

Comparative Metabolism of Mixtures of Chemicals by Animals, Plants, and Microorganisms and Their Significance in Alteration of Pollutants in the Environment

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ABSTRACT

To assess the overall metabolic alteration of organic pollutants through biological systems in the environment, the basic characteristics of metabolic systems in microorganisms, plants, and animals have been examined. While most of their metabolic activities contribute to reduce the levels of pollutant residues and their toxicities, certain of their activities result in the formation of stable, toxic residues (i.e. 'terminal residues'). Conditions which are likely to promote formation of terminal residues are described. There is no single satisfactory microcosm approach to enable scientists to predict overall metabolic fates of pollutants at present. Thus metabolic activities are currently studied by using single species or at best a mixture of related species of organisms. Integration and interpretation of individually collected information, therefore, become rather important tasks. The use of microbial model ecosystems coupled with knowledge on the role of plant and animal systems has provided valuable predictive tools for assessing the nature of toxic residues that accumulate in the environment.

1 INTRODUCTION

Chemicals introduced into the environment are exposed to many weathering and biological forces, all of which could alter the nature of their residues. The purpose of this chapter is to describe those forces which alter the structure of chemicals and thereby affect their movement in the environment and through biological systems and their toxicological properties. Physical interactions such as binding, sequestering, evaporation, solubilization, etc., which do not alter chemical structure are not covered in this chapter.

There is no question about the changing nature of chemical residues in the environment. Mixtures of chemicals given to the environment seldom stay in the

form of the original composition. Two major forces that contribute to alteration processes of organic chemicals in the environment are photochemical (abiotic) and biological (biotic) reactions. The main emphasis of this chapter is on the latter type of reactions.

In this chapter I intend to analyse the basic characteristics of the metabolic systems that are present in three groups of organisms: microorganisms, plants, and animals. In view of the vast knowledge accumulated, no attempt will be made to cover each subject area thoroughly, since there are many excellent review articles published for each metabolic area. Emphasis will be placed on the part of integration of metabolic information separately obtained in different biological systems.

2 MICROBIAL METABOLISM OF CHEMICALS

2.1 Characteristics of Microbial Metabolism

Microorganisms are known to play major roles in metabolizing chemicals in the environment. They are most abundant in soils and aquatic sediments, where the bulk of chemicals accumulate as a result of environmental pollution. Microorganisms do not constitute a large biomass, but they possess extremely active metabolic capabilities. Most organic chemicals introduced into soil are eventually metabolized by microorganisms. The key to the understanding of microbial metabolism is clarification of energy generated or required by their metabolic activities in degrading these chemicals. I have classified microbial metabolism of pollutants into three classes.

- (1) Incidental metabolism: microorganisms do not gain or specifically expend energy in metabolizing pollutants.
 - (a) Non-specific metabolism: metabolic activity is due to wide substrate spectrum enzymes or specific enzymes which are present in many organisms.
 - (b) Analogue metabolism: microorganism possesses a specific enzyme system to metabolize structurally related analogues of the pollutant.
- (2) Catabolism: microorganisms gain energy by metabolizing the whole or a part of the pollutant molecule.
- (3) Detoxification: the pollutants are toxic to microorganisms, and hence a resistant strain(s) develops which degrades pollutant by expending extra energy.

In the case of incidental metabolism the concentration of pollutants could be high or low, as microbes are growing on carbon sources other than the pollutants, while in the case of catabolic activities the pollutants' concentration must be high enough to sustain zymogenous microbial growth. In the detoxification category the concentration of pollutants must be high enough to affect the

microbial population though compounds which are very toxic to microorganisms (e.g. fungicides) could induce detoxification enzymes at rather low concentrations.

Since most pollutants which cause environmental problems are stable, and present at low concentrations (ppm to ppt range), the bulk of microbial metabolism could proceed via 'incidental metabolism' with small percentages of toxic chemicals inducing type (3) (detoxification) metabolism.

There are reasons why incidental metabolism is divided into two subclasses. In 'non-specific metabolism' general enzymes such as hydrolases are present in many species of microbes, and, therefore, the level of metabolic activities is generally related to the total microbial biomass. Another characteristic of this class of metabolism is the increase of metabolic activity accompanying the increase in readily available carbon sources such as glucose. In the case of 'analogue metabolism', by contrast, a specific microbe possessing specialized metabolic enzymes must be developed. A good example is development of microbial strains which are capable of growing on biphenyl or monochlorobiphenyl as a sole carbon source (Ahmed and Focht, 1972; Furukawa and Matsumura, 1975). Such strains are capable of slowly degrading some congeners of PCB (polychlorinated biphenyl) through analogue metabolism. Thus, addition of readily available carbons such as glucose usually stops analogue metabolism, since they promote metabolic activities other than that which supports specific carbon utilization.

2.2 Types of Common Microbial Metabolic Activities

2.2.1 Hydrolytic Metabolism

Microorganisms possess various hydrolytic enzymes (hydrolases). These enzymes are omnipresent and act as the source for non-specific metabolism. Many microorganisms also possess 'exoenzymes' which are excreted to the outside of their cell bodies to digest and pretreat nutrient sources. Most of the exoenzymes are known to be hydrolytic enzymes. Therefore, pollutant molecules possessing ester, ether, amide, peptide, and alkyl halide bonds are potentially susceptible to hydrolytic attacks. A number of examples of hydrolytic metabolism are cited by Matsumura and Benezet (1978) and Matsumura (1982).

2.2.2 Oxidation

Oxidation is a common metabolic activity among aerobic microorganisms. Systems known to affect pollutants are (1) epoxidation (e.g. conversion of aldrin to dieldrin); (2) oxidation of thio ethers to sulfoxides and sulphones (e.g. phorate); (3) oxidative dealkylation (e.g. alkylamines and alkyl ethers); (4) aromatic ring opening (e.g. monochlorobiphenyl); (5) decarboxylation (e.g. dichlorobenzilic acid, Miyazaki *et al.*, 1969); and (6) β -oxidation.

Reactions (1), (2), (3), and (6) have been found to occur in many microorganisms, and, therefore, under aerobic conditions many of these reactions are carried out as non-specific metabolism. The ring opening reactions are very important metabolic capabilities limited to only some microorganisms. Most aromatic pollutants are degraded through ring opening reactions assisted by analogue metabolism. At least in limited cases such activities involve cytochrome P-450-mediated systems. A key point to remember, however, is that microbial monooxygenase systems generally have narrow substrate spectra compared with corresponding systems in animals. Such a characteristic makes it difficult to develop a microbial species as a model for microbial metabolism in the environment.

2.2.3 Reduction

Reductive metabolism is prevalent among microbial species, particularly facultative and obligatory anaerobic microorganisms. So far as is known, all reductive metabolism of pollutants is carried out via non-specific metabolic activities. Well-known reactions are (1) dehalogenation reactions on haloalkanes, (2) nitroreduction, and (3) reduction of carboxylic acids, aldehydes, and ketones, to alcohol. Other less frequently observed reductive reactions are (4) dehalogenation of halogenated aromatics, (5) hydrogenation of alkenes (e.g. DDE to TDE), and (6) reductive *N*-dealkylation (Esaac and Matsumura, 1980). An interesting aspect of reductive reactions is that some readily occurring reactions such as (1) and (2) above may occur under anaerobic conditions through non-enzymatic reactions involving some electron carriers such as flavoproteins, porphyrins and iron-sulphur proteins (e.g. ferridoxins) (see Esaac and Matsumura, 1980) under anaerobic conditions. In such cases, reductive reaction rates on pollutants are also influenced by the degree of the anaerobic condition (i.e. redox potential), the efficiency and the amount of the electron transfer system, and the nature of the substrate.

2.2 Combination of Microbial Actions on Mixtures of Chemicals and Formation of Terminal Residues

As a result of a multitude of reactions occurring with a given pollutant, many reaction products are expected. A good example is that which occurs with DDT (Figure 1). The major degradative force here is reduction which eliminates one of the chlorines from the trichlorocarbon location. Other minor reactions are dechlorination, oxidation including decarboxylation, and a replacement reaction on the trichlorocarbon with a cyano moiety (DDCN).

While one can assume that these reactions take place in a random order by involving many microbial species, two key features may be pointed out: first, the reductive reaction to form TDE from DDT is the key rate-limiting reaction

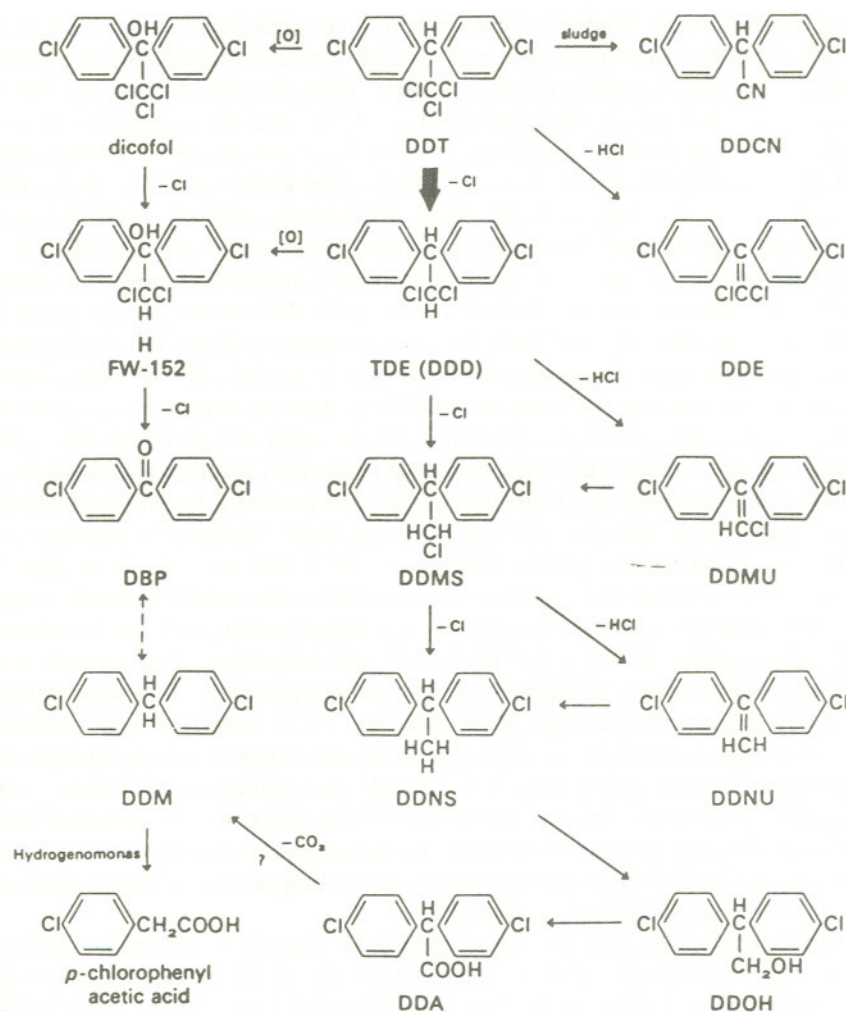


Figure 1 Metabolic conversion of DDT by microorganisms and in the environment. From Matsumura (1975)

without which the bulk of DDT will stay in the form of either DDT or stable toxic metabolites (e.g. DDCN, DDE, dicofol, or DBP), and, second, these stable residues are often called 'terminal residues'. Though the term is somewhat misleading (i.e. no residue is really terminal in the environment), the concept itself is very important as these stable toxic residues are the ones which cause unexpected environmental damages. While in the above example it was minor reactions that produced 'terminal residues', there are other ways microorganisms

produce stable toxic residues. Isomerization is one of the key microbial reactions which produce environmentally problematic residues. Formation of α -BHC (BHC = benzene hexachloride) from γ -BHC (Benezet and Matsumura, 1973), ketoendrin from endrin (Matsumura *et al.*, 1971) and the appearance of two ketone forms of dieldrin (metabolites G and F) in soil as a result of microbial action on dieldrin (Matsumura *et al.*, 1968) are a few known examples. One could raise the question of why microorganisms should carry out such reactions which do not give any benefit to them. The most likely explanation of the event is that the reactions are carried out through incidental metabolism and that some pollutant molecules are accidentally taken up by the enzyme system which is metabolizing some other substrate. Since the pollutant molecule is different from the natural substrate, the enzyme may be able to go partially through the normal reaction. For instance, the enzyme could bind with the pollutant and thereby form an intermediate complex involving the activation of a particular site in the molecule. Since the enzyme cannot proceed to the next reaction the substrate is dissociated. The dissociated substrate is likely to assume a thermodynamically more stable form than the starting isometric form. Microbial conversion of endrin to Δ -ketoendrin (Matsumura *et al.*, 1971) and of dieldrin to photo-dieldrin (Matsumura *et al.*, 1970) may be classified in this category. Another way to form isomers is to have two reactions occurring in exactly opposite directions, e.g. dehydrogenation versus hydrogenation, and dehydration (e.g. on diol) and subsequent hydrolysis. The former scheme has been postulated for isomerization of γ -BHC (Figure 2) (Benezet and Matsumura, 1973). Another way to produce stable residues is to selectively degrade certain labile constituents in mixtures of pollutants leaving stable members behind. Accumulation of β -BHC and nonachlor in the environment where technical BHC and chlordane had been used is due to this type of microbial action. In the same way, in the case of 2,4,5-T residues, 2,3,7,8-TCDD is expected to remain for long periods of time in soil even after 2,4,5-T has completely disappeared.

Mixtures of chemicals are also expected to degrade in a similar fashion, assuming that there are no components which are inhibitory or stimulatory to microorganisms. Labile components are degraded first, leaving more stable components. In the case of PCBs (polychlorinated biphenyls), Furukawa and Matsumura (1975) have shown that the lower chlorinated members of PCB mixtures are readily degraded by *Alcaligenes* sp., and the relative proportions of the higher chlorinated members (e.g. penta-, hexa-, heptachlorobiphenyls) increase at the same time.

If any of the components of the original mixture are inhibitory or stimulatory to microbial activities, it is possible that patterns of degradation could change drastically. Often pesticide mixtures containing fungicides have shown unusual persistence characteristics, indicating that fungicides suppress soil microorganisms which are normally active in degrading other pesticides. In some cases, such interactive properties of pesticides are used for prolonging the active periods of

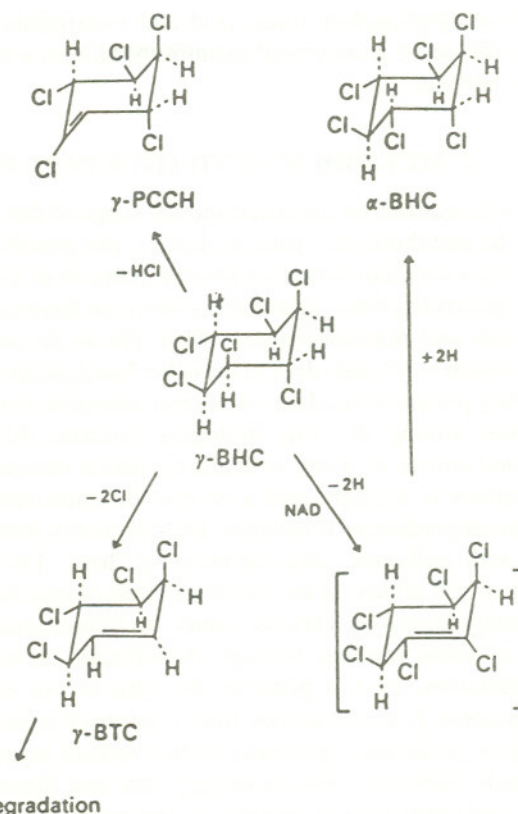


Figure 2 Metabolism of γ -BHC by microorganisms. From Matsumura (1975)

pesticides. A good example is the mixing of propanil, a useful herbicide particularly on rice, with organophosphates or carbamates to extend the period of effectiveness of the former. The latter compounds are known to inhibit hydrolases which are produced by soil microorganisms. In other cases, various materials have been shown to stimulate microbial growth, and thereby the presence of such compounds in the mixture alters the nature of degradation. Carriers for pesticides such as emulsifiers, solvents, and oils may be catabolically metabolized by microorganisms; these activities would in turn contribute to the incidental metabolism of pesticides. The addition of glucose and other readily available carbohydrates to soil is known to increase microbial activities. On the other hand, such increases are often accompanied by the lowering of the redox potential of the immediate environment (i.e. creation of anaerobic conditions), particularly in humid or waterlogged soil environments. This would thereby change the nature of the metabolic activities. For instance, parathion is generally

degraded to diethylphosphorothioic acid and *p*-nitrophenol in soil, but under anaerobic conditions it is converted to aminoparathion which persists in soil for much longer periods.

3 METABOLIC ACTIVITIES IN PLANTS

In view of the limitations on the space and the scope of this paper, an exhaustive coverage of the metabolic activities in plants is not possible. Interested readers are referred to an excellent article by Shimabukuro *et al.* (1982). Metabolism of pollutants by plants has been regarded as a slower or less extensive counterpart of that by animals and microorganisms. Also, plants do not pick up lipophilic pollutants as much as animals do. On the other hand, plants play very significant roles in altering pollutant residues on several accounts: (a) plants represent the largest biomass among all living biological systems; (b) plants serve as the immediate food source to many animals; (c) plants increase the availability of pollutant residues to sunlight and also provide photosensitizing materials to promote photodegradation of residues; and (d) plants form 'terminal residues' by reacting with pollutants and sequestering them. The reason for the last phenomenon is that plants do not excrete pollutant metabolites as animals do. They are usually stored in various tissues or sequestered in non-vital parts. Elimination of some residues through detachment of some parts (e.g. fruit ripening, defoliation, etc.) is possible, but this is not considered an active elimination process. It is well known that in terms of polarity the optimum for plant pick-up of pesticides is generally shifted toward more polar regions than that for animals. However, oil-producing plants and those with extensive root systems (e.g. root crops such as carrots) are known to pick up even highly apolar compounds from soil.

Major metabolic reactions found in plants are oxidation (e.g. epoxidation, hydroxylation, desulphuration, thio ether oxidation, *N*-demethylation), hydrolysis, and reduction, though reduction is much less prevalent than oxidation in plants.

What makes plants very unique, however, is the availability and the versatility of their conjugation systems (i.e. secondary metabolism). As mentioned above, in plants conjugation largely determines the nature of terminal residues since elimination is not a significant factor. The major types of conjugates in plants are simple and complex glucosides, glutathione conjugates, and amino acid conjugates. No glucuronides or sulphates have been observed as in the case of mammalian conjugates. Since differences in conjugation abilities are often attributed to the selectivity of certain herbicides, conjugation processes in general may be regarded as their defence mechanism to phytotoxic agents. Since it is known that a large proportion of indoleacetic acid, a natural plant hormone, is bound as an inactive glucose conjugate, conjugative reactions may also be regarded as one of the natural ways to regulate active agents in plants. However,

not all conjugated compounds seem to be non-toxic. Mumma and Hamilton (1976), for instance, have found that a number of 2,4,-D derived amino acid conjugates are highly phytotoxic.

Finally it is pertinent to point out that large proportions of residues found in plants are often non-extractable by any solvents or aquatic media. Some of these non-extractable residues may be biologically unavailable. Marshall and Dorrough (1977), for instance, found that when insoluble residues of ^{14}C -carbaryl and carbofuran from bean plants were fed to rats, 98 % and 85 % of those were excreted in the faeces, while the rats fed ^{14}C -labelled water-soluble metabolites of the same pesticides from the same plants excreted 80 % to 90 % of the radiocarbon in the urine, indicating that the former residues were not absorbed by the digestive systems of the rats.

As for the nature of interactions of mixtures of chemicals with plant materials, little is known about the actual mechanisms of joint action among various chemicals, though undoubtedly there must be serious interactions. In the field of herbicidal science it has been known for some time that unexpected serious injuries (e.g. leaf burn) occur on crops when the wrong kind of carrier or pesticide mixture is given, indicating that chemicals do interact (Ashton and Crafts, 1973). One of the useful applications of chemical interactions in this field has been the development of 'protectants' for EPTC-type herbicides. A protectant such as naphthyl anhydride is applied to oats as a seed coating prior to treatments with EPTC in the field to protect the oats, and at the same time to kill the wild oats which are similar to cultivated oats. While the mechanism of protection is not known, such examples clearly indicate that chemicals could interact to produce unexpected effects in plants.

The recent tree damages being reported in Germany and other European countries illustrate that complex cause-effect relationships may exist between air pollution and plant stress. Acidity of rain, if it is not accompanied with any other changes, may not cause extensive damage to those trees. However, acidity changes the forms of other chemicals such as metal ions (e.g. Al^{3+} , Hg^{2+} , Ag^{3+}) and alters the binding of those chemicals to soil particles. The toxicities of these metal ions to plants are well known. The stressed plants could in turn become more vulnerable to other plant toxic chemicals, since they do not produce enough glutathione, amino acids, UDPG (uridine diphosphate glucose), and other conjugating agents which act as protective shields for many toxic chemicals.

4 CHARACTERISTICS OF METABOLIC ACTIVITIES IN ANIMALS

Many excellent review articles are available in this field. Some examples are Dorrough and Ballard (1982) and O'Brien (1967). In general, animals have well-developed detoxification systems. Their purpose is to alter foreign compounds (xenobiotics) in such a way that their metabolites may be directly or indirectly

(i.e. after conjugation) excreted outside their bodies. Usually apolar xenobiotics are made more polar and more water soluble. In the case of higher animals, the liver is the organ specialized for detoxification processes. There are very well-developed detoxification enzymes in the liver. Most of them have wide substrate spectra, indicating that they are capable of processing varieties of xenobiotics.

In mammalian species, oxidation through microsomal mixed function oxidase is perhaps the most important detoxification mechanism. This system utilizes cytochrome P-450 as the terminal substrate-binding component. Esterases and other hydrolases play important roles in degrading pollutants, particularly organophosphorus and pyrethroid insecticides. Reduction usually is not as important as oxidation in this regard, though certain compounds such as DDT and toxaphene are largely degraded through reduction reactions. Also, in intestines and other digestive organs (e.g. in ruminants), reductive metabolism is known to be prevalent. Conjugation systems active in mammalian species in general are the glutathione-S-transferase system and the glucuronyl and sulphate conjugation systems (Neal, 1980; Williams, 1959).

'Induction' is a very well-established phenomenon, where administration of chemicals to animals results in an increase in detoxification enzymes, particularly those in the liver or equivalent tissues specialized in detoxification functions. Many lipophilic pollutants are known to cause 'induction', particularly in mixed function oxidase and associated systems such as δ -aminolaevulinic acid synthetase. Stimulation of such detoxification enzymes by one component of a mixture assists the degradation of other components. This aspect of metabolic alterations and resulting chemical interactions has been thoroughly covered by several authors (e.g. Nebert *et al.*, 1972; Poland and Knutson, 1982).

Since metabolism is closely tied to excretion in animals, apolar xenobiotics which are not metabolized are not readily excreted to urine or faeces. An exception is the case with lactating animals, where even apolar (lipophilic) xenobiotics are excreted with milk. Lipolysis which accompanies lactation also facilitates transfer of fat-stored xenobiotic residues to milk. While there are other routes of excretion such as skin and hair shedding, evaporation through exhalation, salivary secretion, etc., they are all minor routes and do not significantly contribute to the overall elimination dynamics. Thus, as far as apolar pollutants are concerned, animals generally do not produce 'terminal residues' *per se* through their excretory routes to pollute the environment. Such generalization, however, does not apply to polar pollutants (e.g. heavy metals) which may be excreted and contaminate some environments.

'Terminal residues' accumulated in animals' fat tissues, on the other hand, could be transferred to other animals through predation and parasitism, or be recycled back to the environment through death and subsequent decay of their carcasses. From the viewpoint of food-chain or food-web transfer of pollutants, metabolic capabilities of animals which serve as food sources become an important issue. In this regard, certain groups of animals have been found to

have a rather low metabolic capability to degrade xenobiotics. For instance, fish and amphibians in general have low metabolic capabilities. Fish is a very important food source for many animals including man and therefore accumulation of unmetabolized 'terminal residues' in fish becomes quite a concern.

5 EXAMPLES OF METABOLIC ALTERATIONS OF POLLUTANTS IN BIOTIC ENVIRONMENTS

When all the metabolic capabilities of various biological systems are considered, an extremely complex picture of biological transformation emerges. From the viewpoint of microcosm technology, approaches to develop models to predict eventual metabolic fates of new chemicals in the environment are very difficult. Early models involved model ecosystems which housed many biological components (see Giesy, 1980). The best example is the one developed by Dr R.L. Metcalf of the University of Illinois (Metcalf, 1977). These models have generated general qualitative data indicating what types of metabolic products might form in the biotic environment. They have provided very valuable information in this regard. In interpreting data generated by such systems, however, one must be aware of two major weaknesses in all model ecosystems housing various heterogeneous organisms (i.e. microbes, plants, and animals). First, the total biomass of animals and plants in such systems is clearly exaggerated no matter how large the system is. Therefore, the metabolic activities of the plants or animals placed in the container predominate in that system. For instance, in the case of Metcalf's model the metabolic activity by the insects (salt marsh caterpillars) predominated. The main residue of DDT in that system is DDE, while in nature TDE and other microbial products are formed along with DDE. Second, the result may be highly variable for the purpose of quantitative assessment, reflecting the problem of maintaining stable ecosystems. Particularly problematic are the changes in microbial activities and biomass such as uncontrolled algal growth.

Since that time many microcosm approaches have been proposed (see, for example, Giesy, 1980; Maki *et al.*, 1980). The general direction of microcosm research on metabolic alteration of pollutants is to study individual components separately, at least at the initial phase. Thus metabolic activities in microorganisms, plants, and animals are studied separately using individual species. This may sound like a tedious approach. However, such studies clearly give an indication of the roles and contribution of individual components.

In the case of microbial microcosm studies, several lines of approach have been made (Bourquin and Pritchard, 1979). One approach is to correlate the level of metabolic activity to general microbial biomass or activities (e.g. respiration, ATP, etc.) (Baughman *et al.*, 1980). This approach may be appropriate for non-specific metabolism in the category of incidental metabolism, particularly for

hydrolytic degradation through wide-spectrum hydrolases. For specific metabolism such as analogue metabolism and catabolism, various researchers have selected certain microorganisms using the pollutants themselves or their structural analogues as selective agents (e.g. Cerniglia *et al.*, 1979; Gledhill and Saeger, 1980). After a certain adaptation period, specific microorganisms which are capable of degrading those carbon sources may develop. While the ease by which such specific microorganisms are selected could be used as an indication for the general degradability of the pollutant, there is no established methodology which may be satisfactorily used for all cases of specific microbial metabolism. These microbial studies in laboratories, however, have yielded valuable data as to the possible nature of terminal residues. With this type of approach, various candidate metabolites such as metabolites G and F of dieldrin (Matsumura *et al.*, 1968), γ -BTC from γ -BHC (Tsukano and Kobayashi, 1972), various chlorinated benzoic acids from PCB (Ahmed and Focht, 1972; Furukawa and Matsumura, 1975), and DDCN from DDT (Albone *et al.*, 1972; Jensen *et al.*, 1972) have been identified. These approaches have also given basic information on the mechanisms of microbial metabolism of pollutants.

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