

**ADVANCES IN**

**Applied  
Microbiology**

**VOLUME 18**

ADVANCES IN

# Applied Microbiology

*Edited by* D. PERLMAN

School of Pharmacy  
The University of Wisconsin  
Madison, Wisconsin

VOLUME 18



1974

ACADEMIC PRESS, New York San Francisco London

A Subsidiary of Harcourt Brace Jovanovich, Publishers

Copyright © 1974, by Academic Press, Inc.

ALL RIGHTS RESERVED.

NO PART OF THIS PUBLICATION MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM OR BY ANY MEANS, ELECTRONIC OR MECHANICAL, INCLUDING PHOTOCOPY, RECORDING, OR ANY INFORMATION STORAGE AND RETRIEVAL SYSTEM, WITHOUT PERMISSION IN WRITING FROM THE PUBLISHER.

ACADEMIC PRESS, INC.

111 Fifth Avenue, New York, New York 10003

*United Kingdom Edition published by*  
ACADEMIC PRESS, INC. (LONDON) LTD.  
24/28 Oval Road, London NW1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 59-13823

ISBN 0-12-002618-X

PRINTED IN THE UNITED STATES OF AMERICA

# CONTENTS

## Microbial Formation of Environmental Pollutants

MARTIN ALEXANDER

I. Introduction .....	1
II. Products of Pesticide Metabolism .....	2
III. Inorganic Nitrogen Compounds .....	13
IV. Simple Carbon Compounds .....	28
V. Acidity and Mine Drainage .....	34
VI. Simple Sulfur Compounds .....	37
VII. Mercury .....	43
VIII. Arsenic .....	46
IX. Selenium and Tellurium .....	47
X. Oxygen .....	49
XI. Nitrosamines .....	50
XII. Other Carcinogens .....	53
XIII. Complex Human and Animal Toxins .....	56
XIV. Other Phytotoxins .....	60
XV. Other Compounds with Odor and Taste .....	62
XVI. Perspective .....	63
References .....	64

## Microbial Transformation of Pesticides

JEAN-MARC BOLLAC

I. Introduction .....	75
II. Mechanisms of Pesticide Transformation .....	77
III. Enzymatic Reactions in Pesticide Metabolism .....	81
IV. Chemical Structure and Microbial Transformation Relationship .....	114
V. Conclusions .....	122
References .....	124

## Taxonomic Criteria for Mycobacteria and Nocardiae

S. G. BRADLEY AND J. S. BOND

I. Introduction .....	132
II. Earlier Classification Schemes .....	133

III. Developing Classification Systems .....	134
IV. Differential Characters .....	136
V. Regulation of Metabolism .....	145
VI. Degradation of Intracellular Proteins .....	153
VII. DNA Analyses .....	162
VIII. Definition of the Genera .....	172
IX. Evaluation of Species .....	176
X. Concluding Remarks .....	184
References .....	185

### Effect of Structural Modifications on the Biological Properties of Aminoglycoside Antibiotics Containing 2-Deoxystreptamine

KENNETH E. PRICE, JOHN C. GODFREY,  
AND HIROSHI KAWAGUCHI

I. Introduction .....	191
II. Relative Activity, Susceptibility to Enzymatic Inactivation, and Toxicity of Naturally Produced and Semisynthetic 2-Deoxystreptamine-Contain- ing Antibiotics .....	217
III. General Conclusions Regarding the Influence of Structural Variation on the Biological Properties of 2-DOS-Containing Compounds .....	283
References .....	299

### Recent Developments of Antibiotic Research and Classification of Antibiotics According to Chemical Structure

JÁNOS BÉRDY

I. Introduction .....	309
II. Antibiotic Research in the Past Decade .....	310
III. Systematization of Antibiotics .....	336
IV. Classification of Antibiotics According to Chemical Structure .....	345
V. Conclusions .....	397
References .....	402
SUBJECT INDEX .....	407
CONTENTS OF PREVIOUS VOLUMES .....	411

# Microbial Formation of Environmental Pollutants

MARTIN ALEXANDER

*Laboratory of Soil Microbiology, Department of Agronomy,  
Cornell University, Ithaca, New York*

I.	Introduction .....	1
II.	Products of Pesticide Metabolism .....	2
III.	Inorganic Nitrogen Compounds .....	13
	A. Ammonia .....	13
	B. Nitrate .....	16
	C. Nitrite .....	20
	D. Hydroxylamine .....	23
	E. Nitrogen Oxides .....	25
IV.	Simple Carbon Compounds .....	28
	A. Ethylene .....	28
	B. Carbon Monoxide .....	29
	C. Carbon Dioxide .....	31
	D. Organic Acids .....	33
V.	Acidity and Mine Drainage .....	34
VI.	Simple Sulfur Compounds .....	37
	A. Hydrogen Sulfide .....	37
	B. Sulfur Dioxide and Carbonyl Sulfide .....	40
	C. Volatile Organic Sulfur Compounds .....	41
VII.	Mercury .....	43
VIII.	Arsenic .....	46
IX.	Selenium and Tellurium .....	47
X.	Oxygen .....	49
XI.	Nitrosamines .....	50
XII.	Other Carcinogens .....	53
XIII.	Complex Human and Animal Toxins .....	56
XIV.	Other Phytotoxins .....	60
XV.	Other Compounds with Odor and Taste .....	62
XVI.	Perspective .....	63
	References .....	64

## I. Introduction

Probably since the first realization that microorganisms are important in communicable disease, individual species of bacteria and, later, viruses, protozoa, and fungi have been acknowledged as important to environmental pollution. At the present time in the technologically advanced countries, but not in most tropical regions, pollution by parasitic microorganisms is rarely of concern. Water pollution in these nations, more often than not, denotes not a water supply contaminated with bacteria, viruses, or protozoa able to parasitize humans but rather one containing unwanted chemicals. Yet, microorganisms are still causing pollution, sometimes to a modest but often to a serious extent, and

the resulting deterioration of environmental quality arises not because the water supply contains a potential parasite able to grow within the body, but a free-living heterotroph or autotroph that proliferates in the water and there synthesizes undesirable or harmful metabolites. Moreover, the biogenesis of chemical pollutants not only affects water quality, but also brings about detrimental changes in soils and the atmosphere.

The present discussion, of necessity, will only highlight some of the metabolites involved and will not delve in depth into the problem areas. It still should be obvious to the reader, however, that little environmental or microbiological research has been done on most facets of the microbial formation of polluting chemicals.

## II. Products of Pesticide Metabolism

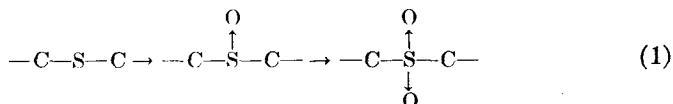
Pesticides are essential in man's arsenal of weapons to protect himself from certain communicable diseases and in his endeavors to provide himself with food and clothing. The enormous progress in the control of malaria and yellow fever, for example, is largely attributable to the effectiveness of insecticides in reducing the populations of insect vectors transmitting the protozoa and viruses. In the technologically advanced countries, the pest-control agents often make the difference between success and failure in food and fiber production and between making a profit or incurring a financial loss. In the developing countries of Latin America, Africa, and Asia, by contrast, regions where the food supply is often insufficient to meet the need of the local population, the use of pesticides may determine whether there is adequate food or widespread famine. The rapid increase in use of these substances in the developing countries is shown by the 40-fold rise in pesticide usage in the cropped area of India in a 13-year period (Pradhan, 1970).

Pesticides have gained prominence not only by virtue of their effectiveness in controlling insects, weeds, and plant pathogens, but because a few are significant environmental pollutants. All these compounds are toxic, or allegedly so, otherwise they would not have been employed in agriculture or public health, but the real or alleged toxicity at the levels used in the field is only to a narrow spectrum of organisms—a small or a large group of animals, rooted plants or microorganisms. Many of the chemicals themselves have little toxicity, however, even to the pest for whose control the substance is designed, and such compounds owe their effectiveness solely to their conversion to the actual toxicant in a process known as activation. Often the activation occurs in susceptible or closely related species, but the toxicant may also be generated microbiologically in certain environments, even when microorganisms are not the group of organisms whose control is sought. Activa-

tion is evident among the organophosphorus insecticides, phenoxy alkanolic acid herbicides, and other pesticides.

Other chemicals marketed as pesticides are indeed toxic, but they are, or can be, transformed microbiologically to new toxicants that act on species also inhibited by the original chemical. Hence, instead of the environment containing just the original pest-control agent, it may contain two or more. In addition, several pesticides that are active in their original forms are converted microbiologically to new inhibitors which act not on the same organisms succumbing to the parent compound, but instead on entirely different species; thus, a fungicide may be converted to a human carcinogen or to a phytotoxin.

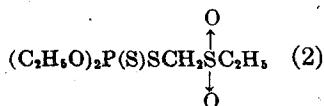
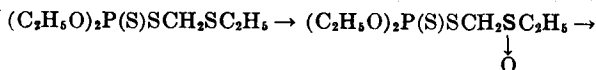
Excellent illustrations of how nontoxic chemicals are made into inhibitory products are found among the insecticidal phosphorothionates and thioethers. The former can be written as  $(RO)_2P(S)OX$ , where R is typically a short alkyl substituent and X can be any of a variety of groups. The latter have a thioether,  $-C-S-C-$ , in the molecule. Compounds with these structures typically have little toxicity, but they become extremely potent if the phosphorothionate is converted to the phosphate,  $RO_2P(O)OX$ , or the thioether-sulfur is oxidized to a sulfoxide,  $-C-S(O)-C-$ , or sulfone,  $-C-S(O_2)-C-$ . The potential activity



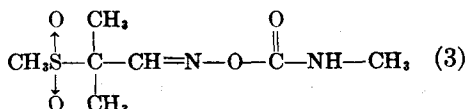
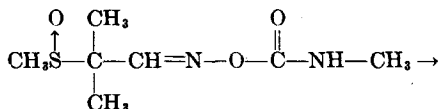
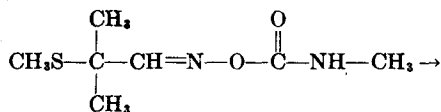
of the product may be about 10,000-fold greater than that of the precursor (O'Brien, 1960). In a typical study, Read (1971) applied the insecticides chlorfenvinphos and dyfonate to a soil, from which samples were taken at regular intervals for bioassay of their capacity to inhibit the larvae of a test insect. The toxicity of the treated samples was low at first, but the inhibitory effect increased with time as the chemicals were transformed and activated. Dimethoate,  $(CH_3O)_2P(S)SCH_2C(O)NHCH_3$ , is a phosphorothionate that is activated in soil by an apparent oxidation to the corresponding oxygen analog (Bache and Lisk, 1966), although a precise role for microorganisms has yet to be established in this sequence.

Several thioethers are converted in soil to the toxic agents, and of considerable importance is the fact that the hazardous product may be quite persistent and fail to succumb rapidly to biodegradation. This is well illustrated for the insecticide known as phorate, *O,O*-diethyl *S*-(ethylthiomethyl)phosphorodithioate, which is oxidized to the corresponding sulfoxide and sulfone.





These products in turn persist for more than 16 weeks (Getzin and Shanks, 1970). A closely related insecticidal thioether is disulfoton,  $(C_2H_5O)_2P(S)SC_2H_4SC_2H_5$ , a compound that, in its unaltered form, shows little toxicity, yet marked increases in toxicity ensue as the thioether-sulfur is oxidized to the sulfoxide and sulfone. Disulfoton sulfoxide and disulfoton sulfone persist for some time, so that soils containing them deleteriously affect insect inhabitants (Takase *et al.*, 1971). A similar transformation is evident with 2-methyl-2-(methylthio)propionaldehyde-(*O*-methylcarbamoyl)oxime, an insecticidal, miticidal, and nematocidal agent sold under the name aldicarb or temik. This molecule is likewise converted to the corresponding sulfoxide and sulfone by oxidation of its sulfur.



The toxicity of this commercially important pesticide has been ascribed largely to the sulfoxide (Coppedge *et al.*, 1967). Although such reactions are probably microbial, no definitive work exists to establish that the subterranean microflora is responsible for producing the insecticidal agents.

Many of the synthetic compounds introduced or transported to soil or water neither are volatile nor are destroyed at appreciable rates by the resident communities, and thus they persist for months, years, or even decades. Some of these chemicals are transformed to long-lived products. The prolonged persistence of a toxicant is of special concern because the stress on susceptible populations is not soon relieved, and

the polluting or pesticidal agents continue to make their presence felt for long periods of time. Possible reasons for the failure of microorganisms to bring about rapid biodegradation and chemical structures that are refractory to attack have recently been reviewed (Alexander, 1973).

Three chlorinated hydrocarbon insecticides—DDT, heptachlor, and aldrin—illustrate quite clearly the genesis of a new and durable toxicant, each of the parent molecules in these instances also persists for considerable time intervals. DDT, the widely known abbreviation for 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane, controls a wide variety of insects important as vectors of microorganisms causing human disease and as pests affecting the food supply, but it is converted to DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane), also abbreviated TDE, which likewise is insecticidal. Indeed, DDD acts on a somewhat different spectrum of insects than its precursor, and it is marketed commercially because of its potency. That such a formation of one toxicant from a second is widespread is suggested by observations that the reaction, involving a simple reductive dechlorination, takes place in samples of soil (Parr *et al.*, 1970), raw sewage (Pfaender and Alexander, 1972), marine waters (Patil *et al.*, 1972), and estuarine sediments (Albone *et al.*, 1972), among other habitats. A high percentage of the bacteria isolated from marine waters and raw sewage possesses the capacity of converting DDT to DDD, often in good yield, and a similar capacity presumably exists among isolates from other localities. The dechlorination probably involves a step in a cometabolic sequence, and some 20–75% of the bacteria from sewage and ocean waters in two studies appear able to cometabolize DDT (Patil *et al.*, 1972; Pfaender and Alexander, 1973). Among the identified organisms effecting the requisite dechlorination are *Aerobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae* (Wedemeyer, 1966), *Fusarium oxysporum* (Engst *et al.*, 1967), *Hydrogenomonas* sp. (Pfaender and Alexander, 1972), *Proteus vulgaris* (Barker *et al.*, 1965), and *Saccharomyces* (Kallman and Andrews, 1963).

Heptachlor (1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-endo-methanoindene) is subject to epoxidation, and the resulting heptachlor epoxide has been found in studies of many different soils (Helling *et al.*, 1971). The epoxide is reported to be the more toxic of the two compounds to animals, including insects, and it is possibly more toxic to humans, too. Heptachlor disappears slowly with time from treated land, but it is replaced by the epoxide, which endures for many years. Indeed, much of the parent substance is recovered in treated soils as the more toxic product (Murphy and Barthel, 1960; Wingo, 1966). The oxidation in soil probably is attributable to activities of the microflora, and a high percentage of the fungi, bacteria, and actinomycetes isolated

from that environment is able to bring about the epoxidation (Miles *et al.*, 1969). *Chlorella pyrenoidosa* also forms the epoxide in good yield (Elsner *et al.*, 1972).

Aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-*endo*-1,4-*exo*-5,8-dimethanonaphthalene) is likewise subject to epoxidation in soil, and both the original pesticide and the product of epoxidation—known as dieldrin—are insecticidal. Dieldrin is also quite persistent, and it can be found many years after its first application to a field. Inasmuch as this conversion occurs in nonsterile, but not in sterilized, soil, the process is apparently microbial (Lichtenstein and Schultz, 1960). The same transformation takes place in an aldrin-containing medium incubated with a soil inoculum (Lichtenstein *et al.*, 1963). Species of *Fusarium*, *Penicillium*, *Trichoderma*, and actinomycetes (Tu *et al.*, 1968) among others are able to synthesize the toxic epoxide. Dieldrin, in turn, can be metabolized to a still more toxic product, given the trivial name of photodieldrin, in samples of water and silt and by a large number of isolates from soil, water, silt, and the gastrointestinal tract (Matsumura *et al.*, 1970).

Numerous other chlorinated molecules are subject to microbial modifications, and some of the metabolites that are generated are toxic and accumulate sometimes for short, sometimes for long periods of time. Included are not only insecticides, but also herbicides and fungicides, such as the chlorophenoxy alkanolic acid herbicides and the chlorophenol fungicides. A well known illustration of activation among members of these groups is the conversion of 2-(2,4-dichlorophenoxy)ethyl sulfate to the phytotoxin 2,4-dichlorophenoxyacetic acid (2,4-D) in soil. The former compound, which is commonly considered to be the herbicide, is in fact phytotoxic only after it is acted on microbiologically. Some evidence suggests that another inhibitor, 2-(2,4-dichlorophenoxy)ethanol, may be produced and accumulate during the transformation. The conversion of the substituted ethyl sulfate to the substituted ethanol can be brought about in culture by *Bacillus cereus* var. *mycoides* (Andus, 1953; Vlitos and King, 1953).

The  $\beta$ -oxidation sequence for the metabolism of fatty acids serves as a novel means by which herbicides are produced by microbial populations. The compound sold as the herbicide 4-(2,4-dichlorophenoxy)butyric acid, 4-(2,4-DB), is itself innocuous at the rates typically used for weed control, but when applied to susceptible plants, the fatty acid portion of the molecule is subject to a  $\beta$ -oxidation sequence to yield the active principle, 2,4-D. Essentially the same reaction sequence is effected by microorganisms in the soil. Not only is the substituted butyric acid metabolized by  $\beta$ -oxidation but so too are the omega-substituted

2,4-dichlorophenoxy pentanoic, hexanoic, heptanoic, octanoic, nonanoic, decanoic, and undecanoic acids. All such compounds having even-numbered fatty acids yield the herbicidal 2,4-D (Table I). Moreover, phenoxy compounds with odd numbers of carbons in the fatty acid moieties are metabolized to 2,4-dichlorophenol, presumably by  $\beta$ -oxidation to yield 2,4-dichlorophenoxyformic acid, which then undergoes decarboxylation.

The identical  $\beta$ -oxidation pathway for the initial phases in the degradation of omega-substituted 2,4-dichlorophenoxyalkanoic acids occurs in axenic cultures of *Nocardia coeliaca* (Taylor and Wain, 1962). Moreover, analogous mechanisms are involved in the metabolism of unsubstituted and monochloro-substituted phenoxyalkanoic acids by cultures of *Aspergillus niger*, *Nocardia opaca*, and *N. coeliaca* (Faulkner and Woodcock, 1966; Taylor and Wain, 1962; Webley *et al.*, 1958). Although appreciable accumulations of 2,4-dichlorophenol were not noted in early studies of soil amended with 2,4-D, which is one of the major phenoxy herbicides in agricultural practice, the phenol has been found as a product of the metabolism of 2,4-D in cultures of an *Arthrobacter* (Loos *et al.*, 1967b) and in enzyme preparations derived from the bacterium (Loos

TABLE I  
METABOLISM OF OMEGA-SUBSTITUTED PHENOXYALKANOIC ACIDS IN SOIL<sup>a</sup>

Substrate added <sup>b</sup>	Products detected <sup>b</sup>				
	2,4-Dichloro-phenol	2,4-D acetic acid	2,4-D propionic acid	2,4-D butyric acid	2,4-D pentanoic acid
Even-numbered acids					
2,4-D butyric	—	+	—	<sup>c</sup>	—
2,4-D hexanoic	—	+	—	+	—
2,4-D octanoic	—	+	—	+	—
2,4-D decanoic	—	+	—	+	—
Odd-numbered acids					
2,4-D pentanoic	+	—	+	—	<sup>c</sup>
2,4-D heptanoic	+	—	+	—	+
2,4-D nonanoic	+	—	+	—	+
2,4-D undecanoic	+	—	+	—	+

<sup>a</sup> From Gutenmann *et al.* (1964).

<sup>b</sup> The substrates and products are the omega-substituted 2,4-dichlorophenoxy alkanolic acids.

<sup>c</sup> The product listed in these combinations is the substrate.

*et al.*, 1967a), and it also can be formed by a strain of *Flavobacterium* incubated with 4-(2,4-DB) (MacRae *et al.*, 1963).

2,4-Dichlorophenol is of especial significance since its formation from an actual or potential phytotoxin would lead to the appearance of a product with an entirely different spectrum of action than its precursor inasmuch as the phenol is not only antifungal to some degree, but it is also an antiviral agent (Ando *et al.*, 1970) and is believed to be a sex pheromone for arthropods (Berger, 1972). Hence, a study was recently made of its possible formation both in a soil (Lima silt loam) and in fresh water-sediment collected from a rural stream. Both 2,4-D and 2,4,5-trichlorophenoxyacetic acid (the herbicide known as 2,4,5-T) were added to initial concentrations of 50 ppm, and the soil and water were incubated under aerobic conditions. The data in Figs. 1 and 2 show that 2,4-D is converted to 2,4-dichlorophenol and 2,4,5-T is transformed to its corresponding phenol, which is fungicidal, in both soil and water. The yields of the chlorophenols were consistently low, and neither persisted as long as the herbicides from which they were generated. Both metabolites were identified by gas chromatography and mass spectrometry. It is noteworthy that, although 2,4-D was destroyed quickly in the fresh water-sediment model ecosystem, an unidentified product appeared in the water on the sixth day of incubation, and it persisted for 12 weeks; thus, a pesticide that is readily susceptible to attack may give rise to a long-lived metabolite, which possibly may be an environmental pollutant (K. W. Sharpee and M. Alexander, unpublished observations).

The herbicide Zytron (*O*-2,4-dichlorophenyl *O*-methyl isopropyl phosphoramidothioate) can also be converted to 2,4-dichlorophenol in soil. Although the phenol is toxic at concentrations greater than 10 ppm, its concentration in nature is not known to be high enough to have an antimicrobial effect. Zytron can be degraded, moreover, by *Aspergillus clavatus* (Fields and Hemphill, 1966).

A chlorophenol used for the control of a multitude of pests is pentachlorophenol. This compound is dehalogenated in soil, apparently by microorganisms, to yield a variety of chlorinated metabolites, many of which have antifungal activity and which probably are toxic to other species as well. The products include 2,3,4,5-, 2,3,4,6-, and 2,3,5,6-tetrachloro-, 2,4,5-, and 2,3,5-trichloro-, 3,4- and 3,5-dichloro- and 3-monochlorophenols (Ide *et al.*, 1972). These phenols are characteristically resistant to microbial attack (Alexander and Aleem, 1961; MacRae and Alexander, 1965) and hence may cause prolonged pollution.

Another environmental difficulty was recently encountered from the use of chlorinated phenols. Penta- and 2,3,4,6-tetrachlorophenol are applied as fungicides to freshly cut timber and hence they appear in saw-

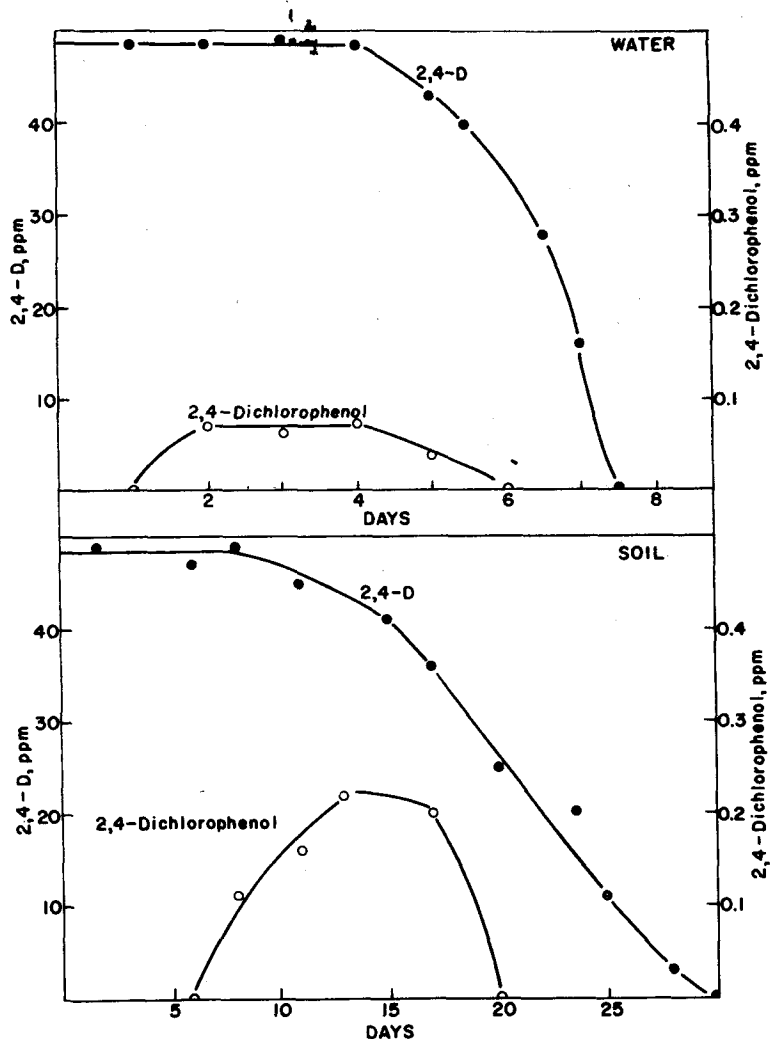


FIG. 1. Changes in the concentrations of 2,4-D and 2,4-dichlorophenol in a soil and in a fresh water-sediment model ecosystem.

dust and wood shavings. The problem came to light owing to the presence of such treated wood products in the poultry house, where the phenols are apparently methylated microbiologically to yield penta- and 2,3,4,6-tetrachloroanisole. The anisoles then appear in the chicken flesh and impart a musty taint to the chickens. The phenols are not methylated in autoclaved poultry litter, attesting to the role of microbial colonists. Such methylation reactions can be carried out in axenic cultures of *Aspergillus*, *Penicillium*, and *Scopulariopsis* (Curtis *et al.*, 1972). The methyla-

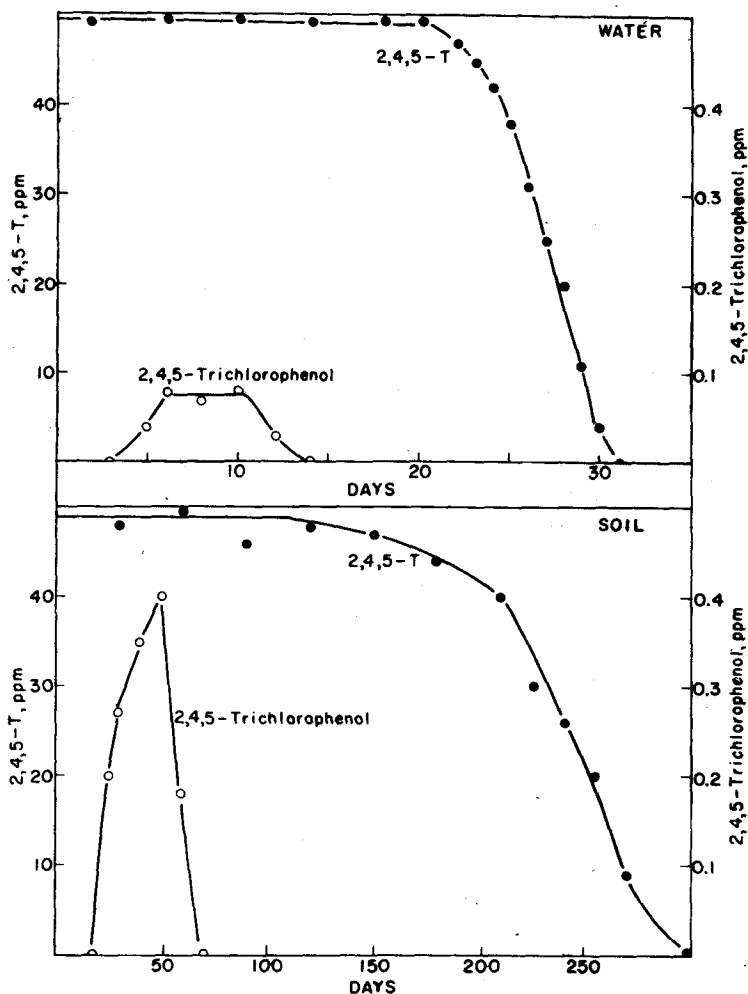


FIG. 2. Changes in the concentrations of 2,4,5-T and 2,4,5-trichlorophenol in a soil and in a fresh water-sediment model ecosystem.

tion is not restricted to the highly chlorinated phenols because even 2,4-dichlorophenol can be *O*-methylated by a strain of *Arthrobacter* to give 2,4-dichloroanisole (Loos *et al.*, 1967b).

Other chlorinated pesticides are subject to enzymatic modification to yield possible environmental pollutants. An interesting case is the herbicide propanil (3',4'-dichloropropionanilide), which is transformed in soil to 3,4-dichloroaniline, and the latter then gives rise to 3,3',4,4'-tetrachloroazobenzene. Formation of the azobenzene requires microorganisms since none is observed in sterilized soil receiving either propanil

or the dichloroaniline (Bartha and Pramer, 1970). The herbicide can be converted to the tetrachloroazobenzene in nutrient media or sterilized soil inoculated with a mixture of *Penicillium piscarium* and *Geotrichum candidum* (Bordeleau and Bartha, 1971), while the azo compound can be formed from 3,4-dichloroaniline in *Fusarium oxysporum* cultures (Kaufman *et al.*, 1972). The reason for potential environmental concern is that both 3,4-dichloroaniline and 3,3',4,4'-tetrachloroazobenzene are mutagenic. It has been proposed that subterranean mutagens might alter the virulence of soil-borne pathogens so that they become more harmful to agricultural crops (Prasad, 1970).

The chlorinated aromatic herbicide known as dichlobenil (2,6-dichlorobenzonitrile) on occasion induces a leaf margin chlorosis in crops. The chlorosis is attributable not to the added chemical, but to the 2,6-dichlorobenzamide generated from it. This synthesis of a phytotoxin with a somewhat different range of susceptible plants than its precursor phytotoxin results from microbial activity, and the new toxicant is apparently quite resistant to further enzymatic modification in nature (Verloop, 1972). It is also possible for microorganisms themselves to synthesize the herbicidal 2,6-dichlorobenzonitrile from the nonphytotoxic  $\alpha$ -amino-2,6-dichlorobenzaldoxime, a process occurring both in soil and in cultures of *Pseudomonas putrefaciens* (Milborrow, 1963).

Diuron, 3-(3,4-dichlorophenyl)-1,1-dimethylurea, is another popular herbicide. This compound is demethylated in soil to give rise to a new phytotoxin, 3-(3,4-dichlorophenyl)-1-methylurea (Dalton *et al.*, 1966). Such dealkylations are common to microorganisms.

A notable instance of a microbiologically induced altered spectrum of action, one which led to a significant change in agricultural practice, is evident in the case of pentachlorobenzyl alcohol. This compound was introduced into Japan for the control of the blast disease of rice. The chemical was effective and showed no phytotoxicity even when applied to the foliage of test plants at concentrations of 2000 to 10,000 ppm. Two years after its introduction, however, reports arrived of deleterious effects on tomato, melon, and cucumber plants resulting from its use. The symptoms were particularly severe if the plants were grown in soil containing compost made from rice plants receiving the fungicide the year before. Analysis of the rice straw inducing these changes demonstrated that it contained pentachloro- and 2,3,4,6- and/or 2,3,5,6-tetrachlorobenzoic acids, all of which are remarkably effective in suppressing the growth of leguminous and solanaceous plants. Laboratory trials then revealed that the same polychlorinated benzoates were formed in soil. The first phase in the chemical transformation involved the oxidation of pentachlorobenzyl alcohol to the corresponding benzoic acid. This oxidation did not occur and no phytotoxicity was evident in sterile soil



receiving the alcohol, and bacteria, actinomycetes, and fungi in culture brought about by the same oxidation; hence, the microscopic community is implicated. As a result of this outcome, the production and marketing of these fungicide preparations terminated in 1968 (Ishida, 1972).

Pentachloronitrobenzene, a fungicide applied to seed and soil for protection of crops against pathogens, is converted in soil, apparently by its residents, to pentachloroaniline. It also can be reduced to the aniline by fungi in culture. The product of the reduction then suppresses the growth of fungi and actinomycetes, although the aniline is less fungitoxic than the nitro compound (Ko and Farley, 1969; Nakanishi and Oku, 1969). Thus, one antimicrobial agent can be generated from a second.

Reactions in nature presumably brought about by microorganisms may convert antimicrobial compounds to phytotoxins. An excellent illustration is the partial dehalogenation of 2-chloro-6-(trichloromethyl)pyridine, a chemical of practical value because it suppresses the nitrifying bacteria. By inhibiting the oxidation of ammonium to nitrate, it can prevent much of the loss of inorganic nitrogen from farm land since nitrate, but not ammonium, can be denitrified and the anion is readily washed through the soil. A recent report provides data showing that 2-chloro-6-(trichloromethyl)pyridine is converted in soil to 6-chloropicolinic acid (Fig. 3). The latter is more toxic than the former to tomatoes, cotton, sugar beets, and legumes (Geronimo *et al.*, 1973). Chloral hydrate, a substance with herbicidal action, is converted rapidly in soil to trichloroacetic acid, the latter likewise being a useful herbicide. Microorganisms are reported to participate in the transformation (Schütte and Stephan, 1969).

The important fungicide thiram (tetramethylthiuram disulfide) is acted on, in part at least biologically, to yield products that are harmful to totally dissimilar organisms. For example, it is converted in the rumen, presumably by the indigenous microbiota, to  $\text{CS}_2$  and probably  $\text{H}_2\text{S}$  (Robbins and Kastelic, 1961). The former is fungicidal; the latter affects a broad range of organisms.  $\text{CS}_2$  is also evolved from soil treated with thiram (Munnecke *et al.*, 1962). As discussed below, thiram can be cleaved and nitrosated, the result being a carcinogen. A product of its biological reduction is dimethyldithiocarbamate, a reduction effected by *Glomerella cingulata* (Richards and Thorn, 1960) and a number of algae (Lindahl, 1964). Dimethyldithiocarbamate is likewise antifungal and is commercially marketed as such as the sodium salt,  $(\text{CH}_3)_2\text{NC}(=$

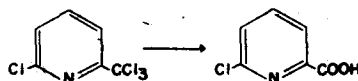


FIG. 3. Conversion of 2-chloro-6-(trichloromethyl)pyridine to 6-chloropicolinic acid.