Biodegradation of Chemicals of Environmental Concern

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Summary. Microorganisms in soils and waters convert many synthetic organic chemicals to inorganic products. Other compounds are transformed only by cometabolism. These microbial processes may lead to environmental detoxication, the formation of new toxicants, or the biosynthesis of persistent products. Type reactions are proposed for major categories of enzymatic transformation of synthetic chemicals in soils, natural waters, and sewage. Some organic molecules are resistant to microbial attack, and explanations for the persistence of such compounds are suggested.

A variety of synthetic chemicals are present in inland and marine waters and in agricultural and other soils. Some of these compounds are toxic or may be converted to hazardous products in nature. Government regulatory agencies have been controlling the use of pesticides for some time, and the U.S. Environmental Protection Agency (EPA) has initiated a program to establish procedures for assessing the environmental impact and health hazards of chemicals not classified as pesticides. In response to public and government concern and because of the intriguing research problems presented, environmental scientists, biologists, and chemists have been giving increased attention to identifying and determining the behavior and fate of organic compounds in natural ecosystems. Progress is hindered by the enormous number of chemicals that are used in industry, farming, and the home; current estimates suggest that tens of thousands of different compounds are used in commerce. Many of these substances are deliberately or inadvertently released into waters and soils. Moreover, many of them represent classes of molecules that biologists and biochemists have not previously investigated.

An organic chemical introduced into a terrestrial or aquatic ecosystem may be subjected to nonenzymatic or enzymatic reactions brought about by the inhabitants of the environment. Several types of abiotic mechanisms for chemical change have been described, and in aquatic environments photochemical reactions may be significant. However, nonenzymatic reactions rarely lead to appreciable changes in chemical structure, and it is the biodegradative sequences that bring about major changes in the structure of the introduced chemical. Current evidence suggests, furthermore, that the indigenous microbial populations are the chief agents of change of molecules that are metabolized in waters and soils. Although plants and animals metabolize a variety of chemicals, the activities of the higher organisms are often modest by comparison with the transformations effected by heterotrophic bacteria and fungi residing in the same habitat.

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Role of Microorganisms

The mineralization, or complete biodegradation, of an organic molecule in waters and soils is almost always a consequence of microbial activity. Few abiotic mechanisms of importance in nature totally convert organic compounds of any degree of complexity to inorganic products, and mineralization sequences characterize the microbial metabolism of several classes of synthetic compounds. As they convert the organic substrate to inorganic products, the responsible populations make use of some of the carbon in the substrate and convert it to cell constituents. At the same time, energy is released, and the populations increase in numbers and biomass as they assimilate some of the carbon and acquire energy for biosynthesis. As a consequence, mineralization is typically a growth-linked process. Detoxication is a common outcome of mineralization except when one of the products itself is of environmental concern, as in the case of nitrate in certain waters or sulfide under anaerobic conditions.

With many chemicals, a microbial conversion quite different from mineralization takes place. Interest in this type of conversion rose markedly when it was found that many compounds are acted on biologically in soils and waters but no microorganisms able to use the compounds as sources of nutrients or energy could be isolated. The evidence for a microbial role in the transformations is the finding that the compound is transformed in nonsterile but not in sterilized samples of the natural environment or is transformed more rapidly in the nonsterile circumstances. Compounds that are thus modified in nonsterile but not sterilized environmental samples, or are acted on more readily when viable organisms are present, include DDT, 2,4,5-T, aldrin, heptachlor (*1*-*3*), and many other chlorinated and nonchlorinated molecules. Microorganisms able to use these chemicals as nutrients or for energy have yet to be isolated. The finding that chemicals are subject to microbial action and yet do not apparently sustain growth of the responsible populations has led to considerable research on the phenomenon, which has been termed cometabolism, or sometimes cooxidation (*4*). The populations presumably are growing on another substrate while performing the transformation known as cometabolism.

Objection to this term has been voiced inasmuch as it does not describe a new metabolic phenomenon (*5*). Granting that from the biochemical viewpoint cometabolism is merely biotransformation, the environmental consequences of cometabolism are such that maintaining a separate term is defensible. Two environmental consequences stand out. First, the populations responsible for the transformation do not increase in numbers or biomass as a result of the introduction of the chemical into water or soil. This lack of increase is a reflection of the inability of the organisms to use the chemical for biosynthetic purposes, and it is in marked contrast to the increase in population size or biomass when a mineralizable substrate is introduced into the same environment. Because populations acting on many synthetic chemicals are usually small, a compound subject to cometabolism is characteristically modified slowly, and the rate does not increase with time, again in contrast with a substrate acted

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on by mineralization (Fig. 1). The points in Fig. 1a represent the commonly observed population increase and chemical disappearance when bacteria are provided with a compound they can use as a carbon and energy source for growth. In Fig. 1b, a hypothetical model for cometabolism is used to show the lack of increase in bacterial population size and the very slow decline in concentration of a chemical that is cometabolized by bacteria which use some other compound in the natural ecosystem as a source of carbon and energy; in the model it is assumed that the size of the population of cometabolizing bacteria is at a steady state because of the continuous availability of small amounts of the growth substrate. Second, products structurally similar to the introduced chemical accumulate because the responsible organisms do not have a sufficient array of enzymes to bring about its extensive transformation, particularly to intermediates in normal metabolic sequences. Accumulations of products of this type have been observed in natural environments, model ecosystems, and microbial cultures in artificial media. Such modest changes in the molecule often do not result in detoxication because they are not sufficiently great to remove the structural features associated with toxicity to one or another species.

Direct evidence for cometabolism in nature has been largely lacking, however. The evidence for its occurrence in natural ecosystems consists of the activity in nonsterile but not sterilized samples of the environment, the inability to isolate a microorganism that uses the compound as a nutrient or energy source, and the demonstration that isolated microorganisms growing on other organic compounds carry out a reaction analogous to that in the natural ecosystem. Recent studies with soil and sewage, on the other hand, have provided direct evidence that this phenomenon is taking place. With sewage amended with certain ^{14}C-labeled herbicides, for example, the added pesticides are converted almost stoichiometrically to organic products and no microbial cell material is formed; failure of microorganisms to use the introduced compound as a carbon source was shown when it was observed that the ^{14}C label was not found in the nucleoside fraction of the organic matter in the sewage model ecosystem (*6*). In investigations of cometabolism in soil, ^{14}C-tagged carbon monoxide was introduced into the gas phase over samples of soil. These studies were initiated to provide additional evidence that microorganisms in soil are major agents in the destruction

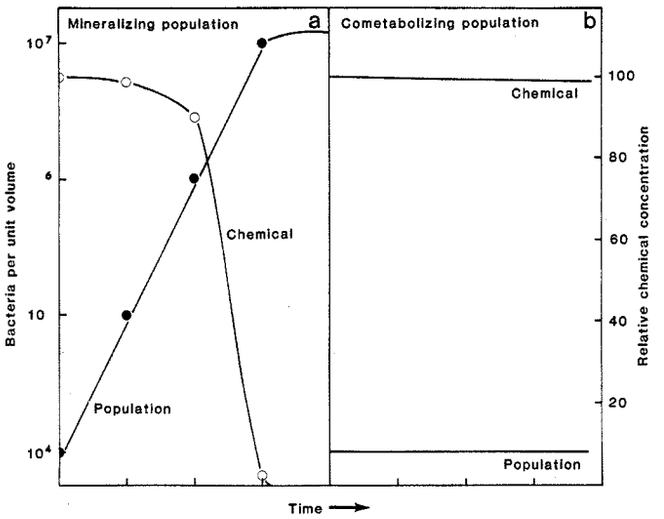


Fig. 1. Hypothetical model for population changes and metabolism of a chemical modified by mineralizing and cometabolizing populations.

of this atmospheric pollutant (*7*). The conversion of CO to CO_2 in these test systems was not the result of microorganisms using the gas as a carbon source, because essentially none of the ^{14}C was recovered in the organic matter-microbial cell fraction of soil. Moreover, the CO was not oxidized by autotrophic bacteria because its oxidation did not enhance the rate of CO_2 fixation by soil microorganisms, a necessary consequence if the reaction was autotrophic. The process is thus brought about by organisms that oxidize CO but obtain neither carbon nor energy from the reaction, that is, by a cometabolic sequence (*8*).

The physiological basis for cometabolism is not clear; that is, why microorganisms, which commonly grow on the substrate they metabolize, are unable to proliferate at the expense of the compounds they act on by cometabolism. In view of the large number of synthetic chemicals that apparently can be cometabolized, establishing the physiological explanation is quite important. The most likely hypothesis is related to enzyme specificity. Many enzymes present in microbial cells catalyze reactions involving several different but chemically related substrates (*9*). If the product of the action of one of these enzymes is not a suitable substrate for any other enzyme in the organism, that compound will accumulate, although the initial enzyme characteristically converts its natural substrate to products that provide energy and a source of carbon for the active species (*4*).

Ecological Consequences

Ecological and public health considerations have resulted in great attention being given to certain categories of microbial transformations. When the chemical introduced into waters or soils is toxic, a common consequence of microbial action is detoxication. In modifying the chemical, the detoxifying microorganisms destroy its actual or potential deleterious influence on one or more susceptible animal, plant, or microbial species. Although mineralization of toxicants is characteristically detoxication, intermediates in the sequence may endure for some time, for example, in the conversions of phenoxy herbicides in soil to yield phytotoxic intermediates (*10*). Some of the intermediates in mineralization that accumulate are known to be nontoxic, but often their potential hazard to animal, plant, or microbial life has not been assessed. Cometabolism of a toxicant may also result in detoxication, and certain synthetic compounds that appear to be cometabolized in nature are converted to products that are not of ecological concern (*11*).

Certain chemicals that are themselves innocuous are converted enzymatically to products that are hazardous to one or another species. Toxicants may be generated from innocuous precursors (by a process that is often called activation) in a mineralization sequence, although the intermediates usually do not persist, and activation also may occur during cometabolism. Microbial activation is evident in the methylation of inorganic mercury in aquatic sediments and other environments to yield the more toxic mono- or dimethylmercury—compounds that are not only harmful but are also readily taken up from the sediments by aquatic animals (*12*). Inorganic arsenic may also be methylated, and the products of this microbial process are the far more toxic methyl arsines; this reaction occurs in microbial cultures as well as in natural ecosystems (*13*, *14*).

We have been investigating a reaction sequence leading to the conversion of innocuous compounds to potent carcinogens. The reactions are in part enzymatic and, apparently, in part nonenzymatic but related to microbial cells or activities. This transformation is the *N*-nitrosation of secondary amines. It is of particular importance because the inorganic and organic precursors of nitrosamines are widespread. The organic precursors are secondary and tertiary amines or quaternary nitrogen compounds, and these substances are present in higher plants, phytoplankton, animal wastes, and a number of synthetic compounds that are introduced in large quantities into natural ecosystems. The inorganic precursor, nitrite, is continually generated in soils and waters from ammonium during nitrification or from nitrate. *N*-Nitrosation has now been demonstrated to take place in samples of soil, water, and sewage, and the secondary amines that can be nitrosated in these model ecosystems to yield the carcinogens include simple dialkylamines and diethanolamine. The nitrosation reaction may be catalyzed enzymatically, but microbial cell constituents or other complex organic materials may also effect nitrosation (*15–17*).

These compounds are of potential environmental concern not only because of their toxicity and the ubiquity of their precursors, but also because the nitrosamines are reasonably long-lived in natural ecosystems. The persistence of some nitrosamines illustrates another feature of certain microbial transformations: they may convert a readily metabolizable compound to a product that is not quickly destroyed. Thus, although the *N*-nitroso derivatives may endure for some time, the dialkylamine precursors are readily transformed microbiologically in natural environments (*18, 19*). Moreover, should the nitrosamines be generated in soil, they could affect humans, because plants may assimilate the carcinogens from soil or the compounds may leach downward through soil and enter the ground water used for drinking (*20*).

Another category of ecologically important transformations involves the conversion of toxicants that are active against one type of organism into products that affect another. Because of such transformations, as well as activation, it is essential to identify environmentally generated products inasmuch as the putative environmental detoxication, in these instances, has not been achieved and new species have been placed under stress. A notable example of this category of transformation is the dehalogenation and oxidation of pentachlorobenzyl alcohol, a compound introduced in Japan to control a fungus pathogenic to rice. This antimicrobial agent was not harmful to the rice plants to which it was applied, but when the plant residues containing the fungicide were incorporated into the soil, tri- and tetrachlorinated benzoic acids were formed. These products were not antifungal, but they suppressed the development of plants sown after the

Table 1. Type reactions for transformation of chemicals of environmental importance.

Category	Reaction*	Example†	Reference
Dehalogenation	$RCH_2Cl \rightarrow RCH_2OH$	Propachlor (S,M)	(*29*)
	$ArCl \rightarrow ArOH$	Nitrofen (S)	(*30*)
	$ArF \rightarrow ArOH$	Flamprop-methyl (S)	(*31*)
	$ArCl \rightarrow ArH$	Pentachlorophenol (S,M)	(*32*)
	$Ar_2CHCH_2Cl \rightarrow Ar_2C{=}CH_2$	DDT (M)	(*33*)
	$Ar_2CHCHCl_2 \rightarrow Ar_2C{=}CHCl$	DDT (G,W,M)	(*3, 33*)
	$Ar_2CHCCl_3 \rightarrow Ar_2CHCHCl_2$	DDT (G,S,M)	(*3, 33, 34*)
	$Ar_2CHCCl_3 \rightarrow Ar_2C{=}CCl_2$	DDT (W,S,M)	(*33, 35*)
	$RCCl_3 \rightarrow RCOOH$	N-Serve (S), DDT (W,M)	(*3, 33, 36*)
	$HetCl \rightarrow HetOH$	Cyanazine (S)	(*37*)
Deamination	$ArNH_2 \rightarrow ArOH$	Fluchloralin (S)	(*38*)
Decarboxylation	$ArCOOH \rightarrow ArH$	Bifenox (S)	(*39*)
	$Ar_2CHCOOH \rightarrow Ar_2CH_2$	DDT (M)	(*40*)
	$RCH(CH_3)COOH \rightarrow RCH_2CH_3$	Dichlorfop-methyl (S)	(*41*)
	$ArN(R)COOH \rightarrow ArN(R)H$	DDOD (S)‡	(*42*)
Methyl oxidation	$RCH_3 \rightarrow RCH_2OH$ and/or $\rightarrow RCHO$ and/or $\rightarrow RCOOH$	Bromacil (S), diisopropylnaphthalene (G), pentachlorobenzyl alcohol (S,M)	(*21, 43*)
Hydroxylation and ketone formation	$ArH \rightarrow ArOH$	Benthiocarb (S), dicamba (W)	(*44*)
	$RCH_2R' \rightarrow RCH(OH)R'$ and/or $\rightarrow RC(O)R'$	Carbofuran (S), DDT (S,W,G)	(*3, 45*)
	$R(R')CHR'' \rightarrow R(R')CHOH(R'')$	Bux insecticide (S)	(*46*)
	$R(R')(R'')CCH_3 \rightarrow R(R')(R'')CCH_2OH$	Denmert (S)	(*47*)
β oxidation	$ArO(CH_2)_nCH_2CH_2COOH \rightarrow ArO(CH_2)_nCOOH$	ω-(2,4-Dichlorophenoxy)-alkanoic acids (S,M)	(*10, 48*)
Epoxide formation	$RCH{=}CHR' \rightarrow RCH(O)CHR'$	Heptachlor (S,M)	(*49*)
Nitrogen oxidation	$R(R')NR'' \rightarrow R(R')N(\rightarrow O)R''$	Tridemorph (S)	(*50*)
Sulfur oxidation	$RSR' \rightarrow RS(O)R'$ and/or $\rightarrow RS(O_2)R'$	Aldicarb (S,M)	(*51*)
=S to =O	$(AlkO)_2P(S)R \rightarrow (AlkO)_2P(O)R$	Parathion (S,M)	(*52, 53*)
	$RC(S)R' \rightarrow RC(O)R'$	Ethylenethiourea (S)	(*54*)
Sulfoxide reduction	$RS(O)R' \rightarrow RSR'$	Phorate (S)	(*55*)
Reduction of triple bond	$RC{\equiv}CH \rightarrow RCH{=}CH_2$	Buturon (S,M)	(*56*)
Reduction of double bond	$Ar_2C{=}CH_2 \rightarrow Ar_2CHCH_3$	DDT (W,M)	(*57*)
	$Ar_2C{=}CHCl \rightarrow Ar_2CHCH_2Cl$	DDT (W,M)	(*3, 33*)
Hydration of double bond	$Ar_2C{=}CH_2 \rightarrow Ar_2CHCH_2OH$	DDT (W,M)	(*57*)
Nitro metabolism	$RNO_2 \rightarrow ROH$	Nitrofen (S)	(*58*)
	$RNO_2 \rightarrow RNH_2$	Pentachloronitrobenzene (S,M), Sumithion (S,W,M)	(*59*)
Oxime metabolism	$RCH{=}NOH \rightarrow RC{\equiv}N$	Aldicarb (S,M)	(*51*)
Nitrile/amide metabolism	$RC{\equiv}N \rightarrow RC(O)NH_2$ and/or $\rightarrow RCOOH$	Bromoxynil (S,M), Dichlobenil (S,W)	(*60*)

*Abbreviations: R, organic moiety; Ar, aromatic; Alk, alkyl; Het, heterocycle. †Reaction demonstrated in sewage (G), microbial culture (M), soil (S), or natural waters (W). ‡3-(3′,5′-Dichlorophenyl)-5,5-dimethyloxazolidine-2,4-dione.

original rice crop was harvested. The process is apparently microbial and cometabolic (*21*). From the standpoint of public health, the conversion of the widely used antifungal compound, thiram, to the carcinogenic dimethylnitrosamine in model ecosystems is noteworthy. The reaction sequence in this instance appears to involve both microbial and nonenzymatic mechanisms (*15*).

Enzymatic "defusing" may also occur. This refers to the conversion to a nontoxic product of a compound that might otherwise be activated; that is, the potential for toxicity is biologically destroyed before the molecule is converted to the inhibitor. Defusing takes place in the microbial cleavage in culture of 4-(2,4-dichlorophenoxy)butyrate to yield the nontoxic 2,4-dichlorophenol before the phytotoxin 2,4-dichlorophenoxyacetate is generated (*22*). Enzymatic defusing in natural ecosystems or in models thereof has yet to be shown.

Type Reactions

A major problem facing investigators who deal with the biodegradation and environmental metabolism of synthetic chemicals is that few biochemical precedents exist for many of the molecules of environmental concern. Even among the priority water pollutants established by the EPA and the compounds or classes of chemicals on the priority list for testing under the Toxic Substances Control Act, few chemicals have been studied to ascertain how they are metabolized in microbial cultures, let alone natural ecosystems. The precedents that have been established are useful in determining what microorganisms may do to other representatives of a particular class of chemicals.

The pathways of metabolism are often delineated in microbial cultures because the investigations are easier to conduct and the data are less equivocal, but the transformations must be established in natural ecosystems or models of them. These ecosystems include sewage, soils, fresh waters, estuaries, and oceans. Sewage transformations are of particular environmental significance, for it is in sewage that many chemicals are first subjected to biological transformation and may be destroyed or converted to new toxicants. Soils receive a multitude of chemicals from agriculture, municipal wastes, food processing industries, and discharges from manufacturing and from the transport of materials from place to place. Many compounds are introduced into fresh, estuarine, or oceanic waters by industries, transportation activities, and municipal or industrial sewage treatment operations in which the compounds are incompletely destroyed.

Investigators dealing with environmental metabolism must use a somewhat different approach from that employed in classical biochemistry and microbiology. They are interested not only in compounds that are generated within the microbial cell but in compounds that are both formed and excreted so that they appear outside the cell. Moreover, they must persist outside the cell long enough for the intermediate to appear in quantities sufficient for detection and potential toxicity. It is still not clear why only certain compounds in a metabolic sequence accumulate outside the active organisms and why others are confined to the interior of the responsible species. Moreover, it is not yet possible to establish generalizations about which of the likely intermediates will accumulate. In addition, few meaningful predictions can be made of which steps in a mineralization or cometabolic sequence will be slow and which fast. Because of these difficulties, predictions of which intermediates will appear outside the cell and accumulate are tenuous at best.

A few of the type reactions are given in Table 1. Several types of cleavage reactions are listed in Table 2. These represent sequences that have been established in model ecosystems or, in some instances, by direct measurements of soils or waters. For some of the reactions or sequences only a single example exists, but for others many different compounds undergo the transformation. The chemical listed may itself be modified as indicated in the type reaction, or the reaction may apply to a late step in the degradation or transformation of the listed chemical. Moreover, in some instances, the data are not sufficiently rigorous to show that the reaction is microbial, because some of the processes may be partly or wholly nonenzymatic. The types of products listed are found only outside the cell and at concentrations that are readily detectable; that is, these are the products that are of potential environmental importance, not just those that are generated intracellularly.

In view of the multitude of chemicals that are discharged and the unfeasibility of testing each one rapidly, the ability to predict metabolic fates and products of

Table 2. Type reactions for cleavage of chemicals of environmental importance.

Substrate	Reaction	Example	Reference
Ester	$RC(O)OR' \rightarrow RC(O)OH$	Malathion (M), phthalates (W,M)	(*61*)
Ether	$ArOR \rightarrow ArOH$	Chlomethoxynil (S), 2,4-D (S,W,M)	(*1, 62, 63*)
	$ROCH_2R' \rightarrow ROH$	Dichlorfop-methyl (S)	(*41*)
C-N bond	$R(R')NR'' \rightarrow R(R')NH$ and/or $\rightarrow RNH_2$	Alachlor (S,M), trimethylamine (G,M)	(*64, 65*)
	$RN(Alk)_2 \rightarrow RNHAlk$ and/or $\rightarrow RNH_2$	Chlorotoluron (S), trifluralin (S,M)	(*66, 67*)
	$RNHCH(R')R'' \rightarrow RNH_2$	Imugam (S)	(*68*)
	$RNH_2CH_2R' \rightarrow RNH_2$	Glyphosate (S,W)	(*69*)
Peptide, carbamate	$RNHC(O)R' \rightarrow RNH_2$ and/or HOOCR'	Benlate (S,M), dimethoate (S)	(*70*)
	$R(R')NC(O)R'' \rightarrow R(R')NH+HOOCR''$	Benzoylprop-ethyl (S)	(*71*)
=NOC(O)R	$RCH=NOC(O)R \rightarrow RCH=NOH$	Aldicarb (S,M)	(*51*)
C-S bond	$RSR' \rightarrow ROH$ and/or HSR'	Benthiocarb (S), Kitazin P (S,M)	(*72, 73*)
C-Hg bond	$RHgR' \rightarrow RH$ and/or Hg	Ethylmercury (S,M), phenylmercuric acetate (S,M)	(*74*)
C-Sn bond	$R_3SnOH \rightarrow R_2SnO \rightarrow RSnO_2H$	Tricyclohexyltin hydroxide (S)	(*75*)
C-O-P	$(AlkO)_2P(S^*)R \rightarrow AlkO(HO)P(S^*)R$ and/or $\rightarrow (HO)_2P(S^*)R$	Gardona (S), malathion (S,M)	(*76*)
	$ArOP(S^*)(R)R' \rightarrow ArOH$ and/or $HOP(S^*)(R)R'$	Diazinon (S), parathion (S,M)	(*53, 77*)
P-S	$RSP(O)(R')OAlk \rightarrow HOP(O)(R')OAlk$	Hinosan (M), Kitazin P (M)	(*73*)
Sulfate ester	$RCH_2OS(O_2)OH \rightarrow RCH_2OH$ and/or $HOS(O_2)OH$	Sesone (S,M)	(*78*)
S-N	$ArS(O_2)NH_2 \rightarrow ArS(O_2)OH$	Oryzalin (S)	(*79*)
S-S	$RSSR \rightarrow RSH$	Thiram (S,M)	(*80*)

*Sulfur or oxygen.

breakdown is critical to government regulators and industrial scientists endeavoring to select which of several chemicals are less likely to be of ecological concern. These type reactions provide a basis for such predictions, but too few type reactions are known.

Considerable progress has been made in defining the pathways of biodegradation of a variety of synthetic chemicals in laboratory cultures of individual microorganisms (*23*). For example, there is much information on how individual microbial species cleave simple aromatic molecules in culture and how they bring about the destruction of aliphatic hydrocarbons. Such studies of single populations in artificial media are reasonably simple, in contrast to environmental investigations of metabolic fate, because the test compound is frequently present in high concentration, the microorganisms are acting individually, and none of the complexing materials and surfaces that characterize soils, sediment, sewage, and even natural waters is present. By use of pure cultures and test solutions, a wealth of information has been gathered on the pathways of breakdown of a number of environmentally important synthetic chemicals, including pesticides, aliphatic and aromatic compounds containing various substituents, and several heterocyclic compounds that appear to be of ecological concern.

Nevertheless, it is difficult to predict the effect of surfaces, which of the several metabolic pathways demonstrated in culture operate in nature, the impact of predation and parasitism on the metabolic functions of the dominant species in soils and waters, and whether the low chemical concentrations in nature will support dissimilar populations using metabolic pathways different from those studied in the laboratory. One of the chief limitations of extrapolations based on studies of axenic cultures in artificial media is the inability to predict which of the products formed during biodegradation will appear outside the microbial cell and accumulate in nature to concentrations sufficient to be of ecological importance. Hence, although studies of metabolism with pure cultures in laboratory media are useful and greatly simplify the establishment of pathways of biodegradation in nature, they are merely the first phase of the testing activity; the final phases include use of model ecosystems and then assessment of products in the natural environment.

Not all the processes effected by microorganisms in soils and waters are degradative. A number of compounds are known to be modified to yield methyl, simple acyl, nitro, and nitroso derivatives. Dimers may be formed, and the formation of nitrogen heterocycles has recently been demonstrated. Type reactions are summarized in Table 3. In addition, oligomers and polymers appear to be generated, giving rise to "bound" residues and uncharacterized brown or black products that are probably polyaromatics.

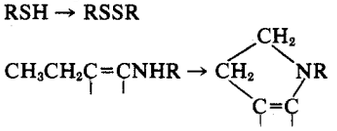
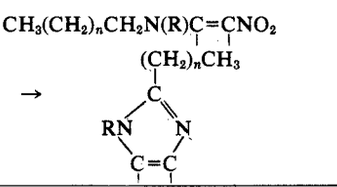
Recalcitrant Molecules

A few years ago, a conflict developed between microbiologists and individuals who monitor synthetic chemicals in waters and soils. Microbiologists were convinced, on the basis of their earlier successes in obtaining active organisms, that every organic compound was able to sustain microbial growth and would be mineralized. Monitoring studies, however, revealed the longevity in soils, waters, or both, of plastics, other synthetic polymers, chlorinated aromatic compounds of various sorts, pesticides, and other industrial chemicals. If microorganisms were so catabolically versatile, it might have been expected that every organic chemical would have been destroyed after sufficient time had elapsed or before the compound was transported for some distance from the original point of discharge. The finding of such an array of dissimilar molecules that persisted in waters and soils revealed the surprising lack of catabolic omnipotence of microbial communities. The weight of field evidence became so great that the conflict has been resolved: many synthetic organic molecules are mineralized very slowly or not at all, and hence they persist for long periods and are of potential ecological concern. Organic chemicals that endure for long periods in natural ecosystems owing to the inability of microorganisms to degrade them rapidly, if at all, are known as recalcitrant molecules. A few of these recalcitrant substances are toxic, and the microflora of waters and soils either does not bring about detoxication or does so slowly. A few of the persistent compounds are not hazardous but are aesthetically undesirable—for example, many plastics and other synthetic polymers.

Some recalcitrant molecules are not toxic at the concentrations found in waters and soils, but they are subject to biomagnification. If the compounds were not persistent, they would not have endured to undergo biomagnification and accumulation in the tissues of higher animals and plants. At the high levels present in such tissues, these recalcitrant molecules have deleterious effects on species at or near the top of food chains. Furthermore, a compound that is not readily destroyed in nature can be transported for some distance; thus, many recalcitrant chemicals introduced originally into soil are present in waters some distance away from the site of first application, and other recalcitrant compounds that are introduced into a river, stream, or sewage treatment plant move to waters far from the original point of introduction. Another problem is that it is not possible to rid natural ecosystems of their content of a recalcitrant chemical should new knowledge in toxicology indicate that a compound that was previously thought to be innocuous is in fact hazardous; in contrast, should a readily mineralizable compound be found to be ecologically unsafe, the supply in natural ecosystems will be destroyed by the indigenous microfloras once the discharge or use of the compound is terminated. Hence, owing to the persistence of toxicants and substances that are aesthetically objectionable, the susceptibility of recalcitrant compounds to biomagnification and distant transport, and the inability to remove them readily, if at all, from natural ecosystems if they are found to be harmful, considerable interest has been focused on the biochemical or ecological bases for molecular recalcitrance. Industrial researchers and technical staffs of regulatory agencies are endeavoring to understand the bases for recalcitrance and to predict which molecules may not be subject to microbial transformation, especially to mineralization.

The persistence and lack of rapid microbial attack on a chemical may be attributable to chemical or environmental causes. The molecule may be completely refractory to microbial destruction. Absolute recalcitrance is probably a property of synthetic polymers such as polyethylene, polyvinyl chloride, and others widely used in manufacturing or in fabrics of importance in the home. Some recalcitrant molecules may be acted on enzymatically, but the reaction is cometabolic; the responsible organisms do not proliferate, the rate of transformation does not increase with time, and the substrates are likely to be persistent. Certain chemicals that are readily biodegradable in one environment are long-lived in another because of environmental factors; thus, the absence of oxygen is occasionally associated with the resistance of even carbohydrates to microbial destruction (*24*). The durability of peats, as long as they are under water, attests to the recalcitrance of organic compounds in cer-

Table 3. Conjugation and other model reactions involving chemicals of environmental concern.

Reaction type	Reaction	Example	Reference
Methylation	$ArOH \rightarrow ArOCH_3$	Pentachlorophenol (S,M)	(*81*)
	$CH_3As(O)(OH)_2 \rightarrow (CH_3)_2AsH$ and/or $(CH_3)_3As$	Methylarsonate (S,M)	(*14*)
	$Hg^{2+} \rightarrow CH_3Hg^+ \rightarrow (CH_3)_2Hg$	Mercuric ions (S,M)	(*82*)
Ether formation	$RCH_2R \rightarrow R(R)CHOCH(R)R$	Diphenylmethane (M)	(*83*)
N-Acylation	$ArNH_2 \rightarrow ArNHC(O)H$	Bifenox (S), Nitrofen (S)	(*30, 84*)
	$ArNH_2 \rightarrow ArNHC(O)CH_3$	DCNA (2,6-dichloro-4-nitroaniline) (S,M), 3,4-dichloroaniline (S)	(*85*)
	$ArNH_2 \rightarrow ArNHC(O)CH_2CH_3$	Chlomethoxynil (S)	(*62*)
Nitration	$ArH \rightarrow ArNO_2$	Chlorodimeform (S)	(*86*)
N-Nitrosation	$(Alk)_2NH + NO_2^- \rightarrow (Alk)_2NNO$	Dimethylamine (S,G,M)	(*16, 87*)
Dimerization	$2ArNH_2 \rightarrow ArN{=}NAr$	Propanil (S,M), trifluralin (S)	(*67, 88*)
	$RSH \rightarrow RSSR$	Denmert (S), Prothiocarb (S)	(*47, 89*)
Nitrogen heterocycle formation		Alachlor (M)	(*64*)
		Dinitramine (S,M), Oryzalin (S)	(*79, 90*)

tain anaerobic deposits (*25*). In addition, certain classes of chemicals, when sorbed to surfaces of colloidal materials present in natural ecosystems, are not readily attacked microbiologically and chemical analysis shows their prolonged persistence (*26*).

Several explanations have been advanced and a number of mechanisms proposed to account for the nondegradability or the longevity of recalcitrant molecules. Biological evolution has explored a narrow range of chemical pathways, and hence it is plausible to believe that various synthetic compounds are too far from the mainstream of catabolic pathways to be substrates for any species. If no enzyme has evolved to convert a molecule to an intermediate in an existing biochemical pathway, it is unlikely that the compound will be acted on biologically. Enzymes are specific in the substrates they act on, and the scope of genetic capabilities of natural populations is great but apparently not all-encompassing; hence, it is likely that certain compounds do not sustain growth of any organism, are not acted on by cometabolism, and do not serve as the basis for the selection of new genotypes able to cope with biochemically novel compounds. A molecule that cannot penetrate the microbial cell and is not modified by an extracellular enzyme is recalcitrant, and the lack of penetration, coupled with the absence of a suitable extracellular enzyme, is a plausible basis for a molecule being refractory. Such an explanation may account for the lack of microbial destruction of polyethylenes, which represent the higher molecular weight homologs of aliphatic hydrocarbons; the latter do penetrate the microbial cell and are degraded by intracellular enzymes.

Microorganisms require energy to maintain themselves; that is, they must carry out oxidations to obtain sufficient energy to carry out their essential functions. Should the ambient concentration of a chemical be very low and the rate of its penetration into the cell thus provide too few molecules per unit time to allow the organism to get enough energy to maintain itself, a species able to metabolize the chemical may not replicate and may even die out; hence, some compounds at minute concentrations may persist. This hypothesis to account for the longevity of water-soluble compounds present at low concentrations may also apply to chemicals having very low water solubilities or to those not emulsified to allow for rapid penetration into the cell; if such a substrate does not enter the cell at a rate sufficient to allow the microorganism to get energy rapidly enough for maintenance as well as growth, persistence is a likely outcome. There is evidence which suggests that certain compounds present in the aqueous phase at trace concentrations are very slowly attacked, although the small amount present should be destroyed readily (*27*).

Other hypotheses to account for the resistance of persistent molecules to microbial breakdown include complexing of the normally available substrate with resistant polyaromatics and inaccessibility of the site on the substrate at which the enzyme should function. A number of compounds that are normally readily degradable are rendered resistant when they are complexed with resistant organic materials. Moreover, resistance may be attributable to inaccessibility of the end of a large molecule belonging to a class of chemicals in which biodegradation is initiated at the terminal portion of the molecule, or it may be ascribed to cross-linkages that mask the site at which the enzyme might otherwise function (*28*).

As industry seeks replacements for persistent compounds and as additional chemicals are sought for new purposes, it is necessary to know which features of the molecule are associated with resistance to biodegradation. These practical interests, coupled with the interest of academic scientists in establishing the bases for recalcitrance, have led to attempts to predict biodegradability from chemical structure. At present, these predictions are somewhat tenuous. Nevertheless, empirical observations provide a basis for suggesting which structural features and substituents underlie resistance and which may make an otherwise resistant molecule into a readily available substrate (*28*).

Conclusion

Interest in biodegradation of chemicals of environmental concern is growing. Regulatory agencies are requiring information, industry is asking questions

that can only be answered experimentally, and university scientists are seeking to establish appropriate generalizations. The presence of industrial chemicals, manufacturing wastes, and other unwanted chemicals in water and of pesticides on land and in crops has convinced the public that pollution by synthetic organic molecules is a problem of major importance. Monitoring studies have demonstrated that industrial effluents are sources of chemicals found in drinking water supplies and that chlorinated organic compounds may be formed from natural products as the water is treated before human consumption. The Toxic Substances Control Act and other federal regulations have played a catalytic role in promoting research on biodegradability. In view of this large amount of activity, one can look forward to new technologies and approaches designed to minimize environmental pollution while maintaining the benefits to society of synthetic chemicals.

References and Notes

1. M. Alexander, *Adv. Appl. Microbiol.* **18**, 1 (1974).
2. J. R. Duffy and N. Wong, *J. Agric. Food Chem.* **15**, 457 (1967); E. P. Lichtenstein and K. R. Schultz, *J. Econ. Entomol.* **53**, 192 (1960).
3. F. K. Pfaender and M. Alexander, *J. Agric. Food Chem.* **20**, 842 (1972).
4. M. Alexander, in *Microbial Degradation of Pollutants in Marine Environments*, A. W. Bourquin and P. H. Pritchard, Eds. (U.S. Environmental Protection Agency, Gulf Breeze, Fla., 1979), p. 67; J. J. Perry, *Microbiol. Rev.* **43**, 95 (1979).
5. M. H. Hulbert and S. Krawiec, *J. Theor. Biol.* **69**, 287 (1977).
6. S. N. Jacobson and M. Alexander, unpublished data.
7. A. N. Nozhevnikova and L. N. Yurganov, *Adv. Microb. Ecol.* **2**, 203 (1978).
8. G. W. Bartholomew and M. Alexander, *Appl. Environ. Microbiol.* **37**, 932 (1979); unpublished data.
9. J. D. Brodie and P. Nicholls, *Biochem. Biophys. Res. Commun.* **32**, 1071 (1968); D. D. Clarke, W. J. Nicklas, J. Palumbo, *Arch. Biochem. Biophys.* **123**, 205 (1968); P. Henkart, G. Guidotti, J. T. Edsall, *J. Biol. Chem.* **243**, 2447 (1968).
10. W. H. Gutenmann, M. A. Loos, M. Alexander, D. J. Lisk, *Soil Sci. Soc. Am. Proc.* **28**, 205 (1964).
11. D. L. Mick and P. A. Dahm, *J. Econ. Entomol.* **63**, 1155 (1970); T. Nakanishi and H. Oku, *Phytopathology* **59**, 1761 (1969); A. Rosenberg and M. Alexander, *J. Agric. Food Chem.* **28**, 297 (1980).
12. A. Jernelöv and A. L. Martin, *Annu. Rev. Microbiol.* **29**, 61 (1975).
13. C.-N. Cheng and D. D. Focht, *Appl. Environ, Microbiol.* **38**, 494 (1979).
14. D. P. Cox and M. Alexander, *Bull. Environ. Contam. Toxicol.* **9**, 84 (1973); E. A. Woolson, *Weed Sci.* **25**, 412 (1977).
15. A. Ayanaba, W. Verstraete, M. Alexander, *Soil Sci. Soc. Am. Proc.* **37**, 565 (1973).
16. A. L. Mills and M. Alexander, *J. Environ. Qual.* **5**, 437 (1976).
17. J. T. Yordy and M. Alexander, unpublished data.
18. J. E. Oliver, P. C. Kearney, A. Konston, *J. Agric. Food Chem.* **27**, 887 (1979); R. L. Tate and M. Alexander, *J. Environ. Qual.* **5**, 131 (1976).
19. A. Ayanaba and M. Alexander, *J. Environ. Qual.* **3**, 83 (1974).
20. D. Dean-Raymond and M. Alexander, *Nature (London)* **262**, 394 (1976); S. U. Khan and P. B. Marriage, *J. Agric. Food Chem.* **27**, 1398 (1979).
21. M. Ishida, in *Environmental Toxicology of Pesticides*, F. Matsumura, G. M. Boush, T. Misato, Eds. (Academic Press, New York, 1972), p. 281.
22. I. C. MacRae, M. Alexander, A. D. Rovira, *J. Gen. Microbiol.* **32**, 69 (1963).
23. P. J. Chapman, in *Degradation of Synthetic Organic Molecules in the Biosphere* (Division of Biology and Agriculture, National Academy of Sciences, Washington, D.C., 1972), p. 17; M. J. Klug and A. J. Markovetz, *Adv. Microb. Physiol.* **5**, 1 (1971).
24. O. W. Kilham and M. Alexander, unpublished data.
25. P. H. Given and C. H. Dickinson, in *Soil Biochemistry*, E. A. Paul and A. D. McLaren, Eds. (Dekker, New York, 1975), vol. 3, p. 123.
26. R. G. Burns and L. J. Audus, *Weed Res.* **10**, 49 (1970); D. Riley, W. Wilkinson, B. V. Tucker, in *Bound and Conjugated Pesticide Residues*, D. D. Kaufman, Ed. (American Chemical Society, Washington, D.C., 1976), p. 301.
27. R. S. Boethling and M. Alexander, *Environ. Sci. Technol.* **13**, 989 (1979); *Appl. Environ. Microbiol.* **37**, 1211 (1979).
28. M. Alexander, *Biotechnol. Bioeng.* **15**, 611 (1973).
29. D. D. Kaufman, J. R. Plimmer, J. Iwan, *Abstr. 162nd Meet. Am. Chem. Soc. (Pestic. Chem.)* (1971), p. 21.
30. H. Ohyama and S. Kuwatsuka, *Annu. Meet. Pestic. Sci. Soc. Jpn.* (1976), p. 220.
31. T. R. Roberts and M. E. Standen, *Pestic. Biochem. Physiol.* **9**, 322 (1978).
32. A. Ide, Y. Niki, F. Sakamoto, I. Watanabe, H. Watanabe, *Agric. Biol. Chem.* **36**, 1937 (1972); R. F. Curtis, D. G. Land, N. M. Griffiths, M. Gee, D. Robinson, J. L. Peel, C. Dennis, J. M. Gee, *Nature (London)* **235**, 223 (1972).
33. G. Wedemeyer, *Appl. Microbiol.* **15**, 569 (1967).
34. T. F. Castro and T. Yoshida, *Soil Sci. Plant Nutr. (Tokyo)* **20**, 363 (1974).
35. F. W. Juengst, Jr., and M. Alexander, *Mar. Biol.* **33**, 1 (1975); R. J. Kuhr, A. C. Davis, E. F. Taschenberg, *Bull. Environ. Contam. Toxicol.* **8**, 329 (1972).
36. C. T. Redemann, R. W. Meikle, J. G. Widofsky, *J. Agric. Food Chem.* **12**, 207 (1964).
37. K. I. Beynon, G. Stoydin, A. N. Wright, *Pestic. Sci.* **3**, 293 (1972).
38. S. Otto, *Environ. Qual. Saf.* **3** (Suppl.), 277 (1975).
39. G. R. Leather and C. L. Foy, *Pestic. Biochem. Physiol.* **7**, 437 (1977).
40. R. V. Subba-Rao and M. Alexander, *J. Agric. Food Chem.* **25**, 855 (1977).
41. A. E. Smith, *ibid.*, p. 893.
42. S. Sumida, R. Yoshihara, J. Miyamoto, *Agric. Biol. Chem.* **37**, 2781 (1973).
43. J. A. Gardiner, R. C. Rhodes, J. B. Adams, Jr., E. J. Soboczenski, *J. Agric. Food Chem.* **17**, 980 (1969); T. Yoshida and H. Kojima, *Chemosphere* **7**, 497 (1978).
44. K. Ishikawa, Y. Nakamura, S. Kuwatsuka, *Nippon Noyaku Gakkaishi* **1**, 49 (1976); C.-C. Yu, D. J. Hansen, G. M. Booth, *Bull. Environ. Contam. Toxicol.* **13**, 280 (1975).
45. C.-C. Yu, G. M. Booth, D. J. Hansen, J. R. Larsen, *J. Agric. Food Chem.* **22**, 431 (1974).
46. B. V. Tucker and D. E. Park, *ibid.* **20**, 412 (1972).
47. H. Ohkawa, R. Shibaike, Y. Okihara, M. Morikawa, J. Miyamoto, *Agric. Biol. Chem.* **40**, 943 (1976).
48. H. F. Taylor and R. L. Wain, *Proc. R. Soc. London Ser. B* **156**, 172 (1962).
49. I. Weisgerber, S. Detera, W. Klein, *Chemosphere* **3**, 221 (1974); E. Elsner, D. Bieniek, W. Klein, F. Korte, *ibid.* **1**, 247 (1972).
50. S. Otto and N. Drescher, *Proc. 7th Br. Insectic. Fungic. Conf.* (Brighton, England, 1973), p. 56.
51. N. R. Andrawes, W. P. Bagley, R. A. Herrett, *J. Agric. Food Chem.* **19**, 727 (1971); A. S. Jones, *ibid.* **24**, 115 (1976).
52. C.-C. Yu and J. R. Sanborn, *Bull. Environ. Contam. Toxicol.* **13**, 543 (1975).
53. D. M. Munnecke and D. P. H. Hsieh, *Appl. Environ. Microbiol.* **31**, 63 (1976).
54. A. Kaars Sijpesteijn, H. M. Dekhuijzen, J. W. Vonk, in *Antifungal Compounds*, H. D. Sisler, Ed. (Dekker, New York, 1977), vol. 2, p. 91.
55. G. Walter-Echols and E. P. Lichtenstein, *J. Econ. Entomol.* **70**, 505 (1977).
56. A. Haque, I. Weisberger, D. Kotzias, W. Klein, *Pestic. Biochem. Physiol.* **7**, 321 (1977); G. M. Tillmanns, P. R. Wallnoefer, G. Engelhardt, K. Olie, O. Hutzinger, *Chemosphere* **7**, 59 (1978).
57. K. C. Patil, F. Matsumura, G. M. Boush, *Environ. Sci. Technol.* **6**, 629 (1972).
58. M. Ichihashi, R. Takahashi, N. Kimura, I. Yokomichi, *Abstr. Annu. Meet. Weed Soc. Jpn.* (1971), p. 35.
59. T. Nakanishi, *Ann. Phytopathol. Soc.* **38**, 249 (1972); T. Nakanishi and H. Oku, *Phytopathology* **59**, 1761 (1969); Y. Takimoto, M. Hirota, H. Inui, J. Miyamoto, *Nippon Noyaku Gakkaishi* **1**, 131 (1976); M. Yasuno, S. Hirakoso, M. Sasa, M. Uchida, *Jpn. J. Exp. Med.* **35**, 545 (1965); R. P. Moody, R. Greehalgh, L. Lockhart, P. Weinberger, *Bull. Environ. Contam. Toxicol.* **19**, 8 (1978).
60. A. E. Smith, *Weed Res.* **11**, 276 (1971); A. E. Smith and D. R. Cullimore, *Can. J. Microbiol.* **20**, 773 (1974).
61. D. F. Paris, D. L. Lewis, N. L. Wolfe, *Environ. Sci. Technol.* **9**, 135 (1975); J. R. Sanborn, R. L. Metcalf, C.-C. Yu, P.-Y. Lu, *Arch. Environ. Contam. Toxicol.* **3**, 244 (1975); G. Engelhardt and P. R. Wallnoefer, *Appl. Environ. Microbiol.* **35**, 243 (1978).
62. Y. Niki and S. Kuwatsuka, *Soil Sci. Plant Nutr. (Tokyo)* **22**, 233 (1976).
63. M. A. Loos, R. N. Roberts, M. Alexander, *Can. J. Microbiol.* **13**, 691 (1967).
64. J. M. Tiedje and M. L. Hagedorn, *J. Agric. Food Chem.* **23**, 77 (1975).
65. R. K. Sethi and S. L. Chopra, *J. Indian Soc. Soil Sci.* **23**, 184 (1975); A. Ayanaba and M. Alexander, *Appl. Microbiol.* **25**, 862 (1973).
66. D. Gross, T. Laanio, G. Dupuis, H. O. Esser, *Pestic. Biochem. Physiol.* **10**, 49 (1979); H. H. Funderburk, Jr., D. P. Schultz, N. S. Negi, R. Rodriguez-Kabana, E. A. Curl, *Proc. South. Weed Conf.* **20**, 389 (1967).
67. T. Golab, W. A. Althaus, H. L. Wooten, *J. Agric. Food Chem.* **27**, 163 (1979).
68. N. Sotiriou, I. Weisgerber, W. Klein, F. Korte, *Chemosphere* **5**, 53 (1976).
69. M. L. Rueppel, B. B. Brightwell, J. Schaefer, J. T. Marvel, *J. Agric. Food Chem.* **25**, 517 (1977).
70. F. J. Baude, H. L. Pease, R. F. Holt, *ibid.* **22**, 413 (1974); A. Fuchs and F. W. de Vries, *Antonie van Leeuwenhoek J. Microbiol. Serol.* **44**, 293 (1978); W. G. Duff and R. E. Menzer, *Environ. Entomol.* **2**, 309 (1973).
71. K. I. Beynon, T. R. Roberts, A. N. Wright, *Pestic. Sci.* **5**, 451 (1974).
72. K. Ishikawa, Y. Nakamura, S. Kuwatsuka, *Nippon Noyaku Gakkaishi* **1**, 49 (1976); C. Tomizawa, *Environ. Qual. Saf.* **4**, 117 (1975).
73. Y. Uesugi, C. Tomizawa, T. Murai, in *Environmental Toxicology of Pesticides*, F. Matsumura, G. M. Boush, T. Misato, Eds. (Academic Press, New York, 1972), p. 327.
74. Y. Kimura and V. L. Miller, *J. Agric. Food Chem.* **12**, 253 (1964); K. Furukawa, T. Suzuki, K. Tonomura, *Agric. Biol. Chem.* **33**, 128 (1969).
75. E. H. Blair, in *Pesticides*, P. Koivistoinen, Ed. (Thieme, Stuttgart, 1975), p. 406.
76. K. I. Beynon and A. N. Wright, *J. Sci. Food Agric.* **20**, 250 (1969); F. Matsumura and G. M. Boush, *Science* **153**, 1278 (1966).
77. J. G. Konrad, D. E. Armstrong, G. Chesters, *Agron. J.* **59**, 591 (1967); N. Sethunathan, *J. Agric. Food Chem.* **21**, 602 (1973).
78. A. J. Vlitos and L. J. King, *Nature (London)* **171**, 523 (1953).
79. T. Golab, C. E. Bishop, A. L. Donoho, J. A. Manthey, L. L. Zornes, *Pestic. Biochem. Physiol.* **5**, 196 (1975).
80. R. Kumarasamy and K. Raghu, *Chemosphere* **5**, 107 (1976); O. Odeyemi and M. Alexander, *Appl. Environ. Microbiol.* **33**, 784 (1977).
81. S. Kuwatsuka and S. Igarashi, *Soil Sci. Plant Nutr. (Tokyo)* **21**, 405 (1975); A. J. Cserjesi and E. L. Johnson, *Can. J. Microbiol.* **18**, 45 (1972).
82. W. F. Beckert, A. A. Moghissi, F. H. F. Au, E. W. Bretthauer, J. C. McFarlane, *Nature (London)* **249**, 674 (1974); M. K. Hamdy and O. R. Noyes, *Appl. Microbiol.* **30**, 424 (1975).
83. R. V. Subba-Rao and M. Alexander, *Appl. Environ. Microbiol.* **33**, 101 (1977).
84. H. Ohyama and S. Kuwatsuka, *Nippon Noyaku Gakkaishi* **3**, 401 (1978).
85. N. K. Van Alfen and T. Kosuge, *J. Agric. Food Chem.* **24**, 584 (1976); *ibid.* **22**, 221 (1974); R. Viswanathan, I. Scheunert, J. Kohli, W. Klein, F. Korte, *J. Environ. Sci. Health* **B13**, 243 (1978).
86. J. Iwan, G. A. Hoyer, D. Rosenberg, D. Goller, *Pestic. Sci.* **7**, 621 (1976).
87. A. L. Mills and M. Alexander, *Appl. Environ. Microbiol.* **31**, 892 (1976).
88. R. Bartha and D. Pramer, *Science* **156**, 1617 (1967); D. D. Kaufman, J. R. Plimmer, J. Iwan, U. I. Klingebiel, *J. Agric. Food Chem.* **20**, 916 (1972).
89. J. Iwan, B. Hertel, J. Radquweit, *Neth. J. Plant Pathol.* **83** (Suppl.), 277 (1977).
90. P. C. Kearney, J. R. Plimmer, W. B. Wheeler, A. Kontson, *Pestic. Biochem. Physiol.* **6**, 229 (1976); T. L. Laanio, P. C. Kearney, D. D. Kaufman, *ibid.* **3**, 271 (1973).

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