiya, M., and Hall, A., 1965. Detergents: effects on the chemical senses of the fish *Ictalurus malates* (le Sueur). *Science, N.Y.*, 148, 1605-7.
- Bengtsson, B. E., 1974. The effects of zinc on the ability of the minnow, *Phoxinus phoxinus* L., to compensate for torque in a rotating water-current. *Bull. Environ. Contam. Toxicol.*, 12, 654-8.
- Benoit, D. A., 1975. Chronic effects of copper on survival growth and reproduction of the bluegill (*Lepomis macrochirus*). *Trans. Am. Fish Soc.*, 104(2), 353-8.
- Berenblum, I. and Shubik, P., 1947. A new quantitative approach to the study of the stages of chemical carcinogenesis on the mouse skin. *Brit. J. Cancer*, 1, 383-91.
- Bingham, E. and Falk, H., 1969. Environmental carcinogenesis. Threshold concentrations of carcinogens and cocarcinogens. *Arch. Environ. Health*, 19, 779-83.
- Bingham, E., Niemeier, R. W., and Reid, J. B., 1976. Multiple factors in carcinogenesis. *Ann. N.Y. Acad. Sci.*, 271, 14-21.
- Blaxhall, P. C. and Daisley, K. W., 1973. Routine haematological methods for use with fish blood. *J. Fish. Biol.*, 5, 771-81.
- Bliss, C. I., 1939. The toxicity of poisons applied jointly. *Ann. Appl. Biol.*, 36, 385-615.
- Boyland, E., 1969. The correlation of experimental carcinogens and cancer in man. *Prog. Exp. Tumour Res.*, 11, 222-34.
- Brett, J. R., 1967. Swimming performance of sockeye salmon (*Oncorhynchus nerka*) in relation to fatigue time and temperature. *J. Fish. Res. Bd. Canada*, 24, 1731-41.
- Brown, E. R., Hazdra, J. S., Keith, L., Greenspan, I., Kwapinski, J. B. G., and Beamer, P., 1973. Frequency of fish tumours found in a polluted watershed as compared to nonpolluted Canadian waters. *Cancer Res.*, 33, 189-98.
- Brown, U. N., 1973. Concepts and outlook in testing the toxicity of substances to fish. In *Bioassay Techniques and Environmental Chemistry* (Ed. E. Glass) Ann Arbor Science Publishers Inc., Ann Arbor, Mich., pp. 73-95.
- Brungs, W. A., 1969. Chronic toxicity of the fathead minnow, *Pimephales promelas* (Rafinesque). *Trans. Am. Fish Soc.*, 98, 272-9.



- Buckley, J. A., Whitmore, C. M., and Matsuda, R. I., 1976. Changes in blood chemistry and blood cell morphology in Coho salmon (*Oncorhynchus kisutch*) following exposure to sublethal levels of total residual chlorine in municipal wastewater. *J. Fish. Res. Bd. Canada*, **33**, 776–82.
- Cairns, J. Jr. and Sparks, R. E., 1971. The use of bluegill breathing to detect zinc. Environment Protection Agency, *Water Pollution Control Research Series 18050 EDQ*, 45 pp.
- Cech, J. J. Jr., Bridges, D. W., Rowel, D. M., and Balzer, P. J., 1976. Cardiovascular responses of winter flounder, *Pseudopleuronectes americanus* (Walbaum), to acute temperature increase. *Can. J. Zool.*, **54**, 1383–8.
- Cech, J. J. Jr. and Wohlschlag, D. E., 1973. Respiratory responses of the striped mullet, *Mugil cephalus* (L.) to hypoxic conditions. *J. Fish. Biol.*, **5**, 421–8.
- Chu, E. H. Y., 1971. Induction and analysis of gene mutations in mammalian cells in culture. In *Chemical Mutagens, Principles and Methods for their Detection* (Ed. A. Hollaender), Vol. 2, Plenum Press, New York, pp. 411–44.
- Cope, O. B., 1971. Interactions between pesticides and wildlife. *Ann. Rev. Ent.*, **16**, 325–63.
- Crocker, J. F. S., Ozere, R. L., Rozee, K. R., Digout, S. C., and Hutzinger, O., 1974. Insecticide and viral interactions as a cause of fatty visceral changes and encephalopathy in the mouse. *Lancet*, **2**, 22–4.
- Davis, C. C., 1972. The effects of pollutants on the reproduction of marine organisms. In *Marine Pollution and Sea Life* (Ed. M. Ruivo), FAO, Fishing News (Books) Ltd., London, England, pp. 305–11.
- Davis, J. C., 1973. Sublethal effects of bleached kraft pulp mill effluent on respiration and circulation in Sockeye salmon (*Oncorhynchus nerko*). *J. Fish. Res. Bd. Canada*, **30**, 369–77.
- Davis, J. C. and Randall, D. J., 1973. Gill irrigation and pressure relationships in rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Bd. Canada*, **30**, 99–104.
- Davis, P. W. and Wedemeyer, G. A., 1971. NaK-activated ATPase inhibition in rainbow trout: a site for organochlorine pesticide toxicity? *Comp. Biochem. Physiol.*, **40B**, 823–7.
- Davy, F. B., Kleerekoper, H., and Matis, J. H., 1973. Effects of exposure to sublethal DDT on the exploratory behaviour of goldfish (*Carassius auratus*). *Water Resources Res.*, **9**, 900–5.
- Durham, W. F. and Williams, C. H., 1972. Mutagenic, teratogenic and carcinogenic properties of pesticides. *Am. Rev. Ent.*, **17**, 123–48.
- Eisler, R., 1972. Pesticide-induced stress profiles. In *Marine Pollution and Sea Life* (Ed. M. Ruivo) FAO, Fishing News (Books) Ltd., London, England, pp. 229–33.
- Epstein, S. S., 1973. The use of dominant-lethal test to detect genetic activity of environmental chemicals. *Environ. Hlth. Perspectives*, **6**, 23–7.
- Esvelt, L. A., Kaufman, W. J., and Selleck, R. E., 1973. Toxicity assessment of treated municipal wastewaters. *J. Water Poll. Control Fed.*, **45**, 1558–72.
- Evans, R. D., 1966. The effect of skeletally deposited  $\alpha$ -ray emitters in man. *Br. J. Radiol.*, **39**, 881–95.
- Falk, H. L., Kotin, P., and Thompson, S., 1968. Inhibition of carcinogenesis: the effect of polycyclic hydrocarbons and related compounds. *Arch. Environ. Health*, **9**, 169–79.
- FAO., 1972. Food and Agriculture Organization of the United Nations. *Marine Pollution and Sea Life* (Ed. M. Ruivo), Fishing News (Books) Ltd., London, England, 624 pp.

- Farley, C. A., 1974. Epizootic neoplasia in bivalve mollusks. *Abs. Symp. Conf. XIth Int. Cancer Congress, Florence*, pp. 219–20.
- FDA, 1970. Food and Drug Administration Advisory Committee on Protocols for Safety Evaluation: panel on reproduction report on reproduction. Studies in the safety evaluation of food additives and pesticide residues. *Toxicol. Appl. Pharmacol.*, **16**, 264–96.
- FDA, 1971. Food and Drug Administration Advisory Committee on Protocols for Safety Evaluation: panel on carcinogenesis report on cancer testing in the safety evaluation of food additives and pesticides. *Toxicol. Appl. Pharmacol.*, **20**, 419–38.
- Finney, D. E., 1971. *Probit Analysis*, 3rd edn., Cambridge Univ. Press, London, England, 333 pp.
- Flamm, W. G., 1974. A tier system approach to mutagen testing. *Mutat. Res.*, **26**, 329–33.
- Fry, F. E. J., 1947. Effects of the environment on animal activity. Univ. Toronto Studies Biological Series 55, *Ontario Fish Res. Laboratory Publ.*, **68**, 62 pp.
- Gaddum, J. H., 1953. *Pharmacology*, 4th edn., Oxford Univ. Press, London, 562 pp.
- Gaddum, J. H., 1957. Drug antagonism. *Pharmacol. Rev.*, **9**, 211–8.
- Gelboin, H. V., 1967. Carcinogen, enzyme induction and gene action. *Adv. Cancer Res.*, **10**, 1–81.
- Giles, M. and Vanstone, W. E., 1976. Changes in ouabain-sensitive adenosine triphosphatase activity in gills of Coho salmon (*Oncorhynchus kisutch*) during parr-smolt transformation. *J. Fish. Res. Bd. Canada*, **33**, 54–62.
- Halter, M. T. and Johnson, H. E., 1974. Acute toxicity of a polychlorinated biphenyl and DDT alone and in combination to early life stages of Coho salmon, *Oncorhynchus kisutch*. *J. Fish. Res. Bd. Canada*, **31**(9), 1543–7.
- Hansen, D. J., Matthews, E., Nall, S. L., and Dumas, D. P., 1972. Avoidance of pesticides by untrained mosquitofish, *Gambusia affinis*. *Bull. Environ. Contam. Toxicol.*, **8**, 46–51.
- Hara, T. J., Law, Y. M. C., and MacDonald, S., 1976. Effects of mercury and copper on the olfactory response in rainbow trout *Salmo gairdneri*. *J. Fish. Res. Bd. Canada*, **33**, 1568–73.
- Harshbarger, J. C., 1974. Integumentary papillomas and carcinomas in fish. *Abs. Symp. Conf. XIth Int. Cancer Congress, Florence*, pp. 218–9.
- Hatfield, C. T. and Johansen, P. H., 1972. Effects of four insecticides on the ability of Atlantic salmon parr (*Salmo salar*) to learn and retain a simple conditioned response. *J. Fish. Res. Bd. Canada*, **29**(3), 315–21.
- Heath, A. G., 1972. A critical comparison of methods for measuring fish respiratory movements. *Water Res.*, **6**, 1–7.
- Herbes, J. E. and Beauchamp, J. J., 1977. Toxic interaction of mixtures of 2 coal conversion effluent components (resorcinol and 6-methyl quinoline) to *Daphnia magna*. *Bull. Environ. Contam. Toxicol.*, **17**(1), 25–32.
- Hewlett, P. S., 1969. Measurement of the potencies of drug mixtures. *Biometrics*, **25**, 477–87.
- Hoar, W. S., 1975. *General and Comparative Physiology*, 2nd edn., Prentice-Hall Inc., New Jersey, 848 pp.
- Houston, A., Madden, J. A., Woods, R. S., and Miles, H. M., 1971. Some physiological effects of handling and TMS anesthetization upon brooktrout *Salvelinus fontinalis*. *J. Gen. Physiol.*, **56**, 342–58.



- Howard, T. E., 1975. Swimming performance of juvenile Coho salmon (*Oncorhynchus kisutch*) exposed to bleached kraft pulpmill effluent. *J. Fish. Res. Bd. Canada*, 32, 789–93.
- Hutchinson, T. C. and Czyrska, H., 1975. Heavy metal toxicity and synergism to floating aquatic weeds. *Verh. Internat. Verein. Limnol.*, 19, 2102–11.
- Ishio, S., 1965. Behaviour of fish exposed to toxic substances. In *Advances in Water Pollution Research* (Ed. O. Jaag), Vol. 1, Pergamon Press, London, pp. 19–33.
- Janicki, R. H. and Kinter, W. B., 1971. DDT inhibits NaK Mg-ATPase in the intestinal mucosae and gills of marine teleosts. *Nature New Biol.*, 233, 148–9.
- Kerr, S. R. and Vass, W. P., 1973. Pesticide residues in aquatic invertebrates. In *Environmental Pollution by Pesticides* (Ed. C. A. Edwards), Plenum Press, London, pp. 134–80.
- Khudoley, V. V., 1972. Induction of liver tumours in aquarium guppies with some nitrogen compounds. *J. Ichthyol.*, 12, 319–24.
- Kirschbaum, A., Strong, L. C., and Gardner, W. U., 1940. Influence of methyl cholanthrene on age incidence of leukemia in several strains of mice. *Proc. Soc. Exptl. Biol. Med.*, 45, 287–9.
- Koeman, J. H. and van Genderen, H., 1972. Tissue levels in animals and effects caused by chlorinated hydrocarbon insecticides, chlorinated biphenyls and mercury in the marine environment along the Netherlands coast. In *Marine Pollution and Sea Life* (Ed. M. Ruivo), FAO, Fishing News (Books) Ltd., London, England.
- Kotin, P., 1976. Dose response relationship and threshold concepts. *Ann. N.Y. Acad. Sci.*, 271, 22–8.
- Kotin, P. and Wisely, A. V., 1963. Production of lung cancer in mice by inhalation exposure to influenza virus and aerosols of hydrocarbons. *Prog. Exp. Tumour Res.*, 3, 186–215.
- LaRoche, G., Gardner, G. R., Eisler, R., Jackim, E. H., Yevish, P. P., and Zarogian, G. E., 1973. Analysis of toxic response in marine poikilotherms. In *Bioassay Techniques and Environmental Chemistry* (Ed. G. E. Glass), Ann Arbor Science Publishers Inc., Ann Arbor, Mich., pp. 199–216.
- Lassiter, D. V., 1976. Prevention of occupational cancer – toward an integrated program of governmental action. *Ann. N.Y. Acad. Sci.*, 271, 214–5.
- Legator, M. S. and Malling, H. V., 1971. The host-mediated assay, a practical procedure for evaluating potential mutagenic agents in mammals. In *Chemical Mutagens, Principles and Methods for their Detection* (Ed. A. Hollaender), Vol. 2, Plenum Press, New York, N.Y., pp. 569–89.
- Lesniak, J., 1977. Histological approach to the sublethal cyanide effects on rainbow trout ovaries. *M.Sc. Thesis*, Concordia University, Montreal, 120 pp.
- Lett, P. F., Farmer, G. J., and Beamish, F. W. H., 1976. Effect of copper on some aspects of the bioenergetics of rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Bd. Canada*, 33, 1335–42.
- Lewis, S. D. and Lewis, W. M., 1971. The effect of zinc and copper on the osmolarity of blood serum of the channel catfish, *Ictalurus punctatus*, Rafinesque and golden shiner, *Notemigonus crysoleucas* Mitchill. *Trans. Am. Fish. Soc.*, 100, 639–43.
- Lindahl, P. E., Olopson, S., and Schwanbom, E., 1977. Rotary-flow technique for testing fitness of fish. In *Biological Monitoring of Water and Effluent Quality* (Eds. J. Cairns, K. L. Dickson, and G. F. Westlake), ASTM, pp. 75–84.
- Lindahl, P. E. and Schwanbom, E., 1971a. A method for the detection and



- quantitative estimation of sublethal poisoning in living fish. *Oikos*, 22(2), 210-4.
- Lindahl, P. E. and Schwanbom, E., 1971b. Rotatory-flow technique as a means of detecting sublethal poisoning in fish populations. *Oikos*, 22(3), 354-7.
- Lloyd, R. and Orr, L. D., 1969. The diuretic responses by rainbow trout to sublethal concentrations of ammonia. *Water Res.*, 3, 335-44.
- Loomis, T. A., 1974. *Essentials of Toxicology*, 2nd edn., Lea and Febiger, Philadelphia, 223 pp.
- Lunn, R. R., Toews, D. P., and Pree, D. J., 1976. Effects of three pesticides on respiration, coughing, and heart rates of rainbow trout (*Salmo gairdneri* Richardson), *Can. J. Zool.*, 54, 214-9.
- Macek, K. J., 1975. Acute toxicity of pesticide mixtures to bluegills. *Bull. Environ. Contam. Toxicol.*, 14(6), 648-52.
- Mawdesley-Thomas, L. E., 1971. Neoplasia in fish: a review. In *Current Topics in Comparative Pathology* (Ed. T. C. Cheng), Vol. 1, pp. 87-170.
- McKee, J. E. and Wolf, H. W. (Eds.), 1963. *Water Quality Criteria*, 2nd edn., Publ. 3-A of Resources agency of the California State Water Control Board, Sacramento, California.
- McKim, J. M. and Benoit, D. A., 1971. Effects of long-term exposures to copper on survival, growth and reproduction of brook trout, *Salvelinus fontinalis*. *J. Fish. Res. Bd. Canada*, 28(5), 655-62.
- McKim, J. M., Christensen, G. M., and Hunt, E. P., 1970. Changes in the blood of brook trout (*Salvelinus fontinalis*) after short-term and long-term exposure to copper. *J. Fish. Res. Bd. Canada*, 27, 1883-9.
- McLeay, D. J., 1973. Effects of 12 hr and 25 day exposure to kraft pulpmill effluent on the blood and tissue of juvenile Coho salmon. *J. Fish. Res. Bd. Canada*, 30, 395-400.
- Menzie, C. M., 1972. Fate of pesticides in the environment. *Ann. Rev. Ent.*, 17, 199-223.
- Mitrovic, V. V., 1972. Sublethal effects of pollutants on fish. In *Marine Pollution and Sea Life* (Ed. M. Ruivo), FAO, Fishing News (Books) Ltd., London, pp. 252-7.
- Mount, D. I. and Stephen, E. C., 1969. Chronic toxicity to the fathead minnow, *Pimephales promelas* in soft water. *J. Fish. Res. Bd. Canada*, 26(9), 2449-57.
- Mulcahy, M. F., 1974. Epizootic neoplasia of northern pike, *Esox lucius* L. in Ireland. *Abs. Symp. Conf. XIth Int. Cancer Congress, Florence*, 218.
- National Academy of Sciences. National Research Council: Food Protection Committee, 1959, *Problems in the Evaluation of Carcinogenic Hazard from Use of Food Additives*, Publ. 749, Washington, D.C.
- Neil, J. H., 1957. Some effects of potassium cyanide on speckled trout (*Salvelinus fontinalis*). *Proc. 4th Ontario Ind. Waste Conf. Ontario Water Res. Comm.*, pp. 74-96.
- Nigrelli, R. F., 1952. Virus and tumours in fishes. *Ann. N.Y. Acad. Sci.*, 54, 1076-92.
- O'Connor, D. V. and Fromm, P. O., 1975. The effect of methyl mercury on gill metabolism and blood parameters of rainbow trout. *Bull. Environ. Contam. Toxicol.*, 13, 406-11.
- Oseid, O. and Smith, L. L. Jr., 1972. Swimming endurance and resistance to copper and malathion of bluegills treated by long-term exposure to sublethal levels of hydrogen sulfide. *Trans. Am. Fish. Soc.*, 101, 620-5.
- Osterman-Golkar, S., 1975. Studies on the reaction kinetics of biologically active

- electrophilic reagents as a basis for risk elements. *Doctorate Thesis*, University of Stockholm, Sweden, 55 pp.
- Osterman-Golkar, S., Ehrenberg, L., Segerbäck, D., and Hällström, I., 1976. Evaluation of genetic risks of alkylating agents. II. Haemoglobin as a dose monitor. *Mutat. Res.*, **34**, 1–10.
- Peterson, R. H., 1974. Influence of fenitrothion on swimming velocities of brook trout (*Salvelinus fontinalis*). *J. Fish. Res. Bd. Canada*, **31**, 1757–62.
- Pfuderer, P. and Francis, A. A., 1973. Effects of phthalate esters on heart rate of goldfish. *Fed. Proc.*, **32**, 224.
- Plackett, R. L. and Hewlett, P., 1952. Quantal responses to mixtures of poisons. *J. Roy. Stat. Soc. B14*, 151–4.
- Reinbold, K. A. and Metcalf, R. L., 1976. Effects of the synergist piperonyl butoxide on metabolism of pesticides in green sunfish. *Pestic. Biochem. Phys.*, **6**(5), 401–12.
- Reish, D. V., 1972. The use of marine invertebrates as indicators of varying degrees of marine pollution. In *Marine Pollution and Sea Life* (Ed. M. Ruivo), FAO, Fishing News (Books) Ltd., London, England, pp. 203–7.
- Renfro, J. L., Schmidt-Nielsen, B., Miller, D., Benos, D., and Allen, J., 1973. Methyl mercury and inorganic mercury: uptake, distribution and effect on osmoregulatory mechanisms in fish. In *Pollution and Physiology of Marine Organisms* (Eds. F. J. Vernberg and W. B. Vernberg), Academic Press, New York, pp. 59–65.
- Roales, R. R. and Perlmutter, A., 1974. Toxicity of zinc and cygon applied singly and jointly to zebra fish embryos. *Bull. Environ. Contam. Toxicol.* **12**(4), 475–80.
- Rosenthal, H. and Alderdice, D. F., 1976. Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae. *J. Fish. Res. Bd. Canada*, **33**, 2047–65.
- Sanders, H. J., 1969. Chemical mutagens: an expanding roster of suspects. *Bull. Chem. and Engin.*, June, pp. 54–68.
- Saunders, N. L. and Sprague, J. B., 1967. Effects of copper-zinc mining pollution on a spawning migration of Atlantic salmon. *Water Res.*, **1**, 419–32.
- Schaumburg, F. D., Howard, T. E., and Walden, C. C., 1967. A method of evaluating the effects of water pollutants on fish respiration. *Water Res.*, **1**, 731–5.
- Scherer, E., 1975. Avoidance of fenitrothion by goldfish, *Carassius auratus*. *Bull. Environ. Contam. Toxicol.*, **13**, 492–6.
- Schmid, W., 1973. Chemical mutagen testing on *in vivo* somatic mammalian cells. *Agents and Actions*, **3**, 77–85.
- Seba, D. B., 1975. Toxicity index for permits. In *Proc. Symposium on Structure–Activity Correlations in Studies of Toxicity and Bioaccumulation with Aquatic Organisms* (Eds. G. D. Veith and D. E. Konasewich), Publ. of I. J. C. Res. Ad. Bd. Windsor, Ontario, pp. 199–259.
- Sellers, C. M. Jr., Heath, A. G., and Bass, M. L., 1975. The effect of sublethal concentrations of copper and zinc on ventilatory activity, blood oxygen and pH in rainbow trout (*Salmo gairdneri*). *Water Res.*, **9**, 401–8.
- Shelton, G., 1971. The regulation of breathing. In *Fish Physiology* (Eds. W. S. Hoar and D. J. Randall), Vol. IV, Academic Press, New York, pp. 293–359.
- Skidmore, J. F., 1970. Respiration and osmoregulation in rainbow trout with gills damaged by zinc sulfate. *J. Exp. Biol.*, **52**, 481–94.
- Sprague, J. B., 1968. Avoidance reactions of rainbow trout to zinc sulphate solutions. *Water Res.*, **2**, 367–72.



- Sprague, J. B., 1970. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. *Water Res.*, **4**, 3-32.
- Sprague, J. B., 1971. Measurement of pollutant toxicity to fish. III. Sublethal effects and 'safe' concentrations. *Water Res.*, **5**, 245-66.
- Sprague, J. B., 1976. Current status of sublethal tests of pollutants on aquatic organisms. *J. Fish. Res. Bd. Canada*, **33**, 1988-92.
- Statham, C. N. and Lech, J. J., 1976. Studies on the mechanisms of potentiation of the acute toxicity of 2-4-D-*n*-butyl ester and 2'-5-dichloro-4'-nitrosalicylanilide in rainbow trout by carbaryl. *Tox. Appl. Pharm.*, **36**(2), 281-96.
- Stich, H. F., Acton, A. B., and Dunn, B. P., 1975. Carcinogens in estuaries, their monitoring and possible hazard to man. *Paper given at Symposium on Environmental Pollution and Carcinogenic Risks*, Lyon, France.
- Swift, D. J. and Lloyd, R., 1974. Changes in urine flow rate and haematocrit value of rainbow trout, *Salmo gairdneri* (Richardson) exposed to hypoxia. *J. Fish. Biol.*, **6**, 379-87.
- Triolo, A. J. and Coon, J. M., 1966. Toxicologic interactions of chlorinated hydrocarbon and organophosphate insecticides. *J. Agr. Food Chem.*, **14**, 544-55.
- Vollenweider, R. A. and Dillon, P. J., 1974. The application of the phosphorus loading concept to eutrophication research. *National Research Council of Canada Report*, #13690, 42 pp.
- Wales, J. H. and Sinnhuber, R. O., 1972. Hepatomas induced by aflatoxin in the sockeye salmon (*Oncorhynchus nerka*). *Nat. Cancer Inst.*, **48**, 1529-30.
- Warner, R. E., Peterson, K. K., and Borogman, L., 1966. Behavioural pathology in fish. *J. Applied Ecol.*, **3** (Suppl.), 223-47.
- Warren, C. E., 1971. *Biology and Water Pollution Control*, W. B. Saunders, Co., Philadelphia, U.S.A., 434 pp.
- Warren, C. E. and Davis, G. E., 1971. Laboratory stream research: objectives, possibilities and constraints. *Am. Rev. Eco. System*, **2**, 111-44.
- Webb, P. W. and Brett, L. R., 1973. Effects of sublethal concentrations of sodium pentachlorophenate on growth rate, food conversion efficiency and swimming performance of under yearling sockeye salmon (*Oncorhynchus nerka*). *J. Fish. Res. Bd. Canada*, **30**, 499-507.
- Wedemeyer, G., and Chatterton, K., 1970. Some blood chemistry values for the rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Bd. Canada*, **27**, 1162-4.
- Weisburger, J. H., Hadidian, Z., Frederickson, T. N., and Weisburger, E. K., 1965. Carcinogenesis by simultaneous action of several agents. *Tox. Appl. Pharm.*, **7**, 502.
- Westlake, G. F. and Kleerekoper, H., 1974. The locomotor response of goldfish to a steep gradient of copper ions. *Water Resources Res.*, **10**, 103-5.
- WHO (World Health Organization), 1964. *Prevention of Cancer Tech. Rept. Ser.*, #276, Geneva, Switzerland.
- Wilson, J. G., 1964. Teratogenic interaction of chemical agents in the rat. *J. Pharm. Exp. Therapeut.*, **144**, 429-36.
- Wolfe, P. H., 1974. Integumentary neoplasms in oysters. *Abs. Symp. Conf. XIth Int. Cancer Congress, Florence*, 219.



