CHAPTER 3

Biotic Processes

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3.1. GENERAL PRINCIPLES OF STRUCTURAL CHANGES OF ENVIRONMENTAL CHEMICALS

In evaluating the enzymatic attack of organisms on environmental organic chemicals, the following situations should be considered: the chemical is easily digested and mineralized without persistent or biologically active intermediates, or it is degraded to low molecular weight fragments circulating in the carbon pool, or it is chemically altered by co-metabolism, without significant breakdown to products joining the natural carbon pool. This chapter is concerned mainly with the last situation since, besides the effects of the parent compounds on organisms and ecosystems, the potential effects of the conversion products have also to be considered.

Evaluation is required for metabolic pathways as well as for the amounts and persistence of the metabolites formed. In general, the principles of structural changes in xenobiotics by enzymes are the same as those of natural compounds.

As examples for enzymatic transformations of inorganic compounds, the metals mercury, lead, and tin are discussed. The transformation of inorganic mercury to methylmercury in biological and related systems (Wood *et al.*, 1968; Jensen and Jernelov, 1969; Neujahr and Bertilsson, 1971; Imura *et al.*, 1971; Landner, 1971) including man (Edwards and McBride, 1975) is well documented. The formation of the volatile dimethylmercury (Wood *et al.*, 1968; Jensen and Jernelov, 1969; Imura *et al.*, 1971), the formation of diphenylmercury from phenylmercury by several soil and aquatic microorganisms (Matsumura *et al.*, 1971), the decomposition of methyl-, ethyl-, and phenylmercury to elemental mercury and the corresponding hydrocarbon by mercury-resistant pseudomonas bacteria (Furukawa *et al.*, 1969), and the vaporization of mercury by activated sludge when treated with mercuric chloride and phenylmercury (Yamada *et al.*, 1969) have all been reported (Fishbein, 1974). For methanogenic bacteria, it was shown that the methylation proceeds via the transfer of a methyl group from a Co^{3+} atom bound in a complex organic molecule, methylcobalamine (a methylated form of vitamin B12; Wood *et al.*, 1968). The methylation of lead has also been observed (Wong *et al.*, 1975). The formation of monomethylmercury is accompanied by an increase of toxicity ('activation'). Furthermore, this derivative may enter biological cycles (Gavis and Ferguson, 1972; Wood, 1974) and may be accumulated in food chains (see also Chapter 5). For lead (Zeman *et al.*, 1951; Cremer, 1959) and tin (Cremer, 1957, 1958), the conversion of the tetraethyl to the triethyl metal has been shown, and also results in an increase of toxicity.

For organic chemicals, primary changes, mainly oxidative, hydrolytic, or reductive, may be accompanied by an increase in toxicity ('activation') or by a decrease ('detoxication') (Klein and Korte, 1970). They are often followed by secondary changes of the primary conversion products, e.g. by alkylation, acetylation, conjugation, or binding with biological molecules. In animals, the secondary processes are frequently accompanied by detoxication through conjugation which results in water-soluble molecules easily eliminated from the body. In plants, detoxication is achieved by fixation of the xenobiotic substances within natural macromolecular structures like cell wall components (Kaufman *et al.*, 1976). This results in the so-called 'unextractable residues', i.e. the incorporated xenobiotic cannot be extracted from the tissue without destruction of the macromolecular bonds. In soil, even more complex reactions occur; the xenobiotic may be involved in humus formation and replace natural constituents in the humic acid macromolecule (Hsu and Bartha, 1976). Oxidative processes are the commonest enzymatic changes of xenobiotics. Mixed-function oxidases are the enzymes generally involved (Mason, 1957; Brooks, 1972). Hydroxylation of aliphatics and aromatics, epoxidation and oxidative cleavage of double bonds, oxidation of phosphorothionates to phosphates, of hydroxyl to keto groups, of thioethers to sulphoxides and sulphones, dehydrogenations, etc., are well known. Some of these

processes, like the epoxidation of cyclodienes, the oxidation of phosphorothionates to phosphates, or the hydroxylation of polychlorinated biphenyls (Yamamoto and Yoshimura, 1973), result in a biological activation. Plant tissues are rich in peroxidase, and the abundance of phenolic products in higher plants suggests generally high oxidase activity (Brooks, 1972), with the result that the occurrence of metabolites with increased toxicity in plants must be taken into account; on the other hand, oxidative processes may lead to a stepwise degradation of the foreign compound. For instance, for cyclodienes (aldrin, isodrin, heptachlor), epoxidation or hydroxylation of the double bond is followed by ring cleavage and by loss of carbon atoms upon decarboxylation (see also paragraph 3.3. iib). Certain microorganisms achieve a ring cleavage of chlorinated aromatics (Furukawa and Matsumura, 1976).

Hydrolytic conversions, e.g. by esterases and amidases which are widely distributed in nature, are also well known, as, for example, organophosphorus compounds, carbamates, aliphatic esters (e.g. 'kelevan'; Sandrock *et al.*, 1974), cyclodiene epoxides, or urea derivatives. The recently discovered degradation of phenylurea herbicides (monolinuron, buturon) to carbamates in plant cultures could be due to the enzyme urease. Since, in hydroponic plant experiments, this conversion was observed to be higher in nitrogen-deficient systems than in normal nutrient media, there arises the question whether plants or microorganisms are able to consume the side-chain nitrogen of the herbicides and excrete the remaining moiety of the molecule as carbamate into the nutrient medium (Haque *et al.*, 1977a).

Reductive conversions are known for xenobiotics containing nitro groups (e.g. the fungicide pentachloronitrobenzene) which are reduced to amines, containing aldehydes and ketones, and containing chloro groups (reductive dechlorination, e.g. DDT → DDD).

The secondary processes in animals, especially conjugations with sulphuric acid, glucuronic acid, or glutathione, which lead to the excretion of the foreign compound, may be regarded as a pathway to elimination for the individual organism but not for the ecosystem of which it is a member, since the xenobiotic continues to exist there and may be taken up by other members of the system. This applies also to the 'unextractable residues' in plants, but very little is known of their biological availability, i.e. whether they are taken up by animals. Even when the xenobiotic is fixed in the soil as a constituent of the polymerizate of humic acids, the possibility that it may become available again for plant uptake cannot be excluded, since the humic acids are constantly undergoing biosynthesis and degradation. Further work on this topic is urgently needed (see also 3.3. iie).

3.2. ORGANOTROPISM

The uptake of a chemical and its distribution within the animal body, its accumulation, remobilization, and excretion are strongly dependent on chemical and biological factors, one of which is the structure of the chemical. Since chemical

alterations may occur by metabolism, not only the organotropism of the parent compound, but also that of the metabolites which often have different physical-chemical properties, must be considered.

(i) Time-course Studies

In order to investigate the time-course of organ distribution, three ^{14}C-labelled model compounds are discussed, 2,2′-dichlorobiphenyl was taken as a lower chlorinated constituent of the commercial PCB mixtures. Dieldrin was chosen as a representative of the cyclodiene group, and hexachlorobenzene as a representative of the chlorinated benzenes and as one of the chemicals most persistent in the environment. The experiments were conducted by oral administration to rats (Iatropoulos *et al.*, 1975).

2,2′-Dichlorobiphenyl is rapidly absorbed from the upper gastrointestinal tract and is transported to the liver mainly by the portal venous system with some participation of lymphatic transport. In the liver it is metabolized rapidly (Milling *et al.*, 1975) and excreted into the intestinal tract without ever reaching the storage depot of adipose tissue.

Dieldrin, like dichlorobiphenyl, is absorbed rather rapidly from the intestinal tract and is transported to the liver mainly through the portal venous system, reaching a peak concentration within the first hour. As the metabolic conversion of dieldrin is much slower than that of dichlorobiphenyl, only part of it can be metabolized and excreted, the major amount being redistributed into the storage depot of adipose tissue. During this redistribution process, the lymphatic system seems to be a major transport pathway; the parallel increases of the contents of lymph nodes and adipose tissue indicate an equilibrium between lymph and depot fat.

Hexachlorobenzene is absorbed more slowly than dichlorobiphenyl or dieldrin; the portal venous transport to the liver seems to be a minor pathway because in spite of its extremely slow metabolic conversion (Rozman *et al.*, 1975), hexachlorobenzene never builds up to high concentrations in the liver. The major part of the ingested hexachlorobenzene is absorbed by the lymphatic system in the region of the duodenum and jejuno-ileum and deposited in the fat, bypassing the systemic circulation and the excretory organs. As with dieldrin, the comparison of lymph nodes and adipose tissue contents indicates an equilibrium between lymph and fat.

(ii) Effects of Chemical Structure on Distribution Between Organs

As examples of the influence of chemical structure on the distribution of residues between organs, the results of the following studies with six radiolabelled chemicals are presented. The chemicals were given daily orally to rats in long-term feeding experiments. 2,2′-Dichlorobiphenyl, 2,5,4′-trichlorobiphenyl, and

2,4,6,2′,4′-pentachlorobiphenyl, three PCB isomers, were chosen to study the influence of chlorine content on organ distribution. Chloroalkylene-9, an isopropylated 2,4-dichlorobiphenyl which was developed as a PCB substitute, was included to observe possible additional effects of the side-chain. Endrin was selected as a representative of the cyclodiene insecticide group, and 'Imugan', a dichloroaniline-derived fungicide, as a so-called 'non-persistent' chemical.

The daily dose was about 1–2 μg/g in the diet, corresponding to 32–75 μg/animal. The application was carried out until a plateau level of accumulation was reached in the body, i.e. until the daily administered dose was daily excreted. Tissue analysis was performed by counting of total radiocarbon 1–3 days after the application was discontinued (exception: in the experiment with 2,4,6,2′,4′-pentachlorobiphenyl, the feeding was discontinued before a plateau level was reached since the formation of a plateau would have taken a very long time). Table 3.1 shows the results of tissue analysis (Klein *et al.*, 1968; Lay *et al.*, 1975; Begum *et al.*, 1975; Kamal *et al.*, 1976b; Lay, 1976; Viswanathan *et al.*, 1976).

The table indicates the variation of tissue concentration with chemical structure. All substances applied are lipophilic and, therefore, should be more concentrated in the fatty tissues. However, this fact was observed only for the medium or higher chlorinated biphenyls and for chloroalkylene (columns 1–3). These three substances show, besides their tendency to be accumulated in the abdominal and subcutaneous fat, also generally a higher level in the other organs than the substances in columns 5 and 6. These results are a consequence of increased chemical stability which prevents catabolism to more hydrophilic products and results in an increased general body concentration. First of all, 2,4,6,2′,4′-pentachlorobiphenyl which is one of the less degradable PCBs (Schulte and Acker, 1974), has the highest tissue concentrations although the experiment was discontinued before the saturation level was reached. The radioactivity in the organs was found to be the unchanged parent compound (exception: liver and kidneys). Additionally, the low excretion rate as well as the relatively low percentage of metabolites in the excreta (Lay *et al.*, 1975) demonstrate that this substance is only slowly metabolized.

Endrin (column 4) is susceptible to metabolic attack by hydroxylation which results in metabolites of increased water solubility. Consequently, the highest residues are not in the abdominal fat but in blood and spleen. In this respect, endrin is not typical of the cyclodiene group. 2,2′-Dichlorobiphenyl and 'Imugan' also give the highest residues in blood and liver respectively. This is in good agreement with their excretion rate and the high percentage of metabolites in the excreta (nearly 100%).

(iii) Differences Between Animal Species

Hexachlorobenzene-^{14}C was administered orally to rats and to rhesus monkeys (about 0.5 mg/kg body weight; Rozman *et al.*, 1975).

Table 3.1 Residues of Environmental Chemicals and Metabolites in Tissues and Organs of Rats after Long-term Feeding (1–2 ppm) was Discontinued. (Normalized to unit concentration of 'Imugan'-^{14}C in muscle, 0.003)

Organ	2,4,6,2′,4′-Pentachloro-biphenyl-^{14}C	2,5,4′-Trichloro-biphenyl-^{14}C	Chloro-alkylene-9-^{14}C	Endrin-^{14}C	2,2′-Dichloro-biphenyl-^{14}C	'Imugan'-^{14}C
Liver	2,040	150	85	90	37	60
Lungs	4,000	120	45	100	40	44
Kidneys	4,000	230	150	100	77	18
Skin and sub-cutaneous fat	1,900	100	100	250	10	14
Blood			30	370	87	9
Abdominal fat	8,700	330	170	130	27	7
Stomach + duodenum		150	35	220	40	6
Spleen		100	45	1,000	50	6
Heart	2,100	130	30	190	80	4
Genitals	4,200	130	90	320	20	3
Brain		20		83	10	1
Muscles	530	60	20	20	10	1

In this experiment, the differences of total radiocarbon content between tissues of rats and rhesus monkeys were small. In some tissues, the concentration was slightly higher for the rhesus monkey. This corresponds to the lower excretion rate of this animal as compared to the rat (Rozman *et al.*, 1975).

However, there may exist differences in the chemical nature of organ residues for different species. For instance, dieldrin is preferably converted to 12-hydroxydieldrin in rats and primates including man (Richardson and Robinson, 1971), but preferably to aldrin-*trans*-diol in mice and rabbits (Müller *et al.*, 1975). The rate of excretion of the diol was by far the highest in the mouse, which must necessarily result in relatively high concentrations of the diol in the mouse liver.

(iv) Sex Differences of Organ Distribution

For the data listed in Table 3.1 and in Rozman *et al.* (1975) differences between male and female animals were small. However, larger differences due to different accumulation capacities are possible. In a long-term feeding experiment with aldrin-^{14}C, females reached the plateau level of body concentration only after 200 days, males after 53 days (Ludwig *et al.*, 1964). This results in a much higher concentration in the abdominal fat at plateau time (females 3.5 μg/g, males 0.29 μg/g, when fed 0.2 μg/g of diet).

3.3. METABOLIC BALANCE OF ENVIRONMENTAL CHEMICALS

(i) *In vitro*

Whereas *in vivo* experiments give information on the balance of distribution and conversion for the whole organism, *in vitro* experiments give information on the possible primary enzymatic attacks on xenobiotics. *In vitro* experiments may be carried out with cell cultures or with various cell fractions, preferably from rat liver or kidney. Experiments with perfused rat liver may accomplish these studies and, when compared with *in vivo* studies, help to localize the site of metabolic attack for the substance in question.

The metabolites detected *in vitro* may not in all cases be the same as those formed *in vivo*. In the isolated cell fractions, metabolic reactions may occur which are not possible in the intact organism. There, competitive reactions may reduce or even prevent the reaction observed *in vitro*. Additionally in the intact organism the primary product formed by a cell fraction may be altered immediately by secondary reactions. The following examples demonstrate the non-applicability of *in vitro* experiments to living animals.

For 2,2′-dichlorobiphenyl, all four theoretically possible monohydroxy derivatives and four dihydroxy derivatives were found *in vitro* in rat cell fractions (Greb *et al.*, 1975a); *in vivo*, however, only three monohydroxy isomers were detected one of which occurred only in very small amounts, and only three dihydroxy

isomers (Kamal *et al.*, 1976a). The only metabolite formed *in vitro* from the cyclodiene insecticide α-(*trans*)-chlordane was dehydrochlordane (Spitzauer, unpublished); *in vivo*, however, the epoxide and hydroxylated products were the major conversion products (Schwemmer *et al.*, 1970; Poonawalla and Korte, 1971; Barnett and Dorough, 1974). In human fat, the epoxide was detected (Biros and Enos, 1973).

These examples show that *in vitro* experiments are suitable for rapid preliminary tests, but that their interpretation should be cautious and that the evaluation of their significance should be made only in conjunction with *in vivo* experiments.

(ii) *In vivo*

(a) Microorganisms

The conversion and degradation of xenobiotics by microorganisms has been the topic of laboratory investigations for many years. Figure 3.1 shows the comparative conversion of model pesticides by the fungus *Aspergillus flavus* (Korte, 1968).

A large number of such studies has been and is being done, and they are usually part of registration procedures for the use of pesticides (Sanborn *et al.*, 1976). For dieldrin, Matsumura and Boush (1967; Matsumura *et al.*, 1968) have found several soil microbial strains capable of degrading dieldrin significantly (Figure 3.2).

However, these results could not be confirmed by using cultures of normal or of dieldrin-contaminated soils, nor by any other microorganisms tested (Vockel and Korte, 1974).

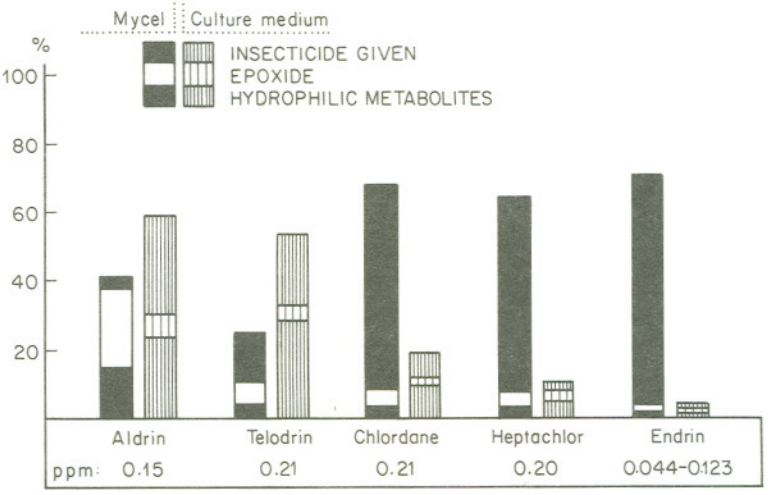

Figure 3.1 Metabolism of cyclodiene insecticides by *Aspergillus flavus*. (Reproduced by permission of Schweizerischer Chemiker-Verband from Korte, 1968)

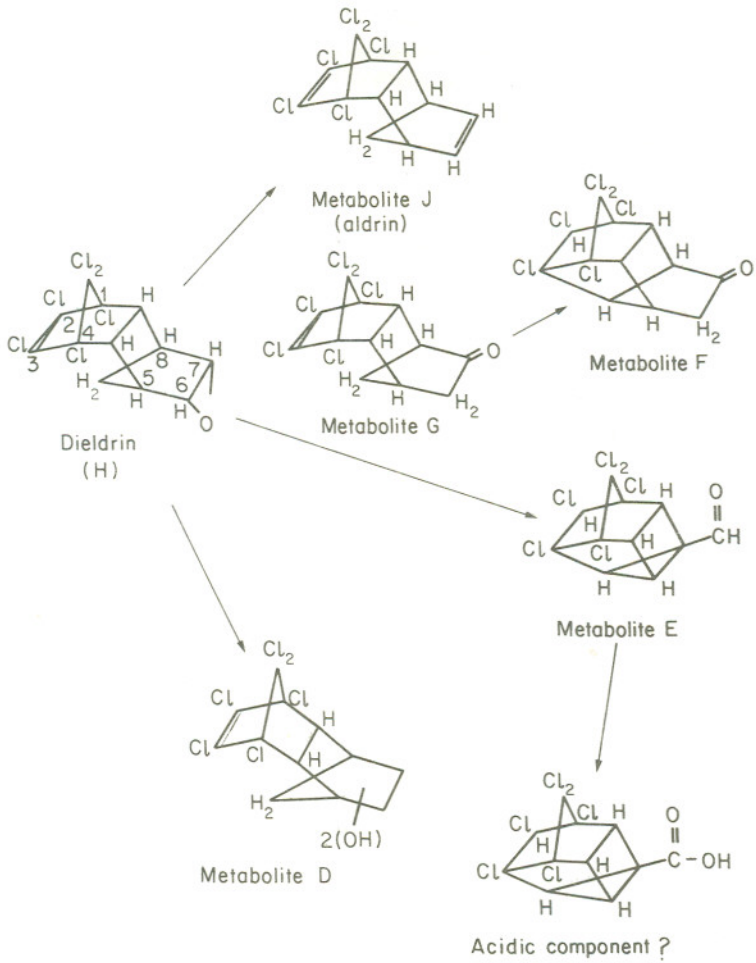

Figure 3.2 Metabolites of dieldrin from microorganisms. Reproduced by permission of Macmillan Journals Ltd., from Matsumura *et al.*, 1968)

Two types of research into microbial degradation of pesticides are of practical importance.

(a) The development of adapted microbial strains that can use pesticides as the sole source of carbon. These can be used in industrial sewage treatment to remove pesticides such as chlorinated phenols which kill the normal bacterial populations.

(b) The identification of chemical intermediates that should be analysed for in environmental samples.

Regulatory agencies frequently require the testing of a pesticide and whole soil reacting together. Under properly controlled conditions such tests yield qualitative information about the nature of metabolites but little quantitative data about the capacity for degradation. They are, however, useful for estimating the relative persistence of pesticides in soil. More information about the environmental impact of pesticides should be obtained from field experiments under outdoor conditions (see 3.3. iie).

The capability of thermophilic microorganisms involved in composting to degrade so-called persistent environmental chemicals present in waste has been investigated by laboratory simulation. It was shown that, during the composting process, most of the organic chemicals tested remained largely unchanged (Müller and Korte, 1974; Müller *et al.*, 1974; Müller and Korte, 1975). Among these were dieldrin and various polychlorinated biphenyls, although degradation of PCB to benzoic acids by *Alkaligenes* sp. was demonstrated to occur (Furukawa and Matsumura, 1976).

(b) Plants

Since in the normal environment, plants grow in soil and form an ecosystem with the soil organisms, it is difficult to investigate plant metabolism in isolation. *In vitro* studies with plant enzymes may give preliminary information on possible

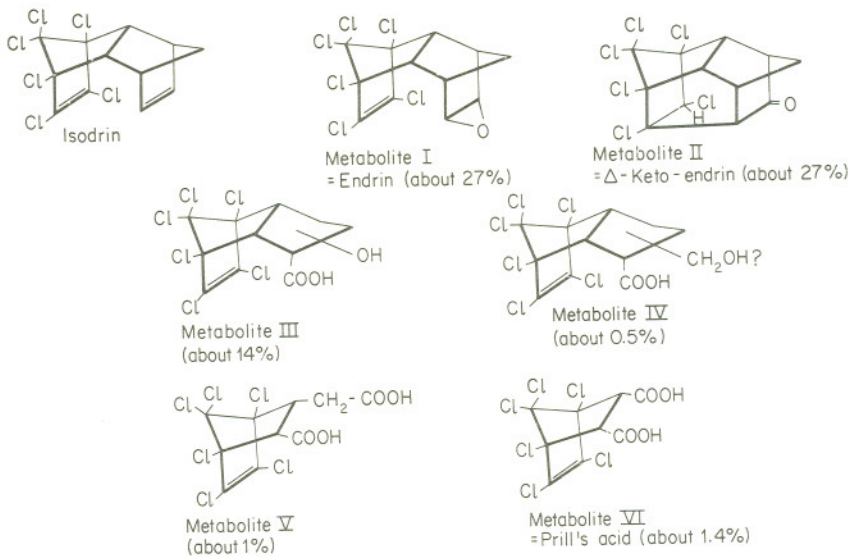

Figure 3.3 Metabolites of isodrin in cabbage. (Reprinted with permission from *Chemosphere*, **4**(2), 99–104. Weisgerber, I., Tomberg, W., Klein, W., and Korte, F. Ecological chemistry. XCV. Isolation and structure elucidation of hydrophilic carbon-14-labelled isodrin metabolites from common cabbage. © 1975, Pergamon Press, Ltd.)

metabolic reactions, but for their interpretation, the same problems are encountered as for animal enzyme investigations (see 3.3. i). In hydroponic culture, the influence of microorganisms can be eliminated by running a blank with the nutrient medium without plants. Plants grown in normal soil can also give good information about plant metabolism when the xenobiotic is applied on the leaves, since the transport from the leaf surface to the soil via roots is very small within the normal laboratory test time.

Figure 3.3 shows the conversion of the cyclodiene isodrin by cabbage plants after foliar treatment. This substance shows how, by means of stepwise oxidation, the molecule is degraded to Prill's acid, a substance with three carbon atoms less than the parent compound (Weisgerber *et al.*, 1975b). This example demonstrates the importance of plant oxidases, mentioned in paragraph 1, for the degradation of xenobiotics in the environment.

Similar experiments with heptachlor showed also, besides epoxidation and hydroxylation, oxidative ring cleavage leading to Prill's acid (Weisgerber *et al.*, 1974a).

(c) Insects

Since insects are the target organisms of insecticides, their metabolism has been widely investigated. All reaction mechanisms discussed in the first paragraph of this chapter are known for insects.

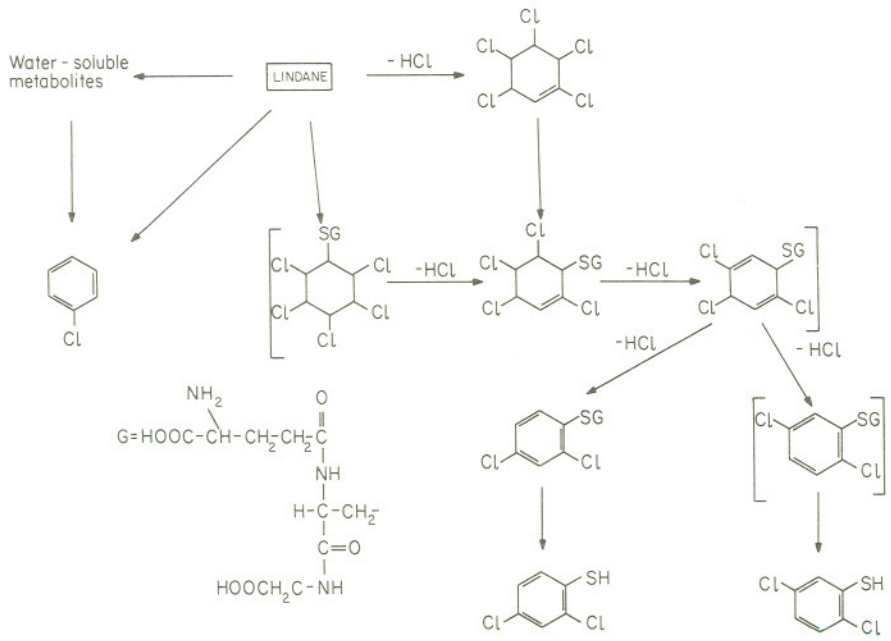

Figure 3.4 Metabolites of Lindane in insects. (Reproduced by permission of Springer-Verlag from Klein and Korte, 1970)

Figure 3.4 presents, as an example, the fate of Lindane in insects (Klein and Korte, 1970). It shows a stepwise dehydrochlorination of the molecule through the intermediacy of glutathione conjugation and results in aromatic compounds.

The problem of resistance to insecticides and its causes has been investigated in many studies (e.g. Klein and Korte, 1970) and will not be discussed here in detail. From the ecotoxicological point of view, it should be mentioned that this phenomenon will lead to an increase in the number and quantity of insecticides used, and, therefore, of effects on the ecosystem.

(d) Animals

Using dieldrin as an example, the dependence of metabolism and excretion by mammals on the animal species and its consequences for the effects of chemicals and for the extrapolation of animal experimental data to man will be discussed.

Dieldrin-^{14}C was given as a single oral dose of 0.5 mg/kg body weight to five animal species: mice, rats, rabbits, rhesus monkeys, and chimpanzees. Besides dieldrin, 12-hydroxydieldrin and 4,5-aldrin-*trans*-diol were found in the excreta; the formulae are shown in Figure 3.5, along with other, minor mammalian metabolites of aldrin. Table 3.2 shows the amounts of the three substances excreted by each animal species within 10 days, in per cent of administered dose (Müller *et al.*, 1975). In all species the faecal excretion of unchanged dieldrin was high in the first 48 hours and then declined rapidly, probably due to the completed excretion of unabsorbed dieldrin. The urine samples contained only metabolites and no dieldrin.

In all five species 12-hydroxydieldrin and 4,5-aldrin-*trans*-diol were the major metabolites. Regarding the ratio of the two metabolites, the rat seems to be comparable to the primates; direct oxidation resulting in the monohydroxy metabolite is their common main metabolic pathway. There is strong evidence that this is also true for man (Richardson and Robinson, 1971). In the mouse and the rabbit, on the other hand, the opening of the epoxide to the diol is the predominant reaction. These findings also demonstrate that, because of metabolism similar to that in man, the rat is the suitable experimental animal for dieldrin and not the mouse.

In fish, the occurrence of methylated metals like methylmercury is well known. However, information is limited on metabolic conversions of organic compounds. Enzymatic hydroxylation seems to be of minor importance in comparison with other vertebrates. For example, the hydroxylation of polychlorinated biphenyls detected for many organisms (summary: Klein and Weisgerber, 1976) has been reported to occur in fish only slowly (Melancon and Lech, 1976; Herbst *et al.*, 1976), or not at all (Hutzinger *et al.*, 1972).

(e) Ecosystems

The most important ecosystems involved in transformation and degradation of environmental chemicals are the plant–soil and the aquatic ecosystems.

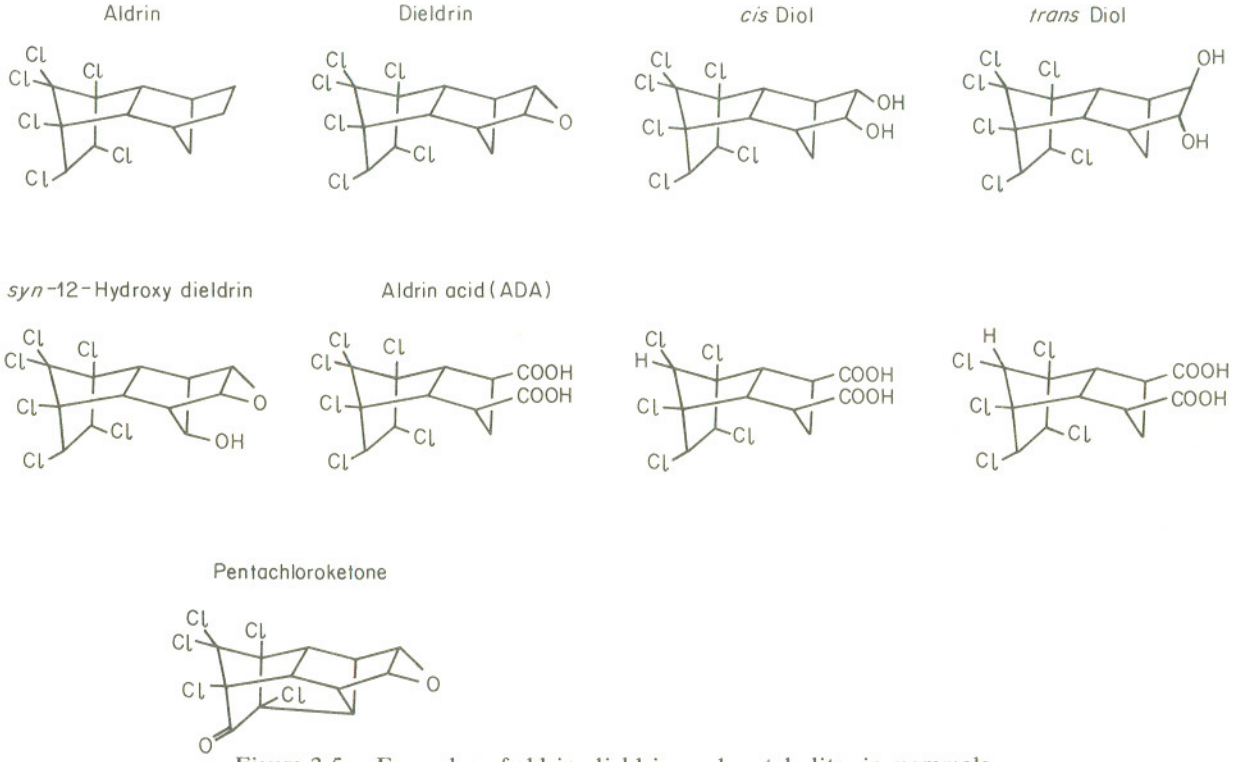

Figure 3.5 Formulae of aldrin, dieldrin, and metabolites in mammals

Table 3.2 Radioactive Material Excreted Within 10 Days After Single Oral Administration of 0.5 mg/kg Dieldrin-^{14}C (per cent of administered dose). (Reprinted with permission from *Chemosphere*, 4(2), Müller, W., Nohynek, G., Woods, G., Korte, F., and Coulston, F. Comparative metabolism of Carbon-14 labelled dieldrin in mouse, rat, rabbit, rhesus monkey and chimpanzee. © 1975, Pergamon Press, Ltd.)

	Mice		Rats		Rabbits		Rhesus	Chimpanzee
	male	female	male	female	male	female	male	female
Dieldrin	5.5	3.2	0.8	2.8	0.3	0.5	9.0	3.2
12-Hydroxy-dieldrin	13.0	7.5	8.8	4.6		0.2	9.4	2.0
Aldrin-*trans*-diol	20.0	26.0	2.3	2.4	1.5	2.0	2.0	1.1
Total	38.5	36.7	11.9	9.8	1.8	2.7	20.4	6.3
Faeces	36.6	35.0	11.3	9.3	0.3	0.5	16.0	5.0
Urine	1.9	1.7	0.6	0.5	1.5	2.2	4.4	1.3

For the plant–soil system the metabolism of environmental chemicals is characterized by uptake of chemicals by plants from soil, distribution within plants and soil, conversion reactions in soil and plants, residue loss by evaporation, leaching, mineralization, or assimilation, and residue fixation in plant or soil macromolecules.

Laboratory tests on the uptake of chemicals from soil by plants are not predictive for the environment, since the uptake is much higher than in the environment when small laboratory pots are used. Under open-air conditions, the uptake of residues by plants is less than one per cent of residue present for the cyclodiene insecticides and other chemicals tested. Although there is strong evidence that plants actively contribute to the disappearance of residues from soil, the reduction of soil burden by harvesting is negligible.

When lysimeters of 60 x 60 x 60 cm size are used under outdoor conditions with ^{14}C-labelled model substances, the quantitative data on uptake and residues in plants and soil are comparable to field data and, therefore, relevant for practical evaluation.

For the conversion of environmental chemicals by the plant–soil system, two examples are presented: the degradation of aldrin in Figure 3.6 (Klein *et al.*, 1973;

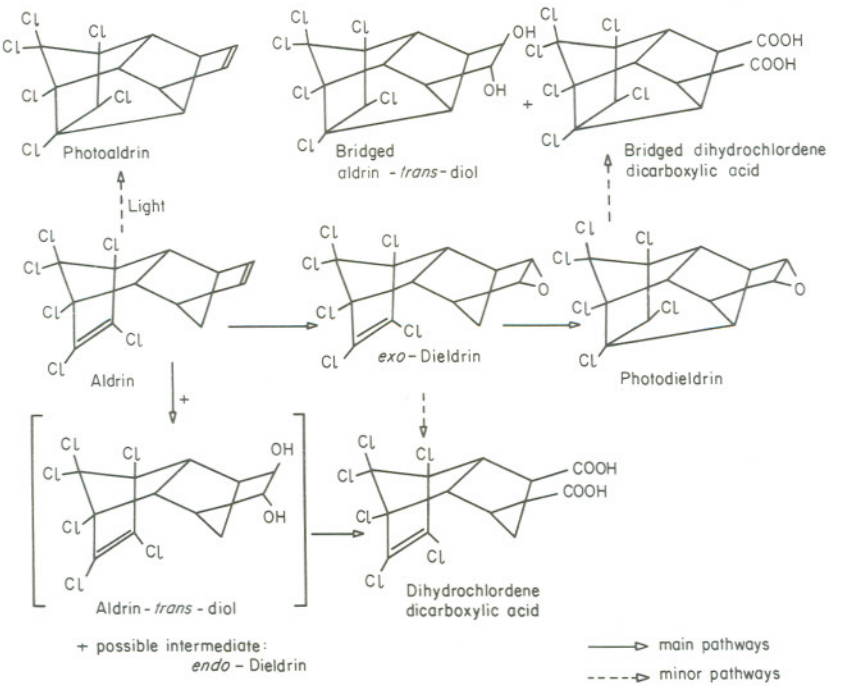

Figure 3.6 Degradation pathways of aldrin in the plant–soil system. (Reproduced by permission of Academic Press, Inc. from Scheunert *et al.*, 1977)

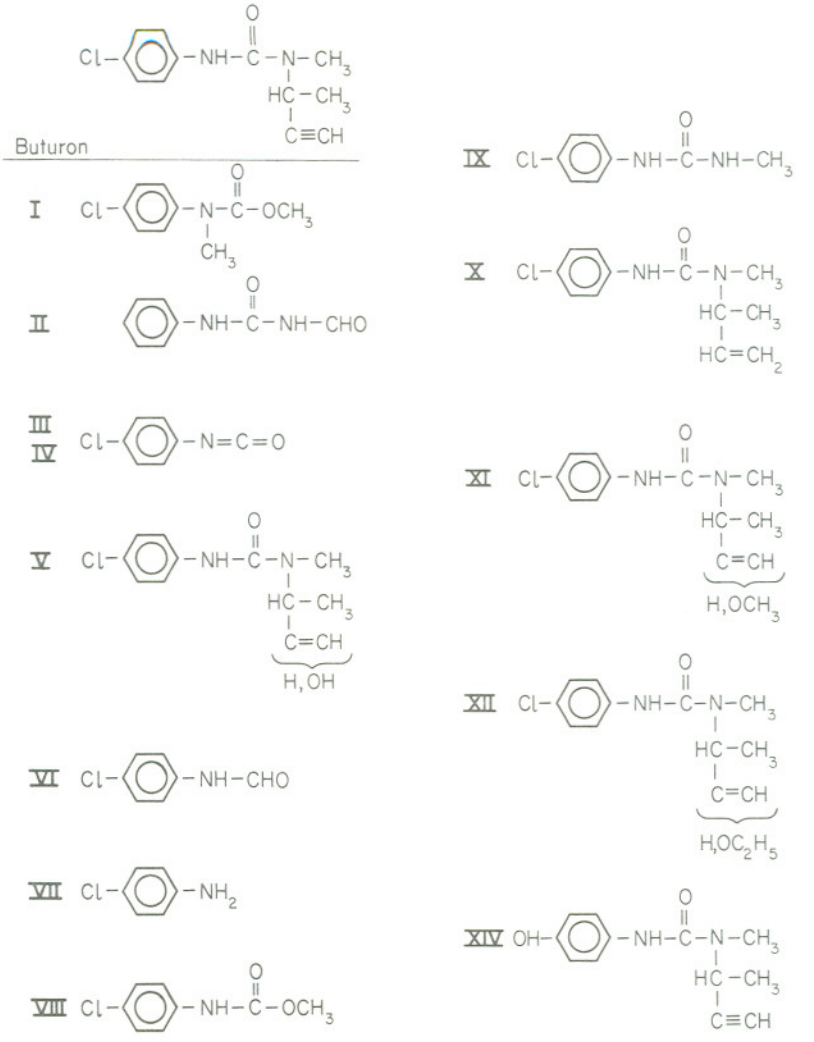

Figure 3.7 Conversion products of buturon in the plant–soil system. (Substances I–IX in wheat plants, I, V, VII–XII in soil, I, VI, XIV in leaching water.) (Reprinted from Hague *et al.*, 1976, by courtesy of Marcel Dekker, Inc.)

Weisgerber *et al.*, 1974b; Kilzer *et al.*, 1974; Weisgerber *et al.*, 1975a), and the conversion products of the phenylurea herbicide buturon in Figure 3.7 (Haque *et al.*, 1976; Haque *et al.*, 1977b).

The substances shown in the two figures are the products of complex combinations of agents like plant enzymes, soil microorganisms, and abiotic effects.

The conversion products in plants may originate from plant metabolism as well as be formed in soil by microorganisms or by abiotic reactions and be taken up by plants. Although the formation mechanisms of individual conversion products cannot be elucidated in detail, the results reported here are significant as they give a realistic description of the nature of residues that must be expected under field conditions and, above all, in human food.

When looking at the material balance of the system after five years, in the case of aldrin the significance of soluble metabolites seems to decrease. After this time, the only important soluble residue is dieldrin; the 'unextractable residues' have increased and are of higher importance than in the first years after application (Tables 3.3 and 3.4).

Furthermore, Table 3.3 demonstrates that within five years half of the radioactivity applied was lost by volatilization. The identity of the volatilized radioactivity was not investigated in this study (Weisgerber *et al.*, unpublished). Thus volatilization is a major pathway for residue loss in ecosystems, and the uptake by plants and the leaching by water are of minor importance. For chemicals other than aldrin, the trend seems to be comparable (e.g. polychlorinated biphenyls; Moza *et al.*, 1976). Little is known of the chemical nature and the fate of volatilized residues; they may be the unchanged compounds, conversion products, or carbon dioxide resulting from total mineralization. Also, information on the 'unextractable residues' is very limited (see also paragraph 1). They may be conversion products bound to macromolecules (lignin, humic acids), or copolymerized with the natural monomers of macromolecules, or they may be ^{14}C reassimilated from $^{14}CO_2$ or its low molecular weight precursors. Further work is required on the possibility of remobilization of these residues and their uptake by plants.

Table 3.3 Material Balance in Plants and Soil Five Years After Application of Aldrin-^{14}C to Soil

Applied in 1969:	103 mg (100%)
Residue in soil 1973:	34.5%
Taken up by plants 1969:	0.1%
Taken up by plants 1970:	0.2%
Taken up by plants 1971:	0.1%
Taken up by plants 1972:	0.1%
Taken up by plants 1973:	0.1%
Leaching water 1969:	1.6%
Leaching water 1970:	4.9%
Leaching water 1971:	2.4%
Leaching water 1972:	1.3%
Leaching water 1973:	0.4%
Recovery after 5 years:	45.7%
Volatilization within 5 years:	54.3%

Table 3.4 Residues of Aldrin-^{14}C and Conversion Products in Soil Five years After Treatment (% of applied radioactivity)

Sample	Aldrin	Metabolite X (unidentified)	Dieldrin	Photodieldrin	Extracted hydrophilic metabolites	Unextractable residue	Total
Soil depth 0–10 cm	0.8	<0.4	11.7	0.4	1.9	4.3	19.4
Soil depth 10–20 cm	0.4	<0.4	5.0	<0.4	1.2	1.9	8.9
Soil depth 20–30 cm	<0.4	<0.4	1.5	<0.4	0.4	1.2	3.1
Soil depth 30–40 cm	<0.4	<0.4	1.5	<0.4	0.4	1.2	3.1
Soil total	1.2	0.4	19.7	0.7	3.9	8.6	34.5

When the balance of metabolism of xenobiotics is to be studied in aquatic ecosystems, the simulation of stable aquatic ecosystems in the laboratory is indispensable. Systems consisting of water and microorganisms, of water, microorganisms, and sediment, of water, microorganisms, sediment, and plants, and also systems including higher animals and fish have been described. In tests with 2,2′-dichlorobiphenyl-^{14}C, 2,5,4′-trichlorobiphenyl-^{14}C, 2,4,6,2′,4′-pentachlorobiphenyl-^{14}C, and chloroalkylene-9-^{14}C, an isopropylated 2,4′-dichlorobiphenyl, the bioaccumulation was highest for chloroalkylene-9 (53.1% of the applied radioactivity in the biomass). This substance also had the highest conversion rate, in the biomass as well as in the water. The toxicity, also, was highest for chloroalkylene-9, especially for Daphnia.

From 2,2′-dichlorobiphenyl, three hydroxylated metabolites were identified in water and biomass, from 2,5,4′-trichlorobiphenyl, one hydroxylated metabolite. From chloroalkylene-9, a number of metabolites were detected in small amounts, six of which could be characterized. These were hydroxylated or methoxylated products or products with partially degraded side-chains. No metabolite could be isolated from the experiment with 2,4,6,2′,4′-pentachlorobiphenyl.

Mixed terrestrial-aquatic model ecosystems including sand with sorghum plants and a 'pond' with water, microorganisms, plankton, Daphnia, snails, algae, mosquito larvae, and fish, have been developed by several authors such as Metcalf (1974). When radiolabelled model chemicals were used, the determination of material balance was possible.

There are only a few reports on studies with radiolabelled compounds in natural-water bodies (see also paragraph 5; Salonen and Vaajakorpi, 1974). Since the radioactivity used has to be very low, an identification of metabolites is not possible; only the concentration of radioactivity is determined in water, sludge, and various organisms.

3.4. LABORATORY SCREENING TESTS

The above procedures to study balances and conversions of chemicals are rather complex and demanding. Therefore, a number of laboratory test methods have been elaborated to overcome such problems. Such test methods may involve the determination of physicochemical characteristics, biochemical determinations of specific results with a chemical, and predictive environmental experiments. Although many of these methods are quick and feasible and appropriate for certain kinds of environmental technology, their value for ecotoxicological evaluations, even on a chemical-to-chemical comparative basis, remains to be proven (see also 3.3. iia). Consequently they cannot be used at present to predict quantitatively the environmental fate of chemicals.

(i) Tests of Biodegradation

Biodegradation tests have been developed for sewage treatment and are related to the analysis of water pollutants by biological oxygen demand (BOD) and

chemical oxygen demand (COD) determinations. The OECD detergent test is widely used and gives results that can be extrapolated to sewage treatment conditions. More feasible flask methods, when used under standardized conditions, may, however, be as good (Zahn and Wellens, 1974). These tests normally include adaptation of the activated sludge for the investigated chemical, thus allowing prediction of degradation upon continuous or long-term exposure. For less polluted aquatic environments, rivers and lakes, these tests are less applicable and feasible, and direct analysis of sources and disappearance of chemicals by BOD, COD, total oxygen demand (TOD), or single compound analysis seems to be more appropriate. Whether laboratory tests, with trace concentrations of pollutants and all the difficulties of nutrients and blanks, allow a quantitative correlation to watersheds remains to be proven. In order to provide metabolic data for aquatic systems comparable to those given in section 3.3, more complicated experiments have to be carried out.

The same applies to standardized degradation tests with soil. Simple laboratory tests, frequently carried out with 100 g of soil, permit measurement of conversion and identification of metabolites but do not give data that are quantitatively related to terrestrial systems (see also 3.3. iia).

(ii) Tests of Bioconcentration

The difficulties of testing for biodegradation also apply to bioaccumulation testing. In addition there is the possible influence of other contaminants. For this reason it would be appropriate to measure the bioaccumulation factor of a certain fish species using river water and not 'clean water' to which was added one chemical only.

(iii) Significance of Screening Tests

For a comparative screening of environmental chemicals for biodegradation and accumulation, tests under standardized conditions and the determination of physicochemical characteristics such as partition coefficients are valuable but it must be emphasized that the sequence of chemicals found in these tests may not be identical to that in the environment.

Due to the limited significance of screening tests for quantitative ecotoxicological investigations of the behaviour of pollutants, no detailed discussion of procedures and results is given here.

One extreme example should be mentioned to underline this statement: In all laboratory degradation tests phthalate plasticizers show only low persistence. In the environment, however, the major amount of these chemicals after use is buried in landfills where they persist for decades and may be slowly released with leaching water.

3.5. FOOD CHAINS

Although the mass of the biota is minute as compared to the mass of the abiotic parts of the biosphere, the biota contain a proportionally greater amount of lipophilic foreign compounds, since these are concentrated in organisms. This bioconcentration in organisms is important for man since it serves as a pathway to human food. For DDT it has been roughly estimated that the biota contain about 0.2% of all DDT ever produced or about 3% of the annual production in the mid-sixties (Woodwell *et al.*, 1971). Provided this estimate is correct within an order of magnitude and also valid for other persistent environmental chemicals it may be concluded that transport and movement in the biota does not play a significant role in the global long-term dispersion of these chemicals. Consequently the global dispersion of organic and inorganic chemicals quantitatively is mainly based on physical and physicochemical factors and it may be easily understood that the global 'distribution patterns' of persistent organics and strontium-90 are alike (Appleby, 1970).

Bioconcentration of nutrients and trace elements from their environment is a general and basic activity of living cells and organisms (Table 3.5).

Nutrient elements are used for the biosynthetic production of the organisms so that this type of bioconcentration sustains life. In contrast bioaccumulation of xenobiotics in higher organisms may be explained as a type of elimination of non-metabolized lipophilic chemicals into internal sinks.

Table 3.5 represents concentration factors of nutrients as well as xenobiotic elements from sea water by plankton and brown algae (Bowen, 1966). The bioconcentration factor of xenobiotics depends on the one hand on the partition coefficient between the releasing (generally aqueous) and the uptaking (generally lipophilic) medium. On the other hand, species-specific factors are also included such as active absorption (penetration through membranes), and metabolic conversion and excretion.

More recent investigations into the accumulation in food chains revealed that higher concentrations of chemicals in organisms at higher levels of the food chains (both aquatic and terrestrial) may result from the slower rate of elimination in the higher levels of the chains (Moriarty, this volume, Chapter 8). On the other hand, there are natural toxins like the compound causing the ciguatera fish disease which is formed by blue-green algae and reaches an edible fish as the tertiary step in a food chain. This chemical is not concentrated in the fish from the aquatic environment (Russell, 1965).

(i) Environmental Data

Data from environmental monitoring of food chains for pollutants are mainly available for mercury, methylmercury, and organochlorine compounds. Most original publications, however, include only one step of the respective chains. As far

Table 3.5 Concentration of Elements from Sea Water by Plankton and Brown Algae[a]. (Reproduced by permission of Academic Press Inc., London – New York – San Francisco from Bowen, 1966)

Element	State in sea water	Plankton CF 1	Plankton CF 2	Brown algae CF 1	Brown algae CF 2
Ag	Anion	210		240	
Al	Particulate?	25,000		1,550	
As	Anion			2,500	200–6,000
Au	Anion?			270	
B	Molecule			6.6	
Ba	Cation?	120		260	
Be	Particulate?				1,500
Br	Anion			2.8	
Ca	Cation	5	10	7.2	0.5–10
Cd	Cation	910		890	
Cl	Anion	1		0.062	
Co	Cation?	4,600		650	450
Cr	Particulate?	17,000		6,500	300–10,000
Cs	Cation		1–5	33	1–100
Cu	Cation	17,000		920	100
F	Anion			0.86	
Fe	Particulate?	87,000	2,000–140,000	17,000	20,000–35,000
Ga	Particulate?	12,000		4,200	
Ge	Particulate?				15–200
Hg	Anion			250	
I	Anion	1,200		6,200	3,000–10,000
K	Cation			34	3–50
La	Particulate?			8,300	
Li	Cation			8?	

Mg	Cation	0.59		0.96	
Mn	Cation?	9,400	750	6,500	6,500
Mo	Anion	25		11	
N	Variable	19,000		7,500	
Na	Cation	0.14		0.78	1
Nb	Particulate?				450–1,000
Ni	Cation	1,700		140	500
P	Anion + organic	15,000		10,000	10,000
Pb	Cation?	41,000		70,000	
Ra	Cation	4,500	2,750	370	100
Rb	Cation			15	5–50
Ru	Anion?		600–3,000		15–2,000
S	Anion	1.7		3.4	10
Sb	Anion?		50		
Sc	Particulate?				1,500–2,600
Si	Particulate?	17,000		120	
Sn	Particulate?	2,900		92	
Sr	Cation	8	9	44	1–40
Th	Particulate?				10
Ti	Particulate?	20,000		3,000	
U	Anion?				10
V	Anion?	620		250	
W	Anion?			87	
Y	Particulate?				100–1,000
Zn	Cation	65,000	1,000	3,400	100–13,000
Zr	Particulate?		1,500–3,000		350–1,000

[a]CF = Concentration factor = ppm in fresh organism/ppm in sea water for the element concerned. CF 1 refers to Bowen (1966), while CF 2 refers to the compilation by Mauchline and Templeton (1964).

Table 3.6 DDT Concentrations in Samples Collected from the Pond, Following a Single Addition of 1 μg/l (ppm in organic matter[a]; seston = plankton + organic matter). (Reproduced by permission of the authors from Salonen and Vaajakorpi, 1974)

		30 days	59 days
Filtered water		0.00004	0.00001
Seston, 8 μm sample		0.0	0.0
Seston, 3 μm sample		0.0	0.0
Sediment		0.69 ± 0.02	0.71 ± 0.02
Bladderwort		1.98 ± 0.04	
Moss		0.38 ± 0.01	0.22 ± 0.01
Dragonfly larvae		0.20 ± 0.02	0.17 ± 0.01
Caddisfly larvae		0.43 ± 0.02	0.38 ± 0.02
Backswimmer		0.24 ± 0.02	
Bivalve mollusc		0.24 ± 0.03	0.17 ± 0.03
Newt		0.58 ± 0.03	
Perch:	muscle	0.44 ± 0.05	0.24 ± 0.03
	gills	3.73 ± 0.19	2.15 ± 0.08
	liver	2.79 ± 1.02	0.52 ± 0.02
	mes. adip.	17.20 ± 4.60	23.80 ± 6.60
Crucian carp:	muscle	0.10 ± 0.01	0.37 ± 0.04
	gills	0.33 ± 0.02	0.83 ± 0.03
	liver	0.09 ± 0.01	0.20 ± 0.01
Crucian carp (whole)		2.06 ± 0.06	2.02 ± 0.05

[a]For most samples the dry weight and the weight of organic matter were identical.

as wildlife is concerned, the work of the Patuxent Wildlife Research Center should be cited (e.g. Anderson and Hickey, 1976; Clark and Lamont, 1976). Further data are given by, for example, Moore and Walker (1964) and Walker *et al.* (1967). Only a few conclusive open-air experiments have been carried out so far. Salonen and Vaajakorpi (1974) have treated a small pond with DDT-^{14}C (1 nCi/l) and analysed the concentrations of ^{14}C as DDT equivalents in water, sediment, many animal species, fish, invertebrates, and plants. Table 3.6 gives a summary of the results. A total balance has not been attempted in this experiment.

(ii) Laboratory Test Models

Several laboratory test models, microcosms or micro-ecosystems have been developed which yield results difficult to interpret for food chain accumulation.

The laboratory model ecosystem of Metcalf (1974) which has been designed for balance studies, including metabolism, bioconcentration, biodegradability and effects on the organisms used, at least permits comparison of chemicals. The experimental procedure has been described in Metcalf *et al.* (1971) with a summary

Table 3.7 Behaviour of Aldrin-^{14}C and Di-2-ethylhexyl Phthalate (DEHP) in a Model Ecosystem (E.M. = ecological magnification). (Reproduced by permission of the author from Metcalf, 1974)

	Concentration (ppm equivalents) in				
	Water	Algae	Snail	Mosquito	Fish
Aldrin					
Total ^{14}C	0.0117	19.70	57.20	1.13	29.21
Aldrin	0.00005	1.95	2.23		0.157
Dieldrin	0.0047	16.88	52.40	1.1	28.00
9-OH-Dieldrin	0.00052	0.12	0.17		0.322
9-C=O Dieldrin	0.0004	0.079	0.217		0.088
Unknown	0.00039	0.585	2.05		0.612
Polar metabolites	0.0040	0.015	0.097		0.004
E.M. values					3,140
DEHP					
Total ^{14}C	0.0078	19.105	20.325	36.609	0.206
DEHP	0.00034	18.322	7.302	36.609	0.044
MEHP	0.00099	0.325	2.541		0.021
Phthalic anhydride	0.00363	0.180	5.772		0.113
Phthalic acid	0.00077	0.094	2.724		0.018
Unknown	0.00190	0.029	0.768		
Polar metabolites	0.00016	0.155	1.218		0.010
E.M. values		53,890	21,480	107,670	130

of data in 1974. Table 3.7 gives an example of the data for aldrin and di-2-ethylhexyl phthalate with this system (from Metcalf, 1974).

3.6. PREDICTABILITY OF THE BEHAVIOUR OF XENOBIOTICS FROM STRUCTURAL CHARACTERISTICS

As examples of the predictability of the environmental behaviour of xenobiotics from structural characteristics of the molecule, the influence of three different structural characteristics is discussed: chlorine content, substituents (epoxy group), and stereochemical configuration.

(i) Influence of Chlorine Content

Among the first organic substances known to be environmental pollutants were chlorinated compounds. It has also been known for many years that substitution with chlorine increases the chemical stability of a compound. When the first polychlorinated biphenyls were detected in the environment and when the gas chromatograph pattern of environmental samples was compared to that of the technical PCB mixtures used, it was observed that the higher chlorinated

components of the mixtures were present. It was concluded that the lower chlorinated components were more easily degraded.

This negative correlation between chlorine content and degradability or convertability to hydrophilic derivatives has been confirmed by many studies (e.g. Matthews and Anderson, 1975).

The urinary excretion of PCB's by mammals may be regarded as a measure of the metabolism to hydrophilic compounds and, consequently, is strongly dependent on the chlorine content. Figure 3.8 shows the excretion of radioactivity during long-term feeding of three selected radiolabelled PCB's by rats: 2,4,6,2′,4′-pentachlorobiphenyl, 2,2′-dichlorobiphenyl, and chloroalkylene-9, an isopropylated 2,4′-dichlorobiphenyl (Begum *et al.*, 1975).

The figure shows that a plateau level of body concentration is reached very slowly for pentachlorobiphenyl. The two less chlorinated compounds, on the other hand, reach the saturation level in a relatively short time and at a relatively low level. The tissue concentrations pertaining to these studies are discussed in section 3.2 and are, as expected, high for the pentachlorobiphenyl and relatively low for the other two compounds. 2,5,4′-Trichlorobiphenyl which is not shown in this figure but included in Table 3.1 on tissue concentrations, is placed between pentachlorobiphenyl and the dichlorobiphenyl.

However, the quantitative excretion pattern is dependent not only on the chlorine content but also on the chemical nature of the metabolites excreted. 2,4′-Dichlorobiphenyl is excreted by rhesus monkeys mainly in monohydroxylated

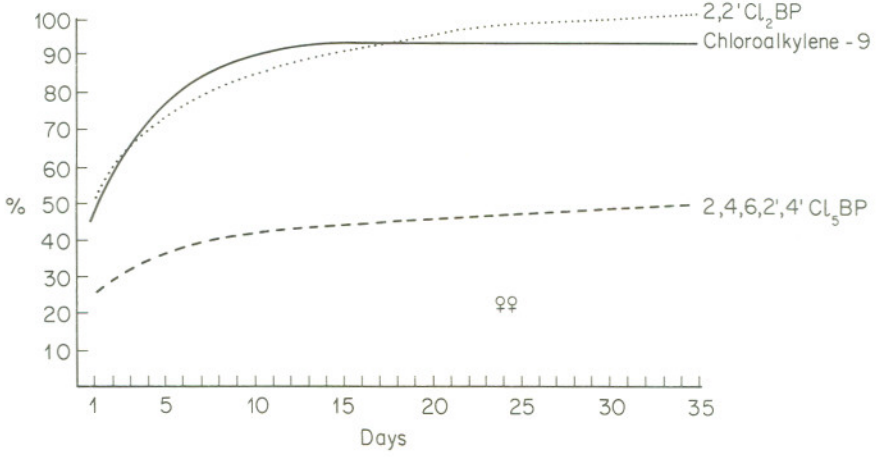

Figure 3.8 Excretion of radioactivity by rats during long-term feeding with 2,4,6,2′,4′-pentachlorobiphenyl-^{14}C, chloroalkylene-9-^{14}C, and 2,2′-dichlorobiphenyl-^{14}C. (Reprinted with permission from *Chemosphere*, 4, 241. Begum, S., Lay, J. P., Klein, W., and Korte, F. Ecological chemistry. CIII. Elimination, accumulation, and distribution of chloroalkylene-9-^{14}C. © 1975, Pergamon Press, Ltd.)

form (66.5% of excreted radioactivity), 2,2′,5-trichlorobiphenyl, however, is mainly in dihydroxylated form (8% of excreted radioactivity). The higher the chlorine content, the lower is the water solubility; therefore, the higher chlorinated isomers need more hydroxy groups to be excreted. When, for highly chlorinated isomers, the introduction of several hydroxy groups is impossible, the excretion becomes more and more difficult, and the substance is accumulated in the body (Greb *et al.*, 1975b).

From these examples it is evident that the metabolism and, consequently, the excretion of PCB's is dependent on the number of chlorine atoms in the molecule. Furthermore, the position of the chlorine atoms has an influence on degradability. Schulte and Acker (1974) have demonstrated that PCB's can be metabolized by mammals, including man, provided that on at least one ring two neighbouring positions are unsubstituted. When these atoms are in *para* and *meta* position, the molecule is metabolized relatively easily, when they are in *ortho* and *meta* position, the degradation takes place more slowly. The preference of the *meta* or *para* position for enzymatic hydroxylation was also reported by Goto *et al.* (1975) and by Sugiura *et al.* (1976).

(ii) Influence of Substituents (Epoxy Groups)

As an example of the influence of the substituents on environmental behaviour, the epoxy group in cyclodienes is discussed.

When, in the cyclodiene insecticides aldrin, isodrin, and heptachlor, the double bond of the non-chlorinated ring is replaced by an epoxy group, the biological activity is increased. Additionally, an *exo*-epoxy group results in stabilization of the molecule and in resistance to enzymatic attack, especially against cleavage of the ring system. In the case of mammals where ring cleavage is of minor importance in the metabolism of these chemicals, the difference between metabolism of olefin and that of epoxide is small. The difference between olefin and epoxide is highest for plants whose metabolism is mainly oxidative and leads to ring cleavage. Thus aldrin (Klein *et al.*, 1973) and isodrin (Weisgerber *et al.*, 1975b) are metabolized by plants to hydrophilic products much faster than dieldrin (Kohli *et al.*, 1973) and endrin (Weisgerber *et al.*, 1968).

(iii) Influence of Conformation

For the influence of stereochemical conformation on the environmental behaviour two examples are selected: the cyclodiene insecticides and BHC isomers.

Aldrin and isodrin are conformational isomers; dieldrin and endrin are the corresponding epoxides. These differences in configuration result in significant differences in metabolic behaviour. The *endo–endo* structure of isodrin and endrin is more susceptible to enzymatic attack than the *endo–exo* structure of aldrin and dieldrin (Klein *et al.*, 1968). In the mammal, endrin is metabolized more rapidly

than aldrin, which means that the stereochemical structure has a greater influence on the rate of metabolism than the substitution with the epoxide. In plants, on the other hand, the percentage of hydrophilic metabolites is higher for aldrin and dieldrin (Weisgerber *et al.*, 1970) than for isodrin (Weisgerber *et al.*, 1975b) and endrin (Weisgerber *et al.*, 1968); however, when looking at the formulae of metabolites, it is evident that isodrin (Figure 3.3, paragraph 3.3. iib) is degraded to smaller molecules than aldrin (Figure 3.6, paragraph 3.3. iie). Isodrin is broken down to substances containing up to three carbon atoms less than the parent compound, aldrin undergoes only ring-cleavage to a relatively stable dicarboxylic acid which still contains the same number of carbon atoms as the parent compound. Thus minute structural changes lead to significant changes in the metabolic behaviour.

Also, for the BHC isomers, there exists a significant difference in environmental behaviour. The fact that the β-isomer is detected in environmental samples in greater concentrations than those corresponding to its occurrence in the technical mixture, indicates that it is more persistent than the α- or γ-isomer. For bacteria as model organisms, it was shown that the adsorption–diffusion mechanism is also different for the three isomers (Sugiura *et al.*, 1975).

3.7. CONCLUSION

It may be concluded from this chapter that, for the ecotoxicological evaluation of a foreign compound, not only the unchanged parent compound but also each conversion product must be considered. The numbers and the quantitative amounts of conversion products are higher for the so-called 'non-persistent' compounds (e.g. buturon, 3.3. iie) than for the persistent ones. The 'unextractable' residues bound in tissues or soil, which have been overlooked for a long time, are also higher for non-persistent compounds. The same applies to the residues which are volatilized, i.e. which escape from the target ecosystem. Therefore, besides the indispensable investigation of persistent xenobiotics, the study of non-persistent compounds should also be stressed.

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