A group of enzymes known as mixed-function oxidases (MFO) are widely found in vertebrates, including fishes, and have been reported in some invertebrates. They show some potential as indicators of sublethal toxic effect, and possibly as a means of monitoring pollutant effects in field populations (Addison, 1984). The normal function of MFO appears to be associated with the metabolism of steroid hormones, but they have been found to be produced at elevated levels in animals exposed to some pollutants, particularly aromatic hydrocarbons and halogenated hydrocarbons. Metabolites of these important pollutants appear to resemble those of some natural steroids, and to induce MFO synthesis. Thus elevated MFO levels may be used to indicate exposure of the animal to pollutants. The technique and its applications are not, as yet, well developed; for example, the link between MFO synthesis and actual toxic effect is not generally established. Mixed-function oxidase production may again, therefore, represent an adjustment whereby 'normal function is maintained without significant cost' (see Figure 4.17). However, it seems likely that enhanced MFO production can lead to another important consequence, genotoxic effects.

The relationship between MFO and genotoxicity is explained by Zahn (1991), and in Figure 4.19. MFO breaks down toxic organic chemicals but the immediate consequence is the production of carcinogenic compounds which can cause alteration of the DNA of the cells, which can be detected by a variety of techniques (Zahn, 1991). Nearly all DNA alterations can be considered as deleterious; and although there is a repair mechanism for restoring damaged DNA, if it becomes overwhelmed DNA damage will accumulate and have consequences either in the present generation (e.g. through tumour formation), or, of course, in future generations (e.g. through the production of mutations).

Lysosomes in cells appear to be deeply involved in the detoxification of or response to toxic substances, and tests based on lysosomal stability have been developed (Moore, 1991). Physiological measurements to determine the effect of exposure to pollutants on the scope for growth of some animals appear to show promise (Axiak, 1991). These have mainly been applied in the rather different circumstances of the marine environment, where they have particular advantages over more conventional approaches (see Section 7.3). Indeed, the range of methods available for assessing sublethal toxicity appears to be limited only by the ingenuity of investigators, and it increases year by year.

4.4.2 Experimental Ecosystems

An alternative approach to the study of sublethal toxicity is that of the experimental ecosystem. Instead of exposing a population of a single species to the pollutant, populations of two or more species, frequently representing different trophic levels, are maintained and exposed together to the pollutant.

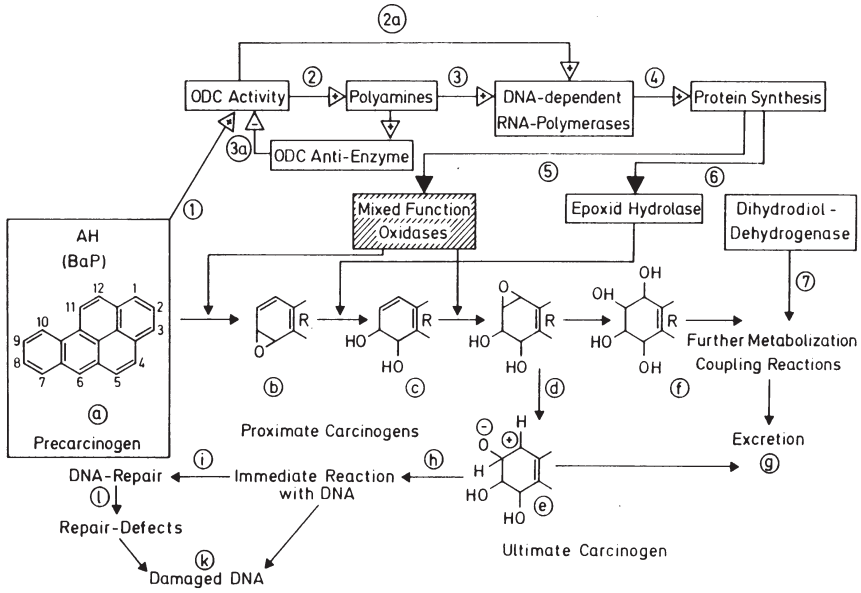

Figure 4.19 Fate and effects of polycyclic aromatic hydrocarbons (PAH). Sequence of events initiated by contact of an inexperienced vertebrate with benzo(a)pyrene (B(a)P). Only a selection of reactions is given. B(a)P is used as an example (+ arrows mean increase, − arrows mean decrease of enzyme activity).

(1) The precarcinogen form provokes a rise in ornithine decarboxylase (ODC) activity (Byus *et al.*, 1976) leading to (2) an increased polyamine synthesis (Russell, 1973) and RNA synthesis through (3) more RNA polymerase activity (Russell, 1971; Jacob and Rose, 1976). The latter is also enhanced by (2a) direct action of ODC (McEnroe and Healy, 1977). RNA synthesis is followed by (4) protein synthesis (DePierre and Ernster, 1978), which enables (5) synthesis of MFO and (6) epoxide hydratase (DePierre and Ernster, 1978). (3a) ODC-antienzyme is induced by high levels of polyamine (Heller *et al.*, 1976). (7) Dihydrodiol-epoxygenase action.

Out of many PAH, B(a)P is one; and from this, one single pathway to an 'ultimate carcinogen' with the capability as an electrophilic to combine, among many others, with DNA, is depicted. The precarcinogen (a) B(a)P, under the influence of MFO is transformed into the (b) 7,8-epoxide and by epoxide hydrolase into (c) the 7,8-trans-diol, which is another substrate for the MFO, thus yielding two diastereomeric forms of (d) 7,8-diol-9,10-epoxide-B(a)P. These 'proximate carcinogens' give rise to 'ultimate carcinogens', which most likely are (e) carbenium ions with the capability to (h) direct action with nucleophilics (Yang *et al.*, 1977) in specific manner (Deutsch *et al.*, 1978; Meehan and Straub, 1979). (f) Some of the compounds arising from the activity of the MFO, epoxide hydrolase, glutathione-*S*-transferase, UDP-glucuronic acid transferase and sulphotransferase may be transformed and/or coupled, thus being detoxified

In part this approach represents an attempt to make the experimental situation more realistic, and hence more directly applicable to the field situation. For example, in the field the ecology of a species is governed not only by its relationship with the physical and chemical environment and by purely endogenous population processes, but also by its relationship with other species (prey, predators, competitors, parasites) with which it shares its habitat. Thus the ecological effect of a pollutant on a species does not depend only upon the properties of the species itself; its influence may be accentuated or attenuated depending upon the nature of interspecific relationships which exist in the environment. Experimental ecosystems are both useful and necessary for studying these and other phenomena, including the distribution of pollutants between water, sediments and living tissues; the biodegradation of pollutants; and the accumulation of pollutants by living organisms from their environment, and the passage of pollutants through the food chain.

Experimental ecosystems vary in their scale and complexity. Generally the smaller-scale systems offer the advantages of more precisely-controlled experimental conditions, but are at best gross simplifications of real systems. Larger-scale systems more closely represent natural systems, but clearly cannot be so precisely controlled and experiments may be difficult or impossible to replicate. The smallest systems are more-or-less enclosed vessels containing populations of microorganisms or plankton. On a larger scale, experimental aquaria or ponds ranging in size from a few litres to a few cubic metres have been used (Giddings, 1983). Experimental river channels, both on a laboratory scale and outdoors, have been widely used (e.g. Arthur *et al.,* 1982; Watton and Hawkes, 1984). Finally, there have been attempts to isolate large water masses in lakes and coastal marine areas, to study the processes which occur within, as it were, a representative sample of the natural environment (Davies and Gamble, 1979; Steele, 1979). Since they vary widely in their scale, complexity and objectives perhaps the only generalisation which can be made about experimental ecosystems is that they can provide valuable information which bridges the gap between laboratory and field studies. Large-scale systems are, however, expensive to maintain, and their use is likely to be confined to aiding the interpretation of the results of more economical methods of study. There appears to have

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and (g) excreted (Oesch, 1982; Yang *et al.*, 1978). (i) Interaction of DNA with ultimate carcinogens leads to strand scissions (Gamper *et al.*, 1977) and to coupling of PAH derivatives (Miller and Miller, 1981). DNA alterations cause repair to start, which to a certain degree may cause (l) some secondary DNA defects (Hanawalt and Friedberg, 1978). This altogether results, in a time-dependent manner, in DNA showing (k) secondary alterations giving rise to mutagenesis/carcinogenesis (Ames *et al.*, 1975; Huberman and Sachs, 1974, 1977)

been some reduction in interest in experimental ecosystems in recent years, perhaps because their results have been less useful and informative than was once hoped. However, this tendency is to some extent counteracted by recent legislative pressure which is leading to the development of artificial ecosystem techniques for regulatory purposes. This arises because, particularly in Europe and the United States, laws are being promulgated which demand that pollutants discharged to the environment are shown to have no unacceptable environmental effect. Such laws are not necessarily very sensible, and they are certainly not scientifically sensible. First, it is not possible to prove that an effect does not exist; second, there is no definition of what is acceptable; and third, experimental ecosystems do not, as we have seen, necessarily simulate the natural environment much more accurately than properly controlled experiments. Nevertheless, if such laws exist some attempt must be made to conform with them, and the volume edited by Hill *et al.* (1994) provides a very full review of recent developments in the applications of experimental ecosystems. (See further discussion in Section 7.2.3.)

4.4.3 Bioaccumulation

Bioaccumulation is an aspect of sublethal toxicity which has received much attention, though many areas of uncertainty remain. Pollutants may, over long time periods, accumulate in tissues to levels which may be harmful to the organism. Since many aquatic species are utilised for human consumption, the public health significance of toxic substances accumulated in their tissues is obvious. Many national and international agencies set concentration limits for pollutants, particularly heavy metals, in tissues for human consumption, and promote research and monitoring programmes. Study of the uptake, metabolism and excretion of pollutants, and of their distribution in the various body organs and tissues, makes an important contribution to understanding their mechanisms of action. Levels of pollutants in the tissues of living organisms are widely used to indicate the degree of contamination of the waters in which they live, particularly when the pollutants are present only intermittently or in very low concentrations, making chemical analysis of the water difficult. Finally, many poisons, particularly heavy metals and refractory organic compounds such as some pesticides, are widely believed to pass from the tissues of prey organisms into those of predators and to attain concentrations there which are several orders of magnitude higher than those in the tissues of the prey species. This phenomenon poses a specific threat to long-lived organisms at the higher trophic levels.

Studies of bioaccumulation are carried out in the laboratory, in experimental ecosystems and in the field. Laboratory investigations are usually concerned initially with determining the 'bioconcentration factor' (BCF), that is, the ratio between the concentration in the animals and the concentration in the water, when the animals

have been exposed for sufficiently long for an equilibrium or steady state to be achieved. This ratio is generally regarded as a valid indicator of the capacity of a pollutant to accumulate in animal tissues. Such limited data as are available (Davies and Dobbs, 1984; Schnoor, 1982) suggest that laboratory-derived BCF values agree reasonably well with those derived from field observations on the animals of polluted waters, at least for certain groups of organic pollutants and provided certain conditions are met in the laboratory determinations. Under certain conditions, there is a good correlation between log P (where P=the octanol-water partition coefficient of the chemical) and the BCF. This offers the possibility that the bioaccumulation potential of a pollutant can be indicated by the result of a relatively simple chemical determination, rather than by the expensive and time-consuming estimation of BCF. However, Davies and Dobbs (1984) in their study of this question, found it necessary to reject determinations of BCF which did not meet certain criteria.

There are many models of bioaccumulation. In the simplest possible model, two compartments are considered: the organism and the environment. Pollutant will enter the organism at a certain rate, which is dependent upon the amount present in the environment. Pollutant will also be lost from the organism, at a rate dependent upon the amount present in the organism. This simple model can be expressed mathematically and predicts that organisms exposed to a constant level of pollutant will eventually reach a 'steady state'; that is, the concentration of pollutant will increase to a certain level and thereafter remain constant. Conversely, the model predicts that in contaminated organisms maintained in clean water, the concentration of pollutant in the organism will decline exponentially. These predictions are generally confirmed by experimental findings.

Such a simple model is of limited practical use, however, and it is not difficult to see why. Most organisms cannot be considered as a single compartment. Studies of the distribution of pollutants in animals invariably show that the pollutant is very unevenly distributed between the various body tissues. Different pollutants behave in different ways. Clearly the animal will begin to suffer harm when the poison concentration in a particular organ reaches a critical level. Thus the concentration of the pollutant in the whole body is not a good indicator of harmful effect. For this reason, models have been derived which treat the organism as a set of interacting compartments. These models treat discrete organs (e.g. liver, kidney, brain) as interacting compartments connected via the blood (itself considered a compartment) with each other and with the external environment. Further, in some models the exchange of pollutants between the compartments is considered as a series of separate processes. For example, uptake of the pollutant from the environment may occur through the body surface, or by ingestion of food or of non-food particulate material. Elimination of the pollutant may occur through outwards diffusion, through renal or gastrointestinal excretion, or by metabolic breakdown. Clearly models which attempt a complete and accurate description of