Appraisal of Tests to Predict the Environmental Behaviour of Chemicals
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4.4
Accumulation in Aquatic Organisms

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4.4.1 INTRODUCTION

A high bioconcentration potential of an anthropogenic chemical (xenobiotic) in biota increases the probability of toxic effects being encountered in man and his environment. Thus, bioconcentration potentials should be assessed in appropriately designed test systems which assist in the prediction of bioaccumulation behaviour in the field. Experiments which rank the bioconcentration potential of various chemicals under similar but limited experimental conditions are not necessarily meaningful, for they may not allow for extrapolations to natural conditions. For clarity, a definition of the terminology associated with bioaccumulation studies should be given:

Bioconcentration. Bioconcentration is the direct uptake of a substance by an organism from water without consideration of the ingestion of contaminated materials. Experimentally, the result of such a process is reported as the bioconcentration factor (BCF), the quotient of the concentration of the substance in the organism and the ambient medium. The BCF can be based on the fresh or dry weight of the material or on the lipid content. The BCF can be determined at any time during an exposure period, but values derived

at conditions reflecting steady state are most useful for comparative purposes. BCF should be determined in an appropriate concentration range where values are independent of the concentration of the compound and ecologically meaningful.

Biomagnification. Biomagnification is the direct uptake of a substance by an organism via food. In aquatic environments, this process operates simultaneously with bioconcentration. *Bioaccumulation* is therefore defined as the uptake of pollutants via food and water.

4.4.2 TEST PROTOCOLS

The majority of experimental work has been carried out with algae, fish, mussels and worms. This reflects demand for representatives of the various components of an aquatic ecosystem. The choice of organism reflects the animal's maintenance requirements, size, habitat, feeding behaviour and mode of pollutant uptake. The extent of bioconcentration of a compound in a given species depends on certain chemical and physical properties of that compound. On the other hand, species differences may exist in addition to differences in physical and chemical conditions of the environment, which may result in different degrees of bioconcentration for a specific compound. Thus, a suitable test method requires: (a) a reliable determination of the accumulation potentials; (b) the choice of a test organism for which results can be accurately extrapolated to the majority of biota.

Analytical requirements in the course of testing need additional consideration. The analytical procedures can be greatly facilitated by the use of radio-labelled compounds, but the availability of labelled substances is limited and their use usually excludes flow-through tests because of the high costs and safety considerations. In addition, when radio-labelled substances are used, complications may occur when metabolites are formed. In this case, the identity of the radioactive compounds has to be determined by substance specific analysis, otherwise calculations of the BCF may be highly erroneous.

Choice of test organisms. The selection of the test organisms can be made using similar criteria to those applied to organisms for monitoring pollutants in natural systems (Phillips, 1978). Some of the most important criteria are that: (a) the organism should accumulate the compound without being killed or adversely affected at the concentration chosen; (b) the organism should be of reasonable size providing sufficient material for analysis; (c) wild animals, when used, should be easy to sample and hardy enough to survive in the laboratory; sedentary organisms are preferred, since they are more representative of an area in question; (d) all organisms of a given species exhibit the same bioconcentration factor or at least a systematic pattern of bioconcentration factors.

4.4.3 BIOCONCENTRATION

When performing bioconcentration tests, the duration of test procedures is an essential factor. According to equation (4), Table 4.4.1, it is necessary to expose the test organisms for four half-lives, to approach >90% of the theoretical BCF (see Figure 4.4.1).

Bioconcentration factors at steady state can be determined using static, semi-static and flow-through tests. The method chosen will depend on the type of substances being tested, their physico-chemical properties, such as water solubility and *n*-octanol:water partition coefficient, the test organisms, the type of environment for which predictions have to be made, and economic considerations.

Flow-through tests have the advantages of providing a relatively constant concentration of test compound and of flushing from the aquaria undesirable excretion products which can influence the results. Large batches of test animals are required in order to provide appropriate subsamples of animals for analysis at different times during the uptake phase. Without such a sampling regime, recognition of the accumulation pattern and that steady state conditions have been established is difficult, particularly for substances with a higher BCF, such as DDT, or the higher chlorinated biphenyls.

Static exposure systems are less costly and, since volumes are comparatively small, experiments with radio-labelled compounds are feasible

Table 4.4.1. Kinetics equations describing uptake and depuration for the determination of bioconcentration factors

$$\frac{dC_A}{dt} = k_1 \cdot C_W - k_2 \cdot C_A \quad (1)$$

$$C_{A_t} = \frac{k_1}{k_2} \cdot C_W (1 - e^{-k_2 t}) \quad (2)$$

$$C_{A_s} = \frac{k_1}{k_2} \cdot C_W = \mathrm{BCF} \cdot C_W \quad (3)$$

$$C_{A_t} = C_{A_s} (1 - e^{-k_2 t}) \quad (4)$$

$$-\frac{dC_A}{dt} = k_2 \cdot C_A \quad (5)$$

$$C_{A_t} = C_{A0} \cdot e^{-k_2 t} \quad (6)$$

$$\ln C_{A_t} = \ln C_{A0} - k_2 t \quad (7)$$

$$t_{1/2} = \frac{\ln 2}{k_2} \quad (8)$$

where

C_A = the level of compound in the animal (ng g^{-1}),
C_{A_t} = the level of compound in the animal at time (t),
C_{A_s} = the level of compound in the animal under steady state conditions,
C_{A0} = the level of compound in the animal at the end of exposure,
C_W = the concentration of compound in water (ng g^{-1}),
k_1 = rate constant for uptake (day^{-1}),
k_2 = rate constant for depuration (day^{-1}), and
$t_{1/2}$ = half-life time (days).

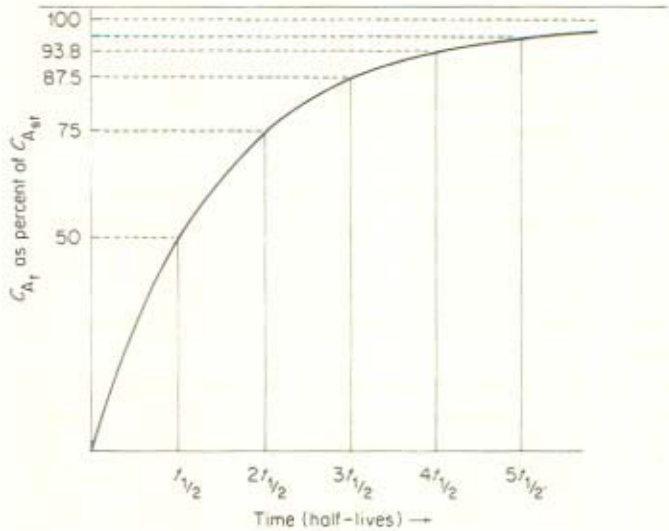

Figure 4.4.1 Level of a compound in a test animal during uptake from water as per cent of the steady state level in relation to time, expressed as half-lives

allowing a simple screen for degradation products. If the concentrations of compounds have to be kept constant during the experiment, redosing of water or exchange of the water is necessary. Sampling and analysis of animals have to be made according to the same schedule as in flow-through tests.

Another approach in static tests is to allow the exposed organisms to take up the compound until steady state conditions are approached without changing or adding the chemical to the water. Steady state is assumed to be established when the concentration of the test compound in the water becomes constant. A prerequisite for this procedure is the use of animals which rapidly achieve steady state, e.g. mussels. An advantage of this method is that only water samples have to be analysed until appropriate steady state conditions are observed. Then the total batch of animals can be used to determine the bioconcentration factor (Ernst, 1977). This provides a more statistically significant sample using a limited number of animals. Generally, static test systems are restricted to less volatile substances. Corrections for loss of substances from the water can be achieved by using blank aquaria.

Substances having high BCFs in the range of 10 000 or more can be tested by an accelerated test procedure, reported by Branson *et al.* (1975). The test examines accumulation in fish muscle using kinetic principles and offers the opportunity to considerably reduce the duration of a single test. Some shortcomings are that (a) only the initial part of the uptake curve is taken into consideration; first order kinetics are assumed but not proven; the depuration

rate may not be constant but can decrease with increasing depuration time; (b) no tissue samples are analysed in the proximity of the steady state; thus the variation of substance levels in tissues of individuals may be more pronounced than in the usual longer test exposures.

4.4.3.1 Determination of the elimination rate constant k_2

The mathematical description of the process of depuration or elimination (equations (5)–(8), Table 4.4.1) is rather simplified and can only be verified in cases where the size and physiological state of test species are optimal and rather non-biodegradable compounds are assessed. The validity of equations (5)–(8), although in many cases fitting the expected curves for the first two half-lives, has occasionally been questioned as a consequence of observed decreases in k_2 with increasing time of depuration. Reasons for this behaviour are that the elimination or decrease rate depends on (a) the actual rate of release of the compound from tissues; (b) the amount to which degradation of the parent compound can be accelerated at the beginning of depuration; and (c) the amount of binding in deeper compartments, from which the release of compounds cannot be described by first order kinetics. Short exposure times have been reported to influence depuration rates, because the uptake and distribution of the compounds are extremely inconsistent and unpredictable (e.g. Macek *et al.*, 1970). These findings and those of Warlen *et al.* (1977) with DDT at different levels in fish are contradictory to results reported by Ellgehausen *et al.* (1980). The latter found that after short exposures of catfish to various compounds, the depuration rates were dependent on the initial level in fish, according to second order reaction kinetics. Furthermore, the lipid pool size has been found to considerably influence the tissue retention in fish. This could be demonstrated in the northern redhorse sucker (*Moxostoma macrolepidotum*) where clearance of chlordane from fish was directly proportional to the adiposity (Roberts *et al.*, 1976). Unfortunately, very often lipid contents of tissues were either not determined or not reported with the majority of the bioconcentration factors presently in the literature.

Also, the chemical composition of the lipid fractions influences the partitioning patterns of xenobiotics. In bioconcentration tests with chlorinated biphenyls in oysters, it was found that PCB concentrations in these animals did not correlate with hexane-extractable lipids. For oysters, it is known that 30–60% of their lipids consist of more polar constituents (Vreeland, 1974; Schneider, 1982). Similarly, in environmental samples pollutant levels were found higher when related to lipids in muscle tissue compared to those in lipids in the liver (Schaefer *et al.*, 1976; Ernst *et al.*, 1976).

When experiments are carried out with compounds having low k_2 values, long periods must elapse before sufficient depuration occurs. In these cases, test animals maintained at normal feeding rates can exhibit apparently faster

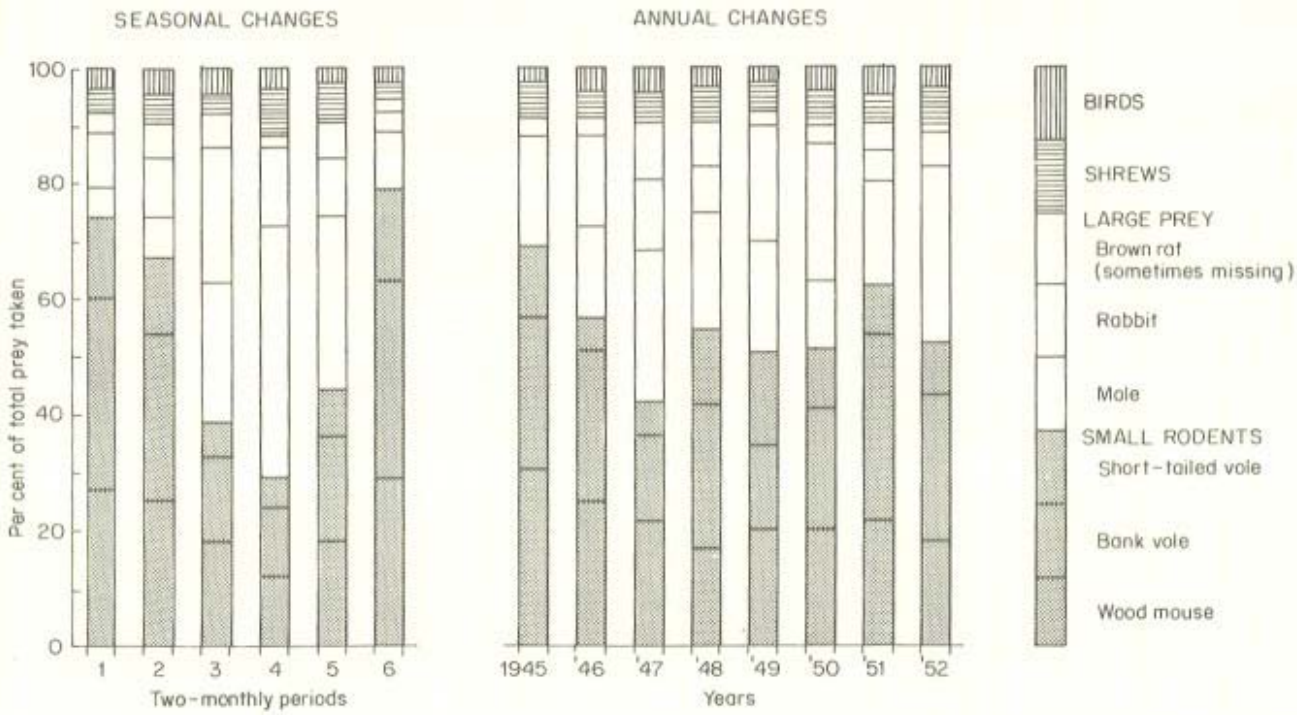

Figure 4.5.1 Variation between seasons and years in the prey taken by tawny owls (*Strix aluco*) during eight years observations in Wytham estate, Oxford. The two-monthly periods numbered 1–6 refer to January–February (1), March–April (2) and so on. (from Southern, 1954)

elimination caused by growth dilution. For reliable predictions and for interspecies comparison it should be noted that k_2 may be species dependent and that the influence of growth dilution must be removed. In view of the importance of the depuration constant k_2 for estimating bioconcentration factors and for the characterization of the biomagnification phenomenon, it seems necessary to determine k_2 with a higher level of precision. Better precision has been obtained through accumulation experiments with marine animals that are fed precisely measured amounts of the compounds on food particles, which subsequently are completely swallowed by the animal (Ernst and Goerke, 1974; Goerke and Ernst, 1977; Goerke, 1979). Animals are held on limited food rations such that body weights are kept constant and the problem of growth dilution is eliminated. The oral route of chemical application has, furthermore, the advantage that both highly volatile and insoluble substances can be introduced into the animals without the need for correction for losses from the ambient medium.

Unless complete recordings of size, source, lipid contents, the conditions and protocols used in bioconcentration tests are available, it is impossible, or at least very difficult, to ascribe the differences in the reported values to specific factors. Differences have been repeatedly reported, for example differences due to sex in fish (Defoe *et al.*, 1978), source of the fish (Veith *et al.*, 1979), and species (Veith *et al.*, 1979) (for additional details see Table 4.4.2).

4.4.3.2 Use of Physico-chemical Data to Predict Bioconcentration Factors

When testing uptake and loss of lindane, dieldrin and DDT in bluegills (*Lepomis macrochirus*) and goldfish (*Carassius auratus*), both processes were found to be related to water solubilities of the compounds (Gackstatter and Weiss, 1967). The inverse relationship of water solubilities with EM (ecological magnification factors as measured in a model ecosystem) and BCFs have been determined by various authors and can be expressed by the equations:

$\log \mathrm{EM} = -0.4732 \log S + 4.4806$	(Metcalf *et al.*, 1975) (mosquito fish, whole)
$\log \mathrm{EM} = -0.3891 \log S + 3.995$	(Lu and Metcalf, 1975) (mosquito fish, whole)
$\log \mathrm{BCF} = -0.523 \log S + 4.53$	(Ernst, 1982) (common mussel, whole tissue)
(S = water solubility in μg/l)	
$\log \mathrm{BCF} = -0.508 \log S + 3.41$	(Chiou *et al.*, 1977)
(S = water solubility in μmol/l)	(trout, muscle)

Besides solubility data, the *n*-octanol : water partition coefficients of organic compounds are used as a measure for bioconcentration in animal tissues,

according to the following equations:

$\log BCF = 0.542 \log P + 0.124$	(Neely *et al.*, 1974) (trout, muscle)
$\log BCF = 0.85 \log P - 0.70$	(Vieth *et al.*, 1979) (fathead minnows, whole)
$\log BCF = 0.74 \log P - 0.535$	(Ernst, 1984) (common mussel, whole tissue)
$\log EM = 0.635 \log P + 0.7285$	(Lu and Metcalf, 1975) (model aquatic ecosystem) (mosquito fish, whole)
$\log EM = 1.1587 \log P - 0.7504$	(Metcalf *et al.*, 1975) (mosquito fish, whole)
$\log BCF^{*}) = 0.980 \log P - 0.063$	(Könemann and van Leeuwen, 1980) (guppies, fat)

(log P = log partition coefficient *n*-octanol : water
*) = calculated on fat weight basis)

BCF–log P linear relationships in fish were reported by Veith *et al.* (1979) up to log P of 7 and by Neely *et al.* (1974) up to log P of 7.62. However, more recent observations suggest a non-linear relationship above log P ~ 6 (Sugiura *et al.*, 1978; Könemann and van Leeuwen, 1980) resulting in a break-down of the linear correlations between log P and log BCF. However, it should be noted that the determination of partition coefficients of highly lipophilic substances is very difficult and calculated log P values may be erroneous (Tulp and Hutzinger, 1978).

A comparison of the correlations reported by various authors cannot be made, since different species and ranges of compounds have been used. By far the most extensive data for one test species have been reported by Veith *et al.* (1979) using fathead minnows for the testing of 30 organic chemicals with log P values ranging from about 3 to 6.5. From their BCF–log P correlation, the log BCF can be estimated to within an order of magnitude from log P values. It should, however, be noted that some compounds, such as hexachlorocyclopentadiene and tris-2,3-dibromo-propylphosphate, did not fit into the correlations reported by Veith *et al.* (1979), presumably because of their unusual behaviour. On the other hand, the correlations are highly dependent on the quality of partition coefficient measurements, a factor which is not firmly established.

Although good correlations can be demonstrated between log BCF and the physico-chemical parameters for an array of compounds and for a specified group of test animals maintained most likely under the same conditions, a generalization of findings will not be possible unless all essential experimental factors are completely recorded.

Because of the above uncertainties, one has to be very cautious in the use of

BCF predictions from these correlations as a replacement for bioconcentration tests. At this time it seems that the estimation of bioconcentration factors from physico-chemical data may be justified for compounds having log P values of up to about 4.5 and water solubilities in excess of 500 $\mu g\ L^{-1}$. Both quantities correspond to a BCF of ~1000 in fresh tissues of fishes or mussels. However, the use of this technique within these ranges has to be better established in future studies before its universal application. Bioconcentration potentials as measured in laboratory tests or as calculated from physico-chemical data should, in final analysis, provide reliable estimates effectively translatable to real populations under natural environmental conditions.

4.4.4 BIOACCUMULATION

Bioaccumulation in nature involves both uptake of xenobiotics from water and from food. Various laboratory experiments have been reported which demonstrate the importance of the food vector. Possibly the fact that biomagnification factors associated with the food vector are very low when compared with BCF associated with the water vector has led to the controversy over the significance of biomagnification. Nevertheless, assuming a steady state concentration for a pollutant compound in the food organism, significant amounts of the compound can be incorporated into the predator at appropriate feeding rates.

The diversity and complexity of food chain relationships in addition to the varying physical and chemical conditions of the environment do not allow an exact quantitative description of substance transfers. In order to approach this problem of the contribution of biomagnification within the accumulation process, some simplifications may be adopted: (a) constant substance concentration in water; (b) same BCF-values for consumer and food; (c) quantitative absorption of the substance associated with food by consumer; (d) persistence of the compound and (e) a constant feeding rate. If contaminated food is the only source of the substance, the concentration of the compound in the predator may be described by:

$$\frac{dC'_A}{dt} = k_3 \cdot BCF_F \cdot C_W - k_2 \cdot C_A$$

where k_3 is the feeding rate constant (day^{-1}), C'_A is the level of compound in the consumer animal, and BCF_F is the bioconcentration factor for the food organism (uptake from water).

Thus

$$C'_{A_t} = \frac{k_3}{k_2} \cdot BCF_F \cdot C_W \cdot (1 - e^{-k_2 t})$$

at time t and a steady state

$$C'_{A_f} = \frac{k_3}{k_2} \cdot \mathrm{BCF_F} \cdot C_W$$

Maximum attainable levels at steady state conditions, considering both uptake from water and from food, will then be described by

$$C_A(\mathrm{S, F, W}) = \mathrm{BCF} \cdot C_W\left(1 + \frac{k_3}{k_2}\right)$$

For fish with a daily feeding rate of 2% of body weight ($k_3 = 0.02\ \mathrm{day}^{-1}$), the biomagnification factor k_3/k_2 for DDT ($k_2 = 0.02\ \mathrm{day}^{-1}$) is 1, i.e., uptake via food equals that from water. By contrast, for a compound exhibiting a high k_2, such as lindane ($k_2 = 0.8\ \mathrm{day}^{-1}$), a biomagnification factor k_3/k_2 of only 0.025 indicates that nearly no biomagnification might be expected. The maximum attainable level by magnification will be limited by the feeding rate constant k_3 and the elimination constant k_2 or half-life ($T_{1/2}$), respectively (Ernst, 1980). Considering only the first consumer level, significant biomagnification will be apparent for substances having $T_{1/2}$ of about 20 days or more, or k_2 values lower than 0.03 at feeding rates of 2–3% of body weight per day. Parallel conclusions were developed by Roberts *et al.* (1979). This trend of half-life dependence is confirmed by a number of analyses of marine samples and by laboratory experiments (Jarvinen *et al.*, 1977).

4.4.5 CONCLUSIONS

Bioconcentration factors can be determined in laboratory tests with reasonable precision if experimental conditions are strictly standardized with regard to the choice of test animals, species and sex; source of animals; maintenance of animals including temperature, feeding regime, water exchange, age, water : biomass ratio; substance concentration including additives; water quality; mode of application. Correlations observed between log BCF and *n*-octanol : water partition coefficient have been established using various methods of exposure suggesting partitioning of the compounds between tissue components and water. Inconsistent results reported in the literature, some of which are presented in Table 4.4.2, may be due to the differences of one or more of these parameters.

It is also necessary to define the ecosystem for which bioconcentration processes have to be predicted. In a river, for example, the parent compound may be thoroughly degraded or modified by chemical reactions or metabolic transformation by microbes and higher plants; and there may be changes in the compound's bioavailability due to adsorption to particulate matter and bottom sediments. Furthermore, fluctuations in the bioavailable concen-

tration of the substance in question may cause severe discrepancies in predicted data, especially for those substances having small k_2-values. These observations also hold true for estuaries; however, having entered oceanic environments, many compounds are present at comparatively constant concentrations in a defined area due to hydrographic relationships and a drastic reduction of the microbial degradation activity. Thus, this environment approaches closely 'ideal' conditions for assessing bioaccumulation patterns.

More precise predictions of total accumulation require the consideration of biomagnification potential at least for compounds having low elimination rate constants, and of other ecological parameters such as species specific feeding behaviour and bioenergetic data. For bottom living animals, sediments are likely to be the source of pollutants and their effects should be assessed. Bioenergetics-based models allow the description of pollutant accumulation if metabolic rates and factors affecting metabolism are known; for example, in fish, body size, growth rate and ambient temperature have to be considered (Norstrom *et al.*, 1976).

For marine organisms bioconcentration results from laboratory tests for those compounds with low BCF, such as δ-HCH, γ-HCH and pentachlorophenol, are comparable to levels occurring under natural environmental conditions (Ernst *et al.*, 1982). For PCBs, similar relationships are suggested from environmental analyses. Biomagnification may be significant in this case but obviously does not have an influence on the order of magnitude of the levels found in organisms in their natural environment.

Standardization of laboratory procedures and verification experiments under natural conditions will be essential in future work.

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