

# **Sulfur in Pesticide Action and Metabolism**



# Sulfur in Pesticide Action and Metabolism

**Joseph D. Rosen, EDITOR**

*Cook College, Rutgers University*

**Philip S. Magee, EDITOR**

*Chevron Chemical Company*

**John E. Casida, EDITOR**

*University of California, Berkeley*

Based on a symposium  
sponsored by the Division  
of Pesticide Chemistry at  
the Second Chemical Congress  
of the North American Continent,  
Las Vegas, Nevada, August  
25–29, 1980.

A C S   S Y M P O S I U M   S E R I E S   **158**

**AMERICAN CHEMICAL SOCIETY**  
**WASHINGTON, D. C.      1981**



Library of Congress CIP Data

Sulfur in pesticide action and metabolism.

(ACS symposium series, ISSN 0097-6156; 158)

Includes bibliographical references and index.

Contents: Low-molecular-weight organosulfur compounds in nature / Eric Block—Chemical mechanisms of the cytochrome P-450 monooxygenase-catalyzed metabolism of phosphorothionate triesters / R. A. Neal—Sulfur in propesticide action / T. R. Fukuto and M. A. H. Fahmy—[etc.]

1. Organosulphur compounds—Physiological effect—Congresses. 2. Pesticides—Physiological effect—Congresses. 3. Organosulphur compounds—Metabolism—Congresses. 4. Pesticides—Metabolism—Congresses.

I. Rosen, Joseph D., 1935— . II. Magee, Philip, 1926— . III. Casida, John E., 1929— . IV. Series: ACS symposium series; 158.

SB952.S94S93

632'.95

81-7916

ISBN 0-8412-0635-X AACR2 ACSMC8 158 1-192

Copyright © 1981

American Chemical Society

All Rights Reserved. The appearance of the code at the bottom of the first page of each article in this volume indicates the copyright owner's consent that reprographic copies of the article may be made for personal or internal use or for the personal or internal use of specific clients. This consent is given on the condition, however, that the copier pay the stated per copy fee through the Copyright Clearance Center, Inc. for copying beyond that permitted by Sections 107 or 108 of the U.S. Copyright Law. This consent does not extend to copying or transmission by any means—graphic or electronic—for any other purpose, such as for general distribution, for advertising or promotional purposes, for creating new collective work, for resale, or for information storage and retrieval systems.

The citation of trade names and/or names of manufacturers in this publication is not to be construed as an endorsement or as approval by ACS of the commercial products or services referenced herein; nor should the mere reference herein to any drawing, specification, chemical process, or other data be regarded as a license or as a conveyance of any right or permission, to the holder, reader, or any other person or corporation, to manufacture, reproduce, use, or sell any patented invention or copyrighted work that may in any way be related thereto.

PRINTED IN THE UNITED STATES OF AMERICA

**American Chemical  
Society Library**

**1155 16th St. N. W.**

In Sulfur in Pesticide Action and Metabolism: Rosen, J., et al.;  
ACS Symposium Series; American Chemical Society: Washington, DC, 1981.

Washington, D. C. 20036

## ACS Symposium Series

**M. Joan Comstock, *Series Editor***

### *Advisory Board*

David L. Allara	James P. Lodge
Kenneth B. Bischoff	Marvin Margoshes
Donald D. Dollberg	Leon Petrakis
Robert E. Feeney	Theodore Provder
Jack Halpern	F. Sherwood Rowland
Brian M. Harney	Dennis Schuetzle
W. Jeffrey Howe	Davis L. Temple, Jr.
James D. Idol, Jr.	Gunter Zweig

## FOREWORD

The ACS SYMPOSIUM SERIES was founded in 1974 to provide a medium for publishing symposia quickly in book form. The format of the Series parallels that of the continuing ADVANCES IN CHEMISTRY SERIES except that in order to save time the papers are not typeset but are reproduced as they are submitted by the authors in camera-ready form. Papers are reviewed under the supervision of the Editors with the assistance of the Series Advisory Board and are selected to maintain the integrity of the symposia; however, verbatim reproductions of previously published papers are not accepted. Both reviews and reports of research are acceptable since symposia may embrace both types of presentation.

## PREFACE

Nearly thirty centuries ago Homer referred to "pest-averting sulfur." Today many organosulfur natural products are known to have pesticidal activity, and more than one-third of the total number of synthetic organic pesticides contain sulfur. Pesticides are categorized commonly as organochlorine or organophosphorus compounds but rarely as organosulfur compounds. This is caused in part by the heterogeneous nature of the organosulfur pesticides and in part by the varied functions of the sulfur in contributing to the biological activity.

Pesticide effectiveness and selectivity often are increased by using apolar and relatively stable compounds which can be converted metabolically to the active toxicants. Most sulfur-containing pesticides are in fact propesticides undergoing metabolic activation by reactions involving or initiated by oxidation. These sulfoxidations increase the reactivity of phosphorothionate insecticides as phosphorylating agents and of thiocarbamate herbicides as carbamoylating agents. Metabolic conversion of sulfides to sulfoxides and sulfones also alters the reactivity, solubility, and ease of translocation of systemic pesticides. Thus, introduction of a sulfur-containing moiety may enhance the selectivity, sometimes with a reduction in mammalian toxicity.

Endogenous sulfur compounds play a critical role in the action of many insecticides, fungicides, and herbicides. The primary target enzyme or receptor may have an essential thiol group and secondary target sites also may have such groupings. The metabolism may be dominated by glutathione-dependent processes thereby allowing interactions with synergists or antidotes that alter the efficiency of these sulfur reactions and thus the toxicity of the pesticides.

Recognition of the importance of sulfur chemistry and biochemistry in relation to pesticide metabolism and action led to a symposium with this title at the August 1980 National Meeting of the American Chemical Society. This symposium volume considers some principles of sulfur chemistry as they affect our understanding of sulfur-containing compounds in biological systems. Expanding knowledge of organosulfur chemistry

and biochemistry will influence significantly the design of pesticides for the future and the evaluation of conditions for the safe and efficient use of these pest control chemicals.

Joseph D. Rosen  
Department of Food Science  
Cook College  
Rutgers University  
New Brunswick, NJ 08903

PHILIP S. MAGEE  
Agricultural Chemicals Division  
Chevron Chemical Company  
940 Hensley St.  
Richmond, CA 94804

John E. Casida  
Pesticide Chemistry and Toxicology Laboratory  
Department of Entomological Sciences  
University of California  
Berkeley, CA 94720

February, 1981



# Low-Molecular-Weight Organosulfur Compounds in Nature: The Search for New Pesticides

ERIC BLOCK

Department of Chemistry, University of Missouri, St. Louis, MO 63121

A rich variety of organosulfur compounds are found in living systems. A list of biochemically notable organosulfur compounds would include, among others, the essential amino acids *cysteine* and *methionine*, peptides such as *glutathione*, and the recently characterized biologically extremely potent glutathione derivative *leukotriene C-1* (1)(1) (Fig. 1) (the "slow reacting substance" of anaphylaxis (SRS) which is known for its role in the lungs during asthma attacks), polycyclic peptide antibiotics such as *bacitracin*, *gliotoxin*, *cephalosporin*, *penicillin*, and the recently discovered  $\beta$ -lactam antibiotic *thienamycin* (2)(2), cofactors and vitamins such as *thiamine*, *biotin*, *coenzyme A* and  $\alpha$ -*lipoic acid*, a biological alkylating agent, *S-adenosylmethionine*, the biological redox systems *ferredoxin* and *rubredoxin* and sulfur-containing bases found in bacterial transfer-RNA such as *4-thiouracil* (3).

A remarkable range of simpler organosulfur compounds are known to be widely distributed throughout the plant kingdom (3,4,5,6). These compounds often make important contributions to the odor and flavor of many of the common comestibles. In some instances these sulfur compounds may also serve by their odor and taste to repel predators or to act for the plant as resistance factors against infection by microorganisms (e.g. as natural pesticides). Not to be outdone, some insects such as the onion maggot, *Hylemya antiqua*, and turnip (or vegetable) weevil, *Listroderes obliquus*, have evolved so that some of the same organosulfur compounds act as attractants and stimulate egg laying and biting (7).

A number of insects and higher animals have also been found to possess unusual small organosulfur molecules which may serve as defensive secretions, sex attractants, or scent markers. In many cases the low molecular weight organosulfur compounds occur in plants (and possibly in animals as well) in an odorless combined form (e.g., as peptides or glycosides) and are released enzymatically when the tissue is injured or stimulated, i.e. during attack by a predator. Finally it should be noted that certain sulfur compounds reported to be of natural origin may

0097-6156/81/0158-0003\$05.00/0

© 1981 American Chemical Society

be artifacts formed during the isolation procedures through reactions of unstable precursors.

In the survey of natural organosulfur molecules that follows I have focused on those lower molecularweight compounds known to show biological activity (or which might be expected to possess such activity), particularly as pesticides and antibiotics, and have emphasized the more current literature with coverage through July 1980. There are valuable lessons for us to learn from a careful study of attempts by other species to use chemicals as pest control agents in their struggle for survival. A few illustrations will be given showing how this knowledge has been put into practice in the design of new pesticides. It is convenient to group the compounds according to whether the sulfur is bonded in an acyclic or heterocyclic manner. The acyclic naturally occurring organosulfur compounds will be discussed first.

### Acyclic Sulfur Systems

Plant Sources. The turnip (*Brassica campestris*), rutabaga (*Brassica napus*), cabbage (*Brassica oleracea*), radish (*Raphanus sativus*) and a number of other plants contain 2-phenylethylisothiocyanate,  $\text{PhCH}_2\text{CH}_2\text{NCS}$ , (3), while the radish, black mustard (*Brassica nigra*), and penny cress (*Thlaspi arvense*) contain related isothiocyanates which possess sharp irritating odors and cause blistering of the skin. Certain of these isothiocyanates such as 3 are insecticidal or antibiotic protecting the plant against parasites (6, 8). At the same time allylisothiocyanate,  $\text{CH}_2=\text{CHCH}_2\text{NCS}$ , found in various *Brassica* species, has been found to be an attractant for the vegetable weevil, *Listroderes obliquus*, which feeds on Brassica species and the flea beetle, *Phyllotreta cruciferae*, and to initiate biting by the former harmful pest (7). The isothiocyanates, or mustard oils as they are called, have been known since the nineteenth century to be secondary products arising from breakdown of mustard oil glucosides (widely distributed in the Cruciferae family) when cellular structure is disrupted (6).

The *Allium* species such as the onion (*Allium cepa*), garlic (*Allium sativum*), shallot (*Allium ascalonicum*), chive (*Allium scordoprasum*), caucas (*Allium victorialis*), and leek (*Allium odorum*) and other members of the Liliaceae family are rich natural sources of biologically active organosulfur compounds. As in the case of the mustard oil from Brassica species, the organosulfur compounds from Allium species are found in the plant tissues in an odorless combined form, e.g. as  $\gamma$ -glutamyl peptides of the S-alkyl- and S-alkenylcysteine sulfoxides 4, Figure 2. Alliin, ( $\text{R} = \text{H}$ ,  $\text{R}' = \text{CH}_2=\text{CH}$ ) the native constituent of garlic is broken down enzymatically to allicin,  $\text{CH}_2=\text{CHCH}_2\text{S}(\text{O})\text{SCH}_2\text{CH}=\text{CH}_2$  (6c), when the plant is crushed. Allicin is bacteriocidal and along with thiosulfinates 5a and 5b from onion or garlic possesses antifungal, antiviral and tumor inhibiting activity (9). Diallyl disulfide,  $(\text{CH}_2=\text{CHCH}_2\text{S})_2$ , and diallyl trisulfide,  $(\text{CH}_2=\text{CHCH}_2\text{S})_2\text{S}$ , two other components of

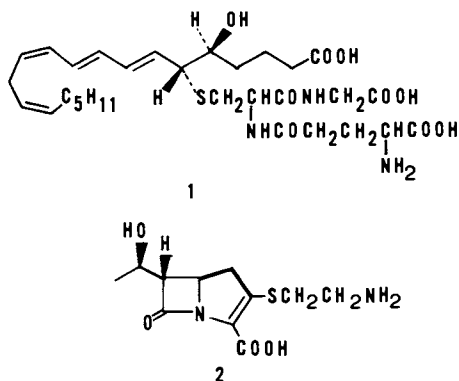


Figure 1. *Leukotriene C-1 (1) and thienamycin (2)*

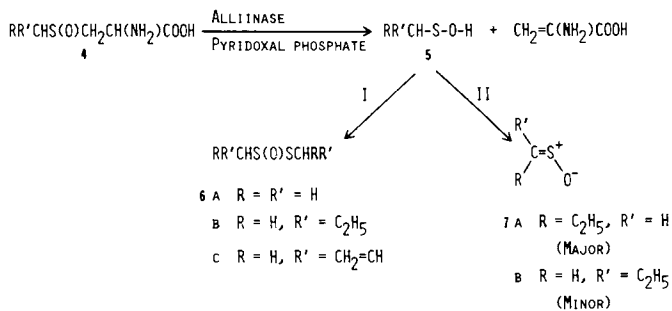


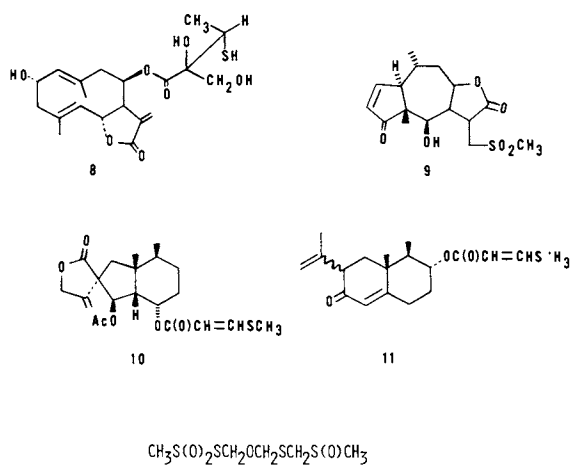
Figure 2. *Organosulfur compounds from allium species*

garlic oil have been found to possess larvicidal activity (fatal at 5 ppm to *Culex pipiens*, a mosquito species) (10). The intensely lachrymatory principle of the onion, shown to be a 20:1 mixture of Z- and E-propanethial S-oxide, 7a and 7b, (11, 12, 13) is derived by enzymatic breakdown of trans-(+)-S-(1-propenyl)-L-cysteine sulfoxide  $\text{CH}_3\text{CH}=\text{CHS}(\text{O})\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$  followed by re-arrangement of the intermediate (E)-1-propenesulfenic acid,  $\text{CH}_3\text{CH}=\text{CHS}-\text{O}-\text{H}$  (11, 13). The onion lachrymator may serve an ecological function in repelling some herbivores or parasites. Di-n-propyl disulfide and other disulfides produced by the onion act as powerful attractants for the onion maggot (*Hylemya antiqua*), and black blowfly (*Phormia regina*) (only the female is attracted!) (7) and stimulate egg laying by the onion maggot and leek moth (*Acrolepiopsis assectella*) (14). Crude onion juice inhibits the growth of *Escherichia coli*, *Pseudomonas pyocyaneus*, *Salmonella typhi* and *Bacillus subtilis* (15).

Recently, the causative agent of a type of allergic contact dermatitis known as "Dogger Bank itch" has been identified as the (2-hydroxyethyl)dimethylsulfoxonium ion,  $(\text{CH}_3)_2\text{S}^+(\text{O})\text{CH}_2\text{CH}_2\text{OH}$ , produced by the marine bryozoan, *Alcyonidium gelatinosum* (16). Related salts are known such as 6-dimethylsulfonium pentanoic acid,  $(\text{CH}_3)_2\text{SCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$ , from the coastal dune plant *Diplotaxis tenuifolia*, and thetin,  $(\text{CH}_3)_2\text{SCH}_2\text{CH}_2\text{CO}_2\text{H}$ , a common component of various marine algae (17).

A variety of other compounds containing acyclic sulfur have recently been isolated from plants although nothing has been reported on their biological activity. These examples would include diethyl tetrasulfide,  $(\text{C}_2\text{H}_5\text{SS})_2$ , and related polysulfides and hydrosulfides from the fruit of the durian, *Durio zibethinus* (a much cherished south-east Asian fruit) (18), germacradienolide thiol 8 from *Eupatorium mikanioides* (19), sesquiterpene lactone sulfone 9 from the roots of *Helentium autumnale* (20), vinyl sulfides 10 and 11 (S-petasin) from *Petasites japonicus* and *Petasites hybridus*, respectively (21, 22), thiosulfonate 12, from the mushroom *Lentinus edodes*, and S-methylthiomethyl 2-methylbutane-thioate,  $\text{C}_2\text{H}_5\text{CH}(\text{CH}_3)\text{C}(\text{O})\text{SCH}_2\text{SCH}_3$ , from the essential oil of hops (23). (See Figure 3.)

Animal Sources. The spray of the striped skunk (*Mephitis mephitis*) which includes trans-2-butenethiol,  $\text{CH}_3\text{CH}=\text{CHCH}_2\text{SH}$ , 3-methylbutanethiol,  $(\text{CH}_3)_2\text{CHCH}_2\text{CH}_2\text{SH}$ , and crotyl methyl disulfide,  $\text{CH}_3\text{CH}=\text{CHCH}_2\text{SSCH}_3$  (24) is obviously very effective in repelling (and making quite miserable) would-be predators. As an aside it may be noted that the sensitivity of the human nose to simple thiols (ca. 0.02 ppb of methanethiol and 0.0007 ppb of 2-methyl-2-butanethiol can be detected (25)) and other small sulfur molecules as well as to low molecular weight amines coupled with the fact that many animals also find their smell offensive supports speculation that natural selection developed this olfactory sensitivity as a form of protection for the organism against the



*Figure 3. Compounds containing acyclic sulfur*

ingestion of decaying food (low molecular weight organosulfur compounds and amines are also products of biological decay). A practical application of this observation is the use of diallyl disulfide,  $(\text{CH}_2=\text{CHCH}_2\text{S})_2$ , as a repellent for injurious birds (26).

The occurrence of  $\Delta^3$ -isopentenyl methyl sulfide,  $\text{CH}_2=\text{C}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{SCH}_3$ , and 2-phenylethyl methyl sulfide,  $\text{PhCH}_2\text{CH}_2\text{SCH}_3$ , as components of the urine scent mark of the red fox (*Vulpes vulpes*) (27), of 5-methylthio-2,3-pentanedione,  $\text{CH}_3\text{C}(\text{O})\text{C}(\text{O})\text{CH}_2\text{CH}_2\text{SCH}_3$ , as a volatile secretion from the anal scent gland of the striped hyena (*Hyaena hyaena*) (28), of di(3-methylbutyl)sulfide,  $(\text{CH}_3)_2\text{CHCH}_2\text{CH}_2)_2\text{S}$ , from the polecat (*Mustela putorius*) (29), and of dimethyl disulfide,  $\text{CH}_3\text{SSCH}_3$ , as an attractant pheromone in the vaginal secretion of the hamster (*Cricetus cricetus*) (30) provide examples of organosulfur compounds involved in chemical interactions between animals.

### Heterocyclic Sulfur Systems

Plant Sources. Fig. 4 displays a broad range of organosulfur compounds found in plants or animals in which the sulfur atom(s) is part of a ring. Many of these compounds show substantial biological activity. Thus, various species of marigold (*Tagetes*) and other members of the Compositae family contain  $\alpha$ -terthienyl, 21, and 5-(3-buten-1-ynyl)-2,2'-bithienyl, 20, which are lethal to bacteria, yeasts and other fungi, nematodes and fish in near-UV light (31,32,33). In the original study in this area, 24 kg of marigold roots were processed to ultimately afford 200 mg of 21 which proved to be nematocidal against *Heterodera rostochiensis* larvae, *Pratylenchus penetrans*, *Ditylenchus dipsaci* and other species of nematodes (34). Other natural thiophene ring containing nematocides include 2-phenyl-5-(1'-propynyl)-thiophene 19 from *Coreopsis lanceolata* (35) and related compounds from *Chrysanthenum vulgare* (36) and other species (37).

A second group of biologically active molecules isolated from natural sources are 1,2-dithiolane derivatives 22-25 which are structurally related to the cofactor,  $\alpha$ -lipoic acid. Asparagusic acid, 22, present to the extent of about 35 ppm in the roots of asparagus (*Asparagus officinalis*) is active against the plant parasitic nematodes *Paratylenchus penetrans* and *P. curvatus*, *Heterodera rostochiensis* and *H. glycines*, and *Meloidogyne hapla*, and is considered to be a major factor in natural resistance of asparagus (38). Asparagusic acid, its syn and anti S-oxide (39) and some related acyclic derivatives are also very effective plant growth inhibitors. Compounds 23 (4-methylthio-1,2-dithiolane) and 32 (5-methylthio-1,2,3-trithiolane) are responsible for the rank, pungent smell associated with the stone-*wort* (*Chara globularis*). Whenever this green alga occurs in a pond it dominates the algal flora of the ecosystem, apparently because 23 and 32 act as extremely active inhibitors of photosynthesis, thus eliminating would-be competitive species (40). The

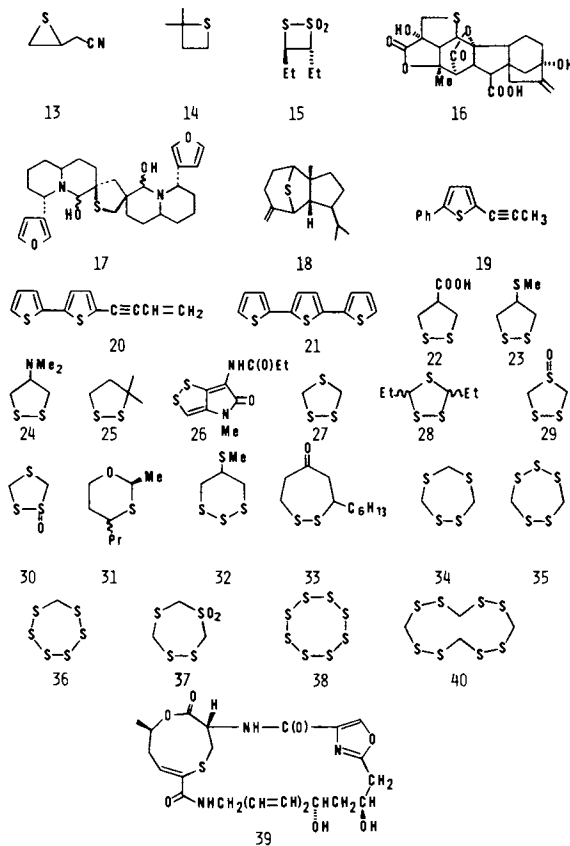


Figure 4. Naturally occurring thiaheterocycles

properties of 1,2-dithiolanes 24 and 25 will be discussed below.

A number of unusual cyclic polysulfides showing antibiotic activity against bacteria and fungi have been isolated from the red alga *Chondria californica* (41) and from the mushroom *Lentinus edodes* (42). Both 34 (1,2,4,6-tetrathiepane) and 35 (1,2,3,5,6-pentathiepane or lenthionine) have been isolated from both sources, while 1,2,3,4,5,6-hexathiepane, 36, was obtained from the mushroom and 27 (1,2,4-trithiolane), 29 (1,2,4-trithiolane 4-oxide), 30 (1,2,4-trithiolane 1-oxide), 37 (1,2,4,6-tetrathiepane 4,4-dioxide) and 40 (1,2,4,5,7,8,10,11-octathiacyclododecane) were obtained from the alga (for a synthesis of 29, see (43); for studies on precursors to 35, see (44)). Trithiolanes related to 27, 29, and 30 have been isolated from steam volatile oil of onion (e.g. 28) (45) and root material of *Petiveria alliacea* (46).

Other biologically active naturally derived thiaheterocycles include 6,6'-dihydroxythiobinupharidine, 17, a fungicide from the spatterdock or yellow pond lily (*Nuphar luteum*) (47,48), griseoviridin, 39, an antiobiotic from *Streptomyces griseus* (49) and the antibiotic ureothricin 26 (50). Other natural thiaheterocycles whose biological activities have not yet been reported include 1-cyano-2,3-epithiopropene, 13, from cabbage, (51) the isomeric 2-methyl-4-propyl-1,3-oxathianes, 31, from the Hawaiian yellow passion fruit (*Passiflora edulis*) (52), the sesquiterpene mintsulfide, 18, from oil of peppermint (*Mentha piperita*) (53), the diterpenoid, pharbitic acid, 16, from the seeds of the Japanese morning-glory (*Pharbitis nil*) (thought to be a gibberellin A regulator) (54), trans-3,4-diethyl-1,2-dithietane 1,1-dioxide, 15, formed by dimerization of the lachrymatory factor of the onion, Z- and E-propanethial S-oxide 1a,b (55), and disulfide 33 from two brown algae of the genus *Dictyopteris* (56,57).

Animal Sources. The marine annelid worm *Lumbrineris heteropoda* produces an insect toxin nereistoxin, 24, 4-N,N-dimethyl-amino-1,2-dithiolane (58). The mink (*Mustela vison*) affords 2,2-dimethylthietane, 14, and 3,3-dimethyl-1,2-dithiolane, 25 (28,63,64), the ferret (*Mustela putorius*) secretes 14, 25, cis- and trans-2,3-dimethylthietane, 2-propyl- and 2-pentyl-thietane, cis and trans-3,4-dimethyl-1,2-dithiolane, and 3-propyl-1,2-dithiolane (65), and the stoat (*Mustela erminea*) contains 2-ethyl-, 2-propyl-, and 2-pentyl-thietane, and 3-ethyl- and 3-propyl-1,2-dithiolane in its anal gland (59,63). These several thiaheterocycles from mustela species probably function as scent markers.

## Conclusion

Fig. 5 gives examples of many of the types of sulfur-containing pesticides which are currently in use or have been used. It is clear that the types of functionalities present, e.g. organophosphate derivatives, chlorocarbons, sulfenamides, dithiocarbamates, oxime derivatives, and cyclic sulfite esters are "unnatural" (or at least presently "unnatural") groupings



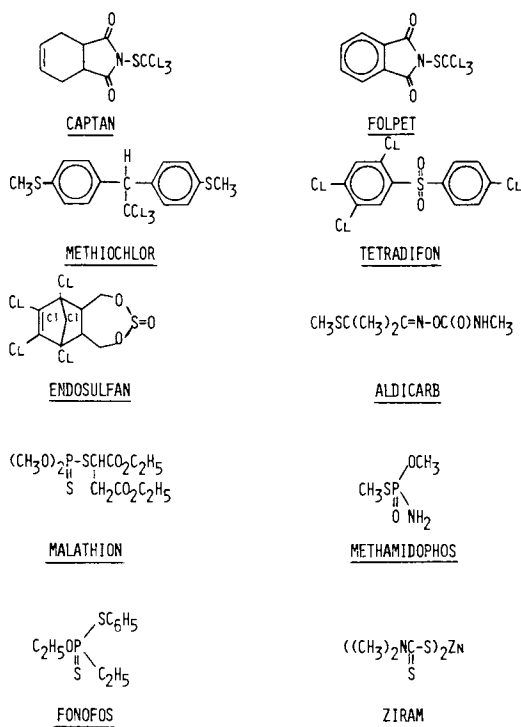


Figure 5. Some common types of sulfur-containing pesticides



Figure 6. Pesticides that were developed based on bioactive natural product models

very different from the structural types covered in the earlier sections of this review. With the aim of avoiding the serious environmental problems associated with the use of some "unnatural" pesticides, synthetic variants of bioactive natural products (such as the pyrethroids) are under active investigation. Excellent examples of the development of new pesticides based on bioactive natural product models are the synthetic insecticides thiocyclam, 5-N,N-dimethylamino-1,2,3-trithiane, 41, (60,61) and cartap, 42, (62) a bithiocarbamate which is converted *in vitro* and *in vivo* to nereistoxin 24 (Note Figure 6 ). A number of organothiocyana-tes and isothiocyana-tes, modeled after the mustard oils, have seen limited use as pesticides. It should be noted here that the "natural" (but not organic) sulfur heterocycle cyclooctasulfur, 38, the native form of elemental sulfur, has been used as an acaricide and fungicide for centuries and is still widely used today (note the occurrence of elemental sulfur in algae (66)).

The isolation of physiologically active natural products and the determination of the chemical basis for their activity, the study of insect biochemistry, and the investigation of the chemical basis for host selection are current areas of research activity which should be of particular interest to the pesticide chemist for it may be anticipated that an important "spin-off" of this research will be the discovery of new and safer organic pesticides. It may also be anticipated that a substantial number of these new pesticides will incorporate that most versatile element, sulfur.

#### Acknowledgments

Support during the preparation of this review by the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the National Science Foundation (CHE 8001490) is gratefully acknowledged.

#### Literature Cited

1. Corey, E.J.; Clark, D.A.; Goto, G.; Marfat, A.; Mioskowski, C.; Samuelsson, B.; Hammarstrom, S. J. Am. Chem. Soc., 1980, 102, 1436.
2. Bouffard, F.A.; Johnston, D.B.R.; Christensen, B.G. J. Org. Chem., 1980, 45, 1130, and accompanying papers.
3. Block, E. "Reactions of Organosulfur Compounds"; Academic Press: New York, 1978.
4. Kjaer, A. Pure Appl. Chem., 1977, 49, 137.
5. Kjaer, A. in "Chemical Plant Taxonomy", Swain, T. Ed; Academic Press: London, 1964, 453.
6. Robinson, T. "The Organic Constituents of Higher Plants"; Burgess Publishing Company: Minneapolis, 1963.

7. Matsumoto, Y. in "Control of Insect Behavior by Natural Products"; Nakajima, M., Ed., Academic Press: New York, 1970; p. 133.
8. Lichtenstein, E.P. in "Natural Pest Control Agents"; Crosby, D.G., Ed., American Chemical Society: Washington, D.C., 1966; p. 34.
9. See references in Block, E.; O'Connor, J. J. Am. Chem. Soc., 1974, 96, 3921.
10. Amonkar, S.V.; Banerji, A. Science, 1971, 174, 1343.
11. Block, E.; Penn, R.E.; Revelle, L.K. J. Am. Chem. Soc., 1979, 101, 2200.
12. Block, E.; Revelle, L.K.; Bazzi, A.A. Tetrahedron Lett., 1980, 21, 1277.
13. Block, E. Pure Appl. Chem., in press.
14. Boscher, J. Compt. rend., 1977, 284D, 635.
15. Abdou, I.A.; Abou-Zeid, A.A.; El-Sherbeeney, M.R.; Abou-El-Gheat, Z.H. Qual. Plant. Mater. Veg., 1972, 22, 29; Chem. Abstr., 1973, 78, 80226b.
16. Carle', J.S.; Christophersen, C. J. Am. Chem. Soc., 1980, 102, 5108.
17. Larher, F.; Hamelin, J. Phytochemistry, 1979, 18, 1396.
18. Moser, R.; Duvel, D.; Greve, R. Phytochemistry, 1980, 19, 79.
19. Herz, W.; Kumar, N.; Blount, J.F. J. Org. Chem., 1980, 45, 489.
20. Kondo, Y.; Yoshizaki, F.; Hamada, F.; Imai, J.; Kusano, G. Tetrahedron Lett., 1977, 2155.
21. Naya, K.; Hayashi, M.; Takagi, F.; Nakamura, S.; Kobayashi, M. Bull. Chem. Soc. Jpn., 1972, 45, 3673.
22. Neuenschwander, M.; Neuenschwander, A.; Steinegger, E.; Engel, P. Helv. Chim Acta, 1979, 62, 609.
23. Moir, M.; Gallacher, I.M.; Hobkirk, J.; Seaton, J.C.; Suggett, A. Tetrahedron Lett., 1980, 21, 1085.
24. Andersen, K.K.; Bernstein, D.T. J. Chem. Ecol., 1975, 1, 493.

25. Ohloff, G.; Flament, I. Progress in the Chemistry of Organic Natural Products, 1979, 36, 231.
26. Tahara, S.; Mitzutani, J. Agric. Biol. Chem., 1979, 43, 2021.
27. Wilson, S.R., Carmack, M.; Novotny, M.; Jorgenson, J.W.; Whitten, W.K. J. Org. Chem., 1978, 43, 4675.
28. Wheeler, J.W.; von Endt, D.W.; Wemmer, C. J. Am. Chem. Soc., 1975, 97, 441.
29. Schildknecht, H.; Wilz, I.; Ensmann, F.; Grund, N.; Ziegler, M. Angew. Chem., Int. Ed. Engl., 1976, 15, 242.
30. Singer, A.G.; Agosta, W.C.; O'Connell, R.J.; Pfaffmann, C.; Bowen, D.V.; Field, F.H. Science, 1976, 191, 948.
31. Chan, G.F.Q.; Towers, G.H.N.; Mitchell, J.C. Phytochemistry, 1975, 14, 2295.
32. Chan, G.F.Q.; Lee, M.M.; Glushka, J.; Towers, G.H.N., Phytochemistry, 1979, 18, 1566.
33. Bohlmann, F.; Zdero, C.; Silva, M. Phytochemistry, 1977, 16, 1302.
34. Uhlenbroek, J.H., Bijloo, J.D. Recl. Trav. Chim. Pays-Bas, 1958, 77, 1004.
35. Kawazu, K.; Nishii, Y.; Nakajima, S. Agric. Biol. Chem., 1980, 44, 903.
36. Guddal, E.; Sorensen, N.A. Acta Chem. Scand., 1959, 13, 1185.
37. Bohlmann, F.; Abraham, W-R. Phytochemistry, 1979, 18, 839.
38. Takasugi, M.; Yachida, Y.; Anetai, M.; Masamune, T.; Kegasawa, K. Chem. Letters, 1975, 43.
39. Yanagawa, H.; Kato, T.; Kitahara, Y. Tetrahedron Lett., 1973, 1073.
40. Anthoni, U.; Christophersen, C.; Madsen, J.O.; Wiium-Andersen, S.; Jacobsen, N. Phytochemistry, 1980, 19, 1228.
41. Wratten, S.J.; Faulkner, D.J. J. Org. Chem., 1976, 41, 2465.
42. Morita, K.; Kabayashi, S. Chem. Pharm. Bull., 1967, 15, 988.

43. Bock, H.; Solouki, B.; Mohmand, S.; Block, E.; Revelle, L.K. J. Chem. Soc., Chem. Commun., 1977, 287.
44. Gmelin, R.; N'Galamulume-Treves, M.; Hofle, G. Phytochemistry, 1980, 19, 553.
45. Kameoka, H.; Demizu, Y. Phytochemistry, 1979, 18, 1397.
46. Adesogan, E.K. J. Chem. Soc., Chem. Commun., 1974, 906.
47. Cullen, W.P.; Lalonde, R.T.; Wang, C.J.; Wong, C.F. J. Pharm. Sci., 1973, 62, 826.
48. Lalonde, R.T. Accts. Chem. Res., 1980, 13, 39.
49. Birnbaum, G.I., Hall, S.R. J. Am. Chem. Soc., 1976, 98, 1926.
50. Yamazaki, M., Maebayashi, Y.; Kato, F.; Koyama, Y. J. Pharm. Soc. Japan, 1975, 95, 347.
51. Kaoulla, N.; MacLeod, A.J.; Gil, V. Photochemistry, 1980, 19, 1053.
52. Winter, M.; Furrer, A.; Willhalm, B.; Thommen, W. Helv. Chim. Acta, 1976, 59, 1613.
53. Yoshida, T.; Muraki, S.; Takahashi, K. Kato, T.; Kabuto, C.; Sukuki, T.; Uyehara, T.; Ohnuma, T. J. Chem. Soc., Chem. Commun., 1979, 512.
54. Yokota, T.; Yamazaki, S.; Takahashi, N.; Iitaka, Y. Tetrahedron Lett., 1974, 2957.
55. Block, E.; Bazzi, A.A.; Revelle, L.K. J. Am. Chem. Soc., 1980, 102, 2490.
56. Roller, P.; Au, K.; Moore, R.E. J. Chem. Soc., Chem. Commun., 1971, 503.
57. Moore, R.E. J. Chem. Soc., Chem. Commun., 1971, 1168; 1972, 326.
58. Sakai, M.; Sato, Y. in "Insecticides, Proceedings of the Second International IUPAC Congress of Pesticide Chemistry," Part 1, Tahori, A.S. Ed.; Gordon and Breach Science: New York, 1972, 163.
59. Crump, D.R. Tetrahedron Lett., 1978, 5233.
60. Watkins, T.I.; Weighton, D.M. Rep. Prog. Appl. Chem., 1975, 60, 404.

61. Berg, W.; Knutti, H.J. Proc. Br. Insectic. Fungic. Conf., 8th, 1975, 2, 683.
62. Konishi, K. in "Insecticides, Proceedings of the Second International IUPAC Congress of Pesticide Chemistry", Part 1, Tahori, A.S. Ed., Gordon and Breach Science: New York, 1972, 179.
63. Crump, D.R. J. Chem. Ecol., 1980, 6, 341.
64. Sokolov, V.E.; Albone, E.S.; Flood, P.F.; Heap, P.F.; Kagan, M.Z.; Vasilieva, V.S.; Roznov, V.V.; Zinkevich, E.P. J. Chem. Ecol., 1980, 6, 805.
65. Crump, D.R. J. Chem. Ecol., 1980, 6, 837.
66. Ikawa, M.; Thomas, V.M., Jr.; Buckley, L.J.; Uebel, J.J.; J. Phycol., 1973, 9, 302.

RECEIVED December 30, 1980.

# Chemical Mechanisms of the Cytochrome P-450 Monooxygenase-Catalyzed Metabolism of Phosphorothionate Triesters

R. A. NEAL

Center in Toxicology, Department of Biochemistry, Vanderbilt University  
School of Medicine, Nashville, TN 37232

One of the most extensively studied phosphorothionate esters from the standpoint of mammalian metabolism is the insecticide parathion. The majority of this paper will center on what is known of the mechanism of mammalian microsomal metabolism of this compound.

Parathion is one of a class of phosphorothionate triesters widely used as insecticides. These compounds exert their toxic effects in insects and mammals by inhibiting the enzyme acetylcholinesterase. The phosphorothionates, in general, are relatively poor inhibitors of acetylcholinesterase but are converted by the cytochrome P-450-containing monooxygenase enzyme systems in insects and mammals to the corresponding phosphate triesters that are potent inhibitors of this enzyme.

What is known concerning the microsomal metabolism of parathion by the rat and other experimental animals is shown in Figure 1. There are five major products of parathion metabolism. One of these is the corresponding phosphate triester, paraoxon, which is formed in a monooxygenase-catalyzed reaction in which the sulfur atom of parathion is replaced by an oxygen atom (1). The paraoxon is subject to hydrolysis by esterases present in various tissues to form diethyl phosphate and *p*-nitrophenol (2). Parathion is not a substrate for these esterases, presumably because the phosphorus atom is not as electrophilic as that in paraoxon. The difference in the electrophilicity of the phosphorus atom in these two compounds is most likely due to the greater electronegativity of oxygen as compared to sulfur.

Parathion is also metabolized to diethyl phosphorothioic acid and *p*-nitrophenol in a reaction requiring a cytochrome P-450-containing monooxygenase enzyme system (3,4). Studies with  $H_2^{18}O$  have indicated that water in addition to molecular oxygen and NADPH is required in this reaction (5). Diethyl phosphate and *p*-nitrophenol can also be formed from parathion in a monooxygenase-catalyzed reaction (6).

0097-6156/81/0158-0019\$05.00/0  
© 1981 American Chemical Society

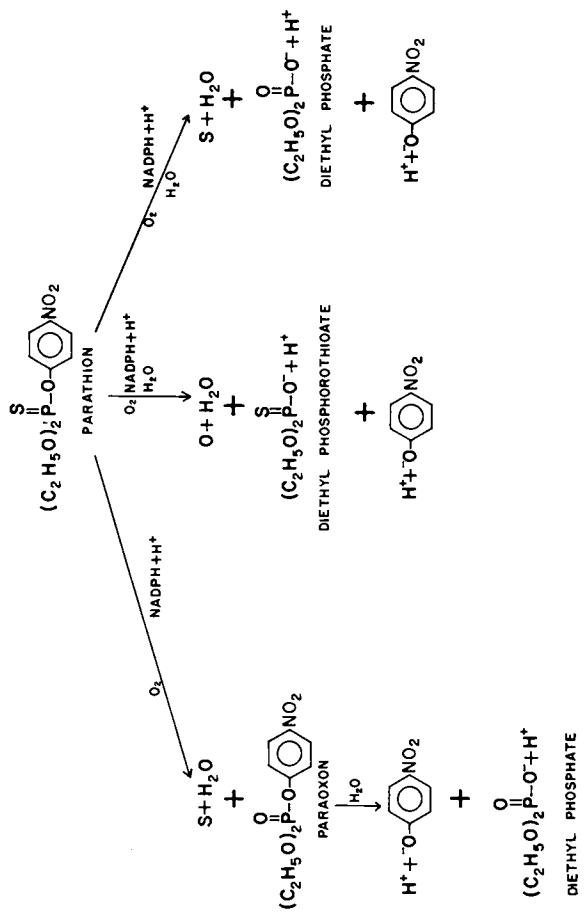


Figure 1. Scheme for the metabolism of parathion by mammalian microsomes



In an examination of the chemical mechanism of the microsomal metabolism of parathion to paraoxon two principal questions were posed. These were, first, what was the initial site of attack of the cytochrome P-450-generated oxygen atom on the parathion molecule and, second, was the attacking oxygen atom retained in the product of the reaction, paraoxon. In regard to the retention of the cytochrome P-450-generated oxygen atom in paraoxon, parathion was incubated with rabbit liver microsomes and NADPH in an atmosphere enriched with  $^{18}\text{O}_2$  or in a buffer enriched with  $\text{H}_2^{18}\text{O}$  (5). The paraoxon formed in these reactions was isolated and examined for an increased content of  $^{18}\text{O}$  using mass spectrometry. No  $^{18}\text{O}$  enrichment was evident in the paraoxon isolated from the incubation carried out in the  $\text{H}_2^{18}\text{O}$ -enriched buffer. However, as can be seen in Table I, when the reaction was carried out in an atmosphere enriched with  $^{18}\text{O}_2$ , the resultant paraoxon contained  $^{18}\text{O}$  to about the same atom-percent excess as the atmosphere in which the incubation took place. These data indicated that the oxygen atom attached to the phosphorus of paraoxon was that which was transferred to parathion by the cytochrome P-450 containing monooxygenase system.

TABLE I

$^{18}\text{O}$  ENRICHMENT IN PARAOXON FOLLOWING INCUBATION OF  
PARATHION WITH RABBIT LIVER MICROSOMES<sup>a</sup>

Experiment	Source of $^{18}\text{O}$ (atoms % excess)	Observed atom % excess $^{18}\text{O}$ in paraoxon
1	$\text{H}_2^{18}\text{O}$ (20)	0.0
2	$^{18}\text{O}_2$ (46)	42.0

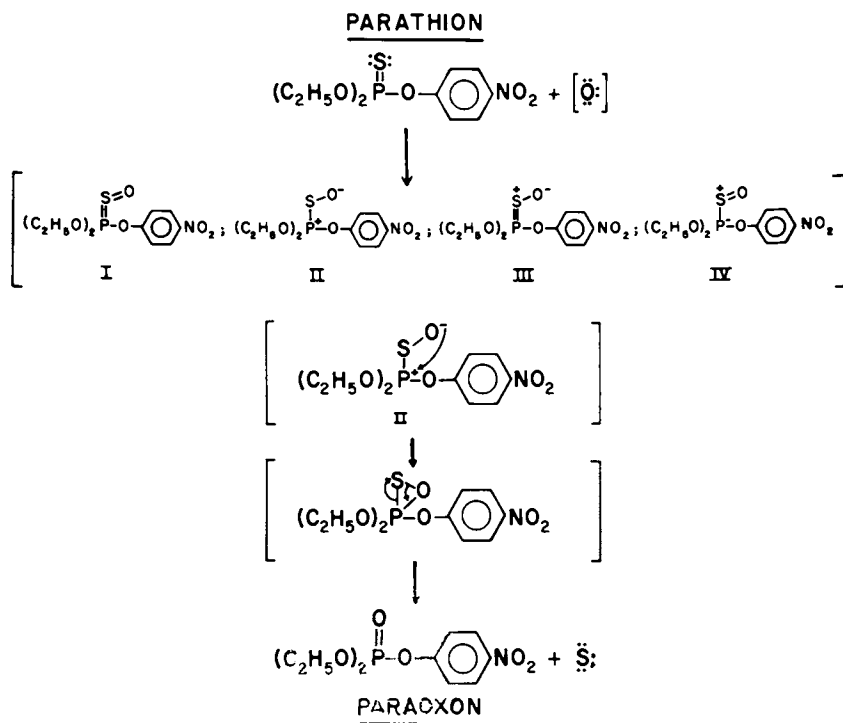
<sup>a</sup>The details of this experiment are described in Reference (5).

Results similar to those described above for parathion were obtained by McBain *et al.* (7,8) in oxygen-18 studies of the metabolism of the phosphorothionate, Dyfonate (O-ethyl S-phenyl ethylphosphonodithioate), using rat liver microsomes.

Additional studies have been carried out to determine the initial site of attack of the cytochrome P-450-generated oxygen atom on parathion. These studies have indicated the sulfur atom is the initial site of attack. Thus, studies of products of the reaction of parathion (9) and menthylmethylphenylphosphinothioate (10) with peroxy acid model systems for the cytochrome P-450-containing monooxygenase systems indicated that these compounds were readily converted to their oxygen analogs (oxygen replacing

sulfur) by these model systems. However, no products were seen on incubation of paraoxon or menthylmethylphenylphosphinate with the peroxy acid (9,10). These results suggested the attack of the oxygen atom of the peroxy acids was on the sulfur atom rather than the phosphorus. The studies of Herriott (10) were carried out using the two diastereoisomers of menthylmethylphenolphosphinothioate. When the reactions were carried out using *m*-chloroperbenzoic acid there was a high degree of retention of configuration about the phosphorus atom but oxidation with peroxytrifluoroacetic acid occurred with predominant inversion. Thus, with the less acidic *m*-chloroperbenzoic acid there was retention of configuration. These results again suggest predominant attack of the peroxy acids on the sulfur atom to form an S-oxide followed by closure to a phosphooxathiiran and concomitant or successive loss of sulfur to produce the oxygen analogs of these phosphothionates. The stereospecificity of the metabolism of the chiral isomers of fonofos (O-ethyl, S-phenyl ethylphosphonodithioate) by mouse liver microsomal monooxygenase systems was examined by Lee et al. (11,12). The oxidative desulfuration of the two isomers of fonofos by mouse liver microsomes proceeded predominantly with retention of configuration. The stereospecific oxidative desulfuration of O-ethyl O-2-nitro-5-methylphenyl N-isopropylphosphoramidothioate to the corresponding oxygen analog by the action of rabbit liver microsomes has also been reported (13). In this case the desulfuration also occurred with retention of configuration. Thus, in these experiments with cytochrome P-450-containing monooxygenase systems the initial attack of the oxygen atom generated in the active site of cytochrome P-450 appeared to be the sulfur atom of the phosphothionates.

From these data, a chemical mechanism for the formation of paraoxon from parathion has been proposed (6). This mechanism is shown in Figure 2. It is postulated that a singlet oxygen atom generated in a cytochrome P-450-catalyzed reaction is donated to the sulfur atom of parathion to yield a compound analogous to the S-oxide formed in the reaction of peracids with thioketones (14,15). The attacking singlet oxygen atom is shown in brackets by way of suggesting that it is more likely transferred from cytochrome P-450 to the phosphorothionate sulfur in a concerted reaction. As shown in Figure 2, there are four different structures that may contribute to the resonance stabilization of the resultant S-oxide. It is proposed that one of these resonance forms (perhaps form II) reacts internally to form a cyclic phosphorus-sulfur-oxygen intermediate analogous to the oxathiiran that has been proposed by numerous investigators to be an intermediate in the reaction pathways of various S-oxides (16). This resultant phosphooxathiiran then undergoes a cyclic electron shift with the loss of sulfur, forming paraoxon. It is proposed that the sulfur atom that is released is in its singlet form. Although there is no evidence to support this hypothesis, it is probable that if the



Drug Metabolism and Disposition

Figure 2. *Chemical mechanism for the metabolism of parathion to paraoxon by the Cytochrome P-450-containing monooxygenase system (6)*

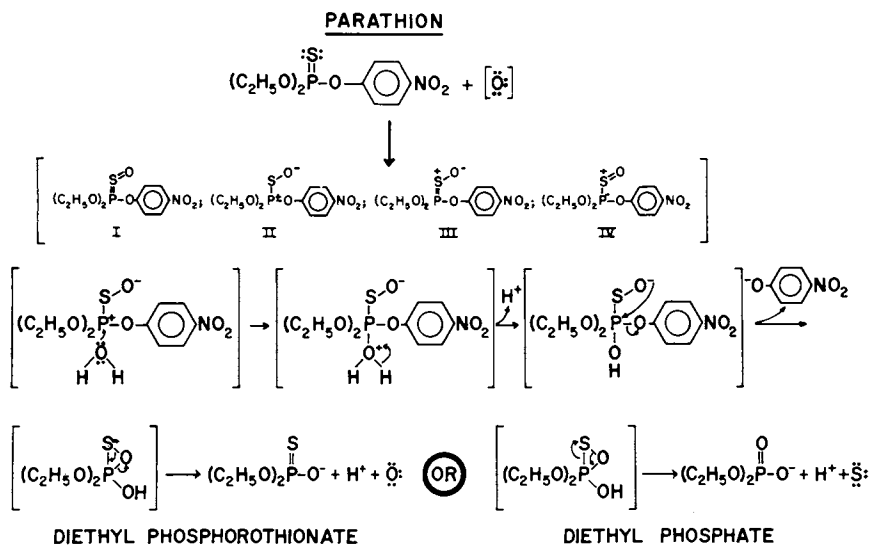
attacking oxygen atom is in its singlet state the departing sulfur atom may also be in its singlet state.

Diethyl phosphorothionate and diethyl phosphate plus *p*-nitrophenol are also products of the cytochrome P-450 monooxygenase-catalyzed metabolism of parathion. Figure 3 shows the proposed mechanism for the metabolism of parathion to diethyl phosphorothioic acid and to diethyl phosphoric acid. It is proposed that the initial reaction is again the transfer of a singlet oxygen atom to one of the unshared electron pairs on the sulfur atom to form the same intermediate S-oxide proposed in Figure 2. One of these resonance forms (perhaps form II) may be directly subject to nucleophilic attack by water as shown in this scheme, followed by loss of a proton and the *p*-nitrophenol group, forming a phosphoxathiiran derivative that may break down with the loss of an oxygen atom, forming diethyl phosphorothioic acid or, with the loss of a sulfur atom, forming diethyl phosphoric acid. Alternatively, the S-oxide may first react internally, as shown in Figure 2, followed by attack by water. Each of these alternatives is possible.

The majority of the diethyl phosphate and *p*-nitrophenol formed in the mammalian metabolism of parathion is undoubtedly derived by the action of esterases or phosphatases (2) on paraoxon formed from parathion in a cytochrome P-450-catalyzed reaction. However, a significant portion of the diethyl phosphate and *p*-nitrophenol must also be the result of the attack of water on the intermediate S-oxide of parathion (6).

The question of what factors control the rate of breakdown of the proposed intermediate S-oxide of parathion to the various products is of interest. With all the systems examined so far, whole microsomes, the reconstituted P-450 monooxygenase system, and the peroxy acid model systems, the predominant product is paraoxon, followed by diethyl phosphorothioic acid and then by diethyl phosphoric acid. It may be that the rearrangement of the intermediate S-oxide to paraoxon is thermodynamically or kinetically more favorable than water attack followed by rearrangement to form diethyl phosphorothioic acid or diethyl phosphoric acid. Alternatively, the limited accessibility of water to the active site of cytochrome P-450 to form diethyl phosphorothioic acid or diethyl phosphoric acid may be a controlling factor. However, it is also theoretically possible that the oxygen atom of the intermediate S-oxide of parathion, which would have a bond angle with the sulfur of approximately  $120^\circ$  (17), may be either *cis* (as shown in Figures 2 and 3) or *trans* to the *p*-nitrophenol group. Whether the S-oxide is *cis* or *trans* may control the route of breakdown of this intermediate to the various products.

If the sulfur atom released from parathion, as proposed in Figure 2, is in its singlet state, it would be a highly reactive electrophile that would bind readily to nucleophiles near the site of its release. The thiono-sulfur atom of parathion has been found to be covalently bound to tissue macromolecules following adminis-



## Drug Metabolism and Disposition

Figure 3. Chemical mechanism for the metabolism of parathion to diethyl phosphorothionate and diethyl phosphate by the Cytochrome P-450-containing monooxygenase system (6)

tration of [ $^{35}\text{S}$ ] parathion *in vivo* (18) and on incubation with hepatic microsomes *in vitro* (4,19). Table II shows the results of an experiment in which double-labeled parathion ( $^{32}\text{P}$ ,  $^{35}\text{S}$ ) was incubated with microsomes isolated from the livers of phenobarbital-pretreated rats. In the absence of NADPH, only a trace of radioactivity could be found bound to the microsomes. However, as shown in Table II, when the incubation was carried out in the presence of NADPH, a substantial amount of sulfur became covalently bound to the microsomes. A small but significant amount of the phosphorus-containing portion of the parathion molecule was also covalently bound to the microsomes. The results of this experiment clearly indicate that the majority of the sulfur bound to the microsomes is free of the phosphorus-containing portion of the molecule and thus must be atomic sulfur released in the metabolism of parathion to paraoxon. This is further substantiated by the finding that the amount of sulfur bound under these conditions is equivalent to the amount of paraoxon formed in the incubation (19). Also shown in Table II are the results of an experiment in which an amount paraoxon, which was approximately five times the amount that would

TABLE II

$^{35}\text{S}$  AND  $^{32}\text{P}$  BOUND TO MICROSOMES FOLLOWING INCUBATION WITH PARATHION AND ITS METABOLITES<sup>a</sup>

Substrate	nmol $^{35}\text{S}$ bound/mg protein/15 min	nmol $^{32}\text{P}$ bound/mg protein/15 min
[ $^{35}\text{S}$ , $^{32}\text{P}$ ] Parathion ( $2.5 \times 10^{-4}$ M)	12.93 $\pm$ 0.37	1.12 $\pm$ 0.21
[ $^{32}\text{P}$ ] Paraoxon ( $2.5 \times 10^{-5}$ M)	--	0.25 $\pm$ 0.03
[ $^{32}\text{P}$ ] Diethylphosphate ( $4 \times 10^{-5}$ M)	--	None
[ $^{35}\text{S}$ , $^{32}\text{P}$ ] Diethyl phosphorothioic acid ( $4 \times 10^{-5}$ M)	None	None

<sup>a</sup> The details of this experiment are described in Poore and Neal (18). This experiment was carried out using hepatic microsomes from phenobarbital-pretreated rats.

be expected to be formed in the incubation containing double-labeled parathion, was incubated with an equal aliquot of the same preparation of microsomes. As can be seen, a much smaller amount of  $^{32}\text{P}$  was bound using [ $^{32}\text{P}$ ] paraoxon than was bound using the double-labeled parathion. Thus the binding of  $^{32}\text{P}$  in a reaction of paraoxon with nucleophilic sites on the endoplasmic reticulum is responsible for only a small portion of the total binding of  $^{32}\text{P}$  seen using double-labeled parathion. It appears that the greater portion of the  $^{32}\text{P}$  binding in the incubation using double-labeled parathion is likely the result of the reaction of one or more of the intermediate S-oxides shown in Figure 2 with nucleophiles on the endoplasmic reticulum. In examining the remainder of the data in Table II, it can be seen that the incubation of the other phosphorus-containing metabolites of parathion with microsomes in the presence of NADPH does not lead to the binding of radioactivity.

TABLE III  
PARATHION METABOLISM BY A RECONSTITUTED MONOOXYGENASE  
SYSTEM FROM RABBIT LIVER<sup>a</sup>

Conditions	Product formation (nmol/nmol P-450/5 min)		
	Paraoxon	Diethyl phosphorothioic acid	Diethyl phosphate
Complete	4.510 $\pm$ 0.424	2.420 $\pm$ 0.156	0.470 $\pm$ 0.107
Minus P-450	0.048 $\pm$ 0.002	0.040 $\pm$ 0.002	0.016 $\pm$ 0.002
Minus reductase	0.018 $\pm$ 0.001	0.070 $\pm$ 0.003	0
Minus lipid	1.790 $\pm$ 0.021	0.816 $\pm$ 0.002	0.206 $\pm$ 0.049
Minus deoxycholate	3.860 $\pm$ 0.679	1.990 $\pm$ 0.347	0.381 $\pm$ 0.100
Minus both lipid and deoxycholate	1.690 $\pm$ 0.120	0.756 $\pm$ 0.008	0.245 $\pm$ 0.005

<sup>a</sup>From Kamataki *et al.* (6).

Drug Metabolism and Disposition

The binding of sulfur and/or an activated intermediate of the phosphorus-containing portion of the parathion molecule to the endoplasmic reticulum leads to a decrease in the amount of cytochrome P-450 detectable as its carbon monoxide complex and to a decrease in the rate of metabolism of substrates such as benzphetamine (19). Neither paraoxon nor any other isolatable metabolite of parathion decreases the amount of cytochrome P-450 or inhibits the ability of microsomes to metabolize substrates such as benzphetamine (19).

We have also examined the metabolism of parathion by purified reconstituted monooxygenase systems isolated from the livers of

phenobarbital-pretreated rabbits and rats. Cytochrome P-450 and NADPH-cytochrome *c*-reductase were purified to apparent homogeneity from both species as previously described (20). Shown in Table III are the results of an experiment examining the metabolism of parathion by a reconstituted system from rabbit liver. The cytochrome P-450 used in this experiment had a specific content of 18.5 nmol/mg protein. As can be seen, there is a requirement for both cytochrome P-450 and the reductase. The activity was also stimulated by addition of dilauroyl phosphatidylcholine. Deoxycholate also has a slight stimulating effect. Another important aspect of the data shown in Table III is that all three of the major phosphorus-containing metabolites of parathion are formed by what appears to be a single species of cytochrome P-450. These data and those from the studies using a peroxy acid model system (9) suggest that the monooxygenase system is only involved in the addition of an oxygen atom to the phosphorothionate sulfur (Figure 2) and that the various products are formed nonenzymatically from a common intermediate. It is believed that this intermediate is one or more of the resonance forms of the intermediate S-oxide shown in Figure 2.

The linearity of the formation of these various parathion metabolites and the linearity of the metabolism of benzphetamine by the reconstituted monooxygenase system from rabbit liver are shown in Figure 4. In these experiments, a reductase-to-P-450 ratio of 3 units to 1 nmol was used and cytochrome P-450 was the rate-limiting enzyme. Using this enzyme system, the rate of formation of the various parathion metabolites departs rapidly from linearity. In contrast, the rate of metabolism of benzphetamine (as determined by formaldehyde production) remained linear throughout the incubation period. As with the data using intact microsomes (19), these data suggest that the binding of some reactive product of parathion to one or both of the enzymes of this reconstituted system is responsible for the departure of the rate of metabolism of parathion from linearity. The inhibition was not due to inactivation of NADPH since an excess of this cofactor did not improve linearity. It was also not due to product inhibition by any of the isolatable parathion metabolites since, as noted previously (19,21), none of these products have an inhibitory effect on parathion metabolism.

We have examined whether the sulfur that was bound to the proteins of a reconstituted system from the liver of phenobarbital-treated rats was bound to both the reductase and cytochrome P-450. In this experiment, the reconstituted system was incubated with [<sup>35</sup>S] parathion. The reaction mixture was dialyzed and applied to a Sephadex G-25 column to remove the last traces of unreacted parathion and its noncovalently bound metabolites. The protein fraction from the Sephadex column was reduced in volume and subjected to SDS-polyacrylamide gel electrophoresis in the absence of either dithiothreitol or mercaptoethanol. The results are shown in Figure 5. There was considerable protein and radioactivity at the origin. This material at the origin represents an aggregate of reductase



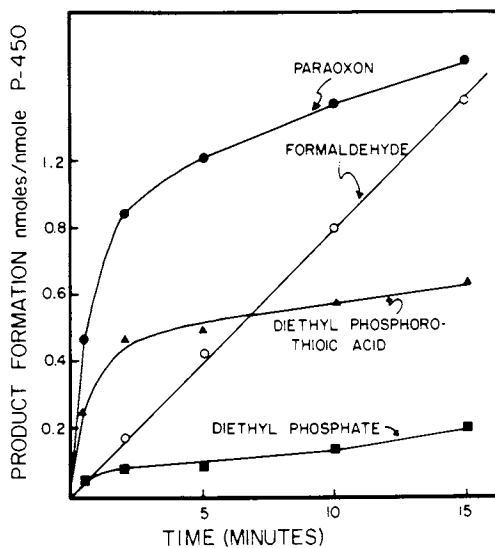
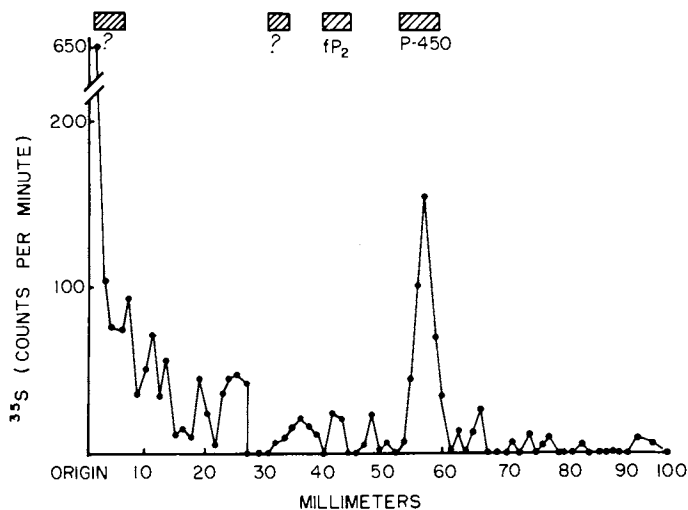


Figure 4. Linearity of the metabolism of parathion and benzphetamine by a reconstituted monooxygenase oxidase enzyme system from rabbit liver. The 0.5-mL reaction mixture contained 50  $\mu\text{g}$  of sodium deoxycholate, 15  $\mu\text{g}$  of dilauroyl L-3-phosphatidylcholine, 1.5 units of NADPH-Cytochrome c reductase, 0.5 nmol of Cytochrome P-450, 0.05M HEPES buffer (pH 7.8), 0.015M  $\text{MgCl}_2$ , 0.1mM EDTA, and  $5 \times 10^{-5}\text{M}$  [ethyl- $^{14}\text{C}$ ] parathion or  $1 \times 10^{-3}\text{M}$  benzphetamine.

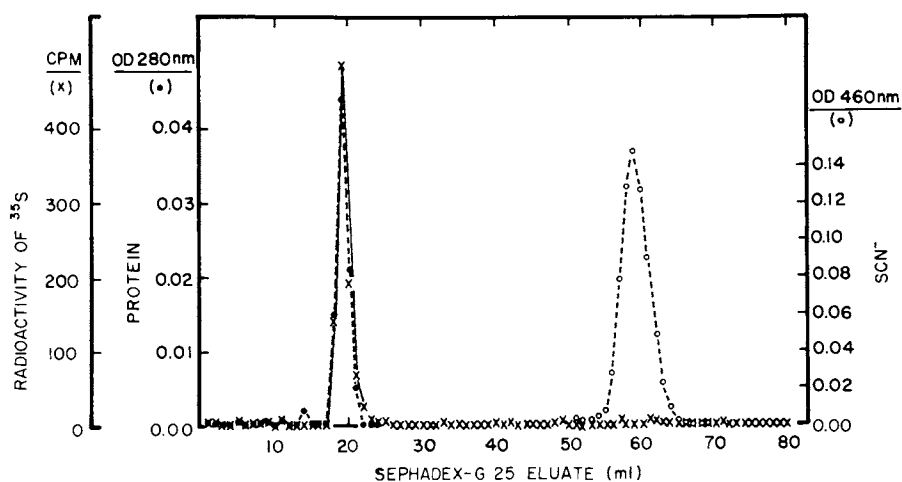
and P-450 molecules caused by the binding of the sulfur atom. Pretreatment of the concentrated eluant from the Sephadex column with 100 mM  $\text{CN}^-$  for 3 hrs. at room temperature prior to gel electrophoresis, a treatment that released most of the bound sulfur, prevents the accumulation of both protein and radioactivity at the origin. Little radioactivity was associated with the position of reductase ( $\text{fP}_2$ ) on the gel, whereas a significant amount was found in the area of the gel corresponding to P-450. This was the case in both the gel of the sample that had been incubated with  $\text{CN}^-$  prior to gel electrophoresis and that which had not been incubated with  $\text{CN}^-$  (Figure 5). These data indicate that the sulfur is bound predominantly or exclusively to cytochrome P-450.

Other work in our laboratory had shown that approximately 50 to 70% of the sulfur bound to rat liver microsomes incubated with  $[\text{}^{35}\text{S}] \text{CS}_2$  was in the form of a hydrodisulfide (22). As noted above, this form of bound sulfur is released on treatment of the microsomes with  $\text{CN}^-$ . The chemical form of the sulfur released is thiocyanate ( $\text{SCN}^-$ ). We therefore examined the ability of  $\text{CN}^-$  to release the sulfur bound to the proteins of the reconstituted system (cytochrome P-450) as  $\text{SCN}^-$ . The results are shown in Figures 6 and 7. In these experiments, a rat liver reconstituted system was incubated with  $[\text{}^{35}\text{S}]$  parathion, dialyzed, and applied to a Sephadex G-25 column to remove the last traces of unmetabolized parathion and its noncovalently bound metabolites. The protein fraction from the Sephadex column was reduced in volume and divided into two portions. One portion was incubated with 10 mM  $\text{CN}^-$  at room temperature for 3 hrs. and the other fraction was incubated for 3 hrs. in the absence of  $\text{CN}^-$ . Figure 6 shows the elution profile of protein, radioactivity, and unlabeled  $\text{SCN}^-$  from a Sephadex G-25 column of the sample not incubated with  $\text{CN}^-$ . The unlabeled  $\text{SCN}^-$  was added just before application to the column. The only peak of radioactivity was that associated with the protein peak. Figure 7 shows the elution profile of the sample incubated with  $\text{CN}^-$ . As can be seen, there was a decrease in the radioactivity associated with the protein and the appearance of a peak that exactly coincided with the colorimetric peak of exogenously added  $\text{SCN}^-$ . Parathion elutes from this Sephadex column in fractions 78-105 and the other sulfur-containing metabolite of parathion, diethyl phosphorothioic acid, elutes in fractions 35-50. The similarity of these data and those obtained with microsomes labeled with  $^{35}\text{S}$  from  $[\text{}^{35}\text{S}]\text{-CS}_2$ , in which the form of the radioactivity was unequivocally identified as  $\text{SCN}^-$  (22), indicates that a portion of the sulfur bound to proteins of the reconstituted system and more likely to cytochrome P-450 is present as a hydrodisulfide. Similar results were obtained by Davis and Mende (23) using rat hepatic microsomes. Figure 8 shows the way in which it is proposed that the hydrodisulfide linkage is formed. A cysteine side chain of the cytochrome P-450 molecule reacts with atomic sulfur to form a hydrodisulfide. Organic hydrodisulfides have been synthesized and are relatively stable (24).



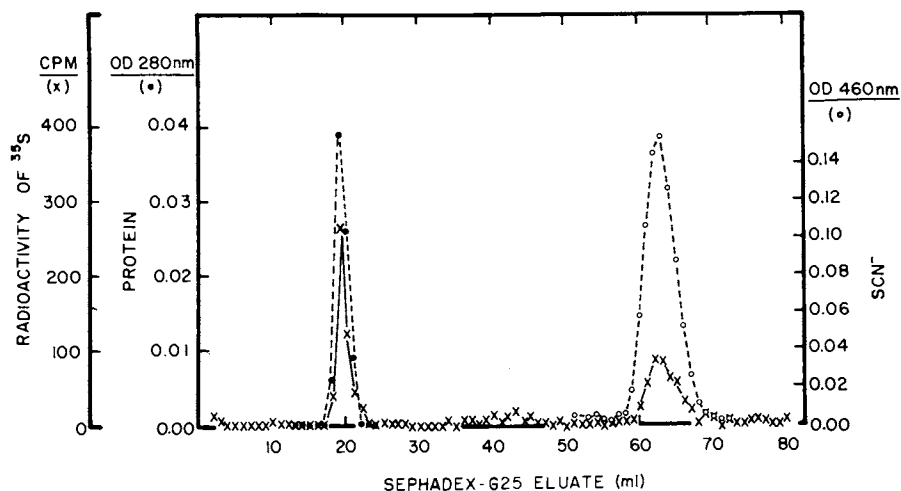
Molecular Pharmacology

Figure 5. SDS-PAGE of a reconstituted monooxygenase system from rat liver that had been labeled with  $^{35}\text{S}$  by incubation with [ $^{35}\text{S}$ ] parathion. The incubation procedures were essentially as described in Figure 4 (20).



Molecular Pharmacology

Figure 6. Elution profile of protein, radioactivity, and thiocyanate from a Sephadex G-25 column of reconstituted monooxygenase system from rat liver that had been incubated with [ $^{35}\text{S}$ ] parathion. The 5-mL incubation mixture contained 20 nmol Cytochrome P-450 (specific activity 16.4 nmol/mg protein), 5 units NADPH-Cytochrome c reductase, 600  $\mu\text{g}$  dilauroyl L-3-phosphatidylcholine, 600  $\mu\text{g}$  sodium deoxycholate, and  $1 \times 10^{-4}\text{M}$  [ $^{35}\text{S}$ ] parathion. The remainder of the incubation mixture is described in Figure 4. The incubation time was 5 min. One-milliliter fractions were collected. The radioactivity (x) represents cpm/0.1 mL. The  $\text{OD}_{280}$  (•) was measured on each 1-mL fraction (20).



## Molecular Pharmacology

Figure 7. Elution profile of protein, radioactivity, and thiocyanate from a Sephadex G-25 column of a reconstituted monooxygenase system from rat liver that had been incubated with [<sup>35</sup>S] parathion followed by incubation with cyanide. The incubation conditions and analytical procedures were as described in Figure 7 except that the labeled protein was incubated with 10mM sodium cyanide for 3 h at room temperature prior to being applied to the Sephadex column (20).

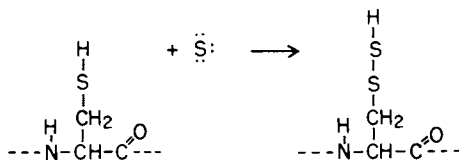


Figure 8. Scheme for hydrodisulfide linkage formation in a reaction of atomic sulfur with the side chain of a cysteine in the Cytochrome P-450 molecule

Additional work in our laboratory using a reconstituted monooxygenase system containing purified cytochrome P-450 has indicated that the sulfur atom released from parathion covalently binds to at least three other, as yet unidentified, amino acids on the cytochrome P-450 molecule. It appears to be clear that the binding of the sulfur atom to cysteine or cysteines is responsible for the loss of cytochrome P-450 detectable as its CO complex and the accompanying loss of monooxygenase activity (25).

The studies described above have been carried out using hepatic microsomes from various mammalian species and purified cytochrome P-450-containing monooxygenases obtained from the livers of rabbits and rats. Additional studies have indicated the microsomes from rabbit (26) and rat lung (27) and rat brain (27) also metabolize parathion in a manner similar to mammalian liver.

In contrast to parathion, certain phosphorothionate triesters bring about the inhibition of hepatic cytochrome P-450-containing monooxygenases when administered in vivo. These include the insecticides fenitrothion, diazinon and methyl parathion (28) and the model phosphorothionate triester diethyl phenyl phosphorothionate (29). The inhibition of the cytochrome P-450-containing monooxygenases by these phosphorothionates is most likely the result of the cytochrome P-450-catalyzed metabolism of these compounds leading to the release and covalent binding of the sulfur atom to cytochrome P-450. The inability of parathion to bring about a similar inhibition of this enzyme is probably related to the acute toxicity of parathion relative to those phosphorothionates noted above. In other words, much larger doses of these compounds may be given in a single dose than parathion without killing the animals as a result of inhibition of acetylcholinesterase.

#### LITERATURE CITED

1. Gage, J.C. Biochem. J., 1953, 54, 426.
2. Aldridge, W.M. Biochem. J., 1953, 53, 117.
3. Neal, R.A. Biochem. J. 1967, 103, 183.
4. Nakatsugawa, T.; Dahm, P.A. Biochem. Pharmacol., 1967, 16, 25.
5. Ptashne, K.A.; Wolcott, R.M.; Neal, R.A. J. Pharmacol. Exp. Ther., 1971, 179, 380.
6. Kamataki, T.; Lin, M.C.M.; Belcher, D.H.; Neal, R.A. Drug Metab. Dispos., 1976, 4, 180.
7. McBain, J.B.; Yamamoto, I.; Casida, J.E. Life Sciences, 1971, 10, 947.
8. McBain, J.B.; Yamamoto, I.; Casida, J.E. Life Sciences, 1971, 10, 1311.
9. Ptashne, K.A.; Neal, R.A. Biochemistry, 1972, 11, 3224.
10. Herriott, A.W. J. Amer. Chem. Soc., 1971, 93, 3304.
11. Lee, P.W.; Allahyari, R.; Fukuto, T.R. Biochem. Pharmacol., 1976, 25, 2671.

12. Lee, P.W.; Allahyari, R.; Fukuto, T.R. Pesticide Biochem. Physiol., 1978, 8, 158.
13. Ohkawa, H.; Mikami, N.; Miyamoto, Agr. Biol. Chem., 1976, 40, 2125.
14. Battaglia, A.; Dondoni, A.; Giogianni, P.; Maccagnani, G.; Mazzanti, G. J. Chem. Soc. (B), 1971, 1547.
15. Strating, J.; Thijs, L.; Zwanenburg, B. Tetrahedron Lett., 1966, 65.
16. Snyder, J.P. J. Amer. Chem. Soc., 1974, 96, 5005.
17. King, J.F.; Durst, T. J. Amer. Chem. Soc., 1963, 85, 2676.
18. Poore, R.E.; Neal, R.A. Toxicol. Appl. Pharmacol., 1972, 23, 759.
19. Norman, B.J.; Poore, R.E.; Neal, R.A. Biochem. Pharmacol., 1974, 23, 1733.
20. Kamataki, T.; Neal, R.A. Mol. Pharmacol., 1976, 12, 933.
21. Neal, R.A. Biochem. J., 1967, 105, 289.
22. Catignani, G.L.; Neal, R.A. Biochem. Biophys. Res. Commun., 1975, 65, 629.
23. Davis, J.R.; Mende, T.J. J. Pharmacol. Exp. Therap., 1977, 201, 490.
24. Karamura, S.; Otsuji, Y.; Nakabayashi, T.; Kitao, T.; Tsurugi, J. J. Org. Chem., 1965, 30, 2711.
25. Halpert, J.; Hammond, D.; Neal, R.A. J. Biol. Chem., 1980, 255, 1080.
26. Neal, R.A. Toxicol. Appl. Pharmacol., 1972, 23, 123.
27. Norman, B.J.; Neal, R.A. Biochem. Pharmacol., 1976, 24, 37.
28. Uchiyama, M.; Yoshida, T.; Homma, K.; Hongo, T. Biochem. Pharmacol., 1975, 24, 1221.
29. Seawright, A.A.; Hrdlicka, J.; De Matteis, F. Br. J. Exp. Path., 1976, 57, 16.

RECEIVED December 30, 1980.

## Sulfur in Propesticide Action

T. R. FUKUTO and M. A. H. FAHMY<sup>1</sup>

Division of Toxicology and Physiology, Department of Entomology,  
University of California, Riverside, CA 92521

It is well known that phosphorothionate insecticides such as parathion (O,O-diethyl O-p-nitrophenyl phosphorothioate) and malathion [O,O-dimethyl S-(1,2-dicarbethoxy)ethyl phosphorodithioate] are intrinsically poor inhibitors of acetylcholinesterase and *in vivo* activation to the respective anticholinesterases paraoxon and malaixon is required before animals exposed to the phosphorothionates are intoxicated. Since metabolic activation is essential to the biological activity of these thiono sulfur-containing organophosphorus insecticides, compounds of this type may be considered as propesticides or, more specifically, proinsecticides.

The introduction of a thiono sulfur in an organophosphorus insecticide has advantages and disadvantages. From a favorable viewpoint, compared to phosphate esters, the phosphorothionates are generally more stable to hydrolysis and, therefore, may have greater insecticidal activity. Perhaps the most important contribution which a thiono sulfur atom may make is the "delay factor" provided by P=S to P=O activation. This factor gives mammals the opportunity to detoxify the toxicant, and in many cases the phosphorothionate is substantially less toxic to warm-blooded animals than the corresponding phosphate ester. A classical example of the "delay factor" is found in the safe organophosphorus insecticide malathion for which it was demonstrated over two decades ago that slow *in vivo* oxidation to the anticholinesterase malaixon provided the opportunity for detoxifying enzymes in mammals, most likely a carboxylesterase, to degrade malathion to nontoxic metabolic products (1). The mouse LD<sub>50</sub> of purified malathion is 3,200 mg/kg (2), compared to 75 mg/kg for malaixon (1).

Among the different classes of organic insecticides in use today, the methylcarbamate esters rank at or near the top in acute mammalian toxicity and in many cases the methylcarbamates are as toxic to mammals as they are to insects (3). For

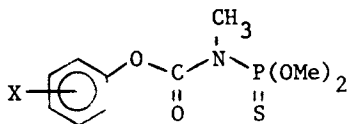
<sup>1</sup> Current address: FMC Corporation, Agricultural Chemical Division, P.O. Box 8, Princeton, NJ 08540.

example, aldicarb and carbofuran, with rat oral LD<sub>50</sub> values of 0.3-0.5 mg/kg and 11 mg/kg, respectively, are among the most acutely toxic pesticides currently being used. The principal reason for the high toxicity of methylcarbamate insecticides to mammals as well as insects probably is attributable to the absence of a "delay factor" (such as that provided by the thiono sulfur in phosphorothionate insecticides), i.e., most methylcarbamate insecticides are direct inhibitors of either insect or mammalian acetylcholinesterase.

### Derivatized Carbamates

During the past decade, a wide variety of new derivatives of toxic methylcarbamate insecticides with improved properties of selectivity have been discovered and several are currently undergoing development as useful insecticides. The first group of compounds of this type were the N-acylated methylcarbamate derivatives (4,5) which were reported to be good insecticides but less toxic to mammals. The N-acyl-N-methylcarbamate derivatives also were appreciably less effective as anticholinesterases, leading to the conclusion that the original methylcarbamate was responsible for intoxication in the insect after in vivo formation from the N-acyl derivative (6). In mice, however, detoxification to nontoxic phenolic products occurred and this accounted for their low toxicity to mammals. One of the N-acyl derivatives, 3-isopropyl-5-methylphenyl N-butyryl-N-methylcarbamate (promacyl) is reported to be highly effective against susceptible and resistant strains of cattle ticks, Boophilus microplus (7).

The basis for the selective toxicity of malathion provided the rationale for the design of the N-dimethoxyphosphinothioyl derivatives of methylcarbamates esters as selectively toxic insecticides (general structure below).



By analogy with malathion, derivatives of this type were expected to be metabolically degraded to nontoxic products in mammals by carboxylesterase action but to the parent methylcarbamate in insects by phosphatase action (8). Thus, these derivatives were expected and found to be toxic to insects and safe to mammals. Subsequent investigations on the comparative metabolism of the N-dimethoxyphosphinothioyl derivative of carbofuran in insects and rodents provided direct support for the rationale used in the design of these derivatives (9).



Aryl- and Alkylsulfenyl Methylcarbamates

The favorable order of selectivity achieved by the dimethoxyphosphinothiyl derivatives prompted the examination of other functional groups for introduction of the "delay factor" in methylcarbamate insecticides. Of the various groups examined, those leading to derivatives which contain an N-S linkage have generated the largest number of new compounds with improved properties of selectivity. These sulfur-containing derivatives may be considered to be carbamate proinsecticides. The first proinsecticides of this type were the N-arylsulfenyl and N-alkylsulfenyl derivatives of toxic methylcarbamate insecticides (10,11). These compounds proved to be highly toxic to house flies and mosquito larvae and were much less toxic to mice than the parent methylcarbamate ester. Typical toxicological data for these compounds are presented in Table I (11).

TABLE I

Toxicological Properties of N-Arylsulfenyl and N-Alkylsulfenyl Derivatives of Insecticidal Methylcarbamates

No.	R	Housefly LD <sub>50</sub> , µg/g	Culex <i>fatigans</i> LC <sub>50</sub> , ppm	Mouse (oral) mg/kg
$\begin{array}{c} \text{O} \\    \\ \text{2,2-Dimethyl-2,3-dihydrobenzofuranyl-7-OCN(CH}_3\text{)-R} \end{array}$				
1	H (carbofuran)	6.7	0.052	2
2	Phenyl-S	9.3	0.0045	25-50
3	4-Tolyl-S	9.0	0.0045	100-125
4	3-Tolyl-S	6.5	0.004	25-50
5	2-Tolyl-S	3.7	0.004	100-125
6	2,4-Xylyl-S	9.0	0.003	50-100
7	4-t-Butylphenyl-S	2.7	0.0025	75
8	2-Me-4-t-Butylphenyl-S	7.5	0.002	75-125
9	MeS	4.9	0.024	20
$\begin{array}{c} \text{O} \\    \\ \text{2-Isopropoxyphenyl-OCN(CH}_3\text{)-R} \end{array}$				
10	H (propoxur)	24.0	0.33	24
11	Phenyl-S	36.0	0.039	300
12	4-Tolyl-S	36.0	0.028	350-400
13	3-Tolyl-S	23.5	0.020	300-400
14	2-Tolyl-S	24.0	0.024	400
15	2,4-Xylyl-S	27.5	0.014	400
16	4-t-Butylphenyl-S	9.0	0.013	750-1000
17	2-Me-4-t-Butylphenyl-S	24.5	0.014	>850
18	MeS	65.0	0.105	300-400

TABLE I (con't)

No.	R	Housefly		<u>Culex</u>	Mouse
		LD <sub>50</sub>	μg/g	<u>fatigans</u>	(oral)
				LC <sub>50</sub> , ppm	mg/kg
		$\text{CH}_3\text{SC}(\text{CH}_3)_2\overset{\text{O}}{\parallel}\text{CH}=\text{NOCN}(\text{CH}_3)-\text{R}$			
19	H (aldicarb)	5.5		0.16	0.3-0.5
20	2-Tolyl-S	12.5		0.084	3-5
21	4-t-Butylphenyl-S	7.5		0.014	10

According to the data, the sulfenylated derivatives were quite effective insecticides and in many cases were more toxic to house flies and mosquito larvae than the parent methylcarbamates. In contrast, the derivatives were without exception less toxic to mice. The arylsulfenylated methylcarbamates generally were highly effective against mosquito larvae. This is probably attributable to their greater lipophilic properties, i.e., compared to the parent methylcarbamate the derivatives are able to partition more rapidly out of the aqueous habitat of the mosquito larvae into the hydrophobic epicuticular wax layer of the larvae. In this regard, in field and laboratory tests, the *N*-phenylsulfenyl derivative of 3-*sec*-butylphenyl methylcarbamate was reported to be outstanding in controlling larvae and adults of susceptible and organophosphate-resistant strains of mosquitoes (10).

The results of a comparative metabolism study of an arylsulfenyl derivative of carbofuran [2,2-dimethyl-2,3-dihydrobenzofuran-7 *N*-methyl-*N*-(2-toluenesulfenyl)carbamate] in the house fly and white mouse indicated the selective action of this compound to be a consequence of different metabolic pathways in insects and mammals (12). The arylsulfenyl group on the carbamate moiety allows the mammal to carry out metabolic reactions leading to less toxic products which are rapidly conjugated, while the toxic parent methylcarbamate is formed in the insect.

Arylsulfenylated methylcarbamates also may have other interesting types of selectivity. An example is given in the honey bee and house fly toxicity data in Table II for several derivatives of propoxur (13). Propoxur and most of the derivatives were highly toxic to the honey bee; in fact, compounds 10-15 were substantially more toxic to the honey bee than to the house fly (also see Table I). The order of toxicity, however, was reversed with the 4-*t*-butylphenyl analog (16) and the 2-methyl-4-*t*-butylphenyl analog (17) was virtually nontoxic to the honey bee, although it was still effective against the house fly.

TABLE II

Toxicity of *N*-Arylsulfenyl Derivatives of Propoxur  
to the Honey Bee and House Fly

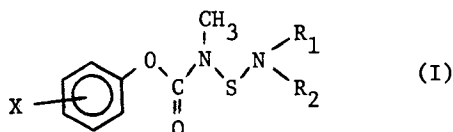
No.	R	2-Isopropoxyphenyl-OCN-SR	
		Honey Bee LD <sub>50</sub> (μg/g)	House Fly LD <sub>50</sub> (μg/g)
10	(Propoxur)	4.48	24.0
11	Phenyl	6.4	36.0
14	2-Tolyl	10.0	24.0
15	2,4-Xylyl	16.8	27.5
16	4- <i>t</i> -Butylphenyl	36.0	9.0
17	2-Me-4- <i>t</i> -Butylphenyl	>800.0	24.5

Examination of the penetration and metabolism of 17 in the house fly and honey bee showed that it penetrated into the honey bee at a much slower rate than into the house fly (14). Further, owing to the combined action of slow conversion rate of 17 to propoxur and rapid rate of degradation of propoxur to nontoxic products, the amount of propoxur in the honey bee remained at a low, non-intoxicating, steady-state level.

A number of reports describing a wide variety of *N*-arylsulfenyl and *N*-alkylsulfenyl derivatives of different types of methylcarbamate insecticides have been disclosed in the patent literature (15,16,17,18,19). Two of these reports (18,19) describe trichloromethyl- or trifluoromethylsulfenyl derivatives of the highly toxic methylcarbamates carbofuran and aldicarb.

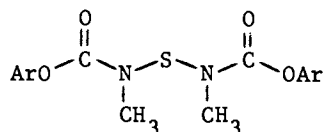
#### *N,N'*-Thiodicarbamates

The discovery of the improved toxicological properties of the aryl- and alkylsulfenyl derivatives of methylcarbamate insecticides stimulated examination of other types of derivatives containing an *N*-S linkage. In most of the cases where highest insecticidal activity is found, the methylcarbamate nitrogen is attached to a sulfur atom which is attached to another nitrogen atom, as shown in the general structure below.



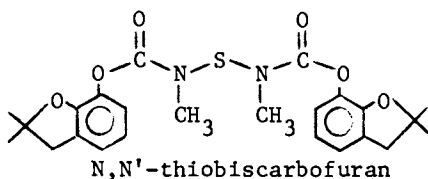
R<sub>1</sub> is generally an alkyl group and R<sub>2</sub> may be alkyl, alkoxy-carbonyl, aryloxy-carbonyl, dialkoxyphosphinothioyl, alkyl-sulfonyl, arylsulfonyl, dialkylaminosulfonyl or (alkyl)(aryl)-aminosulfonyl.

The first methylcarbamate derivative containing an N-S-N linkage was reported by Brown (20) who described compounds of the type below, where Ar is 2-isopropoxyphenyl, 3-isopropylphenyl, 3-s-butylphenyl, 1-naphthyl, etc. These were reported to be



effective in tests against a variety of insects. They were prepared by reaction between two moles of the methylcarbamate and one mole of sulfur dichloride in the presence of a base.

N,N'-Thiobiscarbamates also may be prepared by the reaction between a methylcarbamate ester and sulfur monochloride (21). For example, reaction between two moles of carbofuran and one mole of sulfur monochloride in the presence of pyridine gave N,N'-thiobiscarbofuran instead of the corresponding disulfide.



This compound was much less toxic to the white mouse but comparable to carbofuran in insecticidal activity. Toxicological properties for some of these compounds are presented in Table III. It is apparent from the data that the thiobiscarbamates

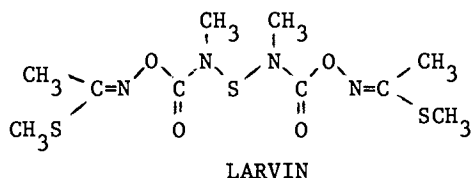
TABLE III

Toxicological Properties of Insecticidal Methylcarbamates and the Corresponding N,N'-Thiobiscarbamates

No.	Compound	House fly	<u>Culex</u>	Mouse oral
		LD <sub>50</sub> ( $\mu\text{g/g}$ )	<u>fatigans</u> LD <sub>50</sub> (ppm)	LD <sub>50</sub> (mg/kg)
1	Carbofuran	6.7	0.052	11
22	<u>N,N'</u> -Thiobiscarbofuran	19	0.007	50-100
10	Propoxur	22	0.33	24
23	<u>N,N'</u> -Thiobispropoxur	35	0.041	700
19	Aldicarb	5.5	0.16	0.3-0.5
24	<u>N,N'</u> -Thiobisaldicarb	8.5	0.17	1.6-2.5

retained much of the insecticidal activity exhibited by the parent methylcarbamates but were 5- to 29-fold less toxic to the white mouse. The improved systemic insecticidal activity of N,N'-thiobisaldicarb (24) was of interest since this compound gave more than a month longer control of cotton aphids and perforator than aldicarb in a laboratory systemic test with cotton plants.

One of the symmetrical N,N'-thiobiscarbamates, N,N'-thiobismethomyl or LARVIN, is currently under development as a new, broad spectrum insecticide (22,23). This compound was



particularly effective in laboratory and field tests for control of lepidopterous larvae which infest a wide variety of crops. Further, with a reported rat oral LD<sub>50</sub> of 160 mg/kg, LARVIN is significantly less acutely toxic to mammals than methomyl (rat oral LD<sub>50</sub> 17-24 mg/kg).

High insecticidal activity is not restricted to symmetrically substituted N,N'-thiodicarbamates and subsequent work revealed a number of unsymmetrical thiodicarbamates with excellent insecticidal activities (24,25,26). In these cases, only one of the two carbamate moieties is represented by an insecticidal methylcarbamate. Table IV provides typical toxicological data for some of these derivatives (24).

TABLE IV  
Toxicological Properties of N,N'-Thiodicarbamates

No.	R	R <sub>1</sub>	House fly LD <sub>50</sub> , μg/g	<u>Culex pipiens</u> LC <sub>50</sub> , ppm	Mouse oral LD <sub>50</sub> , mg/kg
				$  \begin{array}{c}  \text{O} \quad \text{O} \\     \quad    \\  \text{2,2-Dimethyl-2,3-dihydrobenzofuranyl-7-OC-N-S-N-C-OR}_1 \\    \quad   \\  \text{CH}_3 \quad \text{R}  \end{array}  $	
<u>1</u>	(carbofuran)		6.7	0.052	11
<u>25</u>	CH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	9.0	0.016	72
<u>26</u>	CH <sub>3</sub>	C <sub>5</sub> H <sub>11</sub> - <u>n</u>	10.5	0.0022	140
<u>27</u>	CH <sub>3</sub>	C <sub>10</sub> H <sub>21</sub> - <u>n</u>	14.0	0.0012	250
<u>28</u>	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	9.25	0.010	85

TABLE IV (con't)

No.	R	R <sub>1</sub>	House fly LD <sub>50</sub> , µg/g	<i>Culex pipiens</i> LC <sub>50</sub> , ppm	Mouse oral LD <sub>50</sub> , mg/kg
$\text{2-Isopropoxyphenyl-OC-N-S-N-C-OR}_1$ $\begin{array}{c} \text{O} \quad \quad \quad \text{O} \\ \parallel \quad \quad \quad \parallel \\ \text{CH}_3 \quad \quad \quad \text{R} \end{array}$					
10	(propoxur)		24	0.33	62
29	CH <sub>3</sub>	C <sub>5</sub> H <sub>11-n</sub>	35	0.008	>1000
30	CH <sub>3</sub>	C <sub>10</sub> H <sub>21-n</sub>	44	0.009	>1000
31	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	37.5	0.046	>1000
$\text{CH}_3\text{S-C(CH}_3\text{)-CH=N-OC-N-S-N-C-OR}_1$ $\begin{array}{c} \text{CH}_3 \quad \quad \quad \text{O} \quad \quad \quad \text{O} \\   \quad \quad \quad \parallel \quad \quad \quad \parallel \\ \text{CH}_3 \quad \quad \quad \text{CH}_3 \quad \quad \quad \text{R} \end{array}$					
19	(aldicarb)		5.5	0.16	0.4
32	CH <sub>3</sub>	C <sub>10</sub> H <sub>21-n</sub>	13	0.0064	12.5
$\text{CH}_3\text{S-C(CH}_3\text{)-N=O-N-OC-N-S-N-C-OR}_1$ $\begin{array}{c} \text{CH}_3 \quad \quad \quad \text{O} \quad \quad \quad \text{O} \\   \quad \quad \quad \parallel \quad \quad \quad \parallel \\ \text{CH}_3 \quad \quad \quad \text{CH}_3 \quad \quad \quad \text{R} \end{array}$					
33	(methomyl)		3.7	0.64	10
34	CH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	7.3	0.70	310
35	C <sub>2</sub> H <sub>5</sub>	C <sub>6</sub> H <sub>13-n</sub>	7.5	0.08	600
$\text{(CH}_3\text{)}_2\text{NC-C(CH}_3\text{)-N=O-N-OC-N-S-N-C-OR}_1$ $\begin{array}{c} \text{CH}_3 \quad \quad \quad \text{O} \quad \quad \quad \text{O} \\   \quad \quad \quad \parallel \quad \quad \quad \parallel \\ \text{O} \quad \quad \quad \text{CH}_3 \quad \quad \quad \text{R} \end{array}$					
36	(oxamyl)		3.6	0.33	5 <sup>a/</sup>
37	CH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	3.7	0.41	10
38	C <sub>2</sub> H <sub>5</sub>	C <sub>6</sub> H <sub>13-n</sub>	6.0	0.19	12

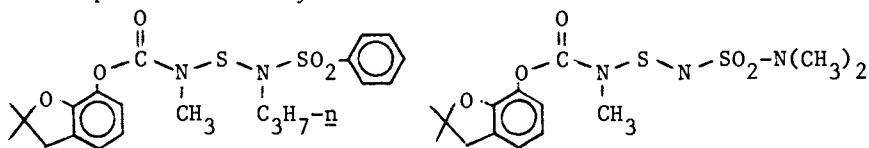
<sup>a/</sup> Rat LD<sub>50</sub>.

As in the case of the examples given in Tables I and III, the N-thiocarbamate derivatives were generally equal to the parent methylcarbamate in toxicity to house flies, particularly after taking into consideration increase in molecular weights. Except for the methomyl and oxamyl derivatives, substantial improvement in mosquito larvicidal activity again appeared to be a function of the lipophilic properties of the derivative. With the exception of the oxamyl derivatives, the data clearly indicate that the N-thiocarbamate derivatives are markedly less toxic to the white mouse than the parent methylcarbamate. Since

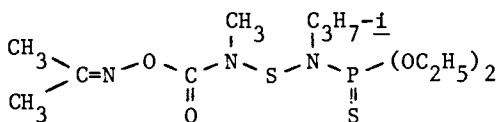
methomyl and oxamyl are somewhat similar in structure and in physical properties, the reason for the high mammalian toxicity of the oxamyl derivatives is not clear. On an overall basis, mouse toxicity was directly related to the hydrophobic properties of the derivative, i.e., derivatives with larger log P values (P = octanol-water partition coefficient) were less toxic to the white mouse.

#### Sulfonamidithio- and Phosphinoaminothiomethylcarbamates

A number of other derivatives also containing the N-S-N linkage have recently been reported in the patent literature. These are compounds where R<sub>2</sub> in the general structure (I) is dialkoxyphosphinothiyl (26), alkylsulfonyl, arylsulfonyl, dialkylaminosulfonyl or (alkyl)(aryl)aminosulfonyl (27). In the case of the sulfonyl compounds, derivatives were prepared of virtually all of the commercially important methylcarbamate insecticides and many of these derivatives were reported to be highly effective against a variety of insects which affect agricultural crops and public health. Typical structures for these compounds are exemplified below by derivatives of carbofuran.



Of the dialkoxyphosphinothiyl derivatives (26), Upjohn experimental insecticide U-47,319, a derivative of methomyl, has shown promise against larvae and ova of lepidopterous insects. Its acute rat oral LD<sub>50</sub> of 278 mg/kg is a significant improvement

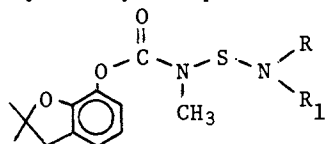


U-47,319

over the LD<sub>50</sub> of 17-24 mg/kg for methomyl.

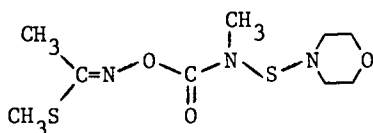
#### Dialkylaminosulfonylmethylcarbamates

Compounds with improved properties of selectivity may also be obtained with derivatives where R<sub>2</sub> in the general structure (I) is alkyl (28,29), exemplified below with carbofuran where R and R<sub>1</sub> are alkyl, cycloalkyl or part of a ring system.



R and R<sub>1</sub> may be the same or different and the compound where R = R<sub>1</sub> = n-butyl (trade name: MARSHAL) is currently undergoing development as a new insecticide. Its acute rat oral LD<sub>50</sub> is 209 mg/kg and it may be used by either soil or foliar application. In contrast, carbofuran with a rat LD<sub>50</sub> of 11 mg/kg, is relatively toxic to mammals and it has been used mainly as a soil insecticide.

A number of aminosulfenyl derivatives of methomyl have been examined for insecticidal activity (30). In general, the derivatives were similar to methomyl in their activities against a number of agriculturally important insects. One of these compounds, U-46,855 showed greater foliar residual life, less

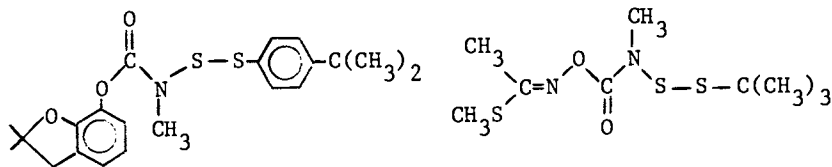


U-46,885

phytotoxicity and lower mammalian toxicity (acute rat oral LD<sub>50</sub> 105 mg/kg) than methomyl. In field trials for the control of the cotton bollworm, use of U-46,855 resulted in significantly higher cotton yields compared to methomyl.

#### Arylthio- and Alkylthiosulfenylmethylcarbamates

A number of methylcarbamate derivatives containing the N-S-S linkage also have been disclosed recently in the patent literature as selectively toxic insecticides (31,32). Typical examples of these compounds are given as follows:



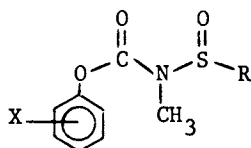
In virtually all examples given, the derivatives were described as being lower in mammalian toxicity compared to the parent methylcarbamate but generally of equal insecticidal activity.

#### N-Sulfinylmethylcarbamates

The sulfur derivatives of methylcarbamate insecticides which have been described are those containing a sulfinyl (or thio) sulfur attached to the methylcarbamate nitrogen atom. N-Alkylsulfinyl or N-arylsulfinyl derivatives (general structure



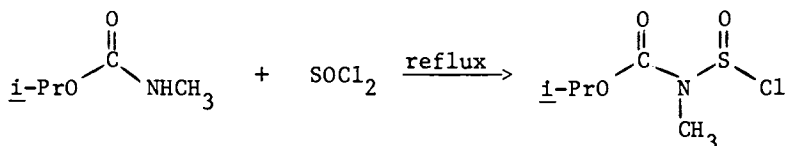
below) of insecticidal methylcarbamates also have been reported as useful insecticides (33).



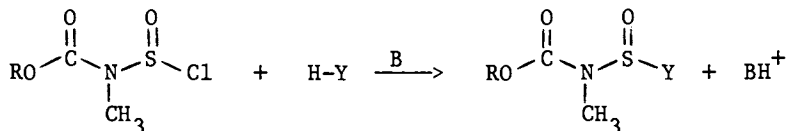
R = alkyl, aryl, or aralkyl

These derivatives were described as having strong insecticidal, acaricidal, and nematocidal activity, along with low mammalian toxicity.

More recently, an interesting reaction was discovered which provides the means for the synthesis of a wide variety of methylcarbamate derivatives containing the *N*-sulfinyl [*N*-S(O)] moiety (34). In attempting to prepare isopropoxy-*N*-methyliminoyl chloride by the reaction between isopropyl methylcarbamate and thionyl chloride, an unexpected product, isopropyl *N*-chloro-sulfinyl-*N*-methylcarbamate was obtained according to the equation below. The same product also was obtained when the

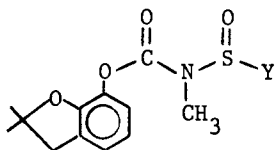


reaction was carried out in the presence of a proton acceptor such as pyridine. Further, the reaction occurred with insecticidal methylcarbamate esters of substituted phenols and oximes. The *N*-chlorosulfinyl-*N*-methylcarbamate intermediates reacted with different nucleophiles (Y) in the presence of a proton acceptor to give a variety of *N*-sulfinyl derivatives as shown below where HY may be an alcohol, phenol, alkanethiol,



arenethiol, *N*-alkylcarbamate, alkyl- or arylsulfonamide, and sulfondiamide. Toxicological properties are given in Table V for examples of each of the different types of derivatives using carbofuran as the parent methylcarbamate.

TABLE V

Toxicological Properties of the N-Sulfinyl Derivatives of Carbofuran

No.	Y	House fly LD <sub>50</sub> ( $\mu$ g/g)	<u>Culex</u> <u>pipiens</u> LC <sub>50</sub> (ppm)	Mouse oral LD <sub>50</sub> (mg/kg)
1	(carbofuran)	6.7	0.052	11
39	-OC <sub>6</sub> H <sub>13</sub> - <u>n</u>	13	0.0025	280
40	-O-phenyl	6.4	0.0055	42
41	-S-C <sub>4</sub> H <sub>9</sub> - <u>t</u>	9	0.01	70
42	-S-phenyl	7	0.05	40
43	-carbofuran <sup>a/</sup>	20	-	165
44	-N(CH <sub>3</sub> )C(O)OC <sub>7</sub> H <sub>15</sub> - <u>n</u>	13.5	0.015	150
45	-N(C <sub>2</sub> H <sub>5</sub> )S(O) <sub>2</sub> CH <sub>3</sub>	12.5	-	75
46	-N(CH <sub>3</sub> )S(O) <sub>2</sub> -phenyl	9.5	-	35
47	-N(CH <sub>3</sub> ) <sub>2</sub> S(O)N-(Bu- <u>n</u> ) <sub>2</sub>	12	-	25

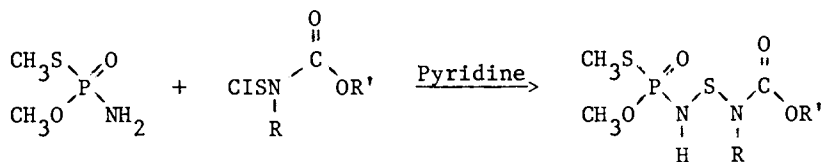
<sup>a/</sup> Attached at the carbamate nitrogen atom of carbofuran.

Most of the N-sulfinyl derivatives in the table show good insecticidal activity along with a significant reduction in mouse toxicity (2- to 25-fold reduction). The number of new derivatives with improved toxicological properties which can be synthesized through the N-chlorosulfinyl intermediates is exceedingly large.

(Alkoxy carbonyl) (alkylamino) sulfenylphosphoramidothioates

The phosphoramidothioate insecticide methamidophos (O,S-dimethyl phosphoroamidothioate) is similar to the methylcarbamates in that it also contains a replaceable hydrogen on a nitrogen atom and, therefore, may be derivatized in an analogous manner. In fact, the N-acetylated product of methamidophos is acephate (O,S-dimethyl N-acetylphosphoramidothioate), a compound which retains the outstanding insecticidal activity of methamidophos but is approximately 45-fold less toxic to the rat (acute rat oral LD<sub>50</sub> of 900 mg/kg compared to 20 mg/kg for methamidophos) (35). With this in mind, a number of (alkoxy carbonyl)-(alkylamino)-sulfenyl derivatives of methamidophos were prepared and examined for toxicological properties (36). These compounds

were readily prepared by the reaction between the *N*-chloro-sulfenyl-*N*-alkylcarbamate (24,37) and methamidophos in the presence of pyridine as shown below.



Housefly and mouse toxicities of some of these compounds are presented in Table VI. All of the derivatives were highly toxic

TABLE VI

Toxicity of *O,S*-Dimethyl  
*N*-(*N'*-Alkoxy-carbonyl-*N'*-Alkylaminosulfenyl)-  
phosphoramidothioates to House Flies and Mice

No.	R	R'	House fly LD <sub>50</sub> , μg/g	Mouse oral LD <sub>50</sub> , mg/kg
48	(methamidophos)		1.3	14
49	C <sub>2</sub> H <sub>5</sub>	CH <sub>3</sub>	2.8	30
50	C <sub>2</sub> H <sub>5</sub>	C <sub>0</sub> H <sub>13-n</sub>	3.8	34
51	C <sub>3</sub> H <sub>7-n</sub>	C <sub>3</sub> H <sub>7-n</sub>	1.9	36
52	C <sub>3</sub> H <sub>7-i</sub>	C <sub>2</sub> H <sub>5</sub>	1.9	50
53	C <sub>3</sub> H <sub>7-i</sub>	C <sub>3</sub> H <sub>7-n</sub>	2.5	50
54	C <sub>4</sub> H <sub>9-t</sub>	C <sub>3</sub> H <sub>7-n</sub>	1.6	50

to house flies with relatively little difference in toxicities. Although the improvement in mouse toxicity was not as large as with most of the methylcarbamate derivatives, in all cases the derivative was less toxic than methamidophos. The possibility remains for greater improvement in selectivity with other derivatives of this type.

**American Chemical  
Society Library  
1155 16th St. N. W.**

### Summary

The foregoing discussion has provided examples of the successful derivatization of methylcarbamate and phosphoramidothioate insecticides with different functional groups to give a variety of sulfur propesticides. In most cases, notable improvement in mammalian toxicity was achieved along with the retention of insecticidal activity. The favorable toxicological properties of the derivatives may be attributed to a combination of: (1) the "delay factor" provided by the functional group, (2) differences in metabolic pathways between different animals and (3) differences in the physical properties of the derivatives. The examples given in this review represent but a small fraction of the modifications which may be made with toxic methylcarbamate insecticides. Further work along these lines is warranted, particularly with those methylcarbamate insecticides having the highest order of insecticidal activity, regardless of their mammalian toxicity. Needless to say, this type of approach is not restricted only to insecticides but may be applied to other pesticides, e.g. herbicides and fungicides which contain the appropriate groups for derivatization.

### Literature Cited

1. Kreuger, H.R.; O'Brien, R.D. J. Econ. Entomol. 1959, 52, 1063.
2. Umetsu, N.; Grose, H.F.; Allahyari, R.; Abu-El-Haj, S.; Fukuto, T.R. J. Agr. Food Chem., 1977, 25, 946.
3. Brooks, G.T., in "The Future for Insecticides," ed. by Metcalf, R.L., McKelvey, J.J., Jr., Wiley-Interscience, New York, 1976, pp. 97-143.
4. Fraser, J.; Clinch, P.J.; Ray, R.C. J. Sci. Food Agr., 1965, 14, 615.
5. Fahmy, M.A.H.; Metcalf, R.L.; Fukuto, T.R.; Hennessy, D.J., J. Agr. Food Chem., 1966, 14, 79.
6. Miskus, R.P.; Andrews, T.L.; Look, M.L. J. Agr. Food Chem., 1969, 17, 842.
7. Gunew, D.S., in "Analytical Methods for Pesticide and Plant Growth Regulators, ed. by Zweig, G; Sharma, J., Academic Press, New York, 1976, p. 207.
8. Fahmy, M.A.H.; Fukuto, T.R.; Myers, R.O.; March, R.B. J. Agr. Food Chem., 1970, 18, 793.
9. Krieger, R.I.; Lee, P.W.; Fahmy, M.A.H.; Chen, M.; Fukuto, T.R. Pestic. Biochem. Physiol., 1976, 6, 1.
10. Schaeffer, C.H.; Wilder, W.H. J. Econ. Entomol., 1970, 63, 480.
11. Black, A.L.; Chiu, Y.C.; Fahmy, M.A.H.; Fukuto, T.R. J. Agr. Food Chem., 1973, 21, 747.

12. Black, A.L.; Chiu, Y.C.; Fukuto, T.R.; Miller, T.A. Pestic. Biochem. Physiol., 1973, 3, 435.
13. Mallipudi, N.M.; Fukuto, T.R. J. Agr. Food Chem., 1979, 27, 261.
14. Mallipudi, N.M.; Fukuto, T.R. Pestic. Biochem. Physiol., 1979, 12, 55.
15. Serban, A.; Gregory, J.M., So. Afr. Pat. 710,959, Feb., 1971.
16. Brown, M.S.; Kohn, G.K., U.S. Pat. 3,663,594, May 16, 1972.
17. Kohn, G.K.; Brown, M.S., U.S. Pat. 3,847,951, Nov. 21, 1974.
18. Farbenfab. Bayer AG, Belg. Pat. 772,634, March 1972.
19. Farbenfab. Bayer AG, Ger. Pat. 2,106,300, Feb. 1971.
20. Brown, M.S., U.S. Pat. 3,679,733, July 25, 1972.
21. Fahmy, M.A.H.; Chiu, Y.C.; Fukuto, T.R. J. Agr. Food Chem. 1974, 22, 59.
22. Sousa, A.A.; Frazer, J.R.; Weiden, M.H.J.; D'Silva, T.D.J. J. Econ. Entomol., 1977, 70, 803.
23. Drabek, J., U.S. Pat. 4,004,031, Jan. 18, 1977.
24. Fahmy, M.A.H.; Mallipudi, N.M.; Fukuto, T.R. J. Agr. Food Chem., 1978, 26, 550.
25. Liang, W.C., Ger. Offen. 2,654,314, June 23, 1977. C.A., 1977, 101, 101,988.
26. Nelson, S.J., U.S. Pat. 4,081,536, March 28, 1978.
27. Hartmann, A.; Kühle, E.; Hamann, I.; Behrenz, W.; Homeyer, B., U.S. Pat. 4,148,910, April 10, 1979.
28. Fukuto, T.R.; Black, A.L.; Chiu, Y.C.; Fahmy, M.A.H. Environ. Quality Safety, 1975, Suppl. Vol. III, 394.
29. Black, A.L.; Fukuto, T.R., U.S. Pat. 4,006,231, Feb. 1, 1977.
30. Gemrich, E.G.; Lee, B.L.; Nelson, S.J.; Rizzo, V.L. J. Agr. Food Chem., 1978, 26, 391.
31. D'Silva, T.D.J., U.S. Pat. 4,122,204, Oct. 24, 1978.
32. D'Silva, T.D.J., U.S. Pat. 4,138,423, Feb. 6, 1979.
33. Ueda, M.; Ooba, S.; Hirano, M.; Takeda, H., U.S. Pat. 3,950,374.
34. Fahmy, M.A.H.; Fukuto, T.R. J. Agr. Food Chem. (submitted).
35. Magee, P.S. Residue Rev., 1974, 53, 3.
36. Fahmy, M.A.H.; Fukuto, T.R. (unpublished work).
37. Brown, M.S.; Kohn, G.K., U.S. Pat. 3,843,689, Oct. 22, 1974.

RECEIVED January 26, 1981.

# S-Oxygenation in Herbicide Metabolism in Mammals

D. H. HUTSON

Shell Research Ltd., Shell Toxicology Laboratory (Tunstall),  
Sittingbourne Research Centre, Sittingbourne, Kent ME9 8AG, U.K.

The sulfur atom in organo-sulfur compounds is very readily oxidized by biological systems to afford sulfoxides and sulfones, and sulfinic and sulfonic acids, depending on the structure of the compound. The importance of the phosphorothionate insecticides has prompted some extensive studies on S-oxidation as has research into the safety assessment of carbon disulfide. However, with the exceptions noted in this Chapter, little is known about the mechanism and the significance of the biological oxidation of carbon-bonded sulfur(1). In view of the enormous amount of information now available on the mechanism of enzymatic oxidation at carbon and nitrogen, it is remarkable that so little attention has been paid to S-oxygenation. Two classes of sulfur-containing herbicides, the alkylthio-s-triazines and the thiocarbamates, have been found recently to have similar modes of biotransformation both involving S-oxygenation. The results of studies on these herbicides form the basis of this Chapter.

## Alkylthiotriazines and Thiocarbamates

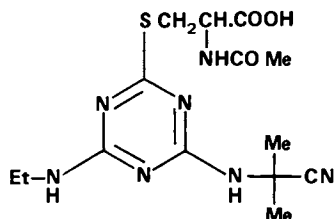
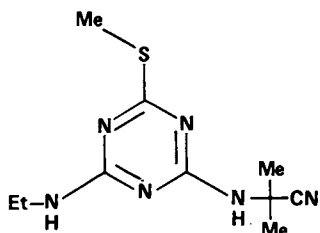
Alkylthiotriazines. In our laboratory we have studied the metabolic fate of 2-(4-ethylamino-6-methylthio-s-triazin-2-ylamino)-2-methylpropionitrile (cyanatryn, 1, Fig. 1). This compound is a member of a class of herbicidal s-triazines which also includes ametryne, prometryne and terbutryne. We were interested to note (2) that two of the major metabolites of cyanatryn were the mercapturic acids 2-[4-ethylamino-6-(N-acetylcysteinyl)-s-triazin-2-ylamino]-2-methylpropionitrile (2.1) and its N-de-ethyl derivative (2.2) (Fig. 2). This pathway had not hitherto been reported for this class of compound.

Mercapturic acid formation in mammals is preceded by interaction with glutathione (GSH), a reaction which may be spontaneous or may be catalyzed, depending on the reactivity of the compound under investigation. The enzymes responsible for the catalysis are found in mammalian liver cytosol, however when cyanatryn was tested under appropriate conditions it neither

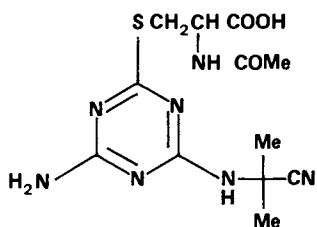
0097-6156/81/0158-0053\$05.00/0

© 1981 American Chemical Society

Figure 1. Cyanatryn



(2.1)



(2.2)

Figure 2. Mercapturic acids derived from the metabolism of cyanatryn in the rat

reacted with GSH nor was a substrate for a rat liver cytosol transferase. It was necessary therefore to examine the reaction using other subcellular fractions of rat liver and possibly other tissues. When cyanatryn was incubated with rat liver microsomes and NADPH, two extra more polar products were detected by thin-layer chromatography (Fig. 3). The minor product was identified as 2-(2-amino-6-methylthio-*s*-triazin-2-ylamino)-2-methylpropionitrile (4, Fig. 4) i.e. de-ethyl-cyanatryn. The major metabolite disappeared on further incubation of the mixture for 10 min with GSH to afford 2-(4-ethylamino-6-glutathionyl-*s*-triazin-2-ylamino)-2-methylpropionic acid (5, Fig. 5). This product, a glutathione conjugate of cyanatryn, had been detected previously (2) in the bile of rats treated with cyanatryn, and was clearly the precursor of the cyanatryn mercapturic acid (Fig. 2). The microsomal product was identified by mass spectrometry as cyanatryn *S*-oxide (6, Fig. 6) and was synthesized (3) by the action of *m*-chloroperbenzoic acid on cyanatryn. The metabolite and the synthetic compound both reacted readily with 3,4-dichlorobenzenethiol in ethanol to afford the 3,4-dichlorothiophenyl derivative shown in Fig. 7. This demonstrates further the reactivity of cyanatryn *S*-oxide with sulfur nucleophiles.

Thiocarbamates. An analogous situation was also discovered recently (4) with the thiocarbamate herbicides which were found to be converted into thiocarbamate sulfoxides by liver microsomes. These products reacted with glutathione (catalysed by a cytosol transferase of liver and also of corn roots and leaves). That these reactions occur in whole animals was shown by the isolation of the appropriate mercapturic acids from the urine of animals treated with the thiocarbamates. For example about 30-40% of an oral dose of *S*-ethyl *N,N*-dipropylthiocarbamate (8.1, Fig. 8) to rats was metabolised via the thiocarbamate sulfoxide (8.2), *S*-(*N,N*-dipropylcarbamoyl)glutathione (8.3), *S*-(*N,N*-dipropylcarbamoyl)cysteine (8.4) and *S*-(*N,N*-dipropylcarbamoyl)mercapturic acid (8.5) (5).

The related compound, molinate (9.1, Fig. 9) has recently been found to undergo the analogous series of reactions in rats (6) and in fish (7).

General Considerations. Both of the *S*-oxidation reactions described above amount to microsomally-mediated bioactivation reactions which allow glutathione-dependent biotransformations to occur with the simultaneous splitting of the molecule and the subsequent elimination of the fragments. The *S*-oxides possess similar reactivities, triazinylating and carbamoylating respectively, and being particularly reactive towards thiol groups. The reactions are important to detoxification in mammals and may also be important in selective toxicity (herbicidal action) in plants (8).



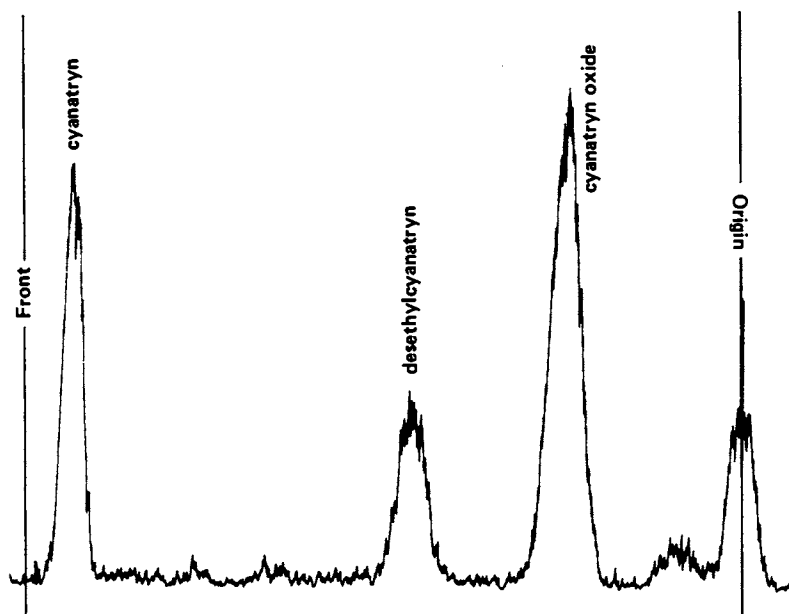


Figure 3. Thin-layer chromatography of the products derived from the incubation of [ $^{14}\text{C}$ ] cyanatryn with rat liver microsomes and NADPH

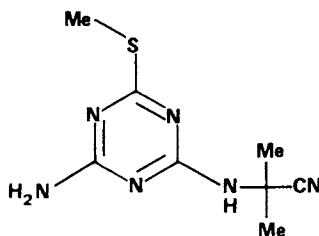


Figure 4. De-ethyl cyanatryn

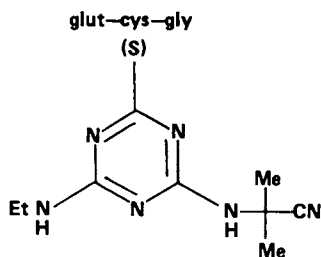


Figure 5. The glutathione conjugate of cyanatryn

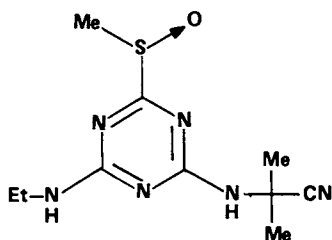


Figure 6. Cyanatryn S-oxide

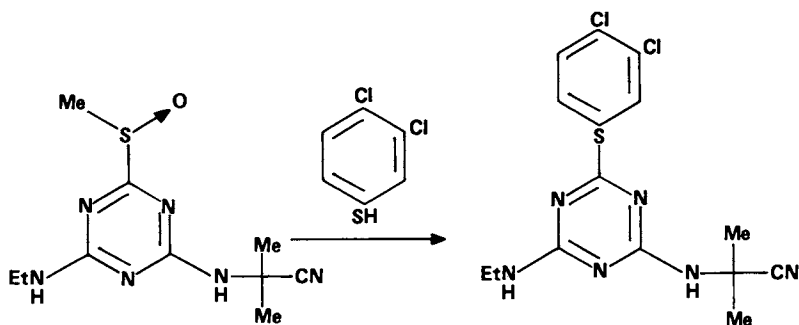
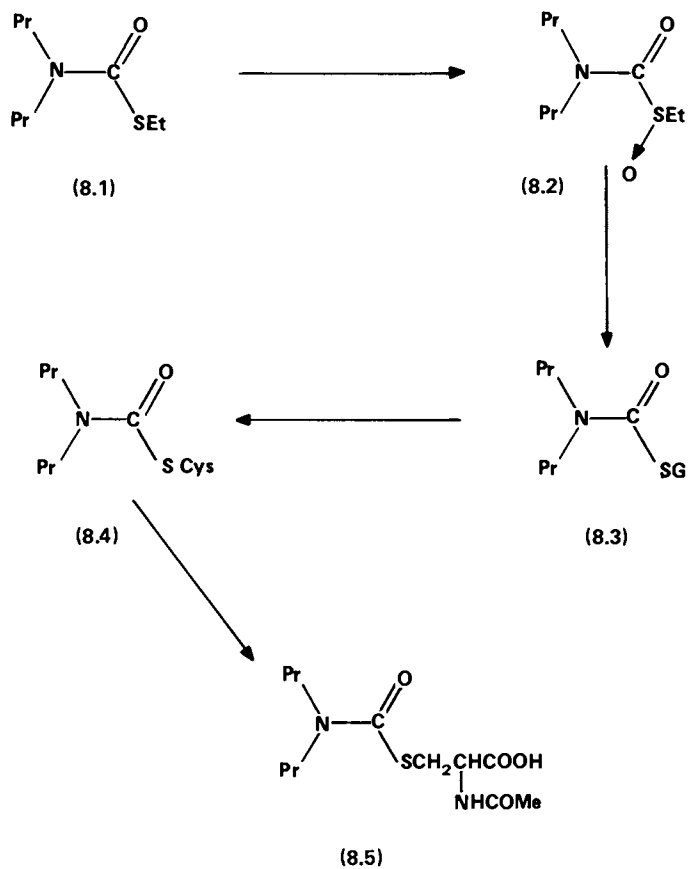


Figure 7. Reaction between cyanatryn S-oxide and 3,4-dichlorothiophenol



GSH = glutathione

Journal of Agricultural and Food Chemistry

Figure 8. Metabolism of S-ethyl N,N-dipropylthiocarbamate via S-oxygenation and glutathione conjugation (5)

In vitro studies on the S-oxygenating enzyme involved in cyanatryn metabolism

The S-oxygenation of cyanatryn occurred readily with liver microsomes and 10,000 g supernatant (the latter was fortified with GSH and the observed product was the glutathione conjugate [5]). The reaction could be detected in 2% liver homogenates but not in homogenates of kidney, lung, intestine, or caecal content. The reaction was readily catalyzed by microsomes from the livers of male and female rats, male and female rabbits and a male human (Table 1) (9). The rat sex difference was much larger for N-de-ethylation than for S-oxidation. Typically, microsomes from male rats were more active than those from females.

Table 1. S-Oxygenation and N-de-ethylation of cyanatryn in three species in vitro

Species	Rate nmoles/min/ mg protein	Ratio S-oxidation : N-de-ethylation	
<u>RAT</u>			
male microsomes	4.5 (2)	1	: 0.4
female microsomes (+ glutathione)	2.0 (4) (2.2)	1 (1)	: 0.025 : 0.025)
male cytosol (+ glutathione)	no reaction (no reaction)		- -
<u>RABBIT</u>			
female microsomes	0.4 (5)	1	: 0.25
male microsomes	0.4	1	: 0.20
<u>HUMAN</u>			
male microsomes	2.4	1	: 0.5

Although it has been assumed for years that S-oxidation involves cytochrome P450 there is no good evidence for this (1). Differences in the species and sex specificity of the S-oxidation and the N-de-ethylation of cyanatryn catalysed by liver microsomes suggested that different enzymes may be involved in the two reactions. Relevant to this problem was the isolation of a cytochrome P450-free enzyme (10) which catalyses the S-oxidation of 2-ethyl-4-thioisonicotinamide. Compounds of similar structure, the thioureylenes, of which methimazole (1-methyl-2-

mercapto-imidazole) (10.1, Fig. 10) is an example, are also oxidised by a cytochrome P450-independent system. This enzyme has been identified by Ziegler and co-workers (11) as the pig liver flavoprotein N-oxygenase which they had previously isolated and characterized. The oxidation products derived from (10.1) were identified as N-methylimidazole and sulfite, probably arising via the di-oxygenated product (10.2).

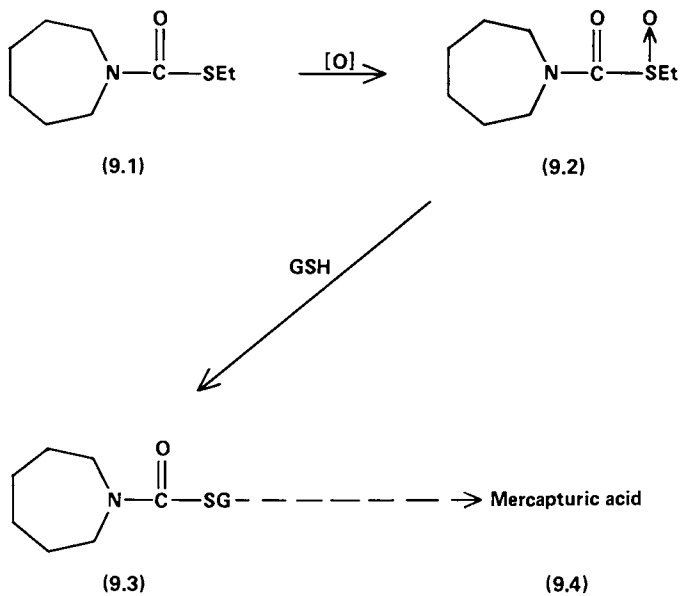
We have recently studied the S-oxidation of cyanatryn further, however, and have come to the conclusion that it is catalysed by the cytochrome P450 system rather than by the flavoprotein S-oxygenase (9). The conclusions were based on the following:

- (a) the reaction (female rat liver microsomes) was completely inhibited by equimolar (0.1 mM) metyrapone;
- (b) the reaction (male rat liver microsomes) was 50% inhibited when carried out in an atmosphere of carbon monoxide/oxygen (4:1, v/v); N-de-ethylation was similarly inhibited, and
- (c) cyanatryn was not a substrate for the flavo-protein S-oxygenase (a gift from Professor Dan Ziegler, Austin, Texas).

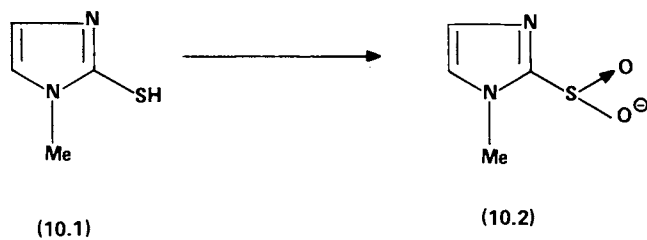
#### Further Reactions of the S-oxides

The reactivity of cyanatryn S-oxide to sulfur nucleophiles has been noted above. This property was also found to account for an interaction between this triazine and a component of rat blood (2). Approximately 1% of a single oral dose (over a wide range, 0.25 - 250 mg/kg) of ring-radiolabelled cyanatryn was bound to the blood of rats and eliminated with a half-life of about 20 days (Fig. 11), i.e. approximately that of the erythrocyte in rat. The radioactivity was located in the erythrocytes, associated with haemoglobin, and covalently bound to the globin. It was released by gentle acid hydrolysis which characteristically cleaved the s-triazinyl-cysteine bond (e.g. as in the glutathione conjugate [5]).

This interaction could be modelled in vitro by incubating washed rat erythrocytes with chemically synthesised [<sup>14</sup>C]cyanatryn S-oxide. The reaction occurred only to a very small extent in both rabbit and dog blood in vivo and in vitro, indicating a difference between the reactivity of the haemoglobin in rat and that in these other species. It is likely that this interaction is general to 6-alkyl-mercapto-s-triazines. A related herbicide (H replacing the CN group of cyanatryn) leaves a higher radiochemical residue in blood than in 10 other tissues of rat 72 hours after dosing (12). The kinetics of elimination were not measured but there is no reason to suppose that the radioactivity would behave differently from that derived from cyanatryn. A report of the reaction between the S-oxide of simetryn



Journal of Agricultural and Food Chemistry

Figure 9. Metabolism of molinate initiated by *S*-oxygenation (6)Figure 10. *S*-oxygenation of methimazole

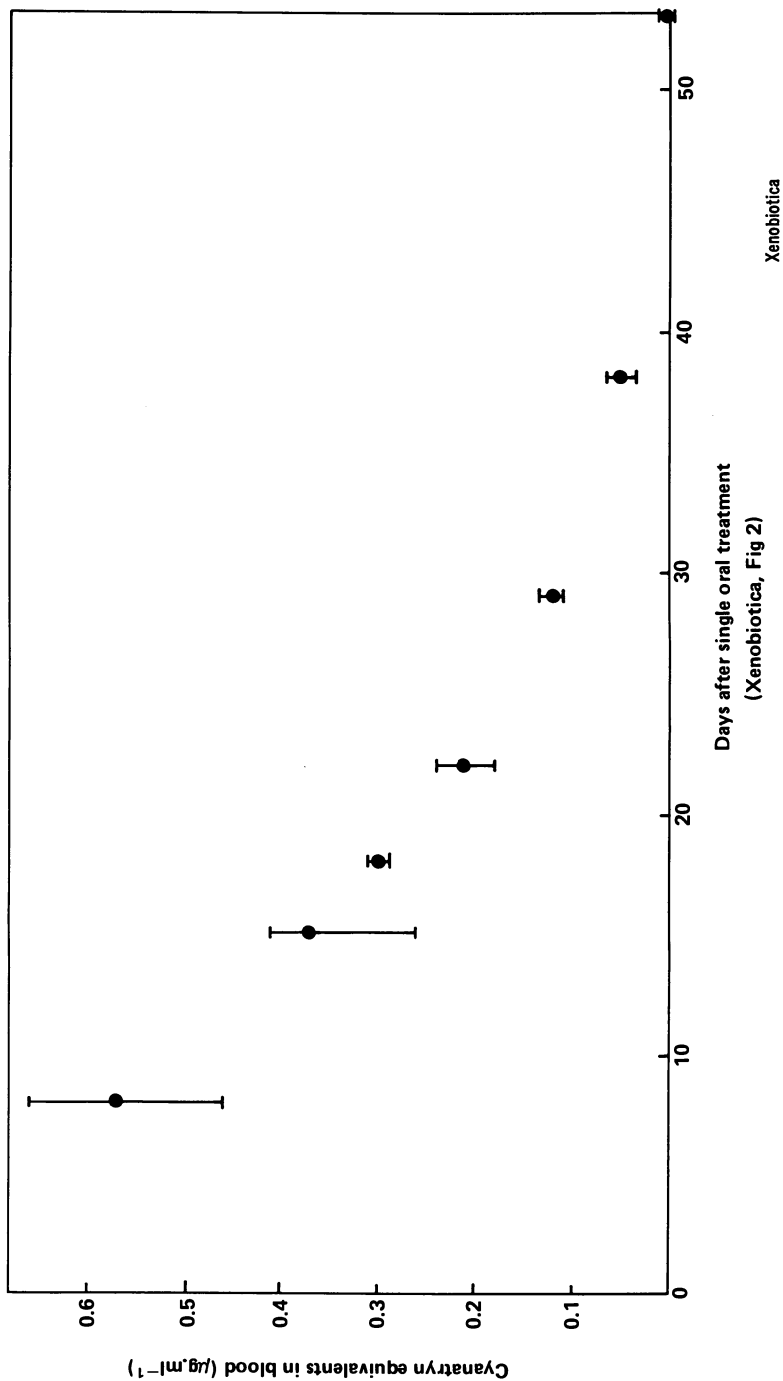


Figure 11. Elimination of radioactivity from the blood of rats dosed orally with [ $^{14}\text{C}$ -ring] cyanatryn (2)

(2,4-bis-ethylamino-6-methylthio-s-triazine) and haemoglobin(13) confirms the species specificity. The reaction was found with rat, guinea pig and chicken bloods (which each contain particularly reactive thiol groups) but not with the blood of dog, cow, sheep, pig or man. Our own studies have also demonstrated very low reactivity with human blood.

As the thiocarbamate S-oxides behave similarly to those of the alkylmercapto-s-triazines in other respects, it would be of interest to investigate their reactivity to haemoglobin.

We have been unable to demonstrate any reaction between cyanatryn S-oxide and DNA *in vitro* or between cyanatryn and liver DNA *in vivo*(2). Three hours after oral doses of [<sup>14</sup>C]cyanatryn giving about 2 (1.98 and 2.41) µg of cyanatryn equivalents per g of liver, the perfused livers were removed, pooled and used to prepare protein, RNA and DNA fractions(14). Values expressed as µg of cyanatryn per g of pooled liver were: total, 2.19; protein, 0.29; RNA fractions, 0.002; DNA fraction, not detectable. Radiochemical in the combusted sample of DNA was not detectable, i.e. less than 1 dpm/mg. This value is equivalent to 1 mol of triazine to 10<sup>7</sup> mol of guanine. Cyanatryn and its S-oxide apparently possess low reactivity towards nitrogen nucleophiles.

### Conclusions

In summary, these investigations into an aspect of sulphur biochemistry have revealed bioactivation reactions via S-oxygenation (leading to mercapturic acid formation and to protein binding) but they have also demonstrated that the rat (cf man) possesses a particularly sensitive sulphur target. The results provide a good example of how further investigation can resolve a problem - in this case the question of the significance of the interaction with haemoglobin.

### Literature Cited

1. Hutson, D. H. in 'Foreign Compound Metabolism in Mammals', Vol. 4, The Chemical Society, London, p.278, 1977.
2. Crawford, M. J.; Hutson, D. H.; Stoydin, G. Xenobiotica, 1980, 10, 169.
3. Bedford, C. T.; Crawford, M. J.; Hutson, D. H. Chemosphere, 1975, 4, 311.
4. Casida, J. E.; Kimmel, E. C.; Ohkawa, H.; Ohkawa, R. Pestic. Biochem. Physiol., 1975, 5, 1.
5. Hubbell, J. P.; Casida, J. E. J. Agric. Fd. Chem., 1977, 25, 404.
6. DeBaun, J. R.; Bova, D. L.; Tseng, C. K.; Menn, J. J. J. Agric. Fd. Chem., 1978, 26, 1098.
7. Lay, M. M.; Menn, J. J. Xenobiotica, 1979, 9, 669.
8. Lay, M. M.; Casida, J. E. Pestic. Biochem. Physiol., 1976, 6, 442.



9. Crawford, M. J.; Hutson, D. H. Xenobiotica, 1980, 10, 187.
10. Prema, K.; Gopinathan, K. P. Biochem. J., 1974, 143, 613.
11. Paulson, L. L.; Hyslop, R. M.; Ziegler, D. M. Biochem. Pharmacol., 1974, 23, 3431.
12. Oliver, W. H.; Born, G. S.; Ziemer, P. L. J. Agric. Fd. Chem., 1969, 17, 1207.
13. Hamboeck, H.; Fischer, R. W.; Winterhalter, K. H. Abstracts of 11th F.E.B.S. Meeting, Copenhagen, August, 1977.
14. Roberts, J. J.; Pascoe, J. M.; Plant, J. E.; Sturrock, J. E.; Crathorn, A. R. Chem.-Biol. Interact., 1971, 3, 29.

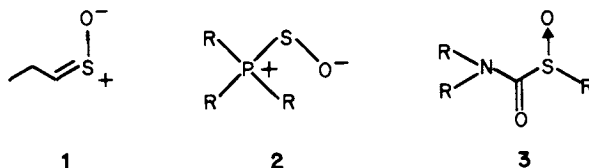
RECEIVED December 30, 1980.

# Toxicological Significance of Oxidation and Rearrangement Reactions of *S*-Chloroallyl Thio- and Dithiocarbamate Herbicides

INGOLF SCHUPHAN<sup>1</sup>, YOFFI SEGALL<sup>2</sup>, JOSEPH D. ROSEN<sup>3</sup>,  
and JOHN E. CASIDA<sup>4</sup>

Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, CA 94720

Many organosulfur compounds undergo biological oxidation at the sulfur atom to yield products which have pronounced physiological activity or serve as intermediates in generating bioactive compounds. Three examples are the lachrymating agent in onions (1) (1), the oxo intermediate (2) in metabolic desulfuration of phosphorothionate insecticides to form potent cholinesterase inhibitors (2), and the sulfoxides (3) produced on metabolism of thiocarbamate herbicides (3).



The herbicidal activity of *S*-alkyl thiocarbamates (3-5) and the mutagenic activity of an *S*-(2,3-dichloroallyl) thiocarbamate (6) are probably due to the reactivity or decomposition products of their metabolically-formed sulfoxides.

<sup>1</sup> Current address: Institut für Pflanzenschutzmittelforschung, Biologische Bundesanstalt, 1 Berlin 33, Federal Republic of Germany.

<sup>2</sup> Current address: Israel Institute for Biological Research, Ness-Ziona, P.O.B. 19, Israel.

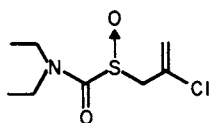
<sup>3</sup> Current address: Department of Food Science, Cook College, Rutgers University, New Brunswick, NJ 08903.

<sup>4</sup> To whom correspondence should be addressed.

This review considers the S-chloroallyl thio- and dithiocarbamate herbicides with particular emphasis on the toxicological significance of their oxidation and rearrangement reactions.

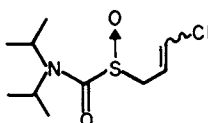
### Thiocarbamate Sulfoxides

**Synthesis.** Oxidation of S-alkyl or S-benzyl N,N-dialkylthiocarbamates with one equivalent of m-chloroperoxybenzoic acid (MCPBA) in chloroform or methylene chloride at  $-25^{\circ}$  to  $25^{\circ}\text{C}$  yields the corresponding carbamoyl sulfoxide (3) in essentially quantitative yield (3-5). The S-chloroallyl thiocarbamate sulfoxides (e.g., 4-7) are obtained in the same manner except that the temperature is maintained between  $-20^{\circ}\text{C}$  and  $0^{\circ}\text{C}$  for the oxidation and extraction of the reaction mixture with 5% sodium carbonate aqueous solution (7, 8).



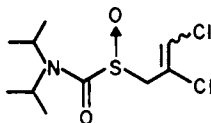
4

2-chloroallyl thiocarbamate sulfoxide



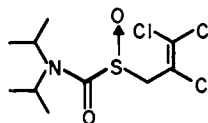
5

3-chloroallyl thiocarbamate sulfoxide



6

2,3-dichloroallyl thiocarbamate sulfoxide  
(diallate sulfoxide, *cis-trans*)



7

2,3,3-trichloroallyl thiocarbamate sulfoxide  
(triallate sulfoxide)

**Spectral Features.** The IR spectra of carbamoyl sulfoxides (3) of S-alkyl, S-benzyl and S-chloroallyl derivatives show the characteristic  $\text{S}=\text{O}$  absorption band near  $1070\text{ cm}^{-1}$ , which is not present in the spectra of the parent compounds (5, 7-9).

The NMR chemical shifts for the carbamoyl sulfoxides (Table I) support their proposed structures. In examining the oxidation reactions, it is convenient to add MCPBA to a solution of the thiocarbamate in  $\text{CDCl}_3$  at  $-20^{\circ}\text{C}$  and take frequent spectra during oxidation as the reaction mixture warms up to  $40^{\circ}\text{C}$ . Comparison of these spectra with that of the parent compound at  $-20$  to  $40^{\circ}\text{C}$  allows recognition of short-lived intermediates and terminal products. With the parent thiocarbamates for compounds 5-7, the methyl group signals appear as one doublet at  $40^{\circ}\text{C}$  but two

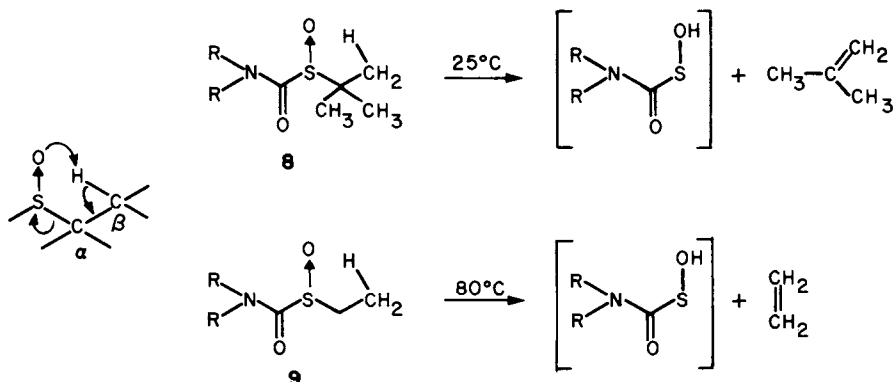
TABLE I

<sup>1</sup>H Chemical Shift Data (ppm) for S-Chloroallyl Thiocarbamates and Thiocarbamate Sulfoxides (7, 8). Solutions in CDCl<sub>3</sub> at 20°-40°C with tetramethylsilane as the internal standard. Proton coupling in Hz: (CH<sub>3</sub>)<sub>2</sub>CH, 6.7 in all cases; CH<sub>2</sub>-C=CH,  $\nu$  < 0.9; CH<sub>3</sub>-CH<sub>2</sub>, 7.1; =CH<sub>2</sub>, 1.1. s = singlet, d = doublet, t = triplet, qa = quartet, qi = quintet, m = multiplet, b = broad

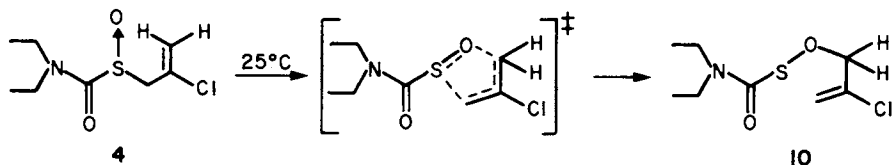
Protons	2-Chloroallyl thiocarbamate <u>12</u>		3-Chloroallyl thiocarbamate		Sulfoxide <u>5</u>		Diallate cis (trans)		Sulfoxide <u>6</u> cis (trans)		Triallate		Sulfoxide <u>7</u>	
	<u>12</u>	Sulfoxide <u>4</u>	Sulfoxide <u>4</u>	Sulfoxide <u>5</u>	Sulfoxide <u>5</u>	Sulfoxide <u>6</u>	Sulfoxide <u>6</u>	Sulfoxide <u>6</u>	Sulfoxide <u>6</u>	Sulfoxide <u>6</u>	Sulfoxide <u>6</u>	Sulfoxide <u>6</u>	Sulfoxide <u>6</u>	Sulfoxide <u>6</u>
1	1.18 (t)	1.26 (m)	1.30 (d)	1.28 (dd) 1.44 (dd)	1.30(1.31) (d)	1.29(1.29) (dd) 1.44(1.45) (dd)	1.31 (d)	1.28 (dd) 1.46 (dd)						
2	3.40 (qa)	3.49 (m)	3.80 (bm)	3.57 (~qi) 4.24 (~qi)	3.82(3.82) (bm)	3.60(3.60) (~qi) 4.36(4.35) (~qi)	3.82 (bm)	3.61 (~qi) 4.37 (~qi)						
3	3.84 (s)	3.90 (s)	3.51 (d) 3.68 (d)	3.70 (d) 3.84 (d)	3.86(4.06) (d)	3.89(4.10) (d)	4.13 (s)	4.16 (d)						
4/5	5.26 (d) 5.48 (d)	5.55 (s)	6.03 (m)	6.23 (m) 3.84 (d)	6.52(6.23) (t)	6.53(6.54) (t)								

doublets are evident at 0°C due to restricted rotation around the amide C-N bond. With the sulfoxide of *S*-propyl *N,N*-diisopropylthiocarbamate, methyl group protons of the two isopropyl substituents appear at 40°C as four doublets (10) as a result of the chiral center at sulfur and the restricted rotation around the amide C-N bond, i.e. the methyl groups are diastereotopic (5, 9); these NMR spectral features are also apparent with sulfoxides 5-7 (Table I) (7, 8). The NMR signals of the sulfoxides are generally at lower field compared with the parent compounds (Table I).

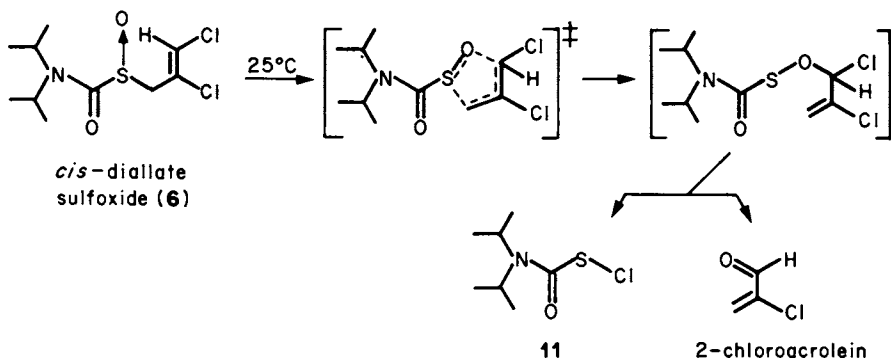
**Rearrangements.** The *S*-alkyl thiocarbamate sulfoxides with a  $\beta$ -hydrogen present (8 and 9) are relatively unstable, decomposing by a *cis*-elimination mechanism. Extensive breakdown occurs at 25°C in the case of the *S*-*t*-butyl thiocarbamate sulfoxides and at 80°C in the case of the *S*-ethyl derivatives (11). The *S*-benzyl thiocarbamate sulfoxides, lacking the  $\beta$ -hydrogen, are the most stable compounds of this type (11).



*S*-(2-Chloroallyl) thiocarbamate sulfoxides (e.g., 4) are thermally unstable and within one hour at 25°C they undergo a spontaneous [2,3] sigmatropic rearrangement to give the *S*-*O*-(2-chloroallyl) thiocarbamate sulfenate esters (10) in quantitative yield (7). This thermal rearrangement is analogous to the reversible rearrangement of *p*-tolyl allyl sulfoxides (12, 13). A sulfenate ester analogous to 10 but with methyl instead of chlorine is formed on MCPBA oxidation of the corresponding *S*-methallyl thiocarbamate; this patent report (14) does not mention any intermediates or speculate on the mechanism of the reaction. Formation of the sulfenate ester is conveniently monitored not only by IR and NMR but also by CI-MS, in the latter case because in contrast to the sulfoxides (7, 9) the sulfenates are sufficiently stable to exhibit a strong molecular ion (7).



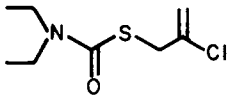
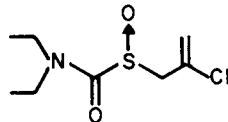
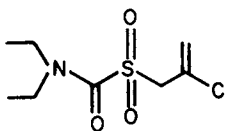
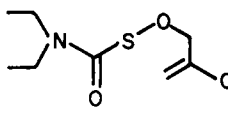
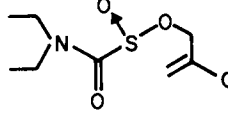
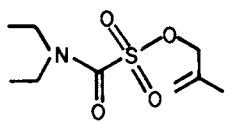
*S*-(3-Chloroallyl) thiocarbamate sulfoxides (e.g., 5-7) undoubtedly rearrange in an analogous manner but in this case the sulfenate quickly undergoes an additional 1,2-elimination reaction (7). The resulting products are the *N,N*-dialkylcarbamoylsulfonyl chloride (11) and the carbonyl compound, i.e. aldehydes from the 3-chloroallyl derivatives (e.g., 5 and 6) and acid chlorides in the case of the 3,3-dichloro analogs (e.g., 7) (7,8). This thermal rearrangement along with the 1,2-elimination reaction is analogous to a sequence previously reported for aryl 3-chloroallyl sulfoxides (15).

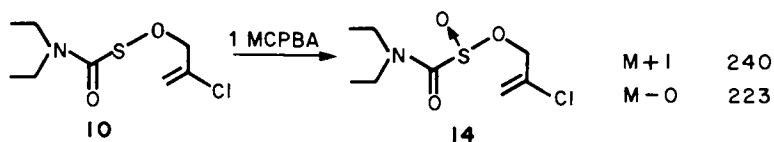
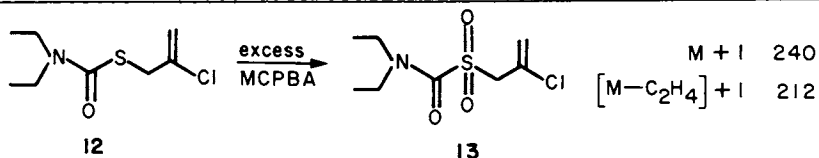


**Oxidations.** The *S*-alkyl and *S*-benzyl thiocarbamate sulfoxides are converted almost quantitatively to the corresponding sulfones on MCPBA oxidation in chloroform (3-5). *S*-(2-Chloroallyl) thiocarbamate 12 or the corresponding sulfoxide (4) with excess oxidant gives the sulfone derivative (13) which is isomeric with the sulfinate ester (14) obtained from the sulfenate ester (10) with 1 mole of MCPBA. Compounds 13 and 14 give characteristic NMR (Table II) and CI-MS spectra (see below). The unusual NMR spectral feature of the *S*-(2-chloroallyl) thiocarbamate sulfoxide (4) in exhibiting a singlet for the two protons of the terminal methylene is retained on further oxidation to the sulfone (13) (Tables I and II). Oxidation of sulfenate ester 10 results in the aforementioned splitting of the CH<sub>3</sub> and CH<sub>2</sub>-N proton signals due to the chiral center and restricted rotation around the amide C-N bond of the sulfinate ester (14) (Table II). Both 13 and 14 give a molecular ion on CI-MS, but sulfone 13 decomposes by loss of ethylene and sulfinate 14 cleaves off oxygen to give the base peak. Further oxidation of 14 results in a new product with completely distinct NMR signals consistent with those expected for the sulfonate ester (Table II).

TABLE II

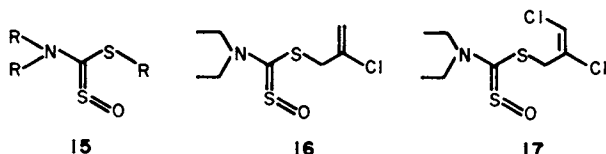
$^1\text{H}$  Chemical Shift Data (ppm) for S-(2-Chloroallyl) N,N-Diethylthiocarbamate and Its Oxidation Products. Solutions in  $\text{CDCl}_3$  at  $20^\circ\text{--}40^\circ\text{C}$  with tetramethylsilane as the internal standard. Proton coupling in Hz:  $\text{CH}_3\text{--CH}_2$ , 7.1;  $=\text{CH}_2$ , 1.1;  $\text{OCH}_2$  in compound 14, 13.1. s = singlet, d = doublet, t = triplet, qa = quartet, m = multiplet.

Compound number and structure	$\text{CH}_3$ (t)	$\text{CH}_2\text{--N}$ (qa)	S- $\text{CH}_2$ (s)	$\text{OCH}_2$ ( $\text{s}$ ) <sup>2</sup>	$=\text{CH}_2$ (d) <sup>2</sup>
<u>12</u> 	1.18	3.40	3.84		5.26 5.48
<u>4</u> 	1.26(m)	3.49(m)	3.90		5.55(s)
<u>13</u> 	1.30(m)	3.46 3.75	4.39		5.68(s)
<u>10</u> 	1.18	3.18		4.48	5.45 5.53
<u>14</u> 	1.23 1.29	3.47 3.58		4.46(d) 4.73(d)	5.46 5.57
	1.25 1.30	3.44 3.70		4.92	5.51 5.66



Dithiocarbamate Sulfines

Synthesis. Oxidation of *S*-chloroallyl dithiocarbamates with equimolar MCPBA in chloroform, methylene chloride or methanol at  $-25^{\circ}\text{C}$  gives a very exothermic reaction leading to the corresponding dithiocarbamate sulfines (15), i.e. sulfallate sulfine (16) and its *trans*-2,3-dichloro analog (17) (16).



Thus, the preferred site of peracid oxidation is the thiono sulfur in dithiocarbamates (16), as with dithio esters (17, 18), rather than the thio sulfur oxidized in thiocarbamates.

Spectral Features. Examination of the equimolar sulfallate-MCPBA reaction mixture reveals a product with a CI-MS peak appropriate for sulfallate plus one oxygen (16).

NMR spectral data (Table III) establish that the peracid monooxygenation products of sulfallate and chloro-sulfallate are sulfines 16 and 17 (16). There is an upfield shift of  $\sim 0.9$  ppm for the *S*-CH<sub>2</sub> protons attributable to "through space" shielding by the negative environment of the sulfine oxygen. Oxidation at the thio instead of the thiono sulfur would have led to a downfield shift for the *S*-CH<sub>2</sub> protons, e.g. 3.84 ppm for the corresponding thiocarbamate (12) and 3.90 ppm for its sulfoxide derivative (4) (Table II). Similar spectral changes occur on conversion of dithio esters to their sulfine derivatives (17). It appears likely that sulfines 16 and 17 are obtained as isomerically-pure materials since only a single *S*-CH<sub>2</sub> signal is evident (Table III). The lack of significant changes in the chemical shifts of the *N*-CH<sub>2</sub>CH<sub>3</sub> protons in contrast to the *S*-CH<sub>2</sub> protons on oxidation of sulfallate and chloro-sulfallate (Table III) provides supporting evidence for the *Z*-sulfines.

Rearrangements. Peracid monooxygenation of dithiocarbamates with 3-chloroallyl substituents (e.g., chloro-sulfallate), in contrast to the analogous thiocarbamates (e.g., diallate), does not lead to [2,3] sigmatropic rearrangement followed by 1,2-elimination reactions, i.e. the equimolar chloro-sulfallate-MCPBA reaction gives no products with NMR signals for =CH<sub>2</sub> protons (16). This lends support to other NMR spectral evidence noted above that only the thiono group is oxidized.

Sulfines 16 and 17 are relatively short-lived in solution, reverting to the starting materials within a few hours at  $40^{\circ}\text{C}$  or rapidly on addition of triphenylphosphine or attempted isolation



TABLE III

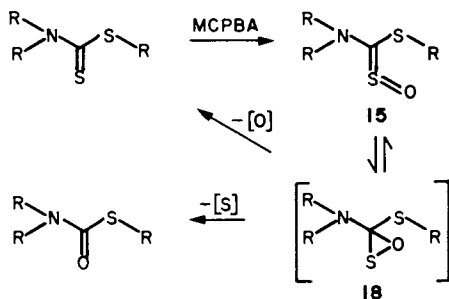
<sup>1</sup>H Chemical Shift Data (ppm) for Sulfallate, Chloro-Sulfallate, Their Sulfine Derivatives and Further Oxidation Products. Solutions in CDCl<sub>3</sub> at 20°-40°C with tetramethylsilane as the internal standard. Proton coupling in Hz: CH<sub>3</sub>-CH<sub>2</sub>, 7.1; =CH<sub>2</sub>, 1.1. s = singlet, d = doublet, t = triplet, qa = quartet.

Protons	Sulfine 16			Sulfine 17		
	Sulfallate	Sulfine 16	Complex 22 <sup>a</sup>	Chloro-sulfallate	Sulfine 17	Complex 22 <sup>a</sup>
1 (t)	1.28	1.29	1.44 1.48	1.30	1.29	1.46
2 (qa)	3.77 4.03	3.87 3.96	3.76 4.15	3.38 4.00	3.88 3.95	3.79 4.16
3 (s)	4.31	3.43	4.48	4.52	3.69	4.74
4/5 (d)	5.33 5.60	5.38 5.54	5.46 5.94	6.31 <sup>b</sup>	6.43 <sup>b</sup>	6.41 <sup>b</sup>
New (s)			10.06			10.03

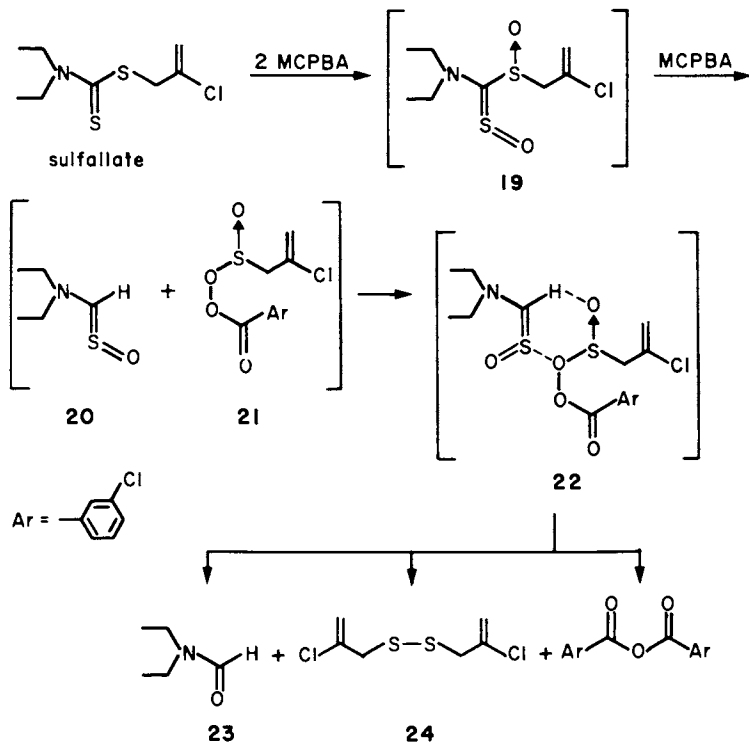
<sup>a</sup>Complex obtained on reaction of sulfallate or chloro-sulfallate with 4 molar equivalents of MCPBA.

<sup>b</sup>Singlet.

involving extraction with aqueous sodium carbonate solution (16). This loss of oxygen probably involves an oxathiiran intermediate 18 which can also extrude sulfur. Desulfuration to form the thio-carbamate (e.g. 12) is normally a minor pathway but becomes major when *p*-toluenesulfonic acid is present in the reaction mixture (16).



**Oxidations.** Treatment of sulfallate or chloro-sulfallate with a 3-5-fold molar excess of MCPBA leads to entirely different products than equimolar MCPBA. This is evident on comparing the NMR spectral features of sulfallate, sulfine 16, and complex 22 or of the

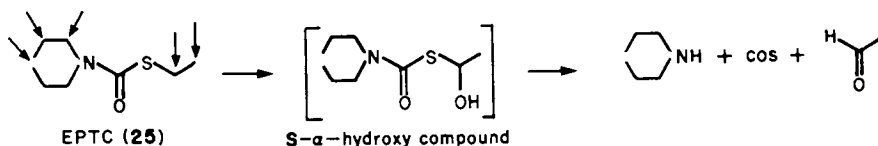


analogous products from chloro-sulfallate (Table III). Complex 22 gives diethylformamide (23), 2-chloroallyl disulfide (24) and the anhydride of *m*-chlorobenzoic acid on TLC isolation using silica gel or on treatment with pyridine, hydrochloric acid, sodium bisulfite or sodium carbonate. However, complex 22 gives completely different  $^1\text{H}$  (Table III) and  $^{13}\text{C}$  NMR signals than any one or combination of all of the compounds obtained on TLC isolation (16). The structure indicated for 22 is one way to rationalize its spectral features and reaction characteristics (16). NMR studies reveal that the low resonating proton ( $\sim 10$  ppm) is directly bonded to a carbon (181.2 ppm) which is split to two lines in the  $^{13}\text{C}$  proton off resonance decoupling mode. This observation is consistent with a sulfine carbon bearing one proton, which indicates that the dithiocarbamate molecule is already cleaved in solution. Sulfine sulfoxide 19 is appropriate for the cleavage reaction due to its excellent leaving group. Isolation of disulfide 24 indicates that the leaving group incorporated into the complex is a sulfoxide rather than a sulfone. A portion of the low temperature  $^1\text{H}$  NMR spectrum of complex 22 is very similar in all respects yet not identical with that of diethylthioformamide sulfine (20) (prepared by MCPBA oxidation of the thioformamide at  $-30^\circ\text{C}$ ). Thioformamide sulfine 20 decomposes very fast above  $-20^\circ\text{C}$  to give diethylformamide (23) and elemental sulfur. Accordingly, sulfine 20 must exist in solution in a complexed form. All properties of complex 22 from sulfallate and the analogous complex "22" from chloro-sulfallate are consistent with the indicated adduct of diethylthioformamide sulfine (20) and a mixed peroxy-anhydride (21).

Other oxidation products of sulfallate are formed via thio-carbamate 12 as discussed before.

### Metabolism of Thio- and Dithiocarbamates

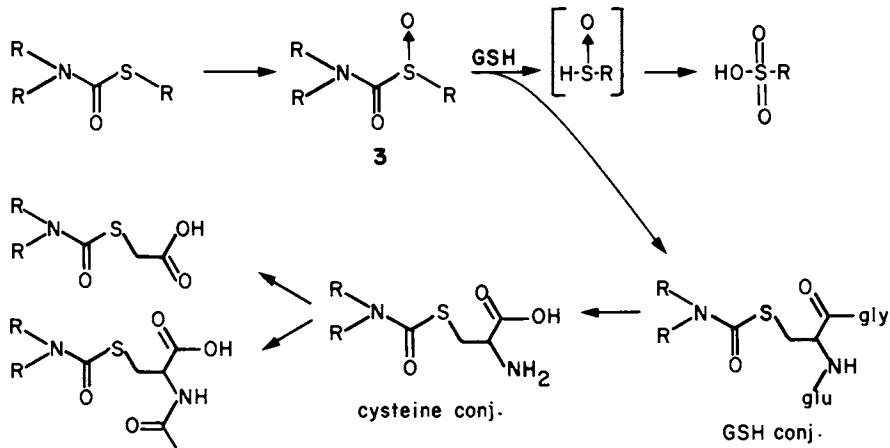
Oxidations and Rearrangements. *S*-Alkyl and *S*-benzyl *N,N*-dialkylthiocarbamates are converted to their sulfoxide derivatives (3) both *in vivo* in rats and on incubation with liver microsomes and NADPH (3-5, 19-21). Studies with EPTC (25) reveal that they may also undergo hydroxylation at each alkyl carbon (designated by arrows) and that carbon hydroxylation at the *S*- $\text{CH}_2$  moiety gives an unstable intermediate which yields acetaldehyde on decomposition (19).



Although diallate sulfoxide (6) is too unstable for possible isolation as a metabolite, it is established as an *in vitro* and

in vivo intermediate in mammals by the detection of two of its derivatives, i.e., the glutathione (GSH) conjugate and its further metabolites formed by an initial carbamylation reaction (10) (see below) and 2-chloroacrolein detected in the microsomal-NADPH system and derived from the rearrangement-elimination reaction sequence discussed above (6). Sulfallate also yields 2-chloroacrolein in the microsomal-NADPH system, presumably by S-CH<sub>2</sub> hydroxylation (22) on analogy with the metabolism of EPTC shown previously.

Carbamylation Reactions. S-Alkyl, S-benzyl and S-chloroallyl thiocarbamates do not readily react with GSH. In contrast, their sulfoxide derivatives (3 and 6) are very effective carbamylating agents for many thiols including GSH (19, 21). The GSH conjugates formed in vivo via 3 and 6 are quickly cleaved, acetylated and further metabolized as follows (19-21, 23, 24).



The sulfenic acid liberated on carbamylation is oxidized in part to the corresponding sulfonic acid based on studies with diallate (6, 8).

### Toxicological Significance of Oxidation and Rearrangement Reactions

Proherbicides. Thio- and dithiocarbamates probably require metabolic activation prior to exerting their herbicidal effects. Sulfoxide metabolites of the S-alkyl thiocarbamates are generally more potent herbicides than the parent compounds (3-5). The herbicidal action of these sulfoxides probably results from their carbamylating action for thiols, although the specific target site or receptor is not defined (23, 24). It is conceivable that the S-chloroallyl thiocarbamate herbicides may act in the same way, since their sulfoxides are also potent carbamylating agents

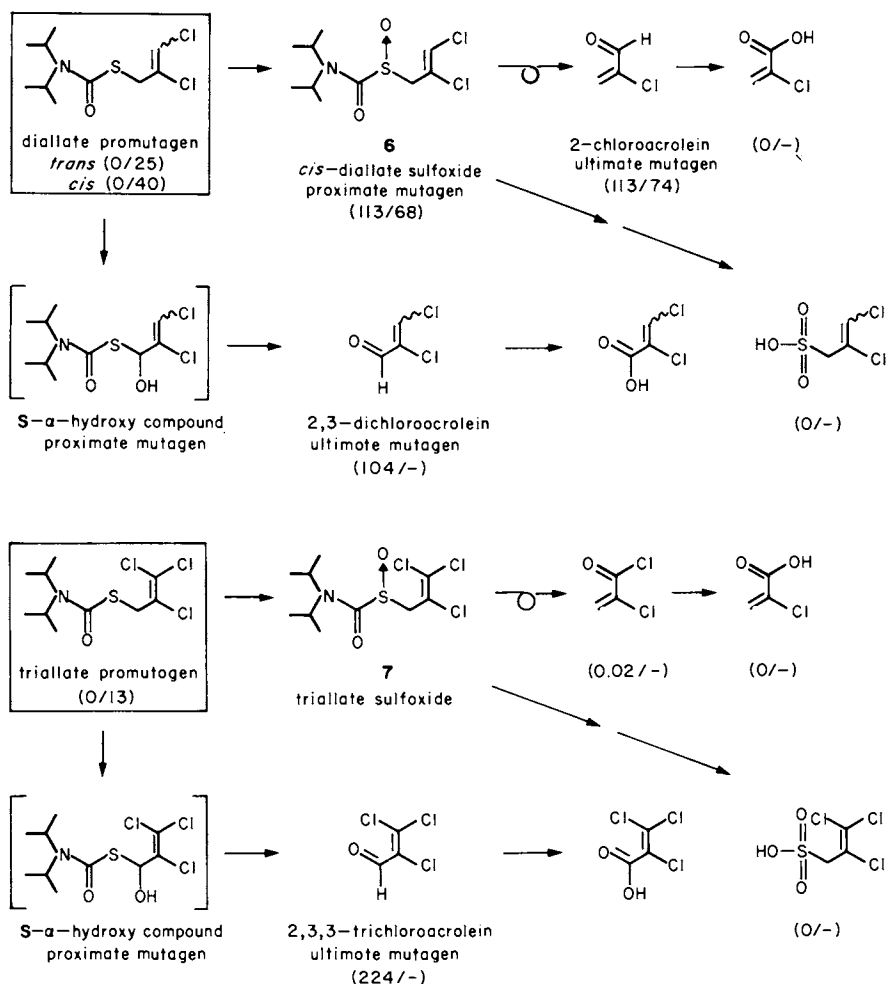
(8, 10). As an alternative, diallate might be a proherbicide and 2-chloroacrolein the ultimate herbicide released on decomposition of diallate sulfoxide (8).

Promutagens. S-(2-Chloroallyl) thio- and dithiocarbamate herbicides, in contrast to the S-alkyl thiocarbamates, show mutagenic activity in the Salmonella typhimurium "Ames" assay; however, they are only mutagenic on metabolic activation, *i.e.*, with the S9 mix (25-27). The requirement for the liver microsomal mixed-function oxidase system for mutagenic activity led to the hypothesis that the S-chloroallyl thio- and dithiocarbamates are promutagens and that an oxidation process is involved in formation of the ultimate mutagens (6). It was therefore of great interest to note that 2-chloroacrolein, an oxidative metabolite of both diallate and sulfallate, and its 2,3-dichloro and 2,3,3-trichloro analogs are extremely potent mutagens (6-8, 22, 28). Various chloroacrolein metabolites are likely to be the ultimate mutagens formed from diallate, triallate and sulfallate as discussed later. Polymer formation occurs on reaction of deoxyadenosine with the difunctional 2-chloroacrolein, probably due to cross linking via Schiff base formation at the carbonyl group and Michael addition at the double bond (28).

Balance of Activation/Detoxification Reactions. The activated intermediates or reactive fragments appear to be carbamoyl sulfoxides or mono-, di- and trichloroacroleins, all of which are relatively unstable compounds. The carbamoyl sulfoxides are rapidly detoxified by reaction with GSH, involving catalysis by a GSH S-transferase in the case of S-alkyl and S-benzyl thiocarbamate sulfoxides (3-5, 21, 23, 24) but probably not with S-chloroallyl thiocarbamate sulfoxides (6, 8, 10). 2-Chloroacrolein is unstable in metabolic systems including in the presence of GSH (28). Highly reactive activated intermediates must act in the same cell or even cellular organelle in which they are formed. Thus, compartmentalization phenomena may be important in the action of the metabolically-activated thio- and dithiocarbamates.

Some Reactions of Diallate, Triallate and Sulfallate of Possible Importance to Their Mutagenic and/or Carcinogenic Properties. Current knowledge of the oxidation and rearrangement reactions of S-(2-chloroallyl) thio- and dithiocarbamate herbicides in relation to their mutagenic activities is illustrated in Figures 1 and 2. The mutagenesis data (6, 22, 28) is from the Ames assay procedure (29) (Figure 3).

Diallate has been examined in greatest detail (Figure 1), in part because it gives the most potent mutagen(s) after activation [*i.e.*, activated cis-diallate gives 40 revertants/nmole and activated trans-diallate gives 25 revertants/nmole (Figure 3); this potency is similar to that of the carcinogen benzo[ $\alpha$ ]pyrene



**Figure 1.** Oxidation and other reactions of diallate and triallate indicating mutagenic activities of the products in the *S. typhimurium* TA 100 assay (revertants/nanomole; without activation/with activation; / designates no data available). 2-Chloroacrolein is a diallate metabolite in the mouse liver microsomes-NADPH system. Dichloroallylsulfonic acid is a urinary metabolite of diallate. The other compounds are potential metabolites of the respective thiocarbamates. The thiocarbamate sulfoxides are unstable at 25°C.

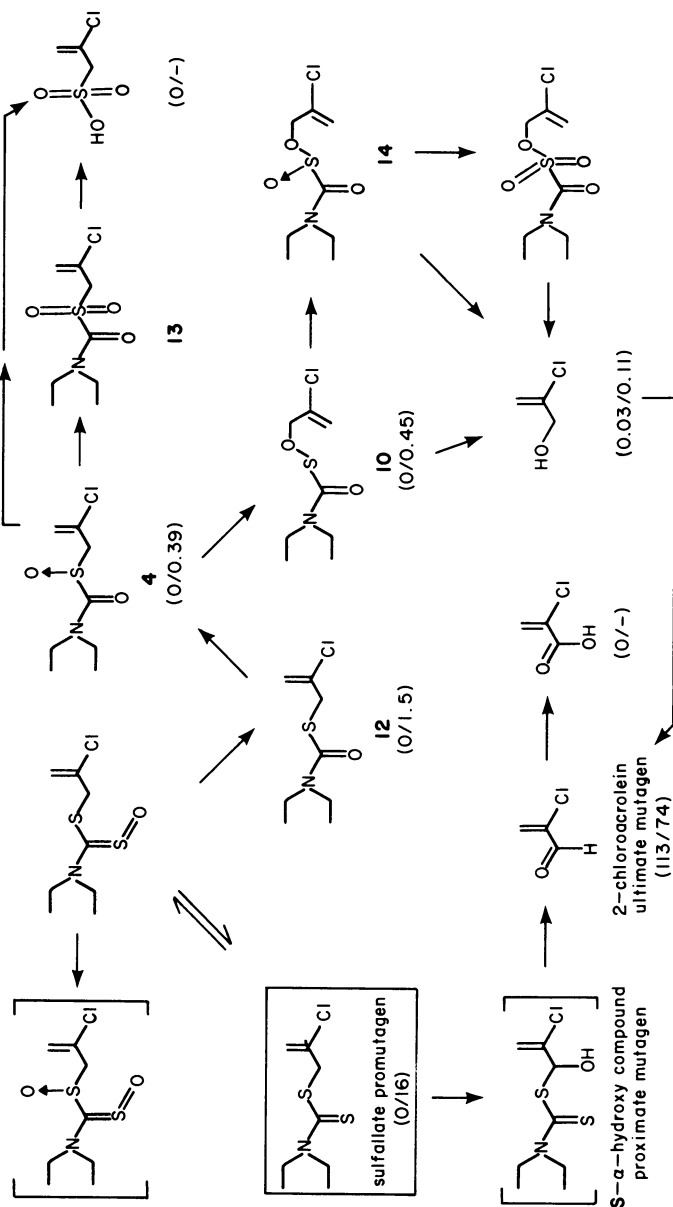


Figure 2. Oxidation and other reactions of sulfamate indicating mutagenic activities of the products in the *S. typhimurium* TA 100 assay (revertants/nmole; without activation/with activation; / designates no data available). All thio- and dithiocarbamates are formed from oxidations with MCPBA except for the  $\alpha$ -hydroxy compound. 2-Chloroacrolein is a metabolite in the mouse liver microsomal NADPH system. The other compounds are potential metabolites. Several of the oxidized thio- and dithiocarbamates are unstable at 25°C.

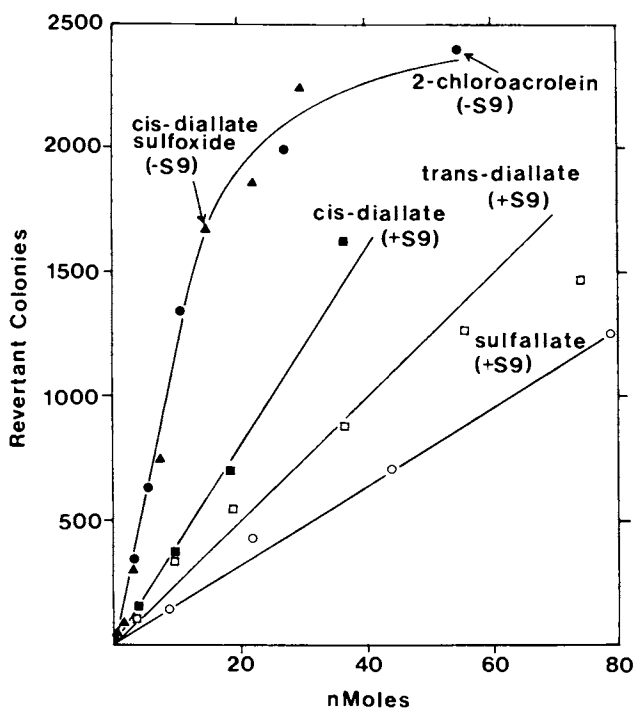


Figure 3. Mutagenic activities of the promutagens *cis*- and *trans*-diallate and sulfallate, the proximate mutagen *cis*-diallate sulfoxide, and the ultimate mutagen 2-chloroacrolein, assayed with *S. typhimurium* strain TA 100 sensitive to base-pair substitution mutagens. The diallate isomers and sulfallate are not mutagenic without the S9 mix. S9 mix refers to a microsomal oxidase system prepared from rat liver and appropriate cofactors. The methodology is detailed in Refs. 6, 22, and 29.



(29)]. Diallate sulfoxide (6) is a very potent mutagen without metabolic activation and it decomposes quickly into 2-chloroacrolein, found to have the same mutagenic activity of 113 revertants/nmole in assays without the S9 mix. Diallate metabolism involves both diallate sulfoxide and 2-chloroacrolein as intermediates, as discussed above. Thus, diallate gives a proximate mutagen, the carbamoyl sulfoxide, and an ultimate mutagen, 2-chloroacrolein, with about three-fold higher mutagenic activity than the bioactivated cis-diallate. Diallate might also yield a second ultimate mutagen, the very potent 2,3-dichloroacrolein, liberated following carbon hydroxylation at the S-CH<sub>2</sub> site.

Triallate and sulfallate are probably activated to mutagenic metabolites from the chloroallyl moieties without the involvement of their sulfoxides as the proximate mutagens (Figures 1-3). Triallate sulfoxide (7) is mutagenic without the S9 mix (6). Somewhat less active is 2-chloroacrylyl chloride obtained from triallate sulfoxide on its rearrangement and elimination reactions. Hydroxylation of triallate at the carbon  $\alpha$  to the sulfur potentially gives the highly potent mutagen 2,3,3-trichloroacrolein on decomposition of the S- $\alpha$ -hydroxy proximate mutagen. Sulfallate oxidation with MCPBA does not yield sulfallate sulfoxide or 2-chloroacrolein. Instead, it gives a variety of sulfines, sulfoxides, sulfones, S-O-sulfenate esters and related products discussed above. Compound 12 and its oxidation products are much weaker mutagens than activated sulfallate. The mutagenic activity of sulfallate is most easily explained by S-methylene hydroxylation to give a proximate mutagen cleaving to 2-chloroacrolein, the ultimate mutagen and a microsomal oxidase metabolite of sulfallate. This hypothesis implies, but does not depend, on the greater ease of S-CH<sub>2</sub> hydroxylation of sulfallate than of its thiocarbamate analog (12).

Diallate is reported to be carcinogenic in mice (30) and sulfallate in mice and rats (31). These herbicides are metabolized to give 2-chloroacrolein, a potent mutagen. S-Methylene hydroxylation may also contribute to the mutagenic activities of diallate and triallate with 2,3-dichloro- and 2,3,3-trichloroacroleins as the ultimate mutagens. These chloroacroleins may be the ultimate carcinogens as well as the ultimate mutagens.

### Summary

N,N-Dialkylthio- and dithiocarbamate herbicides include several S-alkyl and S-benzyl compounds without mutagenic activity and three S-chloroallyl derivatives which are promutagens, i.e. S-(2,3-dichloroallyl) N,N-diisopropylthiocarbamate (diallate), S-(2,3,3-trichloroallyl) N,N-diisopropylthiocarbamate (triallate) and S-(2-chloroallyl) N,N-diethyldithiocarbamate (sulfallate). Diallate and sulfallate are also carcinogens. A large number of

products are identified from the peracid oxidation of diallate, triallate and sulfallate. The initial oxidation products are sulfoxides with diallate and triallate and a sulfine with sulfallate. Sulfoxides of the S-alkyl, S-benzyl and S-chloroallyl thiocarbamates differ greatly in their stability, reactions and biological activities. The S-benzyl and S-alkyl sulfoxides are moderately stable at room temperature. The latter compounds with a  $\beta$ -hydrogen pyrolyze at elevated temperatures by a *cis*-elimination mechanism. Under physiological conditions they readily carbamylate tissue thiols such as glutathione. Sulfoxides of S-(3-chloroallyl), S-(2,3-dichloroallyl) and S-(2,3,3-trichloroallyl) thiocarbamates are thermally unstable and quickly undergo [2,3] sigmatropic rearrangement followed by 1,2-elimination reactions to yield the corresponding N,N-dialkylcarbamoylsulfonyl chloride and acrolein, 2-chloroacrolein and 2-chloroacrylyl chloride, respectively. 2-Chloroacrolein is a metabolite of enzymatic sulfoxidation of diallate and S-methylene hydroxylation of sulfallate. Diallate and triallate might also yield 2,3-dichloroacrolein and 2,3,3-trichloroacrolein on enzymatic S-methylene hydroxylation. These haloacroleins are very potent bacterial mutagens. The herbicidal activity of the S-alkyl thiocarbamates is probably associated with the carbamylating activities of their sulfoxide metabolites, whereas the herbicidal and mutagenic activities of the S-chloroallyl thio- and dithiocarbamates may be due at least in part to the generation of potent chloroacrolein mutagens and herbicides via sulfoxide or S-hydroxymethylene intermediates.

#### Acknowledgment

Supported in part by Grant 5 P01 ES 00049 from the National Institutes of Health.

Acknowledgement is made to the ACS Petroleum Research Fund for partial support of travel costs for I. Schuphan to participate in this Symposium.

#### Literature Cited

1. Block, E. *Amer. Chem. Soc. Symp. Ser.*, 1981, 158, 3.
2. Neal, R. A. *Amer. Chem. Soc. Symp. Ser.*, 1981, 158, 19.
3. Casida, J. E.; Gray, R. A.; Tilles, H. *Science*, 1974, 184, 573.
4. Casida, J. E.; Kimmel, E. C.; Ohkawa, H.; Ohkawa, R. *Pestic. Biochem. Physiol.*, 1975, 5, 1.
5. Casida, J. E.; Kimmel, E. C.; Lay, M.; Ohkawa, H.; Rodebush, J. E.; Gray, R. A.; Tseng, C. K.; Tilles, H. *Environ. Qual. Safety, Suppl.* 1975, Vol. 3, 675.
6. Schuphan, I.; Rosen, J. D.; Casida, J. E. *Science*, 1979, 205, 1013.

7. Schuphan, I.; Casida, J. E. Tetrahedron Lett., 1979, 841.
8. Schuphan, I.; Casida, J. E. J. Agric. Food Chem., 1979, 27, 1060.
9. Tseng, C. K.; Below, J. F., J. Agric. Food Chem., 1977, 25, 1383.
10. Chen, Y. S.; Schuphan, I.; Casida, J. E. J. Agric. Food Chem., 1979, 27, 709.
11. Gozzo, F.; Masoero, M.; Santi, R.; Galluzzi, G.; Barton, D. H. R. Chem. & Ind., 1975, 221.
12. Mislow, K. Rec. Chem. Progr., 1967, 28, 217.
13. Bickart, P.; Carson, F. W.; Jacobus, J.; Miller, E. G.; Mislow, K. J. Am. Chem. Soc., 1968, 90, 4869.
14. Walker, F. H.; Gaughan, E. J. Deutsches Bundespatent Offenlegungsschrift, 1975, 2518544.
15. Lansbury, P. T.; Britt, R. W. J. Am. Chem. Soc., 1976, 98, 4577.
16. Segall, Y.; Schuphan, I.; Casida, J. E., Manuscript in preparation.
17. Zwanenburg, B.; Thys, L.; Strating, J. Tetrahedron Lett., 1967, 3453.
18. Zwanenburg, B.; Kielbasiński, P. Tetrahedron, 1979, 169.
19. Chen, Y. S.; Casida, J. E. J. Agric. Food Chem., 1978, 26, 263.
20. DeBaun, J. R.; Bova, D. L.; Tseng, C. K.; Menn, J. J. J. Agric. Food Chem., 1978, 26, 1098.
21. Hubbell, J. P.; Casida, J. E. J. Agric. Food Chem., 1977, 25, 404.
22. Rosen, J. D.; Schuphan, I.; Segall, Y.; Casida, J. E. J. Agric. Food Chem., 1980, 28, 880.
23. Lay, M.-M.; Hubbell, J. P.; Casida, J. E. Science, 1975, 189, 287.
24. Lay, M.-M.; Casida, J. E. Pestic. Biochem. Physiol., 1976, 6, 442.
25. Carere, A.; Ortali, V. A.; Cardamone, G.; Morpurgo, G. Chem.-Biol. Interact., 1978, 22, 297.
26. Sikka, H. C.; Florczyk, P. J. Agric. Food Chem., 1978, 26, 146.
27. De Lorenzo, F.; Staiano, N.; Silengo, L.; Cortese, R. Cancer Res., 1978, 38, 13.
28. Rosen, J. D.; Segall, Y.; Casida, J. E. Mutat. Res., 1980, 78, 113.
29. Ames, B. N.; McCann, J.; Yamasaki, E. Mutat. Res., 1975, 31, 347.
30. Innes, J. R. M. et al. J. Natl. Cancer Inst., 1969, 42, 1101.
31. "Bioassay of sulfallate for possible carcinogenicity", Natl. Cancer Inst. Carcinogenesis Tech. Rep. Ser., 115, DHEW Publ. (NIH) 78-1370, 1978.

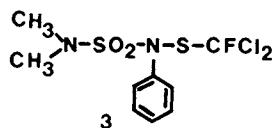
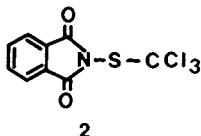
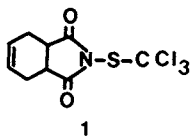
RECEIVED February 17, 1981.

# Biological and Chemical Behavior of Perhalogenmethylmercapto Fungicides: Metabolism and in Vitro Reactions of Dichlofluanid in Comparison with Captan

I. SCHUPHAN, D. WESTPHAL<sup>1</sup>, A. HAQUE, and W. EBING

Institut für Pflanzenschutzmittelforschung, Biologische Bundesanstalt,  
1000 Berlin 33, Federal Republic of Germany

The fungitoxic N-perhalogenmethylmercapto moiety was introduced for plant protection in 1950: The two fungicides captan(1) (N-(trichloromethylthio)- $\Delta^4$ -tetrahydrophthalimide) and folpet(2) (N-(trichloromethylthio)phthalimide) contain a perchlorinated methylthio group (1). About a decade later substitution of fluorine for one of the chlorines in the perchloromethylmercapto moiety led to chemically related fungicides (2,3) of which dichlofluanid(3) ((N-fluorodichloromethylthio)-N'-N'-dimethyl-N-phenyl sulfonyldiamide) was commercialized.



Structural modifications in the amide moiety or the methylmercapto group of these and related compounds result in changes in chemical and biological activity. Thus, the reactivity of the trichloromethyl derivatives against 4-nitrothiophenol decreases in the sequence 1, 2 and 4. A similar trend holds true for the fluorodichloromethyl derivatives 5, 6, and 3. The fluoroanalogues react 4-10 times faster with 4-nitrothiophenol than the trichloro derivatives as shown in Fig. 1 (3). The fluorodichloromethylthio fungicides show similar or greater fungicidal activity than the trichloromethylthio derivatives (2). This may be related to their increased reactivity against biological thiols.

<sup>1</sup> Current address: Max von Pettenkofer Institut, Bundesgesundheitsamt, 1000 Berlin 33, Federal Republic of Germany.

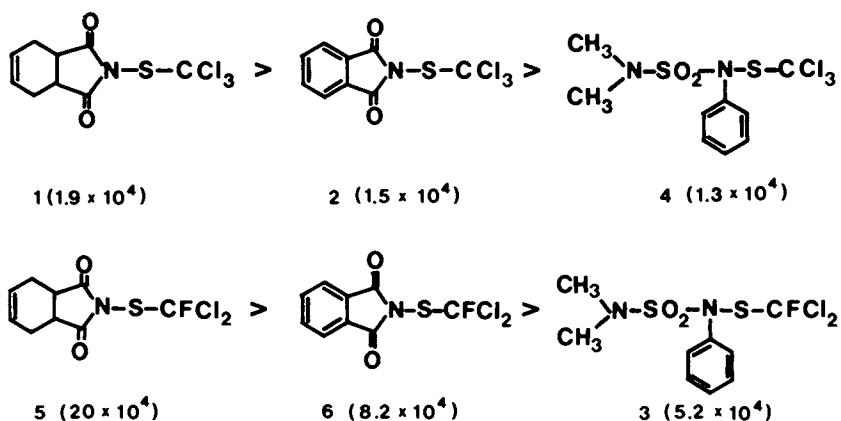


Figure 1. Reaction rate constants  $K(1/\text{mol} \cdot \text{min})$  of perhalogenated methylmercapto derivatives in the reaction with 4-nitrothiophenol at 25°C

The mode of action of the trichloromethylthio fungicides was studied in Saccharomyces. Captan exhibited loss of fungitoxicity in the presence of sulfhydryl compounds (4). In vitro reaction products of captan with cysteine were reported to be tetrahydrophthalimide, hydrogen sulfide, carbon disulfide, thiazolidine-2-thione-4-carboxylic acid and hydrochloric acid (4). From the results it was concluded that the reaction pathway involved thiophosgene as an unstable intermediate and it followed that the fungitoxic properties of captan were related to the trichloromethylthio moiety. This proposal was substantiated by the findings that a variety of trichloromethylthio derivatives had fungicidal properties (5). Reaction of folpet with thiols and its mode of action appear to be similar to those of captan (6). The half-life of captan in water is reported to be about 12 hours, the reaction products being carbon dioxide, hydrochloric acid and sulfur (7). Although captan and folpet are extensively used in agriculture, only one report on captan metabolism in animals has been published (8). Urinary metabolites of orally-administered captan were identified as thiazolidine-2-thione-4-carboxylic acid, a salt of dithiobis-(methanesulphonic acid) and its disulphide monoxide (8). There are no reports concerning metabolic studies in higher plants.

Captan is mutagenic in bacterial assays such as the Salmonella typhimurium "Ames" assay (9,10,11) and

others (12,13). It is a questionable carcinogen in mice, inducing duodenal tumors only at extremely high (8,000-16,000 ppm) dietary levels (14)

Little is known of the biological and chemical behavior of the fluorodichloromethyl derivatives. Photolysis of dichlofluanid results in the formation of *N,N*-dimethyl-*N'*-phenylsulphamide, phenyl isocyanate and isothiocyanates and dimethylamidodisulfonyl chloride (15). GC-MS analysis also indicates the presence of bis-(fluorodichloromethyl) disulfide and two ketones, the latter being artifacts arising from the solvent, acetone. Dichlofluanid metabolism in plants yields *N,N*-dimethyl-*N'*-phenylsulphamide (16), but nothing is

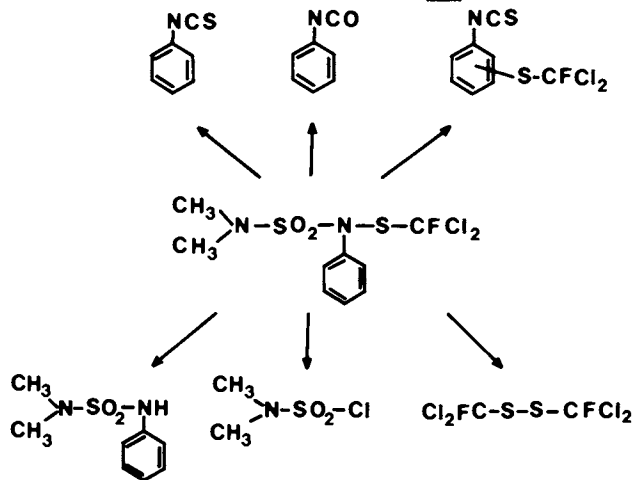


Figure 2. Photolysis of dichlofluanid

known of the fate of the fluorodichloromethylthio moiety. The biological and chemical behavior of the fluorine-containing derivatives as compared to the trichloromethylthio compounds is of interest because chlorine-fluorine substitution normally influences the behavior of organic compounds significantly. Thus, the formation of a fluorinated thiophosgene analogue intermediate might be of toxicological importance.

This report will mainly be concerned with the metabolic fate of dichlofluanid in strawberries, captan in spinach and soil as well as *in vitro* reactions of dichlofluanid with cell thiols and the comparative behavior of metabolites in the Ames assay.

Metabolism of (fluorodichloro<sup>14</sup>C-methyl)Dichlofluanid  
in Strawberries

Spray application of formulated <sup>14</sup>C-dichlofluanid (Euparen) to flowering strawberry plants in a closed controlled ventilated cultivating system resulted in a recovery of 99 % radiocarbon after 36 days (Table I).

TABLE I. Balance Account of [<sup>14</sup>C]Dichlofluanid Radioactivity after Spray Application on Strawberry Plants under Closed Conditions

	Radioactivity recovered from total applied %		Sum
	Extractable	Unextractable	
Fruits	3.53	4.3	7.83
Leaves	49.6	21.2	70.8
Roots	1.55	1.8	3.35
Soil	0.59	3.3	3.89
CO <sub>2</sub>			5.95
COS			0.01
Unknown volatile			0.04
Condensing water			3.59
Washing solutions			3.49
Total			98.95

The major amount of radioactive material (70%) was found in leaves, 7.8% in roots and soil and 6% as <sup>14</sup>CO<sub>2</sub>. Bligh-Dyer extraction of the leaves gave 49.6% of the <sup>14</sup>C-label in the chloroform and methanol-water layers while 21.2% was unextractable. Fruits contained 3.5% radiocarbon in the chloroform and methanol-water fractions while 4.3% remained unextractable.

A parallel experiment in a similar chamber but with the top removed gave a recovery of 45% radio-

carbon. Leaves contained up to 36%, fruits 3.1% and roots plus soil 5.7% of the applied radiocarbon (Table II).

TABLE II. Balance Account of [ $^{14}\text{C}$ ] Dichlofluanid Radioactivity after Spray Application on Strawberry Plants under Open Conditions

	Radioactivity recovered from total applied %		Sum
	Extractable	Unextractable	
Fruits	2.84	0.21	3.05
Leaves	32.85	3.48	36.33
Roots	0.21	0.20	0.41
Soil	1.23	4.02	5.25
Washing solutions			0.18
Total			45.22

Special attention was given to the characterization of the radioactive metabolites found in the strawberry fruits where no parent compound was detectable. Unidentified, very polar metabolites accounted for 83% of the material while 3% was identified as thiazolidine-2-thione-4-carboxylic acid by two-dimensional thin-layer chromatography. Treatment with diazomethane allowed for GC-MS confirmation of the latter as the methylated derivatives of the thiazolidine. Bis-(fluorodichloromethyl) disulfide co-chromatographed with a labeled metabolite but insufficient material was available for confirmation by mass spectrometry.

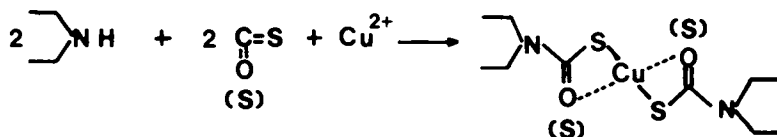
Leaves contained 55% of the label as the parent compound dichlofluanid, 35% as very polar unidentified metabolites and 10% as thiazolidine-2-thione-4-carboxylic acid. A small amount (0.2%) was bis-(fluorodichloromethyl) disulfide as confirmed by two dimensional tlc and MS. This amount is probably less than was actually present as the work up procedures were not optimized to detect such a relatively volatile



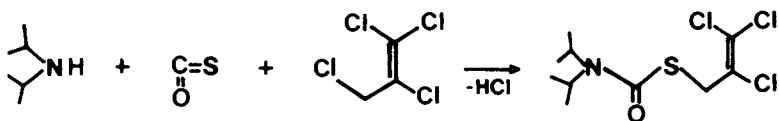
metabolite. The small amount (3.9%) of radioactivity found in soil and 3.4% found in roots were very polar and could not be characterized further even after different enzymatic cleavage reactions or acid hydrolysis.

The volatile metabolites comprised 6% of the radioactivity. Carbon dioxide formed the main component. Other compounds, including carbonyl sulfide, were detected only in traces below 0.04%.

Special attempts were made to identify carbonyl sulfide as a metabolite of dichlofluanid because it would help us understand the metabolic pathway. In a previous investigation into the metabolism of captan,  $\text{CS}_2$  was found as a metabolite (4), while in a latter study, COS was reported (17). Viles' reagent (18) often used by many investigators for this purpose, proved to be unsuitable because of the possible presence of carbon disulfide. Both COS and  $\text{CS}_2$  give colored copper chelates that can not be quantitatively separated by tlc. Furthermore, analysis of the mixture



re by mass spectrometry is futile because neither derivative gives a molecular ion. The formation of COS was proven by passing the air leaving the closed plant chamber through a mixture of diisopropyl amine and 1,1,2,3-tetrachloropropene to give the thiocarbamate, triallate, as a reaction product.



The latter was identified by GC-MS. Using this technique, 0.005% of the total applied radioactivity was shown to be COS. The low yield may have been due to hydrolysis of COS to  $\text{H}_2\text{S}$  and  $\text{CO}_2$ .

The proposed metabolic pathway in strawberries is shown in Figure 3. Five compounds: mixed disulfide, sulfide, sulfenic acid, thiophosgene and the GSH-reaction product have not been identified as a strawberry metabolite but their involvement is very likely based on the formation of bis-(fluorodichloromethyl)

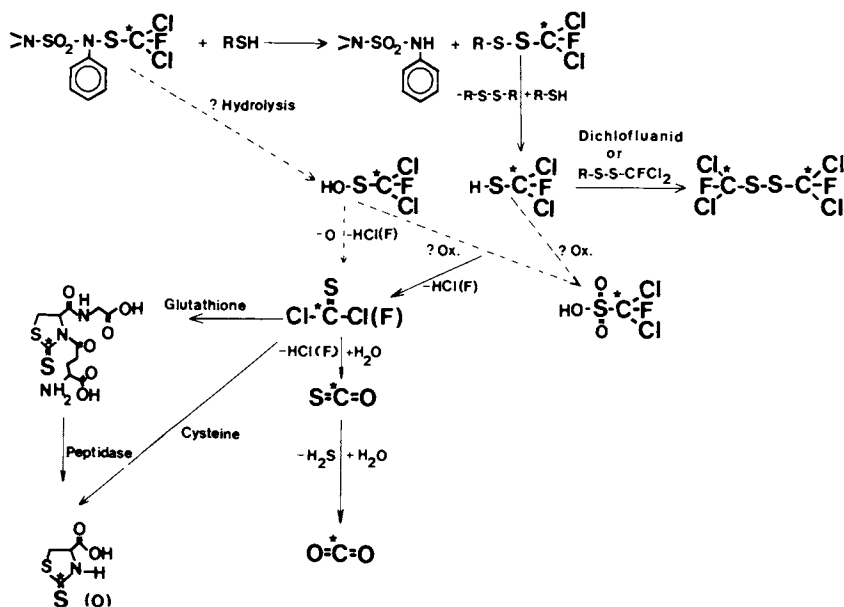


Figure 3. Proposed metabolism of [ $^{14}\text{C}$ ] dichlofluanid in strawberries

disulfide, thiazolidine-2-thione-4-carboxylic acid and carbonyl sulfide. Fluorodichloromethane sulfonic acid co-chromatographed with polar metabolites isolated from leaves. Derivatization via its sulfochloride and reaction with cyclohexene or cyclohexylisonitrile failed to give derivatives in sufficient yield for GC-MS confirmation.

### Metabolism of (trichloro- $^{14}\text{C}$ -methyl) Captan in Spinach and Soil

Our initial studies on the metabolic pathway of captan in spinach point to similarities with dichlofluanid metabolism in strawberries. Preplanting treatment of soil with  $^{14}\text{C}$ -captan followed by spinach cultivation for 34 days in a closed controlled ventilated cultivating system resulted in a recovery of 87% radiocarbon (Table III). The major amount (49%) was found in the soil, 19% in the spinach and 19% as carbon dioxide. Bligh-Dyer extraction of the spinach gave 7.4% of the  $^{14}\text{C}$ -label in the chloroform and metha-

TABLE III. Balance Account of [ $^{14}\text{C}$ ] Captan Radioactivity after Soil Application Followed by Spinach Culture under Closed Conditions

	Radioactivity recovered from total applied %		Sum
	Extractable	Unextractable	
Spinach	7.4	11.5	18.9
Soil	30.7	18.0	48.7
$\text{CO}_2$			19.2
COS			0.02
Unknown volatile			0.18
Condensing water			0.07
Washing solutions			0.04
Total			87.1

nol-water layers while 11.5% was unextractable. Extraction of the soil showed that nearly all the extractable radioactivity (30.7%) was in the chloroform phase while 18% was unextractable. In spinach, 1.3% of the extractable radioactivity was found to be the parent compound while 3% was bis-(trichloromethyl) disulfide and 5.2% was thiazolidine-2-thione-4-carboxylic acid. At least eleven other products were present. These materials were very polar and could not be further characterized.

Captan accounted for 84% of the extractable radioactivity in soil. None of the polar soil metabolites could be identified.

#### In Vitro Studies

To obtain further information concerning the degradation mechanism of the fluorodichloromethyl moiety,

reactions were carried out between dichlofluanid and glutathione or cysteine. These reactions were performed at the 0.01 mmol level in a 1:1 mixture of water/methanol. Molar ratios of dichlofluanid and glutathione reacted immediately to form a very polar derivative. Time dependent tlc analysis of the reaction revealed that the radiocarbon retained at the origin disappeared within two hours in the reaction mixture but a compound identical in tlc behavior to dichlofluanid was formed. The odor of mercapto compounds was evident. Radioactivity from the origin on the tlc plates was lost in open air within 24 hours.

Performing this reaction at 40°C in a closed system fitted with traps for COS and CO<sub>2</sub> absorption and passing nitrogen through it, 2-4% COS and 18 to 22% CO<sub>2</sub> were evolved after 16 hours. A two molar excess of glutathione released up to 40% of CO<sub>2</sub> and 5% of COS. The same experiments carried out with cysteine gave 30-35% CO<sub>2</sub>. Using a two molar cysteine excess, 60 to 70% of CO<sub>2</sub> were released. The remaining radioactivity in the reaction mixture consisted partly of thiazolidine-2-thione-4-carboxylic acid. In all experiments, 5 to 10% of the radioactivity was lost, possibly due to formation of bis-(fluorodichloromethyl) disulfide. This assumption is based on odor comparison between the disulfide and the reaction mixture.

The degradation mechanism proposed from these results (Figure 4) include short-lived intermediates

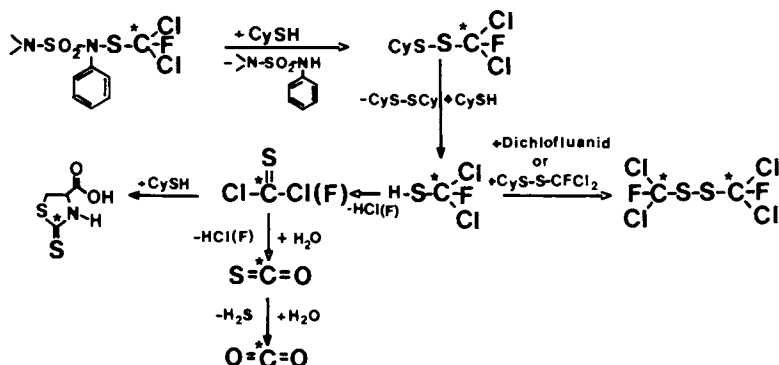


Figure 4. *In vitro* reactions of [<sup>14</sup>C] dichlofluanid with cysteine

not yet detected but necessary to understand the metabolic pathway. These unstable metabolites are analogous to those postulated as a result of in vitro plus in vivo studies of captan metabolism (4,8). In the case of dichlofluanid metabolism the question arises whether fluorine influences the formation of these assumed short-lived intermediates. Thiophosgene especially is assumed to play the major part in the mode of action of captan. Whether thiophosgene or its monofluoro analogue is involved in the degradation pathway of dichlofluanid is not clear.

### Mutagenicity Studies

It is well known that captan is a strong mutagen in the Ames (9,10,11) and other bacterial assays (12, 13) with or without metabolic activation. In contrast, we have found that dichlofluanid and two of its metabolites, thiazolidine-2-thione-4-carboxylic acid and bis-(fluorodichloromethyl) disulfide are not mutagenic to Salmonella typhimurium TA 100. Thiophosgene is not mutagenic when dissolved in dimethyl sulfoxide prior to testing, but gives positive results (2 revertants/nmole) when tested after dissolving in ethylene glycol dimethyl ether or tetrahydrofuran. This indicates that thiophosgene is hydrolyzed by the hydroscopic dimethyl sulfoxide before interacting with the bacteria.

Surprisingly, bis-(trichloromethyl) disulfide was found to be as strong a mutagen as captan, and like captan and folpet, did not need metabolic activation (Figure 5). Comparing these results with the negative response obtained from dichlofluanid and bis-(fluorodichloromethyl) disulfide it can be strongly inferred that the fluorine atom has a fundamental influence on the mutagenic activity of these compounds. Indeed, the trichloromethylthio derivative of dichlofluanid is mutagenic (Figure 6) even in the absence of microsomal activation. In contrast, compounds (5) and (6), the monofluoro analogues of captan and folpet do not show mutagenic activities with and without metabolic activation. Like the other fluoro containing derivatives they have some bactericidal potency in higher concentrations which is completely lost through microsome (S-9mix) addition.

The mutagenic potency of bis-(trichloromethyl) disulfide may be of great significance in the further evaluation of captan. It has been reported (19) and confirmed by us that the disulfide is an impurity in technical captan. Moreover, vide supra, it was found

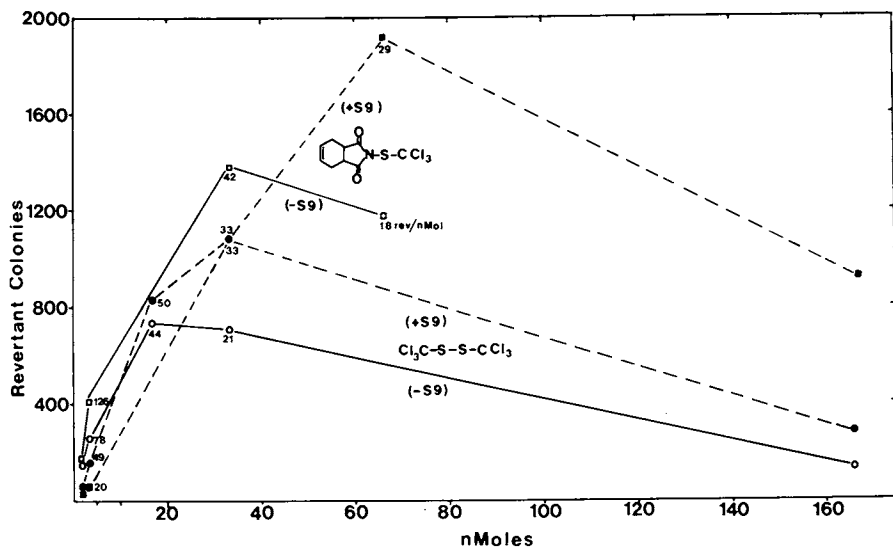


Figure 5. Mutagenic activity of captan and bis-(trichloromethyl) disulfide assayed with *S. typhimurium* TA 100

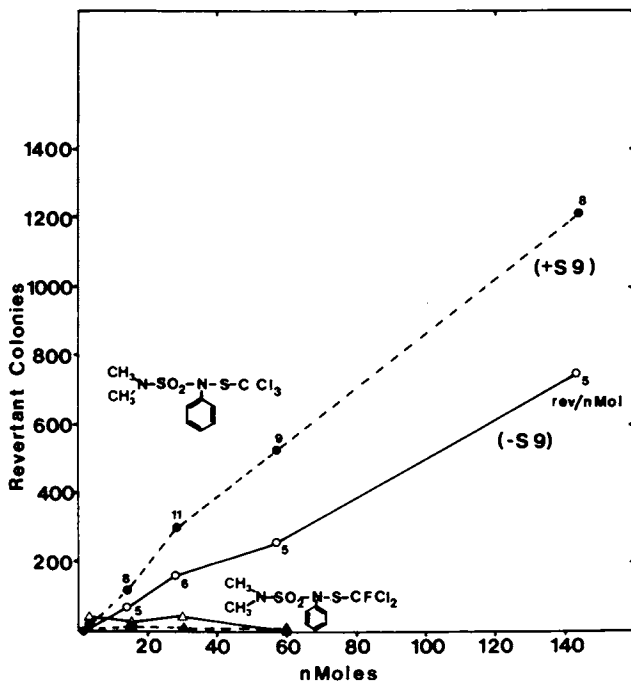


Figure 6. Negative response of dichlofuanid in comparison with its trichloro analog assayed with *S. typhimurium* TA 100

as a metabolite in spinach after soil application of captan and in strawberries after plant application (20,19).

### Acknowledgement

Supported in part by the Ministerium für Forschung und Technologie and the Deutsche Forschungsgemeinschaft.

### Literature Cited

1. DBP 887 506 (17.August 1950): Standard Oil Development
2. DBP 1193 498 (3.November 1960): Farbenfabriken Bayer A.G.
3. Kühle, E.; Klauke, E.; Grewe, F., Angew. Chem., 1964, 76, 807.
4. Lukens, R.J.; Sisler, H.D., Phytopathology, 1958, 48, 235.
5. Lukens, R.J., In "Fungicides". Editor: Torgeson, D.C., 1969, 2, 396. New York and London: Acad. Press.
6. Siegel, M.R., J. Agric. Food Chem., 1970, 18, 823.
7. Wolfe, N.L.; Zepp, R.G.; Doster, J.C.; Hollis, R.C., J. Agric. Food Chem., 1976, 24, 1041.
8. DeBaun, J.R.; Miaullis, J.B.; Knarr, J.; Mihailovskii, A.; Menn, J.J., Xenobiotica, 1974, 4, 101.
9. Marshall, T.C.; Dorough, H.W.; Swim, H.E., J. Agric. Food Chem., 1976, 24, 560.
10. McCann, J.; Choi, E.; Yamasaki, E.; Ames, B.N., Proc. Nat. Acad. Sci. USA, 1975, 72, 5135.
11. Fiesor, G.; Bordas, S.; Stewart, S.J., Mutation Research, 1978, 51, 151.
12. Legator, M.S.; Kelly, F.J.; Green, S.; Oswald, E.J., Ann. N.Y. Acad. Sci., 1969, 160, 344.
13. Bridges, B.A.; Mottershead, R.P.; Rothwell, M.A.; Green, M.H.L., Chem.-Biol. Interactions, 1972, 5, 77.
14. National Cancer Institute 1977; Bioassay of captan for possible carcinogenicity, CAS No.133-06-2, NCI-CG-TR-15 Technical Report Series No. 15.
15. Clark, T.; Watkins, D.A.M., Pestic. Sci., 1978, 9, 225.
16. Vogeler, K.; Niessen, H., Pflanzenschutzachr. Bayer, 1967, 20, 534.
17. Sommers, E.; Richmond, D.V.; Pickard, J.A., Nature, 1967, 215, 214.
18. Viles, F.J., J. Ind. Hyg. Tox., 1940, 22, 188.
19. Wilkes, P.S., Bull. Environm. Contam. Toxicol., 1979, 23, 820.
20. Westphal, D.; Schuphan, I.; Haque, A.; Ebing, W., manuscript in preparation.

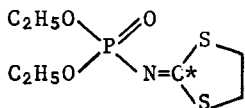
RECEIVED January 29, 1981.

# Comparative Metabolism of Dithiolane Insecticides in Plants, Animals, and the Environment

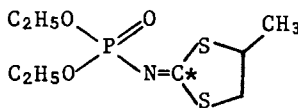
CHIA C. KU<sup>1</sup> and INDER P. KAPOOR

American Cyanamid Company, Agricultural Research Division,  
Princeton, NJ 08540

(I) (Cyolane\* phosfolan, a registered trademark of the American Cyanamid Company) and (II) (Cytrolane\* mephosfolan, a registered trademark of the American Cyanamid Company) are systemic organophosphate insecticides containing the 1,3 dithiolane ring. Phosfolan is useful for the control of several cotton, cabbage and tobacco insects. It is effective against many species of *Spodoptera*, such as the Egyptian cotton leafworm, *S. littoralis*. It is also effective against leafhoppers, aphids, thrips, mites, whiteflies, lygus bugs, leaf miners, cutworms, flea beetles, and alfalfa weevils, while mephosfolan is being used for the control of many lepidopterous and other pests of cotton, corn, rice, sorghum, sugarcane, etc., in several countries, especially in the Middle East and Asia. Among the prominent rice pests controlled are rice hispa (*Hispa armigera*), rice gall midge (*Pachytiplosis oryzae*), and all major rice stem borers like *Chilo suppressalis* and *Tryporyza incertulas*. The investigation of the metabolic fates of the two <sup>14</sup>C-dithiolane insecticides labeled in the imino carbon position was initiated to study the excretion, tissue residue behavior and the nature of the major metabolites in rats. Additional studies were undertaken to determine the nature of the metabolites in cotton, rice paddy and its environment in order to evaluate their similarities or dissimilarities with the rat metabolites. This information is needed by the toxicologist in determining the significance of the metabolites as residues.



I



II

\*Denotes carbon-14 label.

<sup>1</sup> Current address: Mobil Chemical Company, R&D, Crop Chemicals Department, P.O. Box 1028, Princeton, NJ 08540.

0097-6156/81/0158-0097\$05.00/0

© 1981 American Chemical Society



### Metabolism in Rats

1. Phosfolan. When twelve male Royal Hart Wister rats were given a single oral dose of  $^{14}\text{C}$ -phosfolan in peanut oil at 1 mg/kg or 2 mg/kg, which is equivalent to 10 or 20 ppm in the feed and housed in Delmar-Roth metabolism cages for collection of respiration gases and excreta, they excreted approximately 50% of the administered radioactivity and respired about 20% as  $^{14}\text{CO}_2$  within 144 h (Table I). The radioactive residues in the various tissues were found to be distributed throughout the body with lower concentrations residing in fat and muscle (Table II). Chromatographic analysis of urinary and tissue radioactivity showed the presence of only one significant metabolite, which was identified as thiocyanate ion.

Table I. Excretion of radioactivity via respiration gases, urine, and feces by rats treated with a single oral dose of  $^{14}\text{C}$ -phosfolan.

	Radioactivity, % of Dose			
	2 mg/kg		1 mg/kg	
	Trial I <sup>a</sup>	Trial II <sup>b</sup>	Trial I <sup>c</sup>	Trial II <sup>c</sup>
Respiration gases	31.4	18.9	18.2	21.3
Urine	28.5	30.6	31.6	
Feces	15.2	13.7	13.3	51.5 <sup>d</sup>
Cage wash	2.4	1.1	1.6	0.6
Carcass	13.0	23.4	33.1	24.2
Total	90.5	87.7	97.8	97.6
Average	89.1		97.7	

<sup>a</sup>Trial terminated after 192 h.

<sup>b</sup>Trial terminated after 96 h.

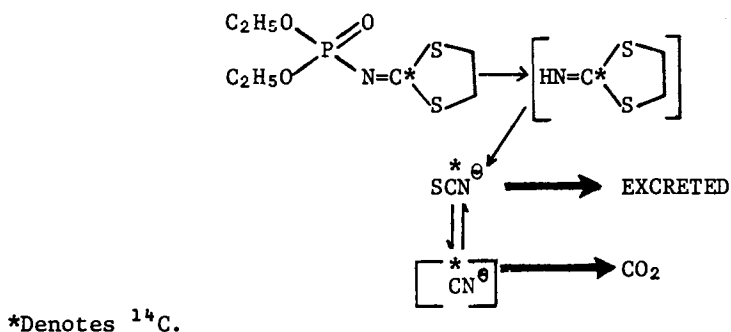
<sup>c</sup>Trial terminated after 144 h.

<sup>d</sup>Urine and feces because mixed and were determined together.

Based on these results, we concluded that the major degradative route of phosfolan in rats was the cleavage of the P-N bond to give iminodithiolane and then to thiocyanate and on to  $\text{CO}_2$ . The formation of iminodithiolane as an intermediate step, although neither salt nor free base were isolated or identified in the formation of thiocyanate, has been postulated by Siegel and Rosenblatt (1). Furthermore, Addor (2) has reported isolation of 2-imino-1,3-dithietane hydrochloride from acid hydrolysis of the related imidocarbonic acid (diethoxyphosphinyl) dithiocyclic methylene ester, which on titration with base, yielded thiocyanate

Table II. Radioactivity levels in tissues of rats following a single oral dose of 2 mg/kg  $^{14}\text{C}$ -phosfolan.

Time Period (Hours)	PPM				
	Liver	Kidney	Muscle	Fat	Blood
0-24	1.14	1.36	0.26	0.24	1.14
24-48	0.71	0.88	0.18	0.22	0.79
48-72	0.46	0.51	0.15	0.16	0.55
72-96	0.42	0.53	0.15	0.16	0.53
96-192	0.15	0.25	0.06	0.06	0.24



Journal of Agricultural and Food Chemistry

Figure 1. Pathways of metabolism of C-14 phospholan in the rat

and thioformaldehyde polymer. A metabolic pathway of phospholan in the rat is proposed in Figure 1. The metabolic fate of thiocyanate and cyanide has already been established (3).

2. **Mephosfolan.** A similar rat metabolism study was carried out as described in phospholan rat metabolism. The results are summarized in Tables III and IV. However, mephosfolan has two metabolic routes: the first, also the major, results in hydrolysis to iminodithiolane and then to thiocyanate and on to  $\text{CO}_2$  which is the same as phospholan. When the metabolic attack is on the methyl group, the second degradative route, oxidation results in the production of the saturated acidic metabolite which is either excreted or possibly degraded further to thiocyanate and to  $\text{CO}_2$ . An alternative route is the hydroxylation of the dithiolane ring, elimination of water and oxidation of the methyl group to yield the unsaturated acidic metabolites. A proposed metabolic pathway of mephosfolan in the rat is shown in Figure 2.

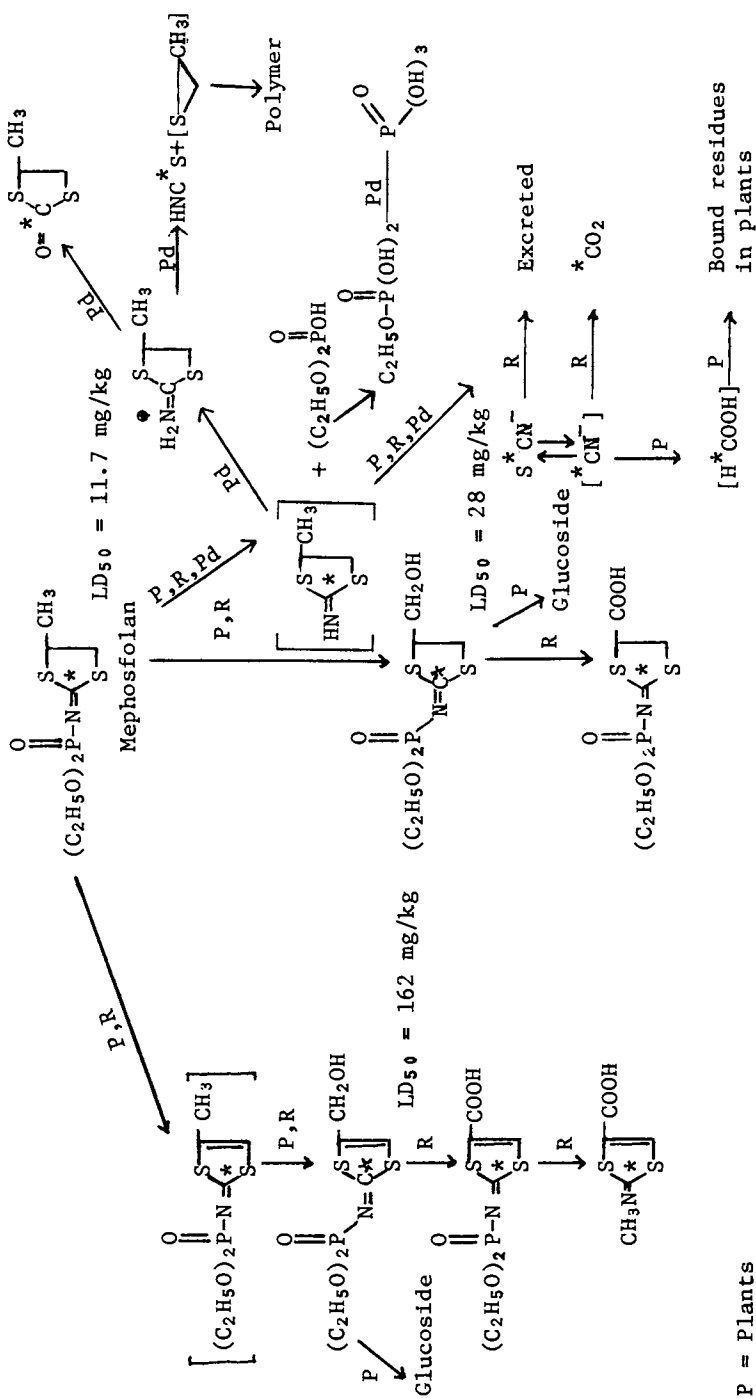


Figure 2. Pathways of metabolism of mephofofolan

Table III. Excretion of radioactivity via respiration gases, urine, and feces by rats treated with  $^{14}\text{C}$ -mephosfolan at 2 mg/kg.

	Radioactivity (% of Dose)			
	Trial 1	Trial 2	Trial 3	Average
Respiration Gases <sup>a</sup>	41.5	31.5	31.3	34.7
Urine	40.8	52.4	43.8	45.7
Feces	<u>15.4</u>	<u>20.7</u>	<u>14.9</u>	<u>17.0</u>
Total	<u>97.7</u>	<u>104.6</u>	<u>90.0</u>	<u>97.4</u>

<sup>a</sup>Trapped in ethanolamine.

Table IV. Radioactivity levels in tissues of rats following a single oral dose of 5 mg/kg  $^{14}\text{C}$ -mephosfolan.

Time Period (Hour)	Liver	Kidney	Muscle	Fat	Blood
0-24	2.20	2.40	0.53	1.03	3.65
0-48	2.02	2.40	0.68	0.24	4.74
0-72	1.69	2.71	0.53	0.14	3.78
0-192	0.40	0.42	0.17	0.15	1.14

### Metabolism in Plants

1. Mephosfolan. The metabolic fate of mephosfolan in cotton plants and in rice plants grown in a paddy environment was studied. Cotton plants were grown from seeds (Delta pine smooth leaf variety). Six-week-old plants containing 4 to 6 leaves were treated foliarly with  $^{14}\text{C}$ -mephosfolan. Plants were analyzed at periodic intervals. The distribution of radioactivity in cotton plants after foliar application of  $^{14}\text{C}$ -mephosfolan is summarized in Table V. The metabolic pathways of mephosfolan in cotton plants involve oxidation, hydrolysis and conjugation to yield glucosides (Figure 2).

For the metabolism study of mephosfolan in rice plants grown in a paddy environment, 4 rectangular polyethylene tanks (60 x 30 x 30 cm) were filled with a silt loam soil to a depth of 20 cm and flooded with water to maintain a level of about 5 cm above the soil surface (Figure 3).



Journal of Agricultural and Food Chemistry

*Figure 3. Simulated rice paddy treated with  $^{14}\text{C}$  mephosfolan at 0.75 kg/ha*

Rice seedlings (approximately 7.5 to 10 cm tall) were obtained by germinating IR-22 rice seeds in two 10 x 40 cm rectangular vermiculite flats for two weeks. Six to eight seedlings were transplanted as bundles into each tank (3 rows and 6 columns) with approximately a 10 cm space between the bundles. The tanks were kept in the greenhouse under artificial light (GTE Sylvania M1000 BU-HOR) on a 12-hour day. Three weeks after transplanting, three of the four tanks were treated with carbon-14 labeled mephosfolan granules by broadcasting the formulated mephosfolan at the rate of 0.75 kg ai/ha. The fourth tank was untreated and served as the control. All tanks were kept flooded to a depth of about 5 cm for 4 months by adding water as needed.

In the rice plants, the distribution of extractable and unextractable residual radioactivity was found to be approximately equal in the rice plants (Table VI). Of this extractable radioactivity, more than 91% was identified as mephosfolan. The unextractable radioactivity has been characterized as  $^{14}\text{C}$ -labeled cellulose and possibly lignin, due to the incorporation of imido  $^{14}\text{C}$ -carbon atom into these natural products.

The results indicate that while mephosfolan is found in the rice plant, it does not accumulate in the rice grain. The radioactive residues found in the rice grain were characterized as  $^{14}\text{C}$ -labeled starch due to the incorporation of the  $^{14}\text{C}$ -imido carbon atom of mephosfolan into glucose and subsequently into the starch. A proposed metabolic pathway of mephosfolan in rice plants is also presented in Figure 2. Although only mephosfolan was found in paddy water, its concentration in water decreased at a rapid rate, presumably due to its degradation by the rice plant and other living organisms in the paddy ecosystem.

2. Role of Glutathione Conjugation. The metabolic fate of dithiolane insecticides in plants suggested the presence of many polar acidic products, presumably glutathione conjugates (4). Work with other dithietane insecticides in tobacco, soybean and peanuts has shown that, in addition to the major route of metabolism, i.e. the cleavage of R-N bond leading to the formation of an unstable dithietane intermediate and then to thiocyanate and further incorporating the imido carbon-14 into natural plant products, an alternative route is the conjugation with either glutathione or serine, followed by the rearrangement of the conjugates into 3 five-membered heterocyclic metabolites. This proposed metabolic pathway is depicted in Figure 4.

### Photodegradation Studies

The photodegradation studies were carried out by exposing approximately equimolar mixtures of either  $^{12}\text{C}/^{13}\text{C}$ -imido-labeled or  $^{12}\text{C}/^{13}\text{C}$ -ethyl-labeled mephosfolan to sunlight. The structures

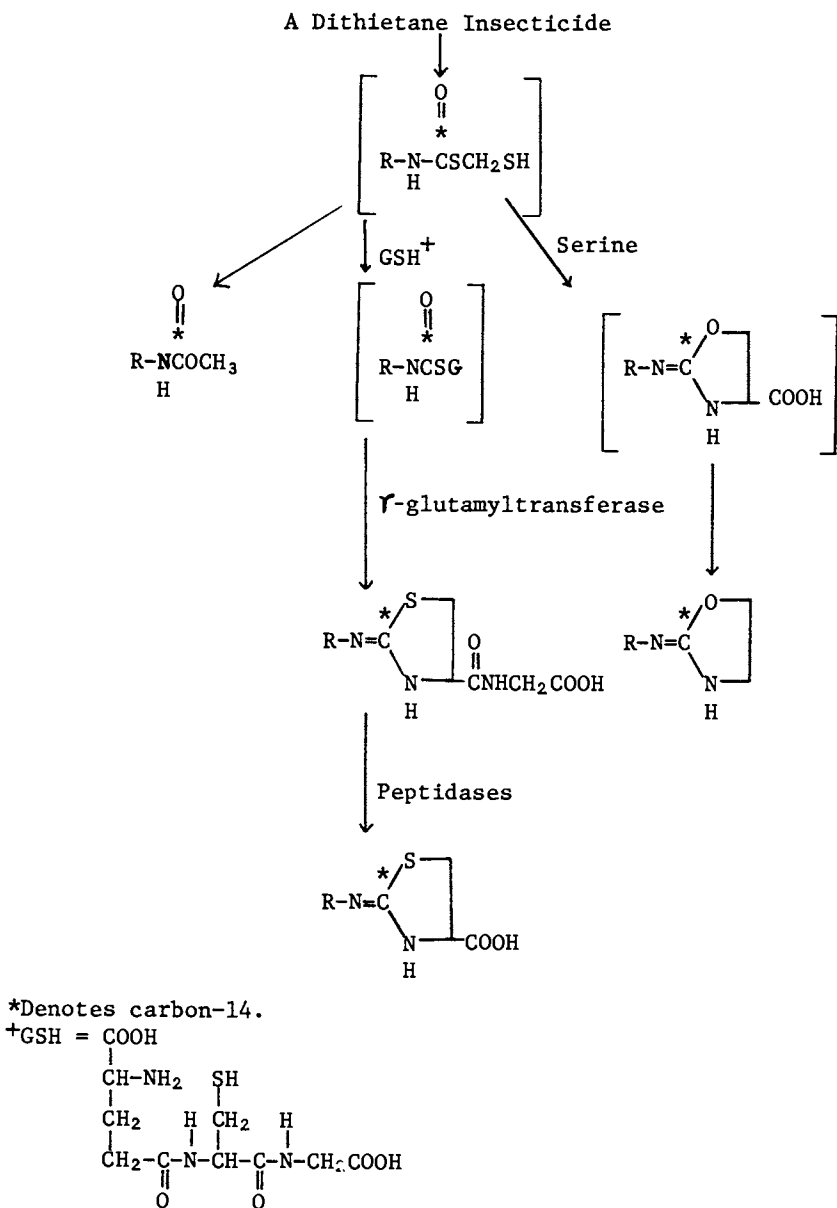


Figure 4. Role of glutathione conjugation in dithietane pesticides

Table V. Distribution of Radioactivity in cotton plants after foliar application of carbon-14 labeled mephosfolan.

Plant Part	% of Applied Dose		
	Days After Treatment		
	7 <sup>a</sup>	21 <sup>b</sup>	42 <sup>c</sup>
<b>Treated Leaf</b>			
Ethanol Extract	81.2	67.5	41.5
Marc	<u>5.1</u>	<u>8.0</u>	<u>11.5</u>
	86.3	75.5	53.0
<b>Root-Stem-Petioles</b>			
Ethanol Extract	0.4	1.2	1.7
Marc	<u>0.6</u>	<u>1.8</u>	<u>2.4</u>
	1.0	3.0	4.1

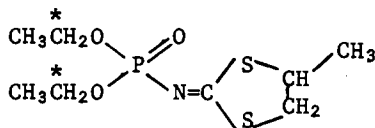
<sup>a</sup>Average of four plants.<sup>b</sup>Average of two plants.<sup>c</sup>One plant only.Table VI. Residual radioactivity (ppm) in rice plants grown in a <sup>14</sup>C-mephosfolan-treated paddy.

Weeks After Treatment	Concentration (ppm)*		
	Methanol Extract	Unextracted	Total
1	2.43	1.71	4.14
2	3.17	3.87	7.04
4	3.44	3.79	7.23
8	2.23	2.39	4.62

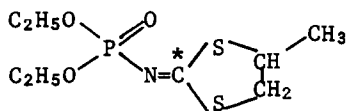
\*ppm expresses as mephosfolan.



The structures of the two  $^{13}\text{C}$ -labeled mephosfolan are given as follows:



Ethyl-label



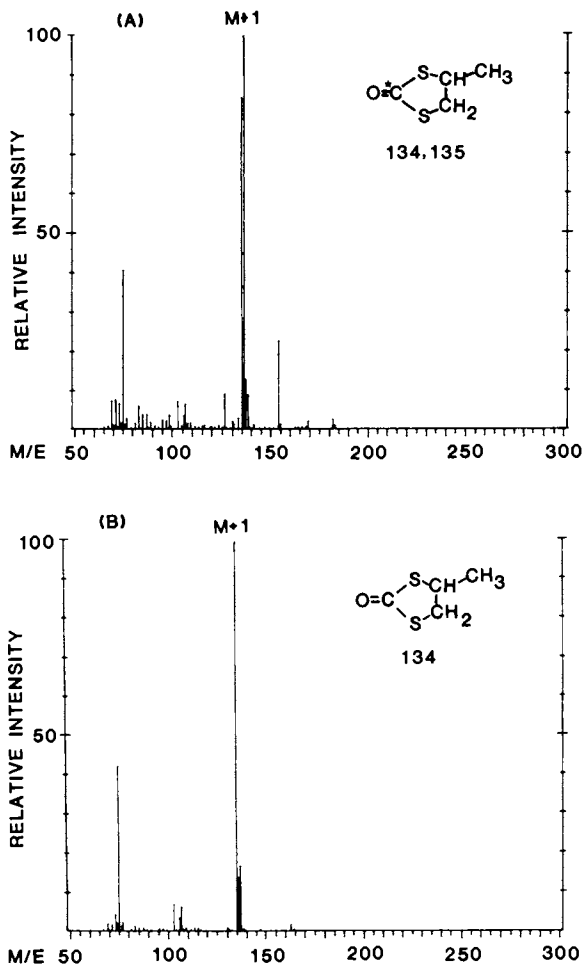
Imido-label

\*Denotes  $^{13}\text{C}$ .

Use of the  $^{12}\text{C}/^{13}\text{C}$  mixtures facilitated identification of photoproducts by gas chromatography chemical ionization mass spectroscopy (GC-CIMS) because molecular and fragment ions in the mass spectra appear as doublets. Solvent artifacts and volatile rice paddy water constituents gave mass spectra containing only singlet ions and were ignored. Irradiations were conducted in distilled water, "natural" water obtained from a flooded rice paddy, and a 2% acetone-water solution. The half-lives for mephosfolan were 18, 14, and 7 days, respectively, and indicated that photosensitization by paddy water constituents and acetone was occurring. The major products identified were cyclic S,S-propylene dithiocarbonate, 2-imino-5-methyl-1,3-dithiolane, and diethyl phosphate. Minor products were ethyl phosphate and phosphoric acid. A suggested scheme for the photodegradation of mephosfolan is also presented in Figure 2. Mass spectrum of the major photodegradation product S,S-propylene dithiocarbonate is presented in Figure 5. When the product was isolated from  $^{13}\text{C}$ -imido-labeled mephosfolan for identification by mass spectroscopy, the chromatogram had doublet M + 1 ions at m/e 135 and 136 (due to carbon-13 label and a fragment at m/e 75 (loss of  $^{13}\text{COS}$ ), as shown in Figure 5A. The spectrum of the same peak from ethyl- $^{13}\text{C}$  labeled mephosfolan (Figure 5B) showed the absence of  $^{13}\text{C}$  doublets. Confirmation of the assigned structure for this material was obtained by cochromatography with synthesized material.

#### Metabolism in Fish in the Rice Paddy Environment

In order to study the uptake and the metabolic fate of mephosfolan in fish reared in the rice paddy environment, fifteen *Carassius auratus* (goldfish), a member of the carp family were introduced to mephosfolan-treated rice paddy tanks one week after the pesticide treatment. Fish were analyzed at periodic intervals. The radioactive residue levels found at various time intervals in the fish kept in the  $^{14}\text{C}$ -mephosfolan-treated rice paddy environment are presented in Table VII. All fish survived during the course of this study. The predominant metabolite in fish tissue was identified as thiocyanate ion. With this data and the data obtained from paddy water analyses, it is appropriate to evaluate



Journal of Agricultural and Food Chemistry

Figure 5.  $\text{CI}(\text{CH}_4)$  mass spectrum of cyclic  $\text{S,S}$ -propylene dithiocarbonate from (A)  $^{13}\text{C}$  imido-labeled and (B)  $^{13}\text{C}$  ethyl-labeled mephosfolan photolysates

Table VII. Recovery of mephosfolan-derived radioactivity from goldfish reared in rice paddy environment in a greenhouse.\*

Exposure Time (Weeks)		Concentration (ppm)		
		Extractable	Unextracted	Total
1	Total $^{14}\text{C}$	0.30	1.06	1.36
	Mephosfolan	0.09		
	SCN ion	0.05		
	Unidentified	0.16		
3	Total $^{14}\text{C}$	0.21	1.0	1.2
	Mephosfolan	0.05		
	SCN ion			
	Unidentified**	0.11		
5	Total $^{14}\text{C}$	0.26	0.42	0.68
	Mephosfolan	0.04		
	SCN ion	Trace		
	Unidentified	0.22		
7	Total $^{14}\text{C}$	0.20	0.57	0.77
9	Total $^{14}\text{C}$	0.11	0.27	0.38

\*Fish were placed in treated paddy water 1 week after treatment.

\*\*Consists of 22 spots; largest 0.008 ppm.

the significance of the relatively minor pesticide uptake by the fish when reared in the paddy environment.

Metcalfe et.al. (5) and Kapoor et.al. (6) have defined the ecological magnification constant as the ratio of the concentration of parent compound in the organism vs. the concentration of parent compound in water. The bio-accumulation constant is defined as the ratio of the total radioactivity concentration in the organism vs. the total radioactivity concentration in water. The authors obtained data for DDT and methoxychlor in a terrestrial-aquatic model ecosystem where the pesticide was applied to terrestrial foliage only. The fish were kept in this system for three days and the highest value for ecological magnification constant for DDT was 84500 and for bioaccumulation was 13500.

The comparative figures for methoxychlor, a biodegradable pesticide, were 1545 and 206, respectively. Although it is

difficult to compare this study with those using the Metcalf system, due to the differences in the two systems, we can examine and compare their data with the mephosfolan study in which fish were exposed for one week in the paddy environment. From a starting concentration of 0.16 ppm, an ecological magnification of 0.56 and a bioaccumulation of 8.5 were found for fish exposed to mephosfolan. Since very low values of ecological magnification (0.56 vs. 84500 for DDT) were obtained, it is evident that mephosfolan is not likely to cause any ecological or bioaccumulation hazards when it is used in a rice-paddy environment.

### Abstract

The metabolism of two  $^{14}\text{C}$ -dithiolane insecticides labeled in the imido carbon position was investigated in rats, cotton plants and a simulated rice paddy environment. Analysis of urine from rats treated with  $^{14}\text{C}$ -mephosfolan, or  $^{13}\text{C}/^{14}\text{C}$  mixture mephosfolan by preparative and thin-layer cochromatography with synthetic standards and by mass spectroscopy showed that the attack occurs at the methyl moiety of the dithiolane ring resulting in a carboxylate ions. Hydrolysis of the P-N bond succeeded by ring opening results in release of the thiocyanate ion. This latter pathway is also the only metabolic route for phospholan. For both insecticides, the predominant tissue metabolite was found to be the thiocyanate ion. When cotton plants were treated, mephosfolan was the predominant radioactive residue. Hydroxylation of the methyl moiety of the iminodithiolane ring and conjugation as glucoside was a significant pathway. Upon treatment of the rice paddy environment with mephosfolan, it was observed that the rice plants assimilate mephosfolan rapidly, degrade it and incorporate the  $^{14}\text{C}$ -carbon into the natural plant products. Fish kept in the paddy environment readily metabolized mephosfolan into thiocyanate and many polar metabolites. The major products of photodegradation of mephosfolan were cyclic S,S-propylene dithiocarbonate, 2-imino-5-methyl-1,3-dithiolane, and diethyl phosphate. Minor products were ethyl phosphate and phosphoric acid.

### Literature Cited

1. Siegel, J. R., Rosenblatt, D. H., J. Am. Chem. Soc. (1958), (80), 1753.
2. Addor, R. W., J. Heterocycl. Chem. (1970), 7, 381.
3. Williams, R. T., "Detoxification Mechanisms", Wiley, New York, N.Y., (1959).
4. Zulalian, J., Blinn, R. C., J. Agric. Food Chem. (1977), 25, 1033.
5. Metcalf, R. L., Sangha, G. K., Kapoor, I. P., Environ. Sci. Technol. (1971), 5, 709.
6. Kapoor, I. P., Metcalf, R. L., Hirwe, A. S., Coats, J. R. Khalsa, M. S., J. Agric. Food Chem. (1973), 21, 310.

RECEIVED February 19, 1981.

# Roles of Iron-Sulfur Proteins in Degradation of Pesticidal Chemicals by Microorganisms

FUMIO MATSUMURA, K. YONEYAMA, and J. MARSHALL CLARK

Pesticide Research Center, Michigan State University, East Lansing, MI 48824

It is well known that in many biological systems, iron-sulfur proteins play important roles as electron transfer agents in oxidation-reduction reactions, e.g., photosynthesis, nitrogen fixation, metabolism of carbohydrates and other organic compounds, etc. Since ferredoxin, one of the iron-sulfur proteins, was isolated from an anaerobic bacterium, Clostridium pasteurianum, by Mortenson et al. (1) in 1962, proteins of this type have been found in a wide variety of living organisms, ranging from microorganisms to higher plants and animals.

The recent progress in the area of biological functions and molecular properties of iron-sulfur proteins has been phenomenal, and comprehensive summaries of these proteins have appeared in many reviews (2,3,4,5) and books (6,7,8). In this paper, the properties of some of the well understood iron-sulfur proteins will be briefly described. An effort will be made then to relate these properties to their possible participation in degradation reactions on organic chemicals, and particularly on pesticide chemicals.

## Properties of Iron-sulfur Proteins

### Low Molecular Weight, Terminal Iron-Sulfur Proteins

Rubredoxins. Rubredoxins are the simplest form of iron-sulfur proteins in which iron is bound to the sulfur atom of cysteine as shown in Fig. 1A. One of the first rubredoxins isolated was from an anaerobic bacterium, Clostridium pasteurianum, by Lovenberg and Sobel (9). The protein is composed of 54 amino acids and has a molecular weight of 6,000. The oxidized form has absorbance maxima at 380 and 490 nm. The biological role of the rubredoxin isolated from C. pasteurianum is still unknown.

Another source of rubredoxins was found in an aerobic bacterium, Pseudomonas oleovorans, utilizing n-hexane as a carbon source (10). This particular rubredoxin differs from those commonly found in anaerobic bacteria in some of its properties: it has a molecular weight of 19,000, and one iron form of the protein is readily converted to a two-iron form (11). The rubredoxin of P. oleovorans functions as a terminal electron transfer component in an enzyme system which participates in the  $\omega$ -hydroxylation of fatty acids and hydrocarbons. The hydrocarbon-oxidizing

0097-6156/81/0158-0111\$05.00/0

© 1981 American Chemical Society

enzyme system is separated into three fractions: rubredoxin, NADH-rubredoxin reductase and  $\omega$ -hydroxylase (10). In the presence of these three proteins, NADH and molecular oxygen, fatty acids and n-alkanes are hydroxylated at  $\omega$ -position during the electron transfer process as shown in Fig. 2 (12). A possible mechanism for hydroxylation is the formation of the alkyl hydroperoxide ( $R-CH_2-OOH$ ) intermediate by  $\omega$ -hydroxylase. The intermediate is then reduced to the hydroxyl group by reductase-rubredoxin system. Also, the  $\omega$ -hydroxylase enzyme system has been shown to catalyze the epoxidation of alkenes (13).

Rubredoxins have been isolated from other anaerobic bacteria such as *Desulfovibrio gigas* (14) and *Peptostreptococcus elsdenii* (15) but these rubredoxins have not been examined to the same extent as those described above.

**Ferredoxins.** Ferredoxins are proteins which contain two or four iron atoms bound to cysteine and inorganic sulfur atoms as shown in Fig. 1B. There are two types of ferredoxins: plant type ferredoxins (top) which consist of two iron and two labile sulfur atoms coordinated to four cysteine residues, and bacterial type ferredoxins (bottom) consisting of four iron and four labile sulfur atoms coordinated to four cysteine residues.

**Plant type ferredoxins.** Tagawa and Arnon (16) described the isolation of a ferredoxin from spinach chloroplast. This ferredoxin is a protein of 12,000 molecular weight, and consists of 97 amino acids (17). Spinach ferredoxin has absorbance maxima at 325, 420 and 465 nm (18). Ferredoxins of this type have been isolated from other sources of plants and algae, e.g., alfalfa (19), taro (20), *Leuceana glauca* (21) and *Scenedesmus* (22). The proteins of these ferredoxins are similar in their properties to ferredoxin from spinach.

The biological functions of chloroplast ferredoxins are to mediate electron transport in the photosynthetic reaction. These ferredoxins receive electrons from light-excited chlorophyll, and reduce NADP in the presence of ferredoxin-NADPH reductase (23). Another function of chloroplast ferredoxins is the formation of ATP in oxygen-evolving noncyclic photophosphorylation (24). With respect to the photoreduction of NADP, it is known that microbial ferredoxins from *C. pasteurianum* (16) are capable of replacing the spinach ferredoxin, indicating the functional similarities of ferredoxins from completely different sources. The functions of chloroplast ferredoxins in photosynthesis and the properties of these ferredoxin proteins have been reviewed in detail by Orme-Johnson (2), Buchanan and Arnon (3), Bishop (25), and Yocum et al. (26).

**Bacterial ferredoxins.** Bacterial ferredoxin was first described in 1962 by Mortenson et al. (1) who found a low-molecular iron protein involved in electron transfer of pyruvate hydrogenase and nitrogenase in *C. pasteurianum*. Subsequently, a number of ferredoxins have been found in widely different types of bacteria such as photosynthetic bacteria and  $N_2$ -fixing bacteria. These bacterial type ferredoxins have molecular

weights ranging from 5,000 to 10,000 as shown in Table 1. They have absorbance maxima at 390-400 nm.

Bacterial ferredoxins function primarily as electron carriers in ferredoxin-mediated oxidation reduction reactions. Some examples are: reduction of NAD, NADP, FMN, FAD, sulfite and protons in anaerobic bacteria, CO<sub>2</sub>-fixation cycles in photosynthetic bacteria, nitrogen fixation in anaerobic nitrogen fixing bacteria, and reductive carboxylation of substrates in fermentative bacteria. The roles of bacterial ferredoxins in these reactions have been summarized by Orme-Johnson (2), Buchanan and Arnon (3), and Mortenson and Nakos (31).

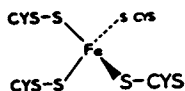
#### Iron-Sulfur Proteins as an Electron Transfer Component to Cytochrome P<sub>450</sub>

Adrenodoxin. Adrenodoxin is the only iron-sulfur protein which has been isolated from mammals. This protein from mitochondria of bovine adrenal cortex was purified almost simultaneously by Kimura and Suzuki (32) and Omura et al. (33). It has a molecular weight of 12,638 (34) and the oxidized form of the protein shows maximal absorbances at 415 and 453 nm. Adrenodoxin acts as an electron carrier protein in the enzyme system required for steroid hydroxylation in adrenal mitochondria. In this system, electron transfer is involved with three proteins: cytochrome P<sub>450</sub>, adrenodoxin and a flavoprotein. Reduced NADP gives an electron to the flavoprotein which passes the electron to adrenodoxin. Finally, reduced adrenodoxin transfers the electron to cytochrome P<sub>450</sub> as shown in Fig. 3. The mechanism of cytochrome P<sub>450</sub> interaction with steroid, oxygen and adrenodoxin in mixed-function oxidase of adrenal cortex mitochondria has been reviewed by Estabrook et al. (35).

Putidaredoxin. Cushman et al. (36) isolated a low molecular iron-sulfur protein from camphor-grown Pseudomonas putida. This protein, putidaredoxin, is similar to the plant type ferredoxins with two irons attached to two acid-labile sulfur atoms (37). It has a molecular weight of 12,000 and shows absorption maxima at 327, 425 and 455 nm. Putidaredoxin functions as an electron transfer component of a methylene hydroxylase system involved in camphor hydroxylation by P. putida. This enzyme system consists of putidaredoxin, flavoprotein and cytochrome P<sub>450</sub> (38). The electron transport from flavoprotein to cytochrome P<sub>450</sub> is similar to that of the mammalian mixed-function oxidase, but requires NADH as a primary electron donor as shown in Fig. 4. In this bacterial mixed-function oxidase system, reduced putidaredoxin donates an electron to substrate-bound cytochrome P<sub>450</sub>, and the reduced cytochrome P<sub>450</sub> binds to molecular oxygen. One oxygen atom is then used for substrate oxidation, and the other one is reduced to water (39, 40).

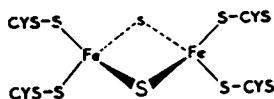
Megaredoxin. Another example of a bacterial mixed-function oxidase was found in the steroid 15 $\beta$ -hydroxylase system of Bacillus megaterium (41). This enzyme system consists of three proteins: FMN-containing flavoprotein (megaredoxin reductase), iron-sulfur protein called megaredoxin, and cytochrome P<sub>450</sub>. The megaredoxin also

## A. RUBREDOXINS ---- 1 IRON SULFUR PROTEIN



## B. FERREDOXINS

## 2 IRON SULFUR PROTEINS (PLANT TYPE FERREDOXINS)



## 4 IRON SULFUR PROTEINS (BACTERIAL TYPE FERREDOXINS)

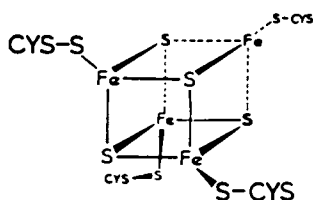


Figure 1. Schematic models of clusters in the iron-sulfur proteins proposed by Orme-Johnson (2)

Figure 2. Role of rubredoxin as an electron carrier in the  $\omega$ -hydroxylation of alkanes of *P. oleovorans* (12)

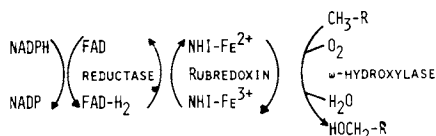
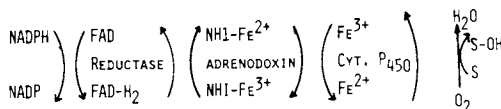


Figure 3. Role of adrenodoxin in the mixed-function oxidation system of adrenal cortex mitochondria (35)





functions as an electron carrier in the same manner as adrenodoxin. The general scheme of the electron transport in the  $15\beta$ -hydroxylase system has been proposed as shown in Fig. 5 (42). In this system, FMN-flavoprotein functions as a primary electron acceptor from NADPH, just as in the case with the FMN-containing flavodoxins as will be mentioned below.

#### Flavodoxins as Iron-Sulfur Protein Mimics

Flavodoxins are a group of flavoproteins which function as electron carriers at low potential in oxidation-reduction systems. The proteins of this group contain one molecule of FMN as their prosthetic group, but, in contrast to ferredoxins, do not contain metals such as iron.

The molecular weight of these proteins ranges from 14,000 to 23,000 as shown in Table 2. Organisms which have been reported to produce flavoproteins include several species of bacteria and alga. However, unlike the case with ferredoxins, these proteins have not yet been found in higher plants and animals.

The first microbial flavodoxin was isolated from an anaerobic bacterium, *Clostridium pasteurianum*, and it was able to replace ferredoxin in the photoreduction of NADP by a chloroplast system (49). Also, the flavodoxin from a blue green alga, *Anacystis nidulans*, has been found to substitute for plant ferredoxin in the photosynthetic system (50). Such interchangeability of flavodoxins with ferredoxins shows a similarity of the metabolic function of both proteins in living organisms. In certain types of plant and microbial systems, the activity of flavoproteins as electron carriers has been shown to be twice as much as that of ferredoxins in terms of stimulating reduction of NADP by chloroplast fraction (49, 50). These facts also support the contention that flavodoxins play an important role in electron transport in the oxidation-reduction systems of organisms. However, the precise metabolic roles of flavodoxins still remain to be established in many respects. A review on the subject of flavodoxins has been written by Mayhew and Ludwig (51).

#### Involvement of Iron-sulfur Proteins in Pesticide Degradation

The above description on iron-sulfur proteins indicates that these proteins are good mediators of electron transfer and as such they could play some role in degradation of organic pollutants where oxidative or reductive reactions are involved. Even without conducting any actual experiment, one could hypothesize that n-hexane and other short, straight chain alkyl hydrocarbons would be oxidized at the terminal position by a type of microorganism such as *Pseudomonas oleovorans* which possess rubredoxins. Also, so far as it is known, all of the microbial oxygenases, involving cytochrome P<sub>450</sub> type hemoprotein, utilize iron-sulfur protein as immediate electron donors. Thus, it is safe to say that wherever organic materials are metabolized by these oxygenases, iron-sulfur proteins are facilitating such reactions.

Table 1. Molecular weight of bacterial ferredoxins

Bacteria	Molecular Weight
<b>Anaerobic Bacteria</b>	
<u>Clostridium pasteurianum</u>	5,200 (27)
<u>Desulfovibrio desulfuicans</u>	6,300 (28)
<b>Photosynthetic Bacteria</b>	
<u>Chromatium vinosum</u>	10,074 (29)
<b>Facultative N<sub>2</sub>-Fixing Bacteria</b>	
<u>Bacillus polymyxa</u>	9,000 (30)

Table 2. Molecular weight of selective flavodoxins (FMN proteins)

Bacteria and Algae	Molecular Weight
<b>Anaerobic Bacteria</b>	
<u>Clostridium pasteurianum</u>	14,600 (43)
<u>Desulfovibrio gigas</u>	16,000 (44)
<u>Azotobacter vinelandii</u>	23,000 (45)
<b>Photosynthetic Bacteria</b>	
<u>Rhodospirillum rubrum</u>	23,000 (46)
<b>Algae</b>	
<u>Anacystis nidulans</u>	20,300 (47)
<u>Chlorella fusca</u>	22,000 (48)

We have accumulated some experimental evidence for the involvement of iron-sulfur proteins in pesticide degradation and now present the data.

#### Degradation of Mexacarbate by Ferredoxin

The most clear-cut demonstration of the ability of iron-sulfur proteins to degrade some pesticidal chemicals has been in the case of ferredoxin on mexacarbate. In this experiment, Esaac and Matsumura (52) incubated spinach ferredoxin (50  $\mu\text{g}$  per tube) obtained from Sigma Chemicals, St. Louis, Missouri with  $^{14}\text{C}$ -mexacarbate in 2.5 ml of standard phosphate buffer and found that 10% of the added mexacarbate degraded after 3 hrs of incubation at 37°C with continuous shaking. The most interesting aspect is that the reaction was highly stimulated when flavin cofactors, such as FMN or riboflavin, were added to the system (Table 3). This stimulatory effect may be interpreted to mean that electrons needed for the reaction are now provided by these flavin cofactors to ferredoxin which then passes them on to the substrate, mexacarbate. It must be noted here that the entire reaction vessel has been kept under nitrogen to provide an anaerobic environment (53). The level of redox potential provided by this treatment was in the neighborhood of 0mV in the presence of ferredoxin as judged by the changes in color of added methylene blue. It is interesting to note that the major reaction product is desmethyl mexacarbate (Fig. 6) which constitutes about 70% of the total radioactivity recovered (Table 3). Previously, all N-desmethylation reactions have been considered to be due to oxidative enzyme systems. However, the above reaction is favored under an anaerobic condition, and, therefore, must be regarded as a reductive reaction (Fig. 7). An analogous reaction has been observed by Esaac and Matsumura (53) who used flavoproteins in place of ferredoxin and found that mexacarbate is reduced in the presence of a flavin cofactor. That flavoproteins play a similar role in this regard to ferredoxin is not entirely surprising, since it has been known that some microorganisms produce a small molecular weight flavoprotein called flavodoxin in place of ferredoxin when they are grown in the absence of ferric or ferrous ions as mentioned above.

#### Metabolic Degradation of Toxaphene by Pseudomonas putida

It has been clearly established that Pseudomonas putida which has been selected to grow on camphor possesses an oxidative metabolic system (54). It contains a hemoprotein, cytochrome P<sub>450</sub><sup>cam</sup>, and an iron-sulfur protein which accepts electrons from NADH via a flavoprotein, putidaredoxin reductase. The scheme was elucidated by Gunsalus and Marshall (54). It was first found in our laboratory that P. putida grown on a camphor-enriched medium is capable of metabolically degrading toxaphene (55). Since toxaphene has been known to be degraded by both oxidative and reductive reactions, an effort was made to distinguish these two types of biochemical reactions using  $^{14}\text{C}$ - and  $^{36}\text{Cl}$ -labelled toxaphene.

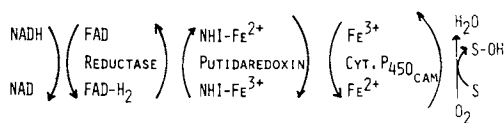


Figure 4. Role of putidaredoxin in the methylene hydroxylation system for camphor (39)

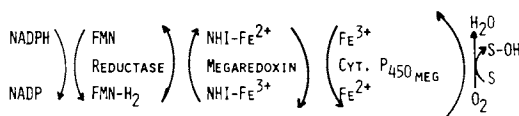


Figure 5. Role of megaredoxin in steroid hydroxylase system of *B. megaterium* (42)

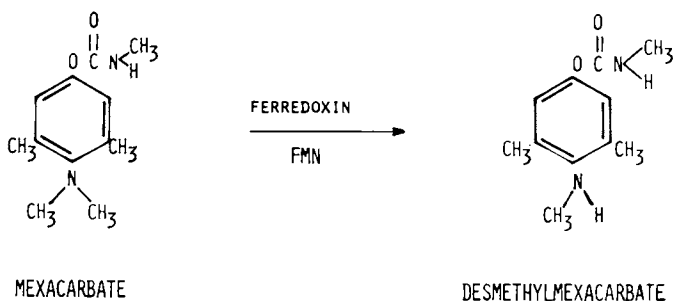


Figure 6. Structures of mexacarbate and desmethylmexacarbate

Figure 7. Degradation pathway of mexacarbate through the flavin cofactor-ferredoxin system

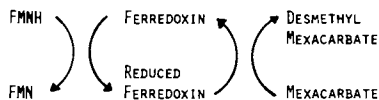


Table 3. Degradation of  $^{14}\text{C}$ -mexacarbamate by ferredoxin in the presence and absence of a flavin cofactor<sup>1</sup>

Cofactors	Percent Non-degraded Mexacarbamate	Degradation Products (%)		
		Water-Soluble	Desmethylmexacarbamate	Ether-Soluble Others
None	90	1	6	3
FMN	4	9	68	19
Riboflavin	4	10	72	14

<sup>1</sup>The reaction was incubated for 3 hrs at 37°C under nitrogen atmosphere in 0.02 M sodium phosphate buffer at pH 6.0.

First, the effects of aerobic and anaerobic culture conditions on toxaphene degradation were studied with washed *P. putida* cells grown on camphor and incubated with no readily usable carbon source. The radioactivities remaining in water after extraction with n-hexane were used as an indicator of metabolic activity. This was further extracted with ethyl acetate after acidification to divide this "total polar metabolites" fraction into aqueous buffer phase and ethyl acetate phase, i.e., the total polar metabolites reported refer to summation of the aqueous buffer and ethyl acetate soluble phases (Table 4). All radioactivities have been corrected by zero time controls and autoclaved 8 hr controls are included in each experiment.

As judged by the radioactivities in terms of "total polar metabolites" increased as the result of 8 hrs incubation,  $^{36}\text{Cl}$ -toxaphene seems to be more labile than  $^{14}\text{C}$ -toxaphene under either of the test conditions. However, it must be noted that the increase in radioactivities in  $^{36}\text{Cl}$ -toxaphene experiments is largely due to those in the aqueous buffer phase. There is a strong possibility that a portion of these  $^{36}\text{Cl}$  radioactivities come from inorganic chloride ions.

Of the total recovered radioactivities after  $\text{AgNO}_3$  treatment (water soluble plus  $\text{AgCl}$ ) of the aqueous buffer phase taken from  $^{36}\text{Cl}$ -toxaphene cultures (Table 5), 30.3% was found as water soluble metabolites and 69.7% as free chloride ion under aerobic conditions. Under anaerobic conditions, 17.0% was found as water soluble metabolites and 83.0% as free chloride ion.

Also in the  $^{14}\text{C}$ -toxaphene cultures (Table 4), the large proportion, if not all, of the radioactivities reported as total polar metabolites were found as ethyl acetate soluble radioactivities in contrast to the  $^{36}\text{Cl}$ -toxaphene cultures, where the radioactivities were partitioned between the aqueous buffer and ethyl acetate phases in approximately a 2.5:1 ratio, respectively. When the  $^{14}\text{C}$  and  $^{36}\text{Cl}$  percentage values in Table 4 from similarly incubated (aerobic or anaerobic) cultures were added, it became apparent that anaerobic cultures were slightly more active in degrading toxaphene than aerobic cultures. However, the above results clearly indicate that at least a portion of toxaphene metabolism by *P. putida* involves oxidative degradation.

#### Effects of Added Cofactors on Toxaphene Metabolism by Washed Cells

Enhancement of metabolism of  $^{14}\text{C}$ -toxaphene by several added cofactors was studied in 8 hrs aerobic cultures of washed *Pseudomonas putida* cells. The results of these experiments are summarized in Table 6. In the NAD group, NADH showed the greatest stimulatory effect on the production of total polar metabolites (1.2 times) over that of the 8 hrs incubate value. Similarly, FAD was responsible for a modest increase (1.1 times). The combination of NADH-FAD showed stimulation above that of either of the unpaired cofactors (2.0 times). Addition of mannitol to NADH-FAD stimulated this combination's total polar metabolite value from 2.0 to 2.3 times that of the 8 hrs incubate. In the sequence NADH to NADH-FAD to NADH-FAD-mannitol, the increase in the radioactivities in the ethyl acetate phase (moderately polar metabolites)

Table 4. Metabolism of  $^{14}\text{C}$  and  $^{36}\text{Cl}$ -toxaphene by washed *P. putida* cells

Fraction <sup>4</sup>	Radioactivities (dpm)/Sample			
	Aerobic		Anaerobic	
	$^{14}\text{C}$	$^{36}\text{Cl}$	$^{14}\text{C}$	$^{36}\text{Cl}$
<u>Aqueous buffer phase</u> <sup>2</sup>				
autoclaved 8 hrs control	0	140	180	478
8 hrs incubate	0	2702	170	3721
<u>Ethyl acetate phase</u> <sup>3</sup>				
autoclaved 8 hrs control	0	1043	0	535
8 hrs incubate	926	1085	1342	1619
<u>(Total polar metabolites)</u>				
autoclaved 8 hrs control	0	1183	180	1013
8 hrs incubate	926	3787	1512	5340
<u>Solvent extract phase</u>				
autoclaved 8 hrs control	96,125	90,575	115,725	98,225
8 hrs incubate	92,212	88,725	110,975	92,704

<sup>1</sup>Data presented in table are averages of two independent experiments.

<sup>2</sup>Very polar metabolites.

<sup>3</sup>Moderately polar metabolites.

<sup>4</sup>All radioactivities have been corrected by zero-time control values.

Table 5.  $\text{AgNO}_3$  complexation of aqueous buffer phase from washed *P. putida* cell incubations with  $^{36}\text{Cl}$ -toxaphene

Fractions	% $^{36}\text{Cl}$ Radioactivities of aqueous buffer phases	
	Aerobic	Anaerobic
<u>Water soluble portion after <math>\text{AgNO}_3</math> treatment<sup>2</sup></u>		
8 hrs incubate	30.3	17.0
<u>Precipitated portion as <math>\text{AgCl}</math><sup>3</sup></u>		
8 hrs incubate	69.7	83.0

<sup>1</sup> Aqueous ( $^{36}\text{Cl}$ ) buffer dpm remaining after acidification and ethyl acetate extraction (see Table 4).

<sup>2</sup> Aqueous buffer supernatant after  $\text{AgNO}_3$  complexation and centrifugation.

<sup>3</sup> Resuspended  $\text{AgCl}$  pellet.

<sup>4</sup> All percentages of radioactivities have been corrected by zero time control values and autoclaved 8 hrs control values.



Table 6. Effects of added cofactors on metabolic fate of  $^{14}\text{C}$ -toxaphene by washed *P. putida* cells<sup>1</sup> incubated aerobically

Treatments <sup>5</sup>	Radioactivities (dpm)/Sample <sup>4</sup>		
	Aqueous Buffer <sup>2</sup>	Ethyl Acetate <sup>3</sup>	Total Polar Metabolites
autoclaved 8 hrs control	160	38	198
8 hrs incubate	615	773	1388
NAD	545	929	1474
NADH	756	833	1589
NADP	627	768	1395
NADPH	413	789	1202
FAD	660	871	1531
Mannitol (Man)	775	1958	2733
NADH-FAD	758	1995	2753
NADH-FAD-MAN	732	2409	3141
NADH-FAD-FMN	1926	1460	3386
NAOH-FAD-FMN-CO	1130	835	1965

<sup>1</sup>Data represented in table are averages of three separate experiments.

<sup>2</sup>Very polar metabolites.

<sup>3</sup>Moderately polar metabolites.

<sup>4</sup>Total  $^{14}\text{C}$ -toxaphene dpm was 96,125 per sample (see Table 4).

<sup>5</sup>All radioactivities reported have been corrected by zero time control values.

was responsible for the overall increase of radioactivities in that no increase was noted in those of the corresponding aqueous buffer phases. Addition of FMN to NADH-FAD resulted in a 2.4 times increase over 8 hrs incubate value of total polar metabolites. However, most of the overall increase is due to an increase in aqueous buffer phase (very polar metabolites). Treatment of the culture containing NADH-FAD-FMN with carbon monoxide gas (NADH-FAD-FMN-CO) during incubation reduced the value 42%.

The ethyl acetate phases of 8 hrs and NADH-FAD-mannitol samples were inspected by gas-liquid chromatography analysis for detection of metabolites (hydroxylated and/or carboxylated) by treatment with TMS-derivatization agent or diazomethane. By comparing the spectra of all cofactor-treated samples to zero time control and autoclaved 8 hrs control spectra, 6 possible metabolic products were indicated and retention times noted (Table 7).

These observations suggest that the oxidative reactions occurring on toxaphene are similar in nature to the system described for camphor degradation (54). It is unfortunate that the above experiments could only be conducted with washed intact cells as cell-free preparations in all cases did not exhibit metabolic capabilities. The reason for this phenomenon has not been found, but to our knowledge no other research group has been able to demonstrate pesticide degrading activities of such oxygenase systems in cell-free preparations.

#### Degradation of Mexacarbate in Cell-free Extracts of *Bacillus megaterium*

It has been well recognized that the mixed-function oxidase system of *Bacillus megaterium* is involved in steroid hydroxylation (41, 42) as already described above. This enzyme system is composed of a NADPH-specific FMN flavoprotein (megaredoxin reductase), an iron-sulfur protein (megaredoxin) and cytochrome P<sub>450</sub>. The megaredoxin protein plays an important role as an intermediate component of electron transfer from reduced flavoprotein to cytochrome P<sub>450</sub>.

An effort was made to investigate the involvement of cell-free extracts of a bacterial mixed-function oxidase system in pesticide degradation. In this experiment, the degradation of <sup>14</sup>C-mexacarbate was examined using the 10,000 g supernatant of lysozyme-treated *B. megaterium* cells in 0.03 M phosphate buffer at pH 7.0. Incubation was carried on for 2 hours at 30°C in the presence of various cofactors. The degradation products were determined as either ether-soluble or water-soluble products according to the method of Esaac and Matsumura (53). As shown in Table 8, the degradation of mexacarbate to ether-soluble products was stimulated by the presence of these cofactors, but the degradation did not produce any appreciable water-soluble products. Since the major ether-soluble product is known as desmethylmexacarbate (53), it is clear that N-desmethylation reaction was stimulated in this enzyme system. The flavin cofactor, FMN, showed the greatest stimulatory effect on the demethylation of mexacarbate when added alone to the incubation system. However, the most mexacarbate degradation was observed in the combined presence of FMN and NADPH where degradation reached about 60% of added mexacarbate in terms of

Table 7. Determination<sup>1</sup> of functional groups of metabolites in ethyl acetate phase<sup>2</sup> by gas-liquid chromatographic analysis

Treatments <sup>5</sup>	Retention time (min)	TMS-derivatization products	Diazomethane treated	Functional group <sup>3,4</sup>
NADH-FAD-mannitol	1.19	+	-	-OH
8 hrs	1.25	-	+	-COOH
NADH-FAD-mannitol	1.50	+	-	-OH
8 hrs	1.69	+	-	-OH
8 hrs	3.13	+	-	-OH
NADH-FAD-mannitol	4.50	+	-	-OH

<sup>1</sup> By TMS-derivatization or diazomethane treatment.

<sup>2</sup> Ethyl acetate phase (see Table 4).

<sup>3</sup> Hydroxylated metabolite = -OH.

<sup>4</sup> Carboxylated metabolite = -COOH.

<sup>5</sup> Apparent oxidative metabolites were recognized by the presence of new peaks after subtraction of zero time control and autoclaved 8 hrs control spectra.

Table 8. Effect of some cofactors on the degradation of  $^{14}\text{C}$ -mexacarbate in the cell-free extracts of Bacillus megaterium<sup>1</sup>

Cofactors	Degradation Products (%)	
	Water-Soluble	Ether-Soluble
None	0	10
FMN	0	40
FAD	0	26
NADH	0	12
NADPH	0	23
NADH + FMN	1	40
NADH + FAD	1	24
NADPH + FMN	1	57
NADPH + FAD	0	28

<sup>1</sup>The reaction mixture was incubated for 2 hrs at 30°C.

radioactivity. Mexacarbate degradation was not stimulated by addition of NADH. It is interesting that mexacarbate is rapidly degraded by a FMN and NADPH cofactor-dependent system from the cell-free extracts of *B. megaterium* because this bacterium has a mixed-function oxidase system which possesses a NADPH-specific FMN-flavoprotein. These facts suggest that mexacarbate degradation may proceed by a oxidative system similar to the one in which hydroxylation of steroids occurs in *B. megaterium*. However, this experiment still remains to be confirmed with the purified mixed-function oxidase.

In summary, various iron-sulfur proteins are known to play important roles as members of electron transfer systems in many biological systems. The roles of these important proteins in degrading xenobiotics such as environmental pollutants have not been investigated in the past. In this paper we have demonstrated, at least in a few cases, that iron-sulfur proteins could indeed facilitate degradation of some pesticides. They are particularly well suited in cases involving redox types of reactions as iron-sulfur proteins naturally possess electron-transferring capabilities. However, it is important to realize that there is a particular difference in the basic organization of mixed-function oxidases found in microorganisms and those isolated from higher animals. With the exception of bovine adrenodoxin, no other animal iron-sulfur protein has been found which functions as an electron donor to terminal cytochrome P<sub>450</sub> type hemoproteins as seen with the microbial systems described above.

It is known that microorganisms play important roles in degrading pesticides and other organics such as pollutants in the environment. Though basic similarities in metabolic patterns between animals and microorganisms have been noted, there are also important basic differences such as the lack of conjugation mechanisms in the microbial systems (56). Studies on environmental fates of chemicals are expected to receive a heavy emphasis in the future as the level of awareness of the problems of toxic chemical wastes increases. Technology of microbial model ecosystems to aid judgement on the biodegradability of untested chemicals, design of microbial disposal systems for toxic waste, etc. are some of the topics that are expected to receive a high priority. To this end our investigation here provides evidence for a basic difference in the organization of the monooxygenase systems, an extremely important type of detoxification enzyme system between the well documented animal systems and microorganisms.

Acknowledgment. We thank Dr. E. G. Esaac of this laboratory for assaying the effect of spinach ferredoxin on photodegradation of mexacarbate. Supported in part by the Environmental Toxicology Research Program, Michigan Agricultural Experiment Station (Journal Article No. 9732), Michigan State University, East Lansing, Michigan.

#### Literature Cited

1. Mortenson, L. E.; Valentine, R. C.; Carnahan, J. E. Biochem. Biophys. Res. Commun., 1962, **7**, 448.

2. Orme-Johnson, W. H. Ann. Rev. Biochem., 1973, 42, 159.
3. Buchanan, B. B.; Arnon, D. I. Advan. Enzymol., 1970, 33, 119.
4. Tsbiris, J. C.; Woody, R. W. Coord. Chem. Rev., 1970, 5, 417.
5. Jensen, L. H. Ann. Rev. Biochem., 1974, 43, 461.
6. Lovenberg, W., Ed. "Iron-Sulfur Proteins" Vol. 1 and 2; Academic Press: New York, 1973.
7. Palmer, G. "The Enzymes" Vol. 22; P. D. Boyer, Ed., Academic Press: New York, 1975, p. 1.
8. Orme-Johnson, W. H.; Orme-Johnson, N. R. "Method in Enzymology" Vol. 53; S. Fleischer and L. Packer, Ed., Academic Press: New York, 1978, p. 259.
9. Lovenberg, W.; Sobel, B. E. Proc. Nat. Acad. Sci. U.S., 1965, 54, 193.
10. Peterson, J. A.; Basu, D.; Coon, M. J. J. Biol. Chem., 1966, 241, 5162.
11. Lode, E. T.; Coon, M. J. J. Biol. Chem., 1971, 246, 791.
12. Peterson, J. A.; Kusunose, M.; Kusunose, E.; Coon, M. J. J. Biol. Chem., 1967, 242, 4334.
13. May, S. W.; Abbott, B. J. J. Biol. Chem., 1973, 248, 1725.
14. LeGall, J.; Dragoni, N. Biochem. Biophys. Res. Commun., 1966, 23, 145.
15. Bachmayer, H.; Yasunobu, K. T.; Peel, J. L.; Mayhew, S. J. Biol. Chem., 1968, 243, 1022.
16. Tagawa, K.; Arnon, D. I. Nature, 1962, 195, 537.
17. Matsubara, H.; Sasaki, R. M. J. Biol. Chem., 1968, 243, 1732.
18. Palmer, G.; Brintzinger, H.; Estabrook, R. W. Biochemistry, 1967, 6, 1658.
19. Keresztes-Nagy, S.; Margoliash, E. J. Biol. Chem., 1966, 241, 5955.
20. Rao, K. K. Phytochemistry, 1969, 8, 1379.
21. Benson, A. M.; Yasunobu, K. T. J. Biol. Chem., 1969, 244, 955.
22. Sugeno, K.; Matsubara, H. J. Biol. Chem., 1969, 244, 2979.
23. Shin, M.; Arnon, D. I. J. Biol. Chem., 1965, 240, 1405.
24. Arnon, D. I.; Tsujimoto, H. Y.; McSwain, B. D. Proc. Nat. Acad. Sci. U.S., 1964, 51, 1274.
25. Bishop, N. I. Ann. Rev. Biochem., 1971, 40, 197.
26. Yocum, C. F.; Siedow, J. N.; Pietro, A. S. "Iron-Sulfur Proteins" Vol. 1; W. Lovenberg, Ed., Academic Press: New York, 1973, p. 112.
27. Mortenson, L. E. Biochim. Biophys. Acta, 1964, 81, 71.
28. Zubieta, J. A.; Mason, R.; Postgate, J. R. Biochem. J., 1973, 133, 851.
29. Dus, K.; DeKlerk, H.; Sletten, K.; Bartsch, R. G. Biochim. Biophys. Acta, 1967, 140, 291.
30. Shethna, Y. I.; Stombaugh, N. A.; Burris, R. H. Biochem. Biophys. Res. Commun., 1971, 42, 1108.
31. Mortenson, L. E.; Nakos, G. "Iron-Sulfur Proteins" Vol. 1; W. Lovenberg, Ed., Academic Press: New York, 1973, p. 37.
32. Kimura, T.; Suzuki, K. Biochem. Biophys. Res. Commun., 1965, 20, 373.

33. Omura, T.; Sato, R.; Cooper, D. Y.; Rosenthal, O.; Estabrook, R. W. *Fed. Proc.*, 1965, 24, 1181.
34. Tanaka, M.; Haniu, M.; Yasunobu, K. T.; Kimura, T. *J. Biol. Chem.*, 1973, 248, 1141.
35. Estabrook, R. W.; Suzuki, K.; Mason, J. I.; Baron, J.; Taylor, W. E.; Simpson, E. R.; Purvis, J.; McCarthy, J. "Iron-Sulfur Proteins" Vol. I; W. Lovenberg, Ed., Academic Press: New York, 1973, p. 193.
36. Cushman, D. W.; Tsai, R. L.; Gunsalus, I. C. *Biochem. Biophys. Res. Commun.*, 1967, 26, 577.
37. Tsibris, J. C. M.; Namtvedt, M. J.; Gunsalus, I. C. *Biochem. Biophys. Res. Commun.*, 1968, 30, 323.
38. Katagiri, M.; Ganguli, B. N.; Gunsalus, I. C. *J. Biol. Chem.*, 1968, 243, 3543.
39. Gunsalus, I. C. "Degradation of synthetic organic molecules," National Academy of Sciences: Washington, D.C., 1972, p. 137.
40. Gunsalus, I. C.; Lipscomb, J. D. "Iron-Sulfur Proteins" Vol. I; W. Lovenberg, Ed., Academic Press: New York, 1973, p. 151.
41. Berg, A.; Carlstrom, K.; Gustafsson, J.; Ingelman-Sundberg, M. *Biochem. Biophys. Res. Commun.*, 1975, 66, 1414.
42. Berg, A.; Gustafsson, J.; Ingelman-Sundberg, M. *J. Biol. Chem.*, 1976, 251, 2831.
43. Knight, Jr. E.; Hardy, R. W. F. *J. Biol. Chem.*, 1966, 241, 2752.
44. Dubourdieu, M.; LeGall, J. *Biochem. Biophys. Res. Commun.*, 1970, 38, 965.
45. Edmondson, D. E.; Tollin, G. *Biochemistry*, 1971, 10, 124.
46. Gusanovich, M. A.; Edmondson, D. E. *Biochem. Biophys. Res. Commun.*, 1971, 45, 327.
47. Smillie, R. M.; Ertseh, B. "Method in enzymology" Vol. 23; S. Pietro, Ed., Academic Press: New York, 1971, p. 504.
48. Zumft, W. G.; Spiller, H. *Biochem. Biophys. Res. Commun.*, 1971, 45, 112.
49. Knight, Jr. E.; Hardy, R. W. F. *J. Biol. Chem.*, 1967, 242, 1370.
50. Smillie, R. M. *Biochem. Biophys. Res. Commun.*, 1965, 20, 621.
51. Mayhew, S. G.; Ludwig, M. L. "The Enzymes" Vol. 12; P. D. Boyer, Ed., Academic Press: New York, 1975, p. 57.
52. Esaac, E. G.; Matsumura, F. *Pharmac. Ther.*, 1980, 9, 1.
53. Esaac, E. G.; Matsumura, F. *Pest. Biochem. Physiol.*, 1979, 10, 67.
54. Gunsalus, I. C.; Marshall, V. P. "Survival in Toxic Environments," M.A.Q. Khan, Ed., Academic Press: New York, 1974, p. 295.
55. Clark, J. M.; Matsumura, F. *Arch. Environ. Contam. Toxicol.*, 1979, 8, 285.
56. Matsumura, F.; Benezet, H. J. "Pesticide Microbiology," Hill, I. R.; Wright, S. J. L., Eds. Academic Press: New York, 1978, p. 623.

RECEIVED March 20, 1981.

# Catabolism of Glutathione Conjugates of Pesticides in Higher Plants

G. L. LAMOUREUX and D. G. RUSNESS

U.S. Department of Agriculture, Science and Education Administration,  
Agricultural Research, Metabolism and Radiation Research Laboratory,  
Fargo, ND 58105

During the last 10 years it has become apparant that many pesticides are converted to glutathione (GSH) conjugates in higher plants (chemical abbreviations are defined in the glossary). Chloro-triazines, hindered  $\alpha$ -chloroacetamides, diphenyl ethers, thiocarbamates, and chlorinated nitrobenzenes are among the pesticides converted to GSH conjugates in vivo in plants (1). Glutathione conjugation is particularly important in the metabolism of herbicides in plants, because it frequently results in the selective detoxification of the herbicide (2). In some plant species, GSH S-transferase activity can be stimulated by the antidote, N,N-diallyl-2,2-dichloroacetamide (3). This increases resistance to certain herbicides that are detoxified by GSH conjugation.

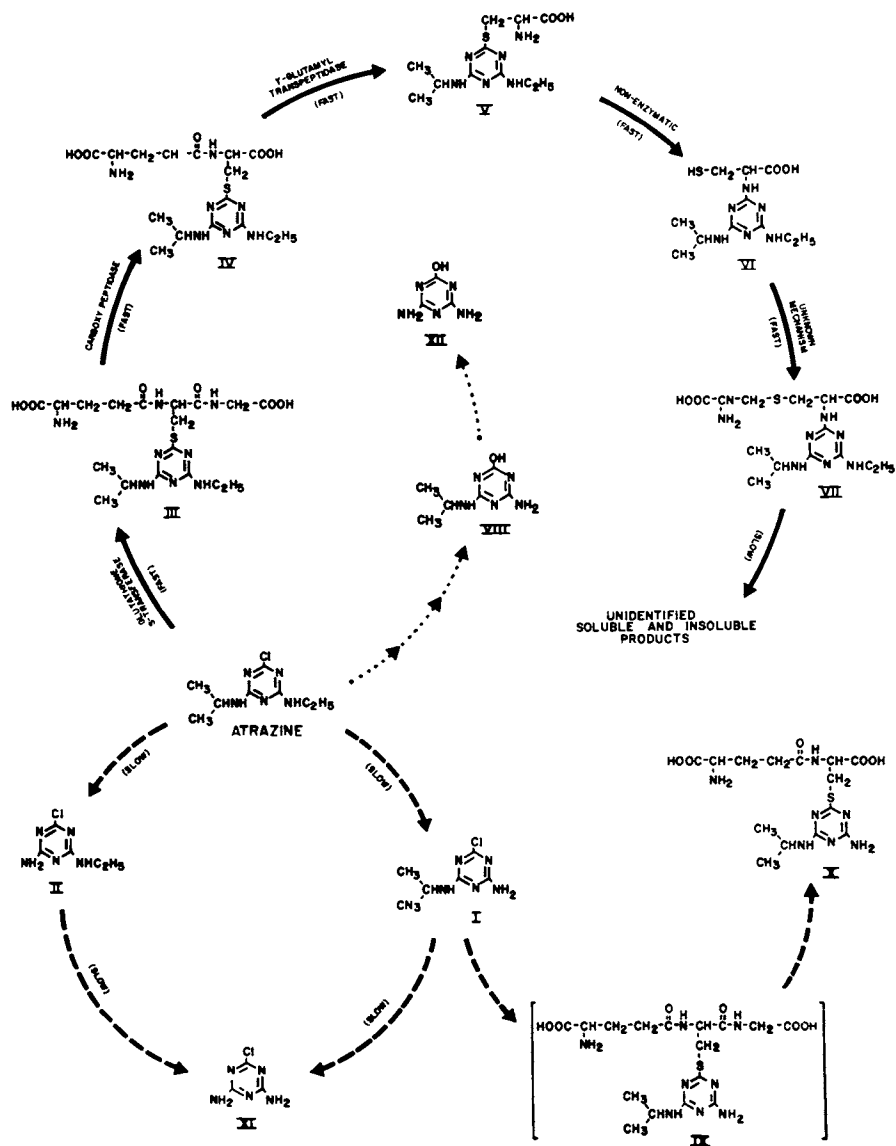
In spite of the importance of the initial GSH conjugtion reaction, very little information is present in the literature regarding the catabolism of GSH conjugates of pesticides in higher plants. The catabolism of the GSH conjugate of atrazine in sorghum has been studied (Figure 1), but there appear to be few similar reports.

Because of the importance of GSH conjugation in pesticide metabolism and the diversity of the plant kingdom, it seemed desirable to study the catabolism of another GSH conjugate in several plant species. Pentachloronitrobenzene-UL-<sup>14</sup>C, [<sup>14</sup>C]PCNB (Figure 2), was chosen as the primary biochemical probe to extend studies on GSH conjugate catabolism in higher plants for the following reasons:

1. In in vitro studies with 10 plant species, all 10 species contained GSH S-transferases that utilized PCNB as a substrate (5).
2. The presence of 5 chlorines on the aromatic ring simplified metabolite identification by mass spectrometry.
3. Preliminary studies with peanut indicated that few competing metabolic reactions were present.
4. The study of several GSH conjugates was possible because several sites on the PCNB nucleus were susceptible to attack by GSH.

This chapter not subject to U.S. copyright.  
Published 1981 American Chemical Society





Journal of Agricultural and Food Chemistry

Figure 1. The metabolic pathway of atrazine in sorghum. The major pathway is indicated by bold arrows, the dashed arrows indicate minor pathways, and the dotted arrows indicate hypothesized reactions. The structure in brackets was not identified (4).

### Methods

[<sup>14</sup>C]PCNB metabolism was studied *in vivo* with 30-day-old peanut plants grown in nutrient solution that contained 17.6 ppm [<sup>14</sup>C]PCNB. Plant tissue was extracted with cold 80% methanol 48 hr after final exposure to PCNB. The extracts were made aqueous and partitioned against chloroform at pH 5.5 and against ethyl ether at pH 2. Water-soluble, chloroform-soluble, and ether-soluble metabolites were isolated by various chromatographic methods and identified by mass spectrometry and/or by synthesis. The details of these studies have been published previously (6, 7).

[<sup>14</sup>C]PCNB metabolism was also studied in barley, corn, cotton, onions, soybeans, and lake water rich in blue green algae. The general techniques used were similar to those previously reported for the metabolism of [<sup>14</sup>C]PCNB in peanut plants (6, 7). In replicated experiments, the higher plant species were grown in nutrient culture and were treated by incorporating a single dose of 3-10 ppm [<sup>14</sup>C]PCNB into the nutrient solution. After 3 days, the roots of these plants were extracted with cold 70% acetone (1X) and acetone (2X). The acetone extracts were diluted 2:1 with water and partitioned against methylene chloride. The methylene chloride-soluble extracts were analyzed by comparative TLC (7). The aqueous phases were concentrated to dryness and dissolved in water that contained 5% acetonitrile and 0.2% acetic acid. These solutions were applied to SEP-PAK C<sub>18</sub> cartridges (Waters Associates) and the cartridges were washed with 5-10 ml of water and eluted with 5 ml of 50% acetonitrile. From 90 to 95% of the <sup>14</sup>C was recovered from the cartridges in the 50% acetonitrile eluate. The eluates were concentrated to dryness and dissolved in 18% acetonitrile/1% acetic acid and subjected to HPLC as previously described for PCNB metabolism in peanut roots (6).

Blue green algae (primarily *Gloetrichia echinulata*) was collected from a Minnesota lake with a plankton net and washed with 100 ml of filtered lake water into a 500-ml culture bottle. [<sup>14</sup>C]PCNB was added (1 ppm) and the system was incubated at 19°C in full sun (10 AM to 7:30 PM). The bottle was gently agitated during this period and was opened hourly for aeration. The incubation was stopped by adding 250 ml of acetone. The entire system, algae and lake water, was extracted and analyzed as previously described for the higher plants.

Precursor-product relationships were studied in peanut cell cultures grown in B-5 Medium (8) that contained 2.8 ppm [<sup>14</sup>C]PCNB and 100 ppm 2,4-D. The liquid shake cultures were harvested after 3 hr, 9 hr, 24 hr, 3 days, 7 days and 14 days. The cells were extracted with 80% methanol and the extracts were made aqueous and partitioned against methylene chloride. Water-soluble metabolites were purified by various chromatographic methods and identified by mass spectrometry in a manner similar

to that used with peanut roots (6). Methylene chloride soluble metabolites were identified by co-chromatography.

Precursor-product relationships were also studied in peanut plants treated with  $^{14}\text{C}$ -labeled metabolites and harvested 1 to 21 days later (6, 7).

In vitro studies were conducted with enzymes extracted from peanut (7), pea (6), and onion (9). The enzymes were fractionated by ammonium sulfate precipitation, dialyzed, and stored frozen until used. The enzymes were assayed for various activities as described in the Results and Discussion.

## Results and Discussion

Water-soluble residues. Peanut plants treated with [ $^{14}\text{C}$ ]PCNB for 48 hr and harvested after a 48 hr post-treatment incubation, absorbed all but 1.2% of the  $^{14}\text{C}$ . The roots contained 88.9% of the applied  $^{14}\text{C}$ , the shoots 7.2%, and total recovery was 97.2%. The distribution of  $^{14}\text{C}$  in peanut root and the other plant tissues was examined as a function of solubility. Although some differences were noted among the various tissues, the water-soluble extracts generally contained about 1/3 of the  $^{14}\text{C}$  (Figure 3). Half of the  $^{14}\text{C}$  in the water-soluble fraction from peanut root was removed by partitioning with ethyl ether. This procedure was not used with extracts from the other tissues. The  $^{14}\text{C}$  that remained in the water-soluble fraction from peanut roots (13.6%) was purified by ion exchange and HPLC. These chromatographic methods indicated that 75% of the  $^{14}\text{C}$  in this fraction was present as four metabolites: S-(pentachlorophenyl)-glutathione [S-(PCP)GSH], S-(tetrachloronitrophenyl)glutathione [S-(TCNP)GSH], S,S'-(tetrachlorophenylene)diglutathione [S,S'-(TCP)diGSH], and S,S'-(tetrachlorophenylene)dicysteine [S,S'-(TCP)diCys] (Figure 4). These metabolites were identified by mass spectrometry (6). S-(Pentachlorophenyl)cysteine [S-(PCP)Cys] was a minor metabolite in this fraction and was identified by chromatography. The three glutathione conjugates were also produced by an enzyme system from pea and by a base-catalyzed reaction between [ $^{14}\text{C}$ ]PCNB and GSH (6).

The water-soluble extracts from barley, lake water rich in blue green algae, corn, cotton, onion, peanut cell culture, and soybean all contained high levels of GSH or  $\gamma$ -glutamylcysteine conjugates (Figure 5). Extracts from barley and blue green algae contained only one GSH conjugate, S-(PCP)GSH. Extracts from the other tissues contained both S-(PCP)GSH and S-(TCNP)GSH. The concentration of S-(TCNP)GSH was actually greater than the concentration of S-(PCP)GSH in soybean and peanut. Corn and cotton were low in S-(PCP)GSH, but contained large amounts of S-(pentachlorophenyl)- $\gamma$ -glutamylcysteine [S-(PCP)- $\gamma$ -GluCys]. The  $\gamma$ -glutamylcysteine conjugate was probably formed by catabolism of the GSH conjugate. Products that chromatographed in the region of the diglutathione and dicysteine conjugates were present in

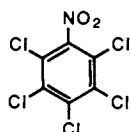


Figure 2. Pentachloronitrobenzene (PCNB, tetrachlor, brassicol, quintozene, etc.)

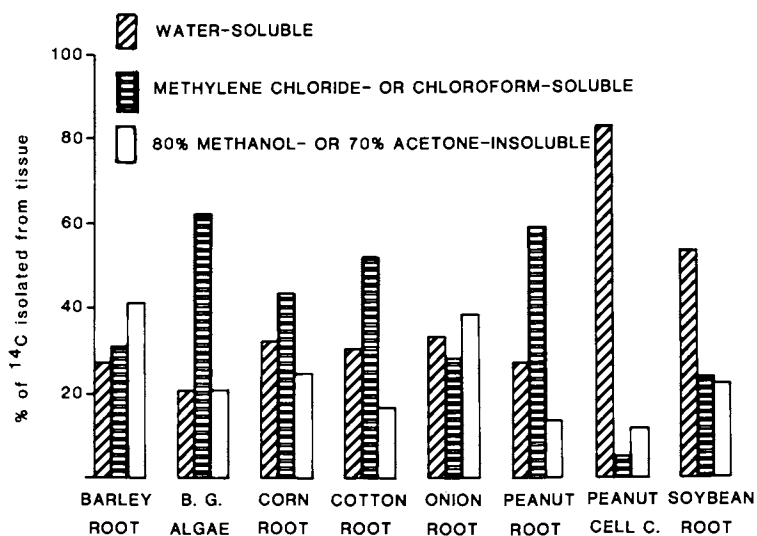


Figure 3. Distribution of  $^{14}\text{C}$  in plant tissues as a function of solubility. Barley, corn, cotton, peanut cell cultures, and soybeans were treated with  $[^{14}\text{C}]$  PCNB for 3 days. Lake water rich in blue green algae was treated for 9 h. Peanut plants were treated for 2 days and subjected to a 2-day post-treatment incubation.

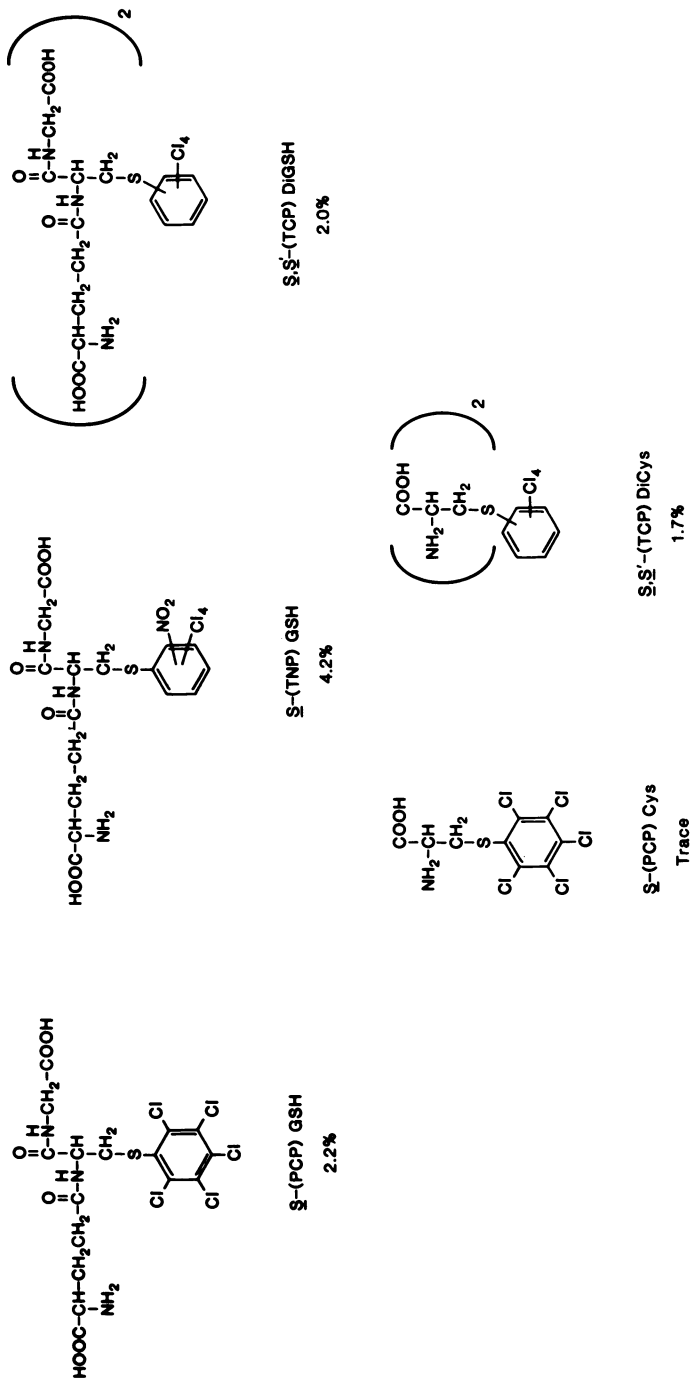


Figure 4. Water-soluble metabolites isolated from the roots of peanut plants treated with [ $^{14}\text{C}$ ] PCNB. This represents 13.6% of the  $^{14}\text{C}$  isolated from the roots.

most of these tissues; however, so many different disubstituted products were possible that tentative assignments of these products were not made.

Ether-soluble residues. The ether-soluble fraction from the roots of [ $^{14}\text{C}$ ]PCNB-treated peanut plants contained 13.7% of the radioactivity in the roots. Ion exchange chromatography indicated that 90% of the radioactivity in this fraction was in the form of S-(pentachlorophenyl)-N-malonylcysteine [S-(PCP)MalCys] and S-(tetrachloronitrophenyl)-N-malonylcysteine [(S-(TCNP)MalCys)] (Figure 6). These metabolites were identified by mass spectral studies of various esters (6). N-Malonylcysteine conjugates were important metabolites in all of the plant systems examined except onion (Figure 7). They accounted for 37% of the radioactivity in the aqueous extracts of peanut cells, 44% in peanut roots, and 35% in cotton roots. Species that produced primarily S-(PCP)GSH, barley and blue green algae, also produced primarily S-(PCP)MalCys. Species that produced significant amounts of S-(TCNP)GSH, cotton, peanut, and soybean, also produced significant amounts of S-(TCNP)MalCys. Onion, which did not produce an N-malonylcysteine conjugate, and corn, which produced only small amounts, were the two species that produced large amounts of S-(PCP)- $\gamma$ -GluCys.

N-Malonylcysteine conjugate formation appeared to involve the GSH conjugation pathway via dipeptide and cysteine conjugate intermediates as shown in Figure 8. The precursor-product relationship between the GSH conjugates and the N-malonylcysteine conjugates was demonstrated in peanut cell cultures (Figure 9). S-(Pentachlorophenyl)glutathione and S-(TCNP)GSH were initial products of metabolism in peanut cell culture and appeared to be converted to S-(PCP)MalCys and S-(TCNP)MalCys, respectively. Intermediates in these conversions were not clearly evident. Peanut cell cultures treated with S-[( $^{14}\text{C}$ )PCP]Cys and harvested 24 hr later produced S-[( $^{14}\text{C}$ )PCP]MalCys in almost quantitative yield. Peanut plants treated with S-[( $^{14}\text{C}$ )PCP]Cys and harvested 20 days later produced S-[( $^{14}\text{C}$ )PCP]MalCys in approximately 28% yield (based on  $^{14}\text{C}$  present in the roots at harvest). This was consistent with a cysteine conjugate intermediate and suggested that the N-malonyl transferase reaction was very fast. The N-malonylcysteine conjugates of PCNB were stable in the peanut cell cultures and they appeared to be end-products of metabolism (Figure 10).

S-(Tetrachloronitrophenyl)-N-malonylcysteine was not produced in sufficiently high yield in peanut cell culture to be the only product of S-(TCNP)GSH metabolism (Figure 9). S,S'-(Tetrachlorophenylene)dicysteine may have been produced from S-(TCNP)GSH after a second reaction with GSH. The metabolite at fraction 75 was not identified in the aqueous extracts from peanut cell cultures. It appeared to be formed from S,S'-(TCP)diCys and it may have been an N-malonylcysteine conjugate.

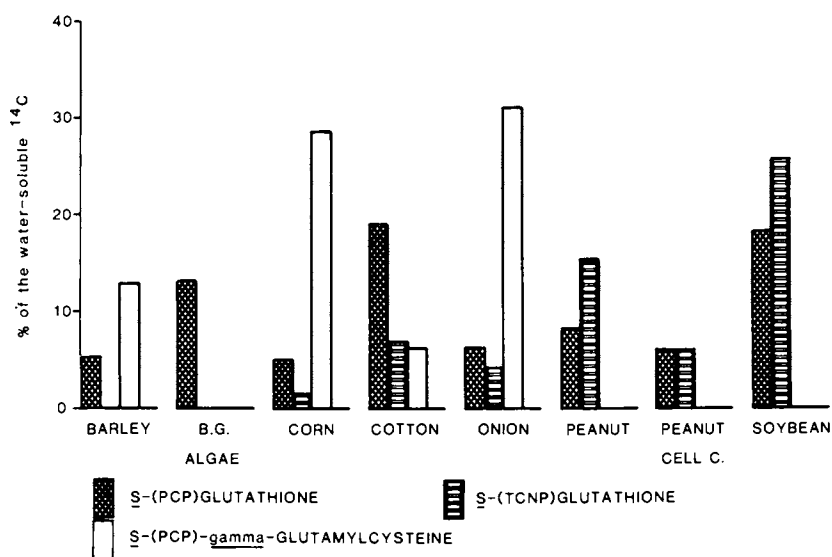


Figure 5. The percent S-(PCP)GSH, S-(TCNP)GSH, and S-(PCP)- $\gamma$ -GluCys in the water-soluble fractions from plant tissues treated with [ $^{14}$ C] PCNB. Treatment times are as described in Figure 3.

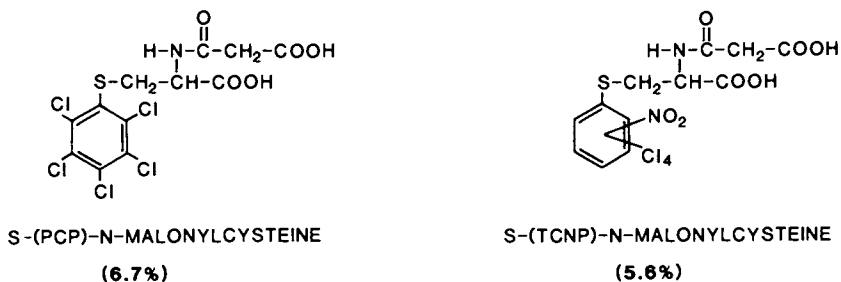


Figure 6. Ether-soluble metabolites isolated from the roots of peanut plants treated with [ $^{14}$ C] PCNB. This represents 13.7% of the  $^{14}$ C isolated from the roots.

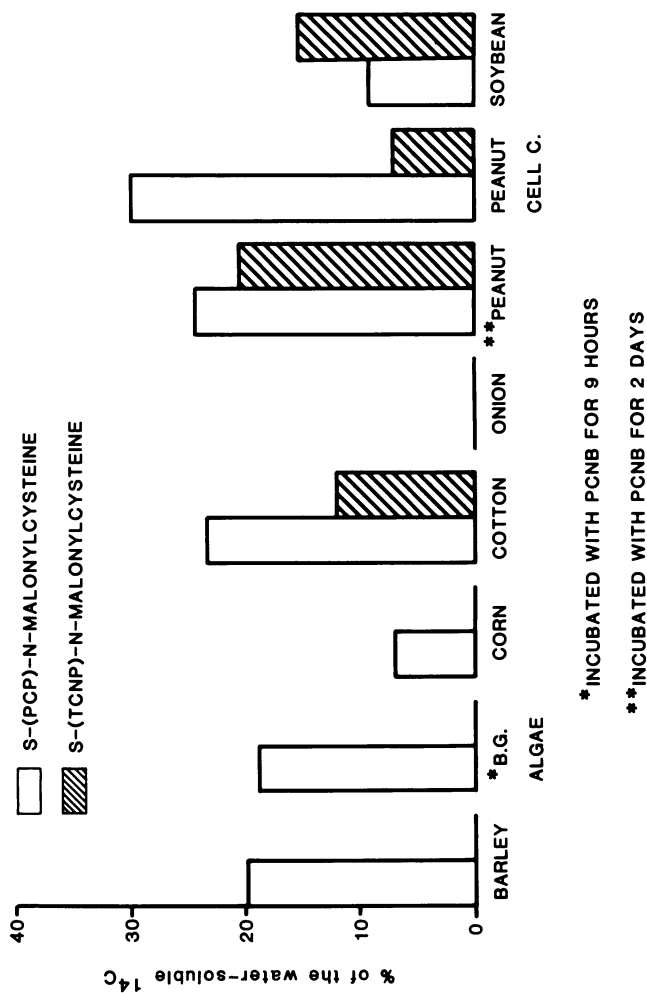


Figure 7. Percent S-(PCP)MalCys and S-(TCNP)MalCys in the water-soluble fractions from plant tissues treated with [ $^{14}\text{C}$ ] PCNB. Treatment times are as described in Figure 3.



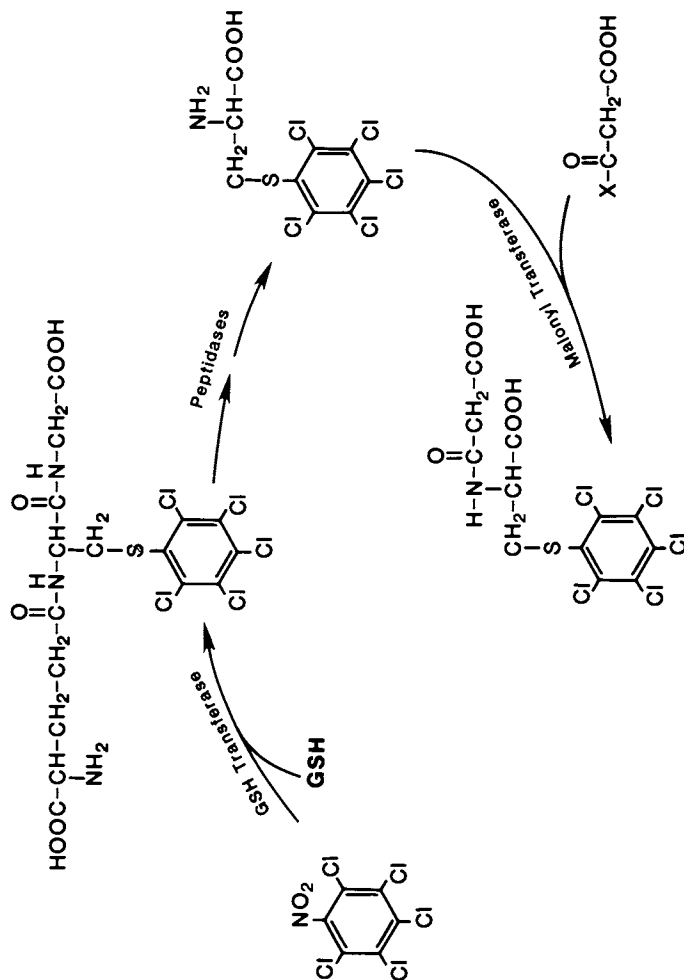


Figure 8. Probable origin of N-malonylcysteine conjugates in plants

The formation of *N*-malonyl conjugates of a variety of *D*-amino acids has been demonstrated in many plant species and is thought to be a detoxification mechanism (10, 11, 12). *N*-Malonylcysteine conjugates have not been commonly reported as metabolites of pesticides in plants. Shimabukuro *et al.* (13) previously reported *N*-(malonyl)-*S*-(2-nitro-4-trifluoromethylphenyl)cysteine as a metabolite of fluorodifen in peanut. The corresponding GSH conjugate was also detected (14). The *N*-malonylcysteine conjugates of PCNB and fluorodifen were probably derived from structurally similar precursors; therefore, it seemed desirable to determine whether a very different GSH conjugate would be metabolized to an *N*-malonylcysteine conjugate in a different plant species. Propachlor had been previously shown to be metabolized to a GSH conjugate in several plant species (15). Therefore, propachlor was used as a biochemical probe to test for *N*-malonylcysteine conjugate formation in soybean.

Soybeans grown in hydroponics were treated with [ $^{14}\text{C}$ -carbonyl]propachlor by incorporation of the herbicide into the nutrient solution. The plants were harvested after 4 and 21 days and the water-soluble metabolites in the 80% methanol extracts from the roots were examined by HPLC. The GSH conjugate and the  $\gamma$ -glutamylcysteine conjugate, major metabolites after 4 days, were identified by chromatographic comparison to standards. The *N*-malonylcysteine conjugate, a major metabolite after 21 days, was identified by mass spectrometry of the diethyl ester. The *N*-malonylcysteine conjugate, the glutathione conjugate, and insoluble residue were the most abundant products after 21 days (Figure 11).

Insoluble residues. Insoluble residue in the roots of peanuts accounted for 13.5% of the applied  $^{14}\text{C}$  48 hr after treatment with [ $^{14}\text{C}$ ]PCNB. This increased to 21% after 33 days. The other plant systems examined also produced insoluble residue after short-term treatment with [ $^{14}\text{C}$ ]PCNB (Figure 3). The values indicated for insoluble residues in systems other than peanut and peanut cell culture may be too high because these tissues were extracted with 70% acetone (1X) and acetone (2X) rather than with 80% methanol (3X).

Insoluble residues have been reported as important end-products in the metabolism of other pesticides known to be metabolized by GSH conjugation in higher plants (4, 6, 14, 15). Some insight into the mechanisms of insoluble residue formation was obtained by comparing the metabolism of [ $^{14}\text{C}$ ]PCNB, *S*-[( $^{14}\text{C}$ )PCP]Cys and pentachlorothiophenol-UL- $^{14}\text{C}$  ([ $^{14}\text{C}$ ]PCTP) in peanut root and peanut cell culture (Figure 12).

Insoluble residue accounted for only 12% of the applied  $^{14}\text{C}$  in peanut cell cultures harvested 14 days after treatment with [ $^{14}\text{C}$ ]PCNB; however, insoluble residue accounted for 37% of the  $^{14}\text{C}$  isolated from the roots of peanut plants 33 days after treatment. When peanut cell cultures were treated with

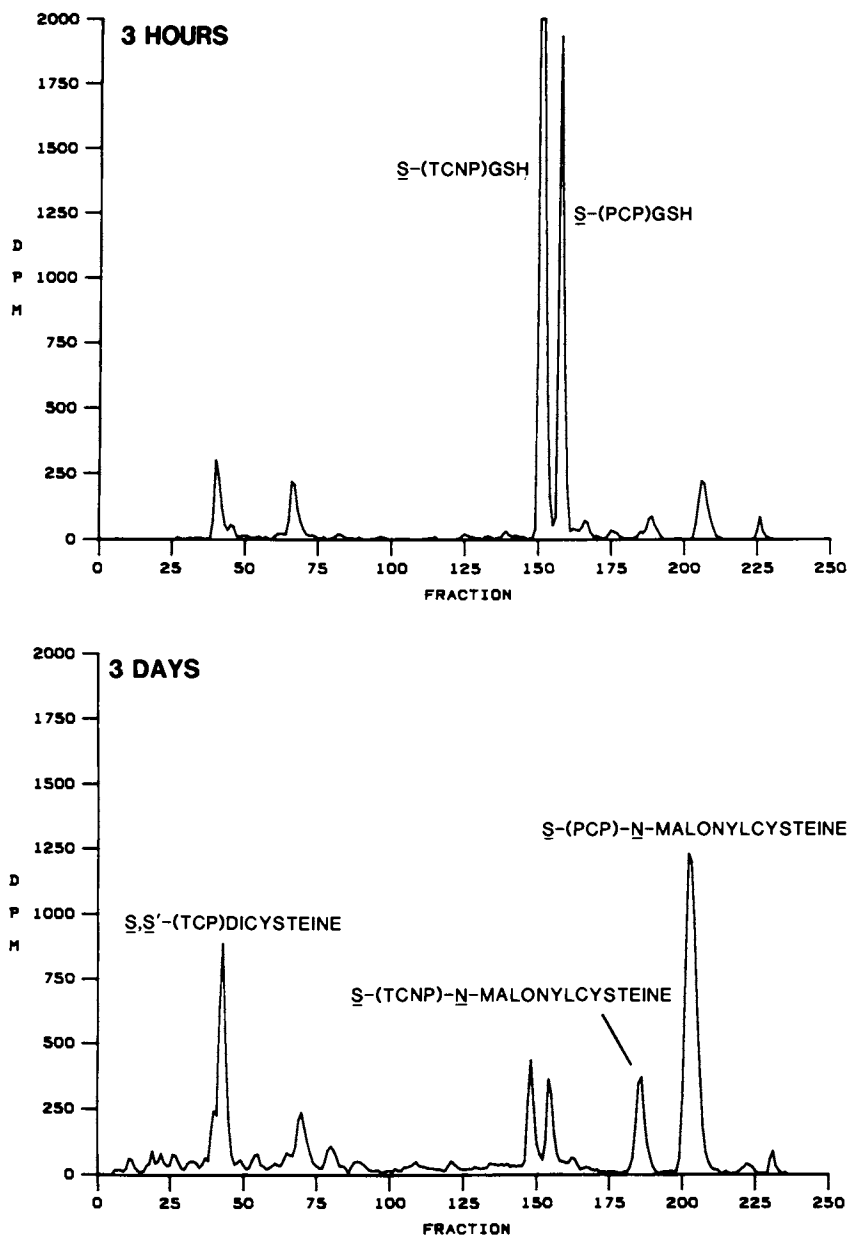
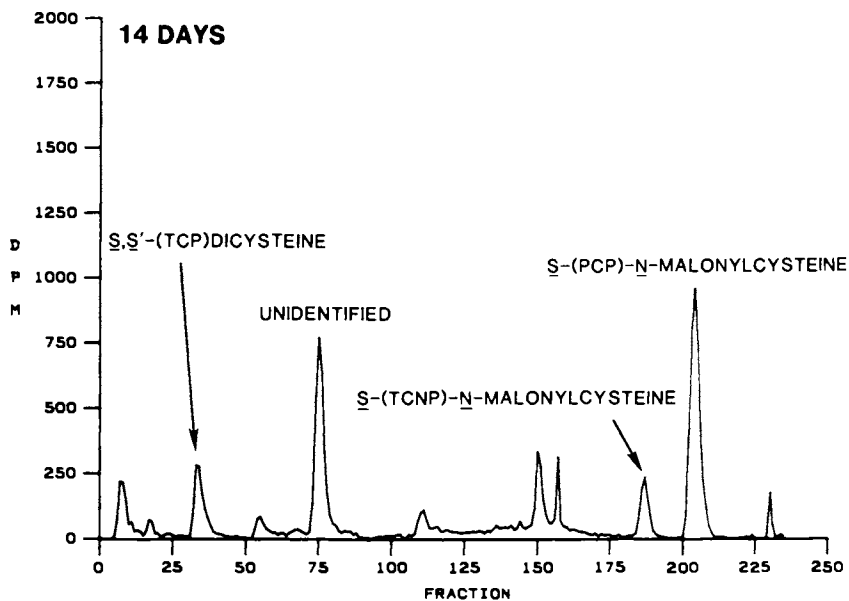
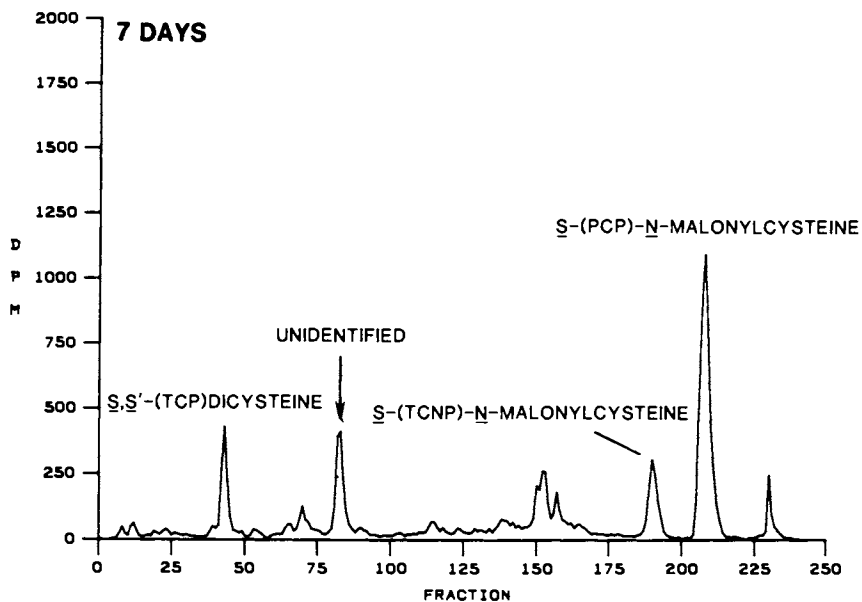


Figure 9. HPLCs of water-soluble extracts from peanut cell cultures treated with  $[^{14}\text{C}]$  PCNB. Extracts were chromatographed on a column of  $\text{C}_{18}$  with a water/acetonitrile/acetic acid gradient similar to that described previously (6).



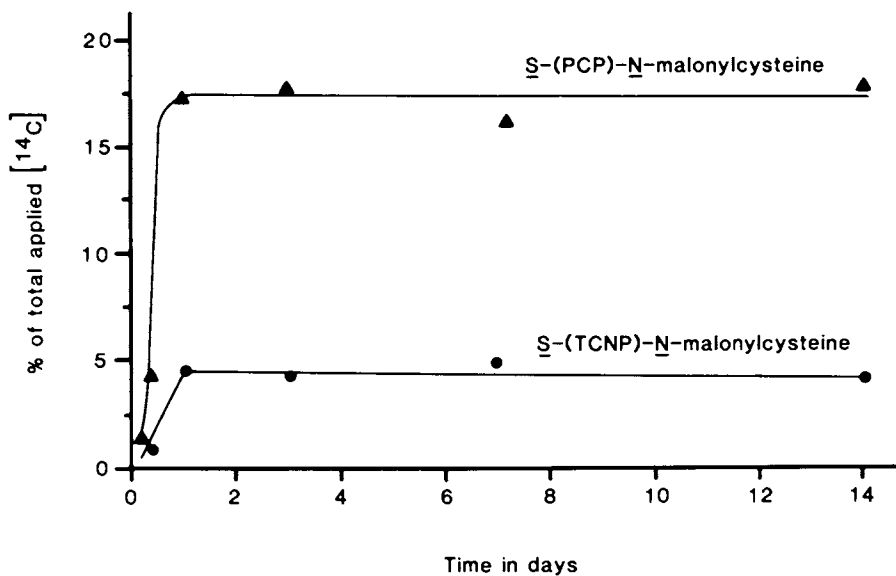
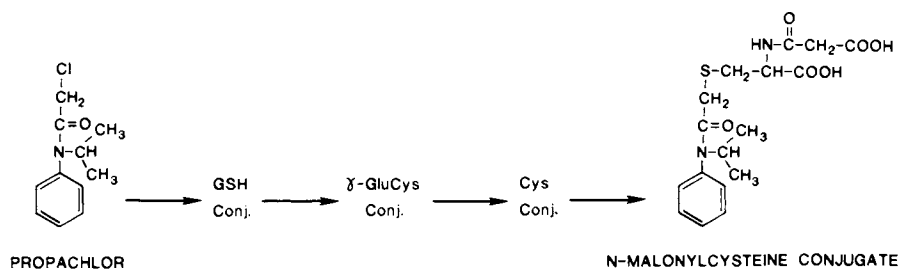


Figure 10. Formation and stability of S-(PCP)MalCys and S-(TCNP)MalCys in peanut cell cultures treated with [<sup>14</sup>C] PCNB. Concentrations were measured by HPLC and structures were verified by MS.



NATURE OF <sup>14</sup> C RESIDUE	PERCENT DISTRIBUTION IN THE ROOTS	
	AFTER 4 DAYS	AFTER 21 DAYS
INSOLUBLE RESIDUE	19	38
GLUTATHIONE CONJUGATE	44	22
γ-GLUTAMYL-CYSTEINE CONJUGATE	32	3
N-MALONYLCYSTEINE CONJUGATE	3	25

Figure 11. Metabolism of propachlor to an N-malonylcysteine conjugate in soybean root. Soybeans treated with [<sup>14</sup>C-carbonyl] propachlor were harvested after 4 and 21 days. The glutathione and γ-glutamylcysteine conjugates were identified by HPLC and the N-malonylcysteine conjugate was identified by the MS of the diethyl ester.

American Chemical  
Society Library  
1155 16th St. N. W.

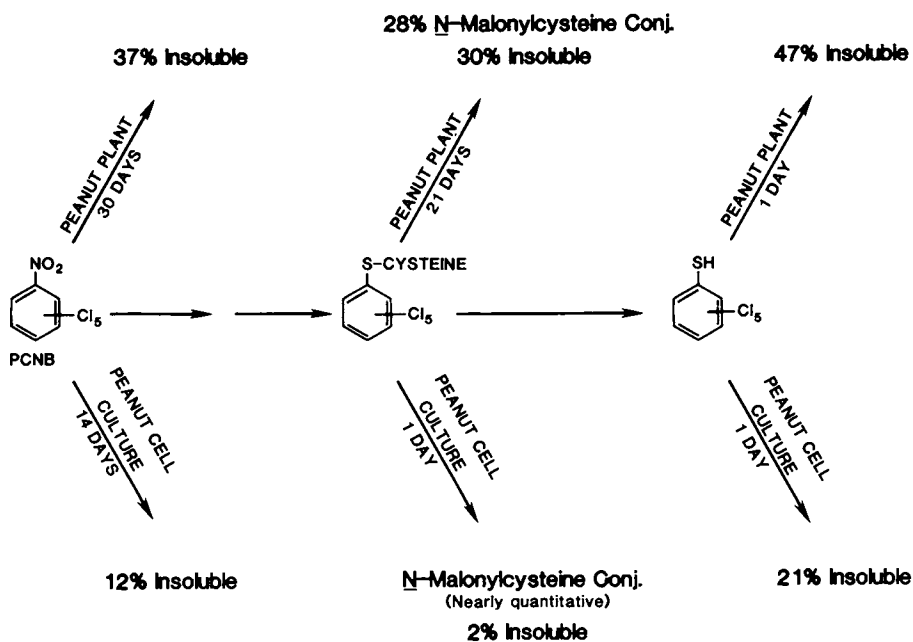


Figure 12. Formation of 80% methanol-insoluble residues in peanut plants (root) and peanut cell suspension cultures treated with  $[^{14}\text{C}]$  PCNB, S- $[(^{14}\text{C})\text{PCP}]\text{Cys}$ , and pentachlorothiophenol- $\text{UL-}^{14}\text{C}$

S-([<sup>14</sup>C]PCP)Cys, only 2% insoluble residue was formed and conversion to S-(PCP)MalCys was nearly quantitative. Since S-(PCP)MalCys is stable in peanut cell culture, it would not be expected to give rise to large amounts of insoluble residue. In contrast, 21 days after peanut plants were treated with S-([<sup>14</sup>C]PCP)Cys, 30% of the <sup>14</sup>C was converted to insoluble residue and 28% was converted to S-(PCP)MalCys. Peanut plants treated with pentachlorothiophenol-UL-<sup>14</sup>C for 1 day produced 80% methanol-insoluble residue in 47% yield (7). Comparable results were observed in peanut cell cultures treated for 1 day with pentachlorothiophenol-UL-<sup>14</sup>C (21% insoluble residue). The results are consistent with a pathway in which S-(PCP)Cys is converted to insoluble residue through a pentachlorothiophenol intermediate. Differences in the amount of insoluble residue formed from PCNB or S-(PCP)Cys in peanut cell culture versus peanut roots could be explained by differences in the relative activities of two competing enzymes, cysteine C-S lyase (produces pentachlorothiophenol from S-(PCP)Cys) and N-malonyltransferase (produces N-malonylcysteine conjugates from S-(PCP)Cys or S-(TCNP)Cys). Barley and onion, which apparently contain very active C-S lyase systems, were the two species that formed the highest levels of insoluble residues. Other mechanisms for the formation of insoluble residues from GSH conjugates probably exist and additional research is needed to determine the nature and relative importance of these pathways.

Methylene chloride-soluble residues. Methylene chloride- or chloroform-soluble <sup>14</sup>C-labeled products were major residues in all of the plant tissues examined except peanut cell cultures (Figure 3). Chloroform-soluble <sup>14</sup>C accounted for 59.2% of the radioactivity isolated from peanut roots 48 hr after treatment with [<sup>14</sup>C]PCNB. The radioactivity was in the form of PCNB (28.7%), pentachloroaniline (22.5%), pentachlorothiophenol (2.6%); pentachlorothioanisole (3.1%); pentachlorothioanisole sulfoxide (0.5%); S-(pentachlorophenyl)-2-thioacetic acid [S-(PCP)ThioAcetate] (0.5%); and S-(pentachlorophenyl)-3-thio-2-hydroxypropionic acid [S-(PCP)ThioLactate] (0.2%); and S-(PCP)Cys (trace) (7). The structures of these compounds are shown in Figure 13. Based on TLC, the last three compounds in this list were classified as polar chloroform- or methylene chloride-soluble residues and the remaining compounds were classified as nonpolar residues.

The amount of chloroform-soluble <sup>14</sup>C in the roots of peanut plants grown in hydroponics decreased greatly as a function of time. After 33 days, chloroform soluble <sup>14</sup>C accounted for only 5% of the <sup>14</sup>C in the roots. This was probably due to metabolism of the remaining PCNB, volatilization of some metabolites, translocation to foliar tissue, and additional metabolism of non-polar metabolites to polar metabolites or insoluble residue. Because of volatility, it is possible that chloroform-soluble



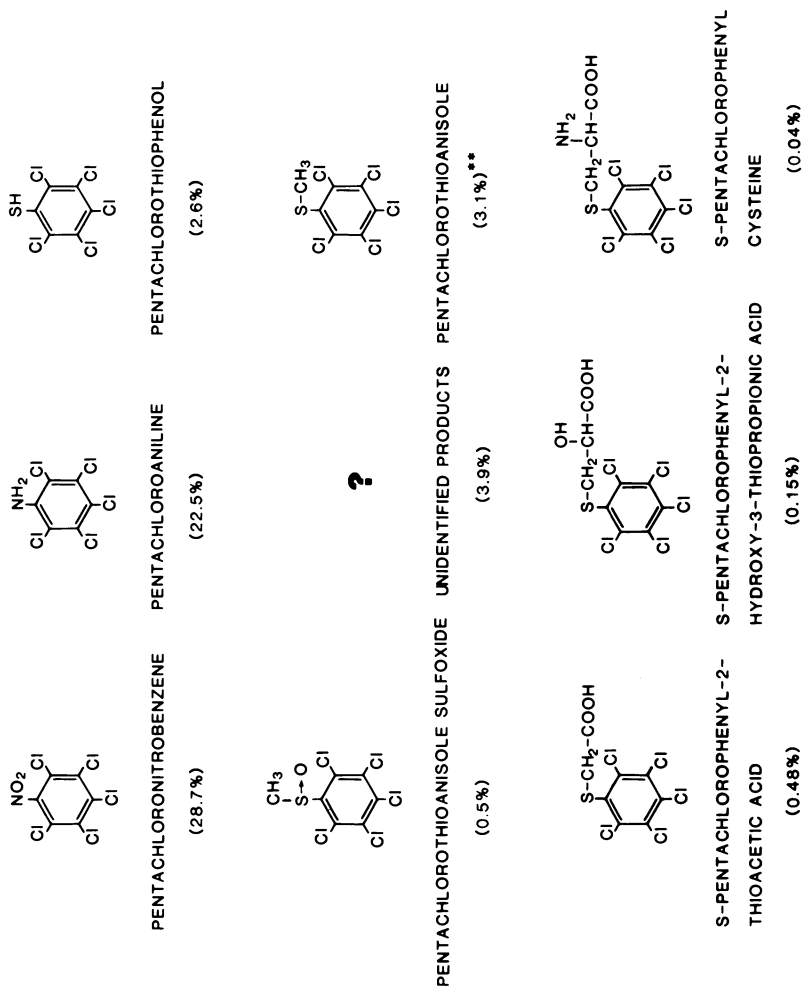


Figure 13. Structures of chloroform-soluble residues isolated from peanut roots treated with [ $^{14}\text{C}$ ] PCNB. Chloroform-soluble  $^{14}\text{C}$  accounted for 59.2% of the  $^{14}\text{C}$  in peanut roots.

residues would continue to be more important in plants grown in soil than plants grown in aerated nutrient solution.

Methylene chloride-soluble radioactivity accounted for only 4-5% of the applied  $^{14}\text{C}$  in peanut cell cultures harvested 3 days after treatment with [ $^{14}\text{C}$ ]PCNB. This value remained fairly constant between 3 days and 14 days. Pentachloroanisole and pentachloroaniline accounted for 1.1% and 3.9% of the applied  $^{14}\text{C}$  in the cultures 24 hr after treatment. Pentachloroaniline increased to 10.5% of the applied  $^{14}\text{C}$  in cultures that were not shaken during the 24-hr treatment, indicating that pentachloroaniline formation was favored under conditions of low oxygen tension. The enzymatic formation of pentachloroaniline from PCNB in the presence of NADPH, FAD, and an enzyme from peanut occurred only under anaerobic conditions (7).

Pentachloroaniline metabolism. Pentachloroaniline represented an important nonpolar residue in many of the plant species examined (Figure 14). Since pentachloroaniline is formed by a reaction initially competitive with GSH conjugation, this residue was considered separately from the nonpolar chloroform-soluble residues. The metabolism of pentachloroaniline was examined briefly in peanut plants treated with pentachloroaniline- $^{14}\text{C}$  and harvested after 20 days. The roots of these plants contained 41.9% of the  $^{14}\text{C}$  as pentachloroaniline, 30.4% as insoluble residue, and 27.7% as polar conjugates. The polar conjugate fraction was analyzed by HPLC. Two products chromatographed in the region of the GSH conjugates of PCNB and two products chromatographed in the region of the *N*-malonyl-cysteine conjugates. When esterified and acetylated, the suspected GSH conjugates yielded derivatives that had TLC properties consistent with esterified, acetylated GSH conjugates. The products that chromatographed in the region of the *N*-malonyl-cysteine conjugates were ether-soluble at pH 2, a property consistent with *N*-malonylcysteine conjugates. After esterification, these metabolites had TLC properties consistent with esters of *N*-malonylcysteine conjugates. Additional studies are needed, but these preliminary data would suggest that pentachloroaniline may be slowly metabolized by GSH conjugation.

Polar methylene chloride-soluble residues. Polar methylene chloride-soluble residues were found in most of the plant tissues treated with [ $^{14}\text{C}$ ]PCNB (Figure 14). These products were only identified in peanut (7). The polar methylene chloride-soluble metabolites from peanut, *S*-(PCP)Cys, *S*-(PCP)ThioAcetate, and *S*-(PCP)ThioLactate, were probably produced from *S*-(PCP)GSH by the pathway shown in Figure 15. Intact peanut plants treated with *S*-[( $^{14}\text{C}$ )PCP]Cys and harvested 20 days later yielded *S*-[( $^{14}\text{C}$ )PCP]ThioAcetate in 7.3% yield; however, *S*-[( $^{14}\text{C}$ )PCP]ThioLactate was not detected. An *S*-substituted 2-thioacetic acid metabolite has also been reported in the metabolism of EPTC in the rat (16).

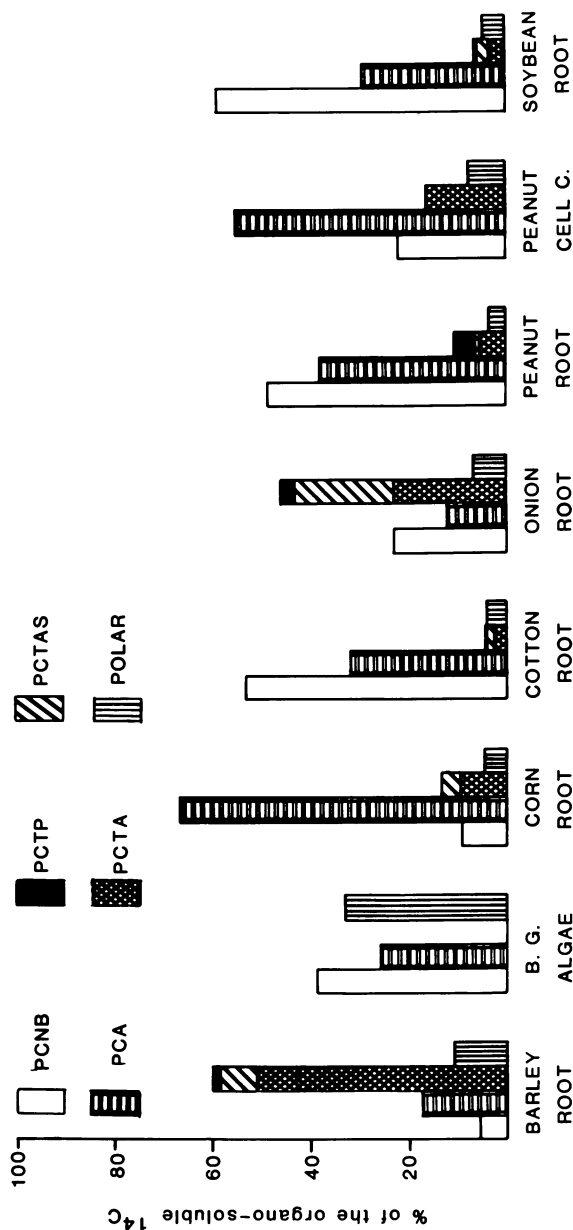


Figure 14. Methylene chloride- or chloroform-soluble residues were isolated from plant tissues treated with [<sup>14</sup>C] PCNB. All tissues were treated for 3 days except lake water which is rich in blue green algae (9 h), peanut plants (2-day treatment/2-day post-treatment), and peanut cell cultures (1 day).

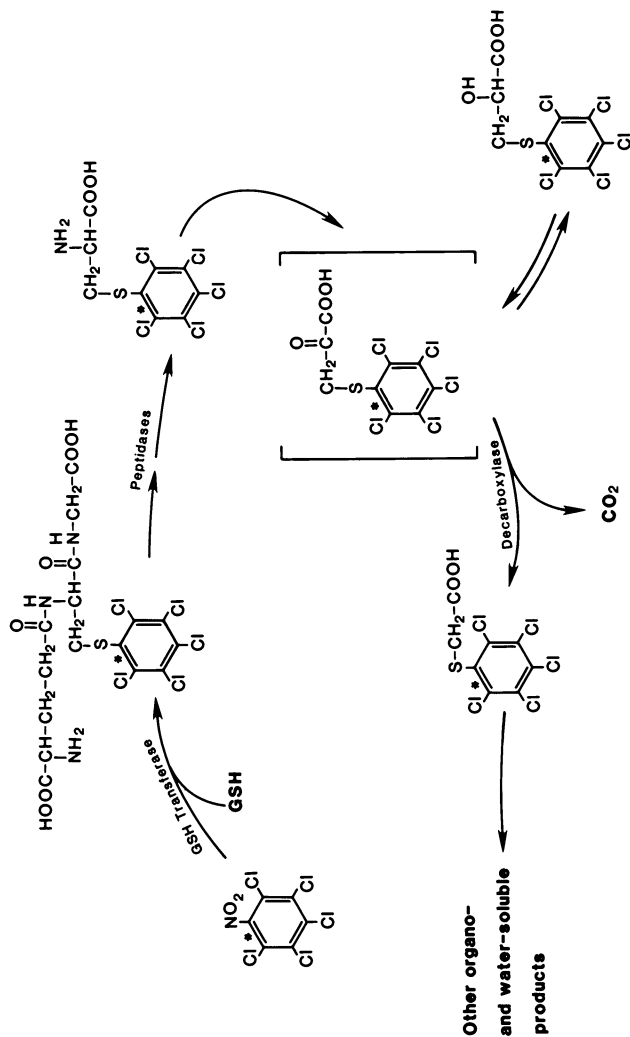


Figure 15. Origin of S-(PCP)thioacetate and S-(PCP)thiolactate in peanut roots

Therefore, a similar pathway appears to operate in certain mammals. When  $S$ -(PCP)ThioAcetate was introduced into peanut plants, pentachlorothioanisole was not formed. However, other metabolites were detected, possibly glucose and amino acid conjugates similar to those reported for 2,4-D (17).

Nonpolar methylene chloride-soluble residues. Pentachlorothioanisole and pentachlorothioanisole sulfoxide were present in the nonpolar methylene chloride-soluble fraction from each of the plant systems examined (Figure 14). In addition, pentachlorothiophenol was detected in some of these extracts. Pentachlorothioanisole has been reported as an important residue of PCNB in almost every biological system that has been examined for PCNB metabolism and pentachlorothiophenol has also been reported as a residue in several of these systems (6). The formation of these residues from  $S$ -(PCP)GSH via the pathway shown in Figure 16 was considered highly probable. Recent in vivo studies indicated that such a system also operates in mammals in the metabolism of propachlor (18, 19) and pentachlorothioanisole (20). In vitro studies with rat liver preparations also suggested that such a system operates in the metabolism of bromazepam (21).

An in vitro enzyme system from onion was used to show that this pathway was operative in plants (9, 22). Onion was chosen as the source of enzymes because pentachlorothioanisole was an important metabolite of PCNB in onion (23).

An active glutathione  $S$ -transferase system was detected in the onion enzyme system when it was assayed with [ $^{14}\text{C}$ ]PCNB and GSH (9). An initial rate of 14 nmol product/mg protein/hr was observed and a yield of 18% was obtained in 17 hr. HPLC indicated that  $S$ -(PCP)GSH was the only major conjugated product of this reaction. This was consistent with the in vivo studies with onion that showed that  $S$ -(PCP)GSH was the dominant GSH conjugate formed. In contrast, an enzyme from pea produced  $S$ -(PCP)GSH,  $S$ -(TCNP)GSH, and what appeared to be two isomeric  $S, S'$ -(TCP)diGSH conjugates (6).

$S$ -(Pentachlorophenyl)glutathione was rapidly degraded to methylene chloride-soluble products when it was incubated with the onion enzyme system in the absence of GSH and PCNB (Figure 17). The initial rate of this reaction, based on substrate disappearance, was 57 nmol/mg protein/hr. The primary methylene chloride-soluble product was the disulfide dimer of pentachlorothiophenol (9). The degradation of  $S$ -(PCP)GSH by this enzyme system was strongly inhibited by GSH,  $\gamma$ -glutamylglutamate, and phenylalanyl glycine. The primary water-soluble component remaining after each of these reactions was shown by HPLC to be  $S$ -(PCP)GSH; however, a significant amount of  $S$ -(PCP)- $\gamma$ -GluCys was detected in the reaction inhibited by  $\gamma$ -glutamylglutamate, and traces of this metabolite were found in most of the reaction mixtures.  $S$ -(Pentachlorophenyl)cysteine was not detected in any of the reaction mixtures. If the formation of pentachlorothio-

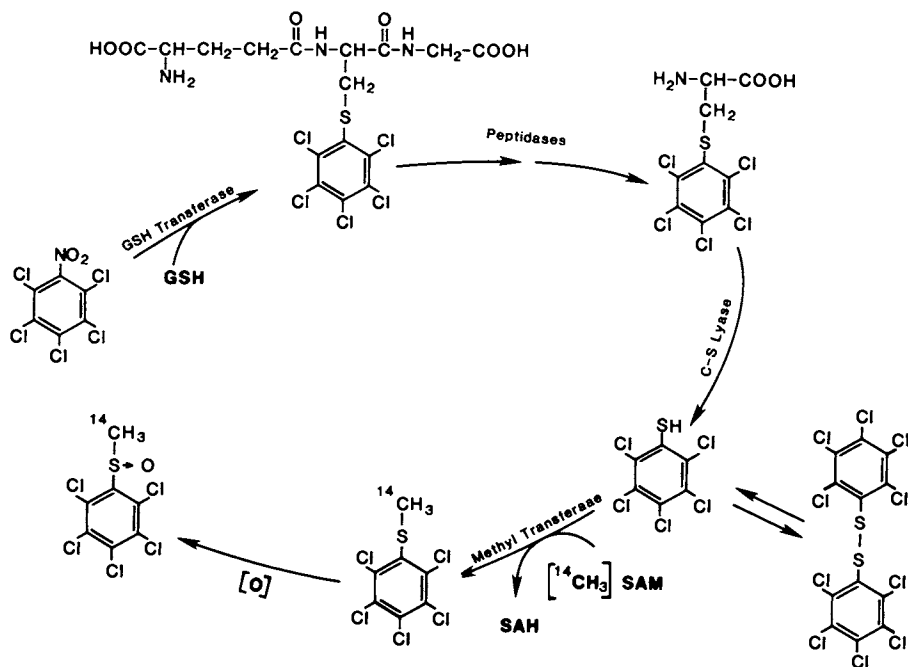
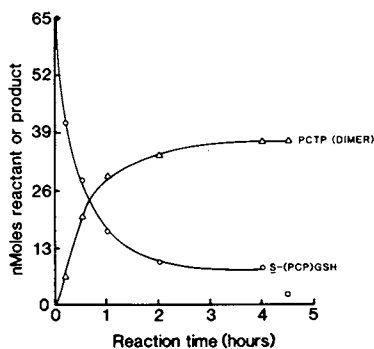


Figure 16. *In vitro* formation of pentachlorothiophenol, pentachlorothioanisole, and pentachlorothioanisole sulfoxide from PCNB



Pesticide Biochemistry and Physiology

Figure 17. Breakdown of S-(PCP)GSH in the presence of the enzyme from onion (9)

phenol proceeded in the manner described in Figure 16, peptides would be expected to be inhibitors. Failure to demonstrate S-(PCP)Cys as an intermediate would not be inconsistent with this reaction sequence if the C-S lyase reaction was much faster than the peptidase reactions.

The immediate precursor of pentachlorothiophenol was assumed to be S-(PCP)Cys. Cysteine C-S lyase enzymes that convert S-aryl and S-alkyl derivatives of cysteine to pyruvate and a thioalcohol have been detected in some plant species (24, 25). When the onion enzyme system was assayed for C-S lyase activity with S-(2,4-dinitrophenyl)cysteine as the substrate, pyruvate and 2,4-dinitrothiophenol were liberated in a nearly 1:1 ratio (9). The initial reaction was very fast (420 nmol product/mg protein/hr) and was complete within 5 min. S-(Pentachlorophenyl)cysteine was also a substrate for this enzyme system and yielded an initial rate of 120 nmol product/mg protein/hr. The primary radioactive product from this reaction, identified by TLC and mass spectrometry, was the disulfide dimer of pentachlorothiophenol. The cysteine C-S lyase reactions with S-(PCP)Cys and S-(2,4-dinitrophenyl)cysteine as substrates were both stimulated by GSH and  $\gamma$ -glutamylglutamate, peptides that inhibited the release of 2,4-dinitrothiophenol and pentachlorothiophenol dimer from their corresponding GSH conjugates. Peptide inhibition of pentachlorothiophenol formation from S-(PCP)GSH, but not from S-(PCP)Cys, is consistent with the pathway outlined in Figure 16.

The immediate precursor of pentachlorothioanisole was assumed to be pentachlorothiophenol. The onion enzyme system was assayed for pentachlorothioanisole synthesis with pentachlorothiophenol and [ $^{14}$ C-methyl] S-adenosyl-methionine ([ $^{14}$ C]SAM) as the substrates. An enzyme dependent reaction with an initial rate of 22.8 nmol product/mg protein/hr was observed (9). After 1 hr the yield of pentachlorothioanisole was 13% and after 17 hr it was 16.5%. The reaction was stimulated by dithiothreitol and was inhibited by S-adenosyl-homocysteine. The methyl transferase reaction was easily coupled with the cysteine C-S lyase reaction by using S-[( $^{14}$ C)PCP]Cys and nonradioactive SAM as the substrates. The rate of formation of pentachlorothioanisole from S-(PCP)Cys was the same as from pentachlorothiophenol. Large amounts of pentachlorothiophenol were formed during the coupled reaction. Apparently the methyl transferase reaction was rate-limiting in the coupled system.

The direct *in vitro* synthesis of pentachlorothioanisole from PCNB, GSH, and [ $^{14}$ C]SAM in the presence of the onion enzyme system was attempted. After an initial lag period of about 30 min, a moderate rate of pentachlorothioanisole formation was observed, 2.3 nmol/mg protein/hr. After 16 hr, a yield of approximately 17% was observed (9). It was concluded that pentachlorothioanisole detected in various plant tissues could easily be produced from the GSH pathway.

Methylthioethers have not been commonly reported as metaboli-

tes of pesticides in plants; therefore, it is difficult to assess their importance as residues arising from the GSH pathway. If the postulated mechanism of pentachlorothioanisole formation in plants is correct, the formation of methylthioethers as pesticide metabolites would depend upon the presence of GSH S-transferases, peptidases, cysteine C-S lyases, and methyltransferases.

Methyltransferase enzymes are widespread in the plant kingdom, but they are frequently very substrate specific. The substrate specificity of the methyl transferase from the onion enzyme system was tested with 18 different substrates (Table I). Pentachlorothiophenol was the best substrate tested; however, four other substrates showed high levels of activity and only nine substrates showed less than 5% of the activity of pentachlorothiophenol. The three most active substrates were ortho substituted thiophenols.

### Summary and Conclusions

The sequence of reactions that apparently occurs in the metabolism of PCNB are shown in Figure 18. The first step, conjugation with glutathione, occurred at several different sites. The glutathione conjugates appeared to be converted to dipeptide conjugates. In onion and corn, S-(PCP)- $\gamma$ -GluCys was a major residue after three days and the  $\gamma$ -glutamylcysteine conjugate of propachlor was an important transitory intermediate in soybean. These results were consistent with a previous observation on the metabolism of the GSH conjugate of atrazine through a  $\gamma$ -glutamylcysteine conjugate in sorghum (Figure 1).

The cysteine conjugates appeared to be key metabolites, occupying pivotal positions in the pathway. S-(Pentachlorophenyl)cysteine was not demonstrated in vitro, but it was a minor metabolite in peanut plants. This anomaly appeared to be due to the kinetics of the various reactions. A cysteine conjugate was clearly shown to be a key intermediary metabolite in the metabolism of the GSH conjugate of atrazine in sorghum (Figure 1).

The N-malonylcysteine conjugates were important metabolites of PCNB in all of the species except onion and the formation of S-(PCP)MalCys from S-(PCP)Cys was demonstrated in both peanut roots and in peanut cell cultures. Propachlor was also converted in high yield to an N-malonylcysteine conjugate and an N-malonylcysteine conjugate of fluorodifen was reported previously (13). N-Malonylcysteine conjugates are probably common end-products in the metabolism of glutathione conjugates, perhaps analogous to the mercapturic acids produced in animals.

Pentachlorothiophenol was formed in vitro from S-(PCP)Cys by a C-S lyase enzyme from onion root. This enzyme was active with S-(PCP)Cys, S-(2,4-dinitrophenyl)cysteine, and the cysteine conjugate of propachlor. A C-S lyase from Albizia lophanta was previously shown to utilize a broad range of cysteine derivatives (24).



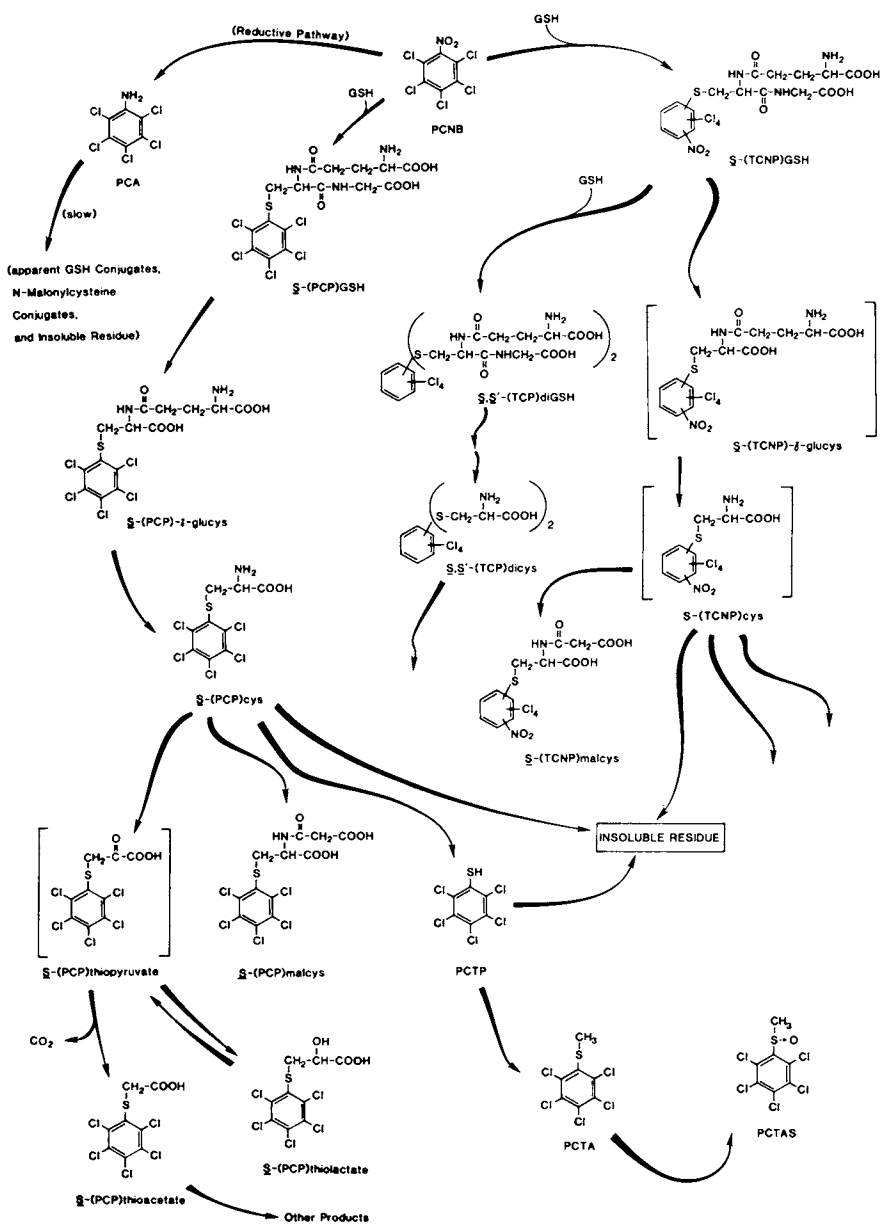


Figure 18. Metabolic pathway of PCNB in higher plants

Table I. ACTIVITY OF VARIOUS SUBSTRATES FOR THE METHYL TRANSFERASE SYSTEM FROM ONION ROOT<sup>a/</sup>

<u>Substrate</u>	<u>Relative Activity</u>
1. Pentachlorothiophenol	100
2. 2-Methoxythiophenol	54
3. 2,5-Dichlorothiophenol	52
4. 3-Methoxythiophenol	30
5. 4-Methoxythiophenol	20
6. 4-Acetamidothiophenol	15
7. 4-Nitrothiophenol	11
8. 2-Mercaptopyrimidine	7
9. 3,4-Dichlorothiophenol	6
10. 4-Methylthiophenol	5
11. 4-Chlorothiophenol	4
12. Thiophenol	2
13. 4-Hydroxy-3-methoxycinnamic acid	2
14. 4-Chlorobenzylmercaptan	0
15. 2-Mercaptoacetic acid	0
16. 3-Mercaptopropionic acid	0
17. Pentachloroaniline	0
18. Pentachlorophenol	0

<sup>a/</sup> The reaction mixtures contained 100  $\mu\text{M}$  thiol substrate, 500  $\mu\text{M}$  DTT, 100  $\mu\text{M}$  [ $^{14}\text{C}$ -methyl]SAM, 25 mM potassium phosphate buffer (pH 7.9), and 1 mg enzyme/ml. The reaction mixtures were incubated at 30° C for 2 hr, diluted with water, and partitioned with methylene chloride. The radioactivity in the methylene chloride phase was used as an index for the reaction. Relative activity was compared using pentachlorothiophenol as 100. All reactions were run in duplicate with appropriate controls in a manner similar to that previously described for pentachlorothiophenol (9).

Insoluble residue was the most abundant product of pentachlorothiophenol metabolism in peanut. The importance of this process is obviously dependent upon the presence of an active C-S lyase system. S-(Pentachlorophenyl)cysteine was also a precursor of insoluble residue in peanut, but the extent to which this involved pentachlorothiophenol as an intermediate was not determined. Additional studies are needed to determine if insoluble residues are commonly formed from GSH conjugates by other routes.

Pentachlorothioanisole was an important metabolite of PCNB in onion and barley and it was also present in most of the other tissues. Pentachlorothiophenol was an intermediary metabolite in the in vitro conversion of PCNB to pentachlorothioanisole. The methyl transferase reaction was demonstrated with pentachlorothiophenol and a number of other substrates. The importance of this reaction in pesticide metabolism needs further evaluation. Some conversion of pentachlorothioanisole to pentachlorothioanisole sulfoxide was observed in vivo, but this reaction was not specifically investigated.

S-(Pentachlorophenyl)-2-thioacetic acid and S-(PCP)ThioLactate were thought to be produced from S-(PCP)Cys by a transamination reaction. S-(Pentachlorophenyl)-2-thioacetic acid accounted for 7.3% of the  $^{14}\text{C}$  in peanut roots treated with S-(PCP)Cys. S-(Pentachlorophenyl)-2-hydroxy-3-thiol-propionic acid and S-(PCP)ThioAcetate were minor metabolites and their presence was not definitively established in tissues other than peanut root.

The metabolism of PCNB to S-(TCNP)GSH ultimately gave rise to S-(TCNP)MalCys in a manner that no doubt paralleled the metabolism of S-(PCP)GSH. The corresponding minor products of metabolism of S-(PCP)GSH were not observed with S-(TCNP)GSH. Several disubstituted metabolites were identified in peanut root. These were thought to be produced by a second reaction of glutathione with S-(TCNP)GSH or some metabolite derived from S-(TCNP)GSH. This would suggest that even polar compounds are not immune to conjugation with glutathione. bis-Methylmercaptotetrachlorobenzene was an important metabolite of PCNB in the Rhesus monkey (26). This metabolite might be formed from a dicysteine conjugate in the manner described for the formation of pentachlorothioanisole from S-(PCP)Cys in Figure 16 or by conversion to pentachlorothioanisole followed by a second reaction with GSH as described by Bakke et al. (19).

The only pathway competitive with GSH conjugation, aryl nitroreduction, gave rise to pentachloroaniline. Pentachloroaniline was very slowly metabolized in peanut roots, 42% of the  $^{14}\text{C}$  in peanut roots was still pentachloroaniline 20 days following treatment with pentachloroaniline-UL- $^{14}\text{C}$ . The major products of metabolism of pentachloroaniline appeared to be insoluble residue and products that had characteristics consistent with glutathione and N-malonylcysteine conjugates.

Evidence that pentachloroaniline may enter the GSH conjugation pathway is interesting in lieu of the fact that tetrachloroaminothioanisole was reported as a metabolite of PCNB in onion (23) and Rhesus monkey (26).

These studies provided strong evidence that N-malonylcysteine conjugates may be produced from a variety of pesticide GSH conjugates in a variety of important plant species. These conjugates appeared to be stable end-products of metabolism. Evidence was also provided that insoluble residues may be produced from GSH conjugates via cysteine conjugate or thiol intermediates. These studies also suggested that certain reactions should be studied in greater detail to assess their importance in pesticide metabolism: i.e., the C-S lyase reaction, the methyl transferase reaction, and the transamination reaction.

The possibility that the onion enzyme system might be used to classify GSH conjugates should be considered.  $\beta$ -Glucosidases have been used to classify pesticide metabolites as glucosides, but there has not been a comparable method for the classification of GSH conjugates. The enzymatic conversion of S-(PCP)GSH to the dimer of pentachlorothiophenol occurred under mild conditions and was presumably specific for GSH-related peptide conjugates. The ease of isolation and stability of the onion enzyme would make it ideally suited for this purpose. Likely deficiencies in such a system would be inhibition of the peptidase reactions by contaminating peptides and failure of N-malonylcysteine conjugates to undergo the reaction.

The methods used for the isolation and derivatization of the metabolites as well as the results from the detailed mass spectral studies presented in the original manuscripts (6, 7) should have broad application in studies dealing with GSH conjugates of pesticides in plants.

#### Glossary of Chemical Abbreviations

2,4-D	2,4-dichlorophenoxy acetic acid
DTT	dithiothreitol
FAD	flavin adenine dinucleotide
GSH	glutathione
NADPH	nicotinamide adenine dinucleotide phosphate
PCA	pentachloroaniline
PCNB	pentachloronitrobenzene
PCTA	pentachlorothioanisole
PCTAS	pentachlorothioanisole sulfoxide
PCTP	pentachlorothiophenol
SAM	S-adenosyl-L-methionine
S-(PCP)Cys	S-(pentachlorophenyl)cysteine
S-(PCP)- $\gamma$ -GluCys	S-(pentachlorophenyl)- $\gamma$ -glutamylcysteine
S-(PCP)GSH	S-(pentachlorophenyl)glutathione
S-(PCP)MalCys	S-(pentachlorophenyl)-N-malonylcysteine
S-(PCP)ThioAcetate	S-(pentachlorophenyl)-2-thioacetic acid

<u>S</u> -(PCP)ThioLactate	<u>S</u> -(pentachlorophenyl)-3-thio-2-hydroxypropionic acid
<u>S</u> , <u>S</u> '-(TCP)diCys	<u>S</u> , <u>S</u> '(tetrachlorophenylene)dicysteine
<u>S</u> , <u>S</u> '-(TCP)diGSH	<u>S</u> , <u>S</u> '-(tetrachlorophenylene)diglutathione
<u>S</u> -(TCNP)Cys	<u>S</u> -(tetrachloronitrophenyl)cysteine
<u>S</u> -(TCNP)- $\gamma$ -GluCys	<u>S</u> -(tetrachloronitrophenyl)- $\gamma$ -glutamylcysteine
<u>S</u> -(TCNP)GSH	<u>S</u> -(tetrachloronitrophenyl)glutathione
<u>S</u> -(TCNP)MalCys	<u>S</u> -(tetrachloronitrophenyl)- <u>N</u> -malonylcysteine

### Acknowledgment

The authors thank Jean-Marie Guout of Rhone-Poulenc for his research on [ $^{14}$ C]PCNB metabolism in peanut cell culture.

### Abstract

Pentachloronitrobenzene (PCNB) was used as the primary biochemical probe to study glutathione conjugate metabolism in plants. In peanut plants, twelve metabolites arising from glutathione conjugation of PCNB were identified. Peanut cell suspension cultures were used to study some of the precursor/product relationships of these metabolites. Pentachloronitrobenzene metabolism was also studied in soybean, cotton, corn, barley, blue green algae and onion. N-Malonylcysteine conjugates were common metabolites of PCNB in all of the species except onion. The N-Malonylcysteine conjugates of PCNB were stable in peanut cell suspension cultures for 14 days. Propachlor was also used as a biochemical probe to study glutathione conjugation metabolism. Propachlor also formed an N-malonylcysteine conjugate via glutathione and  $\gamma$ -glutamylcysteine conjugate intermediates in soybean. The N-malonylcysteine conjugates appeared to be the plant kingdom equivalent to the mercapturic acids produced via glutathione conjugation in the animal kingdom. All of the species examined in this study produced significant amounts of nonextractable residue. In peanut, precursors of the nonextractable residue included S-(pentachlorophenyl)cysteine and pentachlorothiophenol. Pentachlorothioanisole was one of the metabolites formed from the metabolism of a glutathione conjugate of PCNB. The biosynthesis of pentachlorothioanisole from PCNB was demonstrated with an enzyme complex isolated from onion root. The enzyme complex had glutathione S-transferase, peptidase, cysteine C-S lyase, and S-methyl transferase activities. The synthesis of pentachlorothioanisole from PCNB, glutathione, and S-adenosylmethionine was catalyzed by this enzyme complex. In the absence of glutathione, the enzyme complex catalyzed the synthesis of pentachlorothioanisole from S-adenosylmethionine and either S-(pentachlorophenyl)cysteine or pentachlorothiophenol. A variety of thiophenols were tested as substrates for the methyl transferase activity. Several of these were also active.

Literature Cited

1. Lamoureux, G. L.; Frear, D. S. in "Xenobiotic Metabolism: In Vitro Methods" (G. D. Paulson, D. S. Frear, and E. P. Marks, Ed.) ACS Symposium Series 97, p. 102, American Chemical Society, Washington, D.C. (1979).
2. Shimabukuro, R. H.; Lamoureux, G. L.; Frear, D. S. in "Chemistry and Action of Herbicide Antidotes" (F. M. Pallos and J. E. Casida, Ed.) p. 133, Academic Press, New York, 1978.
3. Lay, M. M.; Casida, J. E. in "Chemistry and Action of Herbicide Antidotes" (F. M. Pallos and J. E. Casida, Ed.) p. 151, Academic Press, New York, 1978.
4. Lamoureux, G. L.; Stafford, L. E.; Shimabukuro, R. H.; Zaylskie, R. G. J. Agr. Food Chem. 1973, 21, 1020.
5. Lamoureux, G. L.; Frear, D. S. in "Xenobiotic Metabolism: In Vitro Methods" (G. D. Paulson, D. S. Frear, and E. P. Marks, Ed.) ACS Symposium Series 97, p. 104, American Chemical Society, Washington, D.C. (1979).
6. Lamoureux, G. L.; Rusness, D. G. J. Agr. Food Chem. 1980, 28, 1057.
7. Rusness, D. G.; Lamoureux, G. L. J. Agr. Food Chem. 1980, 28, 1070.
8. Child, J. J.; LaRue, T. A. in "Plant Tissue Culture Methods" (O. L. Gamborg and L. R. Wetter) p. 90, National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada, S7N 0W9 NRCC 14383 (1975).
9. Lamoureux, G. L.; Rusness, D. G. Pest. Biochem. Physiol. 1980, 14, in press.
10. Rosa, N.; Neish, A. C. Can. J. Biochem. 1968. 46, 797.
11. Zenk, M. H.; Scherf, H. Planta 1964, 62.
12. Ladesic, B.; Pokorny, M.; Keglevic, D. P. Phytochem. 1970, 9, 2105.
13. Shimabukuro, R. H.; Walsh, W. C.; Stolzenberg, G. E.; Olson, P. A. "Abstract of Paper," Weed Sci. Soc. Amer. Meeting, Denver, Colo., Feb. 3-6, 1976.

14. Shimabukuro, R. H.; Lamoureux, G. L.; Swanson, H. R.; Walsh, W. C.; Stafford, L. E.; Frear, D. S. Pest. Biochem. Physiol. 1973, 3, 483.
15. Lamoureux, G. L.; Stafford, L. E.; Tanaka, F. S. J. Agr. Food Chem. 1971, 19, 346.
16. Hubbell, J. P.; Casida, J. E. J. Agr. Food Chem. 1977, 25, 404.
17. Shimabukuro, R. H.; Lamoureux, G. L.; Frear, D. S. Pesticide Metabolism in Plants: Reactions and Mechanisms, Proceedings of the US-India Seminar on Biodegradable Pesticides, April 16-19, 1979, Lucknow, India.
18. Larsen, G. L.; Bakke, J. E. J. Environ. Sci. Health, 1979, B14, 495.
19. Bakke, J. E.; Gustafsson, J. A.; Gustafsson, B. E. Science, in press.
20. Bakke, J. E.; Aschbacher, P. W.; Feil, V. J.; Gustafsson, B. E. Xenobiotica, in press.
21. Tateishi, M.; Suzuki, S.; Shimizu, H. Biochem. Pharmacol. 1978, 27, 809.
22. Lamoureux, G. L.; Rusness, D. G. "Abstract of Paper," 178th National Meeting of the American Chemical Society, Sept., 1979.
23. Begum, S.; Scheunert, I.; Haque, A.; Klein, W.; Korte, F. Pest. Biochem. Physiol. 1979, 11, 189.
24. Schwimmer, S.; Kjaer, A. Biochim. Biophys. Acta 1960, 42, 316.
25. Mazelis, M.; Fowden, L. Phytochem. 1973, 12, 1287.
26. Kogel, W.; Muller, W. F.; Coulston, F.; Korte, F. Chemosphere 1979, 8, 97.

RECEIVED December 30, 1980.

## Role of Gut Microflora in Metabolism of Glutathione Conjugates of Xenobiotics

J. E. BAKKE, G. L. LARSEN, and P. W. ASCHBACHER—Metabolism and Radiation Research Laboratory, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Fargo, ND 58105

J. J. RAFTER and J. A. GUSTAFSSON—Department of Medical Nutrition, Karolinska Institute, Stockholm, Sweden

B. E. GUSTAFSSON—Department of Germfree Research, Karolinska Institute, Stockholm, Sweden

In this chapter we survey recent findings that describe the metabolic fate of the xenobiotic moieties of mercapturic acid pathway (MAP) metabolites that are excreted from the liver with the bile. We show that the products of the MAP undergo an enterohepatic circulation that is mediated by intestinal enzymes and/or intestinal microflora.

The conjugation of xenobiotics with glutathione is an important detoxication mechanism for both plants (1) and animals (2) including insects (3). In animals, these glutathione conjugates are further metabolized to mercapturic acids (*S*-substituted-*N*-acetylcysteine conjugates); in plants, the glutathione conjugates are further metabolized to *S*-substituted-*N*-malonylcysteine conjugates (4). In animals, evidence for metabolism of a xenobiotic in the MAP has usually been the isolation of the mercapturic acid of the xenobiotic from the urine, however, the metabolic incorporation of other carbon-sulfur bonds into xenobiotics and the excretion of these metabolites as *S*-glucuronides in the urine (5) and/or as methylthio-, methylsulfinyl-, or methylsulfonyl-containing metabolites in the urine (6, 7) and feces (8) can also result from the catabolism of MAP metabolites. The metabolic introduction of methylthio-, methylsulfinyl- and methylsulfonyl-groups into xenobiotics has been reported in at least eighteen cases (6, 9-25), and caffeine has been shown to be metabolized to a methylthio-containing metabolite (26). Colucci and Buyske (5) and Tateishi et al. (27) showed that rat liver contains enzyme systems that can produce thiols from MAP metabolites, and the latter authors showed that the corresponding methylthio-containing metabolites were formed when liver microsomes and *S*-adenosylmethionine were added to their cysteine conjugate  $\beta$ -lyase system. DeBaun et al. (9) described a pathway in which methionine supplied the methylthio-group in the metabolism of *N*-acetylaminoflourene. Sumio and Mio (28) have proposed a mechanism in which methionine reacted with an arene oxide to form the methylthio-containing metabolites from



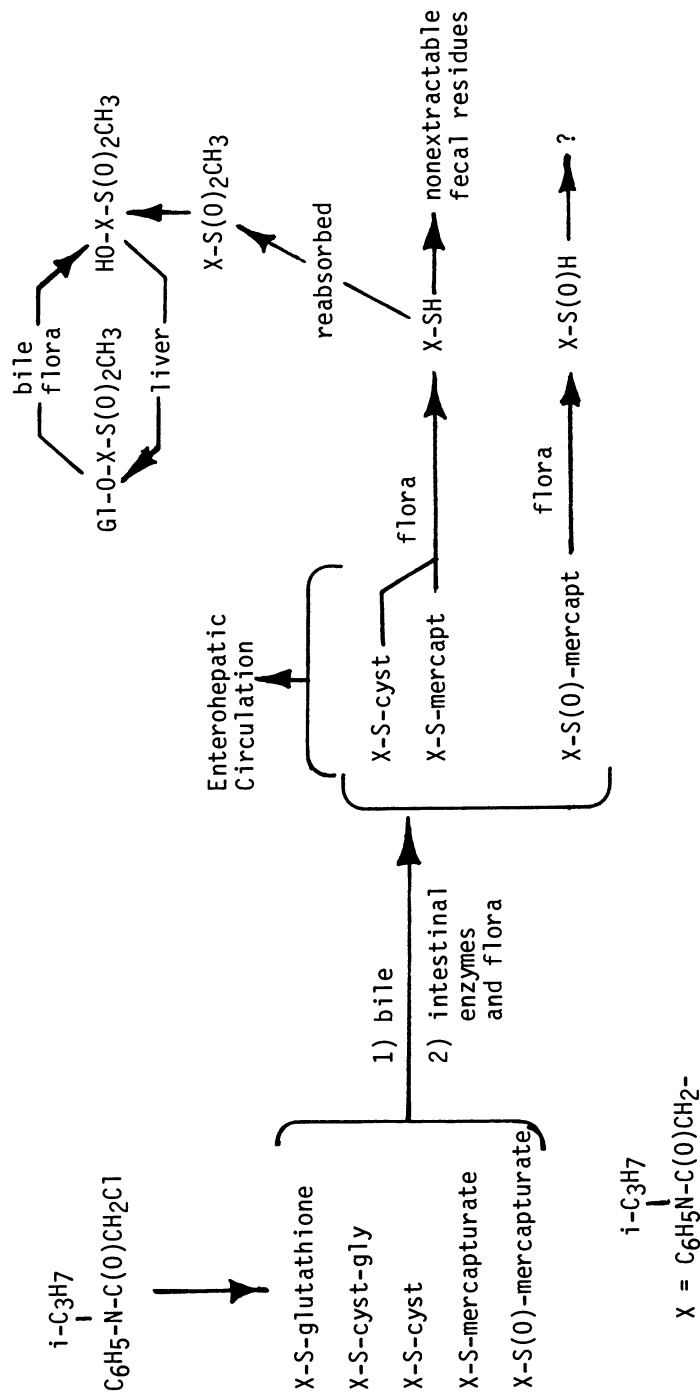


Figure 1. Proposed metabolic pathway for propachlor in rats

2,5,2',5'-tetrachlorobiphenyl. Stillwell et al. (29) have suggested mechanisms for methylthio-group introduction into the naphthalene nucleus based on the mechanism proposed by Tateishi (27) and a mechanism that involves reaction of the epoxide with 2-keto-4-thiomethylbutyric acid. The production of methylthio-containing metabolites of naphthalene was decreased in rats fed neomycin which indicated an involvement of the intestinal flora in the production of these metabolites (29). Recently, we have injected a mercapturic acid of naphthalene, isolated from urine from rats dosed with  $^{14}\text{C}$ -naphthalene, into the cecum of rats and isolated a dihydronaphthalene that contained both a methylthio group and an *O*-glucuronide substituent (34).

Recent studies from our laboratories have shown the presence of two pathways for the catabolism of MAP metabolites that involve enterohepatic circulation and microfloral metabolism (8, 30, 31, 32).

#### Catabolism Utilizing Microfloral C-S Lyases

Propachlor (2-chloro-N-isopropylacetanilide, Fig. 1) is metabolized by rats, pigs and probably mice and sheep by the pathways outlined in figure 1.

In the first pass propachlor is absorbed from the gastrointestinal tract and quantitatively metabolized in the MAP. This conclusion is based on the near quantitative recoveries of propachlor doses as MAP metabolites (Table I) when propachlor was given to germfree rats (30), and bile duct cannulated conventional (33) and germfree rats (34). The major biliary metabolite is the glutathione conjugate which is metabolized by the intestinal microflora to 2-thio-N-isopropylacetanilide either directly or through the cysteine conjugate as shown in figure 2. The cysteinyl-glycine conjugate would follow the same pathways.

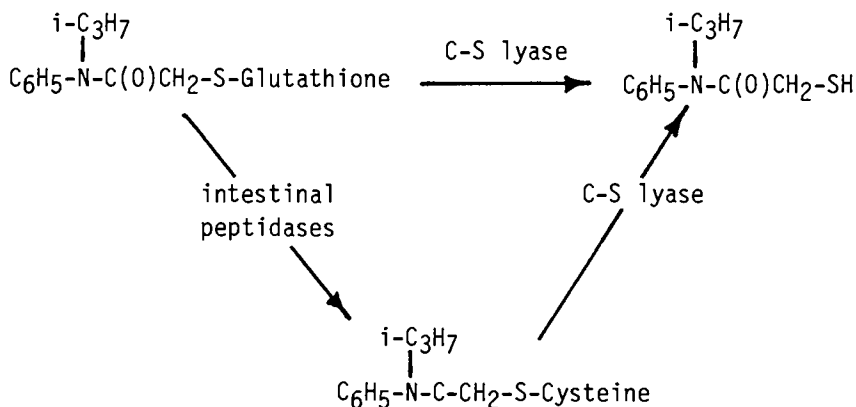


Figure 2.

Table I. Comparisons of the metabolism of propachlor by conventional, germfree and antibiotic treated rats

	Recovery % of dose								
	Conventional		Germfree		Antibiotic treated				
	urine	feces	bile <sup>c</sup>	urine	feces	bile <sup>c</sup>			
glutathione	0.0 <sup>a</sup>	nd	37	nd	nd	28.5	0.0	0.0	42.9
cysteinyl-glycine	nd	nd	nd	nd	nd	14.6	0.0	0.0	nd
cysteine	0.0	nd	nd	nd	19.0	1.5	0.0	38.7	4.8
mercaptopuric acid	17.6	nd	12	63.1	3.7	5.4	19.7	nd	5.3
mercaptopuric acid sulfoxide	9.5	nd	4	5.7	9.4	nd	7.8	21.8	6.4
methylsulfonyl containing	22.7(6) <sup>b</sup>	nd	nd	nd	nd	nd	nd	nd	nd
other	13.0	nd	nd	nd	nd	1.7(1)	nd	nd	nd
total <sup>14</sup> C excreted	68	19	66	68.8	32.1	53	27	65.5	67

a none detected

b number of metabolites

c biliary excretion of <sup>14</sup>C from separate experiments using bile duct cannulated rats

It is probable that the peptide hydrolysis reactions are accomplished by both digestive enzymes and microfloral enzymes. Digestive enzymes are implicated because the major metabolite in germfree rat bile was the glutathione conjugate (28.3% of the dose) while the cysteine conjugate was the major metabolite (19%) present in germfree rat feces; no glutathione conjugate was present in the germfree feces.

The experiments with germfree rats also indicated that MAP metabolites were able to undergo enterohepatic circulation. The bile duct cannulated germfree rats excreted about 53 percent of oral doses in the bile as MAP metabolites and the intact germfree rats excreted only 32 percent of single oral doses in the feces. Enterohepatic circulation of the intact mercapturic acid of propachlor was demonstrated in germfree rats. Germfree rats given single oral doses of dual labeled mercapturic acid of propachlor ( $^{14}\text{C}$ -propachlor- $\text{C}^2\text{H}_3$ -N-acetate mercapturic acid) excreted 78.6 percent of the dose as the mercapturic acid. Only 7.1 percent of the isolated mercapturic acid contained acetate without deuterium. Preliminary results from a similar study with dual labeled cysteine conjugate ( $^{14}\text{C}$ -propachlor- $^3\text{H}$ -cysteine) showed that it can also be absorbed from the gastrointestinal tract and excreted in the urine and bile as the dual labeled mercapturic acid. We have not determined if the glutathione, cysteinyl-glycine or, if present, the glutamyl-cysteine conjugates can be reabsorbed without prior cleavage of peptide bonds.

We have in vitro evidence that the intestinal C-S lyase activity is associated with the microflora. Pig cecal contents metabolize the glutathione and cysteine conjugates and the mercapturate to 2-thio-*N*-isopropylacetanilide and nonextractable residues. The extractable product of the reaction was trapped as the adduct with iodoacetic acid (33) after the incubation. Without the addition of iodoacetic acid, we were unable to recover any identifiable radioactivity from the incubates because of an apparent reactivity of the products. The reactivity of 2-thio-*N*-isopropylacetanilide became apparent during the synthesis of the compound. When a solution of 2-thio-*N*-isopropylacetanilide was allowed to stand at room temperature overnight the corresponding bis-thioether was formed. Such reactivity could be a mechanism by which the nonextractable fecal residues are formed.

We have not determined where the *S*-methylation of the C-S lyase cleavage products occurs. It could take place in the flora, although no 2-methylthio acetanilides were detected in the pig cecal content incubations, and no methylthio-, or methylsulfonyl-containing *N*-isopropylacetanilides were extractable from any rat feces. The conventional rat feces contained only nonextractable residues. The methylation could also take place in the tissues upon or after absorption of the intestinal metabolites because thiol *S*-methyltransferase is present in

many mammalian tissues (36). We can only report that S-methylation and S-oxidation occurs at some point after the microfloral C-S Lyase and before the biliary excretion of glucuronide conjugates of the hydroxylated 2-methylsulfonyl acetanilides in the second pass of the propachlor metabolites through the liver (33). The generation of these glucuronides results in an enterohepatic circulation of the methylsulfonyl containing aglycones until they are excreted in the urine as the aglycones, glucuronides, and other metabolites of the aglycones (20). None of these second pass metabolites were produced in germfree rats or antibiotic treated rats.

The oral administration of antibiotics resulted in the production of germfree characteristics with respect to propachlor metabolism in rats (37) and pigs (31), i.e. no 2-methylsulfonyl acetanilides were formed and only MAP metabolites were excreted. This observation may have economic implications. It is possible that the growth stimulation observed upon incorporation of antibiotics into animal feed could be effected by the suppression of such mechanisms. This could be accomplished either by the prevention of the metabolic formation of new xenobiotics of unknown biological activities or by the conservation of detoxication energy or both.

Species differences in the metabolism of propachlor are summarized in Table II. All species studied metabolized propachlor in the MAP. Obvious, but unexplained differences are that the rat excreted no cysteine conjugate and the chicken formed no methylsulfonyl-containing metabolites. The absence of methylsulfonyl formation by chickens is thought due to the low biliary secretion of first pass metabolites. The ruminant (sheep) excreted large amounts of cysteine conjugate in urine which is also not explained. We do not know if the intestinal flora are involved in the formation of the methylsulfonyl acetanilides isolated from sheep urine.

Pentachloromethylthiobenzene (PCMTB) is metabolized in rats to bis-(methylthio)tetrachlorobenzene (bis-MTTCB) by a microflora dependent pathway that involves biliary excretion of the mercapturate precursors and the action of a microfloral C-S lyase (8). The results of metabolism studies in rats are outlined in table III. Eighty-one percent of single oral doses of <sup>14</sup>C labeled PCMTB were excreted with the feces as bis-MTTCB and nonextractable residues in about equal amounts. Germfree rats excreted about 88 percent of the dose with the feces as at least two mercapturic acids (I, II, fig. 3). The major metabolite in the urine from both conventional and germfree rats was the mercapturic acid.

Table II. Metabolism of propachlor in various species and antibiotic treated pigs

	Recovery % of dose					
	Rat urine	Mice urine	Pigs urine	Antibiotic treated pigs	Chickens	
Cysteine conjugate	0.0	13.1	11.9	15.5	57.5	28.2
Mercapturic acid	17.6	22.9	30.8	48.1	7.5	34.8
Mercapturic acid sulfoxide	9.5	21.7	8.8	16.3	0.0	12.8
Methyl sulfonyl containing	22.7(6) <sup>a</sup>	8.2(2)	28.3(2)	0.0	11.0(2)	0.0
Other	13.0(3)	5.5(2)	0.0	0.0	0.0	9.0(2)
Urine	68	79	82	81	79	73
Feces	19	22	9	9	23	28
Bile	57	--b	--	--	--	3

<sup>a</sup> Number of metabolites

<sup>b</sup> Not determined

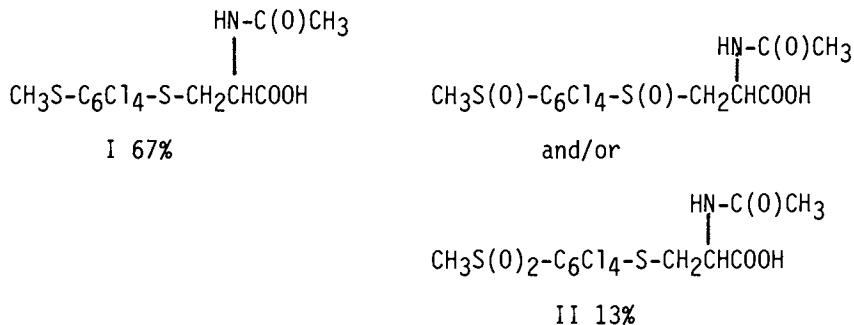
Table III. Comparison of the metabolism of pentachloromethylthiobenzene in conventional and germfree rats

	Recovery % of dose			
	Conventional urine	Conventional feces	bile <sup>c</sup>	Germfree urine feces
Glutathione				
Cysteinyl-glycine	nd <sup>a</sup>	nd	at least 25	nd
Cysteine				
Mercapturic acid	1	nd	nd	2.7
Oxidized mercapturic acid	nd	nd	nd	13.5
Bismethylthio tetrachlorobenzene	nd	34	nd	nd
Uncharacterized	4(1) <sup>b</sup>	nd	38(2)	nd
Nonextractable residues		40	nd	nd
Total <sup>14</sup> C excreted	5.3	80.7	62.8	2.7

a None detected

b Number of metabolites

c Biliary excretion of <sup>14</sup>C from separate experiments using bile duct cannulated rats

*Figure 3.*

Seventy-four percent of oral doses of PCMTB- $^{14}\text{C}$  were excreted in the bile by conventional rats. Most of this (50 to 70%) has been characterized to be products of the MAP (8). Neither of the mercapturates shown in fig. 3 were secreted in the bile therefore, the mercapturic acids that were excreted with the germfree rat feces had to have been formed either by metabolism of the precursors of the mercapturic acid by the intestinal mucosa, or by the tissues during enterohepatic circulation of these precursors. Comparison of the rates of excretion of oral doses of PCMTB- $^{14}\text{C}$  given to germfree and conventional rats indicate that there was enterohepatic circulation of the  $^{14}\text{C}$  in the germfree rats. Conventional rats excreted more than 80 percent of the dose in the feces within two days while it took at least eight days for the germfree rats to excrete 80 percent of the dose in the feces.

The presence of nonextractable residues in the conventional feces indicates a metabolic mechanism for PCMTB in conventional rats that is similar to that for propachlor, but the excretion of the bis-MTTCB with the feces indicates a mechanism that is different. Nothing is known about either the nature of the organisms containing the C-S lyase and their physical location in the intestines, or whether the C-S lyase cleavage product is methylated by the flora or the tissues. If methylation is accomplished by tissue enzymes, then the bis-MTTCB that is excreted with the feces must be resecreted into the gut by some unknown mechanism. Because no bis-MTTCB was present in the bile on the first pass, it may be possible that the proposed C-S lyase cleavage product (a methylthiotetrachlorobenzene thiol) is reabsorbed, methylated and resecreted in the bile on a second pass metabolism. This possibility has not been investigated as yet. A similar overall mechanism could be involved in the formation of the methylsulfides of polychlorinated biphenyls (PCB) that are excreted in the feces of rats dosed with PCBs (17, 18), because we found evidence for the presence of a mercapturic acid precursor (glutathione con-



jugate or cysteinyl-glycine conjugate) in the bile from rats dosed with 2,4',5-trichlorobiphenyl. There was no evidence for any methylsulfide of this PCB in the bile, and the bile contained 95 percent of the dose. We also dosed germfree and conventional rats (oral) with 2,4',5-trichlorobiphenyl-<sup>14</sup>C and measured the tissue residue levels of <sup>14</sup>C. The subcutaneous and omental fat from the conventional rats had tissue residues from 3 to 15 times higher than the germfree rats. A mechanism involving enterohepatic circulation could explain the persistence of such hard residues especially since it is known that methylsulfones of PCBs and DDE do accumulate in the fat (17).

We do not know whether the S-oxidized mercapturic acid of PCMTB (II) is formed in the conventional rat or if it may be a PCMTB metabolite that is unique to the germfree state. It could result from tissue metabolism during the enterohepatic circulation of the mercapturate precursors or the mercapturate (I). If it is formed in the conventional rat, it may be a precursor for the nonextractable fecal residues because no extractable metabolite that contained an oxidized sulfur was found in conventional rat excreta.

As in the case of propachlor mercapturic acid sulfoxide, the biological significance of xenobiotic mercapturic acids that contain oxidized sulfur is not known. Casida et al. (39) have reported that sulfoxidation of some thiocarbamate herbicides is a beneficial step in the detoxication process. However, cysteine conjugates can exhibit adverse biological activities. Smith (40) has reviewed work on the metabolism of the toxic principle in kale and has shown that C-S lyase action on S-methylcysteine sulfoxide produces the toxic principle. Virtanen (41) has reviewed the processes in other plants that lead to the production of compounds with biological activity from S-substituted cysteine sulfoxides.

#### Catabolism Utilizing Tissue C-S Lyases And Microfloral S-Glucuronidases In Addition To The Microfloral C-S Lyase

To our knowledge, Colucci and Buyske (5) had the first report of MAP metabolites being metabolized by a hepatic C-S lyase that led to the formation of an S-glucuronide. Studies on the metabolism of 2-acetamido-4-chloromethylthiazole (32) have extended this pathway to include the excretion of the S-glucuronide with the bile. These studies showed that both the S-glucuronide and the mercapturic acid are first pass biliary metabolites of this thiazole, and that both of these conjugates are metabolized by microfloral mediated deconjugation pathways that ultimately yielded the methylthiomethyl-compounds that were excreted in the urine. Our conception of this pathway is outlined in figure 4. The involvement of the intestinal microflora in this metabolism was shown in three ways. No

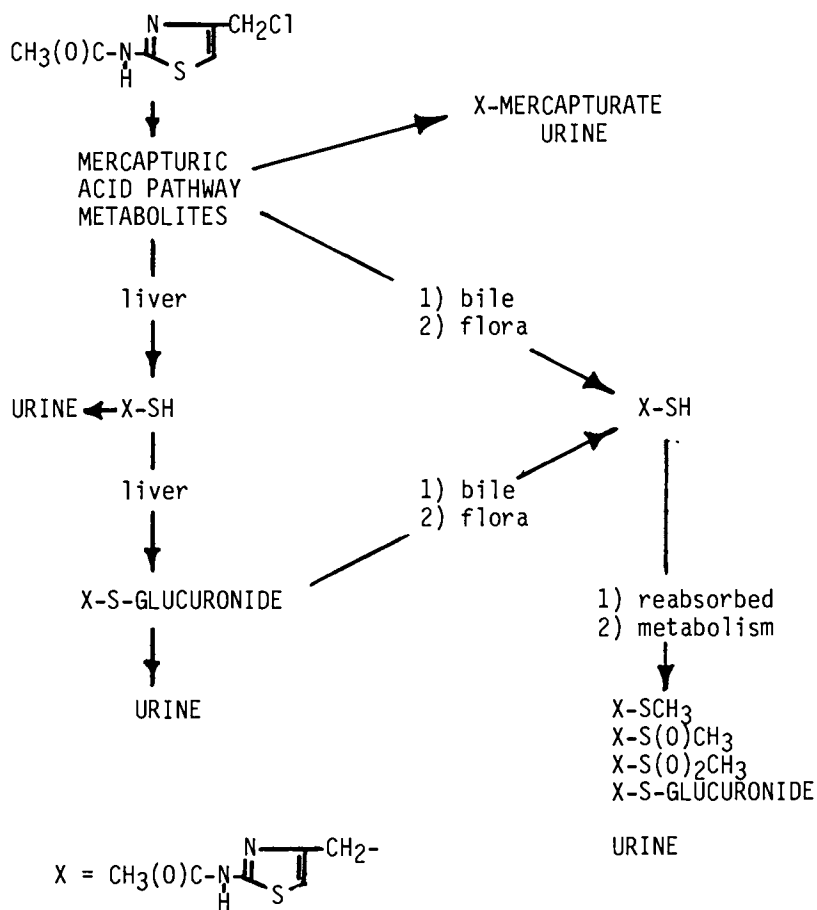


Figure 4. Proposed metabolic pathway for 2-acetamido-4-chloromethylthiazole in rats

4-methylthiomethyl-, 4-methylsulfinylmethyl-, or 4-methyl-sulfonylmethyl-containing 2-acetamido thiazoles were produced by germfree rats. The S-glucuronide and the mercapturic acid were isolated from the bile, and when the S-glucuronide and the mercapturic acid were separately injected into the cecum of rats, the major urinary metabolite was 2-acetamido-4-methyl-sulfinylmethylthiazole (65 and 45 percent of the doses, respectively). We have no evidence where the S-methylation or oxidations occur.

The existence of pathways for the in vivo catabolism of MAP metabolites to compounds that require further metabolism before the xenobiotic moiety is excreted from the body indicates that metabolism of xenobiotics by the MAP may, in some cases, represent only a transient detoxication. The thiols and methylated thiols that are formed represent new xenobiotics. The significance of the formation of these new xenobiotics in the colon is not known. In a recent study, Plummer et al. (42) showed that MAP conjugates of the 4,5-epoxide of benzo(a)pyrene were major biliary metabolites when the epoxide was infused into the portal vein of rats. If the microbial C-S lyase system is operative in the catabolism of benzo(a)pyrene MAP metabolites, the products formed may be involved in carcinogenic properties attributed to the presence of this compound in the environment. Until both the metabolic fates of MAP metabolites and S-glucuronides that are excreted with the bile and the biological properties of the products of the microbial metabolism are known, we cannot necessarily consider these conjugates to be of no biological significance to the host.

#### LITERATURE CITED

1. Shimabukuro, R. H., Lamoureux, G. L., Frear, D. S.: Eds. Pallos, F. M., Casida, J. E., "Chemistry and Action of Herbicide Antidotes"; Academic Press: New York, 1978; p. 133.
2. Chasseaud, L.F.: Eds. Arias, I. M., Jakoby, W. B., "Glutathione: Metabolism and Function"; Raven Press: New York, 1976; p. 79.
3. Brooks, G. T.: Eds. Bridges, J. W., Chasseaud, L. F., "Progress in Drug Metabolism", John Wiley and Sons: New York, 1979; Vol. 3, p. 151.
4. Lamoureux, G. L., see chapter in this publication.
5. Colucci, D. F.; Buyske, D. A. Biochem. Pharmacol., 1965, 14, 457.
6. Chatfield, D. H.; Hunter, W. H. Biochem. J., 1973, 134, 879.
7. Bakke, J. E.; Feil, V. J.; Price, C. E. Biomed. Mass Spectrom., 1976, 3, 226.
8. Bakke, J. E.; Aschbacher, P. W.; Feil, V. J.; Gustafsson, B. E., Submitted to Xenobiotica 1980.

9. DeBaun, J. R.; Miller, E. C.; Miller, J. A. Cancer Res. 1970, 30, 577.
10. Focella, A.; Heslin, P.; Teitel, S. Can. J. Chem., 1972, 50, 2025.
11. Kaul, R.; Hempel, B.; Schafer, W. Arzneim.-Forsch., 1976, 26, 489.
12. Ou, T.; Talsumi, K.; Yoshimura, H. Biochem. Biophys. Res. Commun., 1977, 75, 401.
13. Stillwell, W. G.; Bouwsma, O. J.; Thenot, J-P.; Horning, M. G. Res. Commun. Chem. Pathol. Pharmacol., 1978, 20, 509.
14. Stillwell, W. G. Pharmacologist, 1977, 19, 169.
15. Tateishi, H.; Shimizu, H. Xenobiotica, 1976, 6, 431.
16. Stock, B.; Spiteller, G. "Mass Spectrometry and Combined Techniques in Medicine, Clinical Chemistry, and Clinical Biochemistry", Eggstein, M.; Liebich, H. M., Eds" Tubingen, F.D.R. 1977; p. 379.
17. Jenson, S.; Jansson, B. Ambio, 1976, 5, 257.
18. Mio, T.; Sumio, K.; Mizutani, T. Chem. Pharm. Bull., 1976, 24, 1958.
19. Miller, J. A. Cancer Res., 1970, 30, 559.
20. Bakke, J. E.; Price, C. E. J. Environ. Sci. Health, 1979, B14, 439.
21. Paulson, G. D.; Jacobsen, A. M.; Zaylskie, R. G. Pestic. Biochem. Physiol., 1977, 7, 62.
22. Kuchar, E. J.; Geenty, F. O.; Griffith, W. P.; Thomas, R. J. J. Agr. Food Chem., 1969, 17, 1237.
23. Bray, H. G.; Hybs, Z.; James, S. P.; Thorpe, W. V. Biochem. J., 1953, 53, 266.
24. Koss, G.; Koransky, W.; Steinbach, K. Arch. Toxicol., 1979, 42, 19.
25. Walie, T. Federation Proceedings, 1977, 36, 961.
26. Kamei, K.; Matsuda, A.; Momose, A. Chem. Pharm. Bull., 1975, 23, 683.
27. Tateishi, M.; Suzuki, S.; Shimizu, H. J. Biol. Chem., 1978, 253, 8854.
28. Sumio, K.; Mio, T. Proceedings of the First Meeting of the Japanese Society for Medical Mass Spectrometry, 1976, 1, 67.
29. Stillwell, W. G.; Bouwsma, O. J.; Horning, M. G. Res. Commun. Chem. Pathol. Pharmacol., 1978, 22, 329.
30. Bakke, J. E.; Gustafsson, J-A.; Gustafsson, B. E. Science, 1980, 210, 433.
31. Bakke, J. E., G. L. Larsen, and P. W. Aschbacher. The Effect of Oral Lincomycin on the Metabolism of the Herbicide Propachlor by the Pig. American Society of Animal Science. 1980. (Abstract).
32. Bakke, J. E.; Feil, V. J.; Lindeskog, P.; Rafter, J. J.; Gustafsson, J-A.; Gustafsson, B. E., submitted to Biochem. Pharmacol.

33. Larsen, G. L.; Bakke, J. E. The Role of Enterohepatic Circulation in the Formation of the Metabolites of 2-chloro-N-isopropylacetanilide (Propachlor), unpublished.
34. unpublished
35. Cole, R. D.; Stein, W. H.; Moore, S. J. Biol. Chem., 1958, 1359.
36. Bremer, J.; Greenberg, D. M. Biochim. Biophys. Acta, 1961, 46, 217.
37. Larsen, G. L.; Bakke, J. E. The Effect of Antibiotic Treatment on the Metabolism and Enterohepatic Circulation of Propachlor Metabolites in the Rat, unpublished.
38. Bakke, J. E.; Price, C. E. Metabolism of 2-Chloro-N-Isopropylacetanilide (Propachlor) in the Sheep and Milk Goat, J. Environ. Sci. Health 1979, B14, 291.
39. Casida, J. E.; Gray, R. A.; Tilles, H. Science, 1964, 184, 573.
40. Smith, R. H. "Reports of the Rowett Institute", 1974, 30, 112.
41. Virtanen, A. I. Phytochemistry 1965, 4, 207.
42. Plummer, J. L.; Smith, B. R.; Ball, L. M.; Bend, J. R. Drug Metab. Disp., 1980, 8, 68.

RECEIVED February 5, 1981.

# INDEX

A	B
AgNO <sub>3</sub> complexation of aqueous buffer phase from washed <i>P. putida</i> cell incubations with <sup>36</sup> Cl-toxaphene .....	<i>Bacillus megaterium</i>
122t	degradation of mexacarbate in cell-free extracts of .....
Acephate .....	124-127
46	effect of cofactors on the degradation of <sup>14</sup> C-mexacarbate in the cell-free extracts of .....
2-Acetamido-4-chloromethylthiazole in rats, metabolic pathway for .....	126t
175f	mixed-function oxidase system of ..
Acyclic sulfur, compounds containing ..	124
7f	role of megaredoxin in steroid hydroxylase system of .....
Acyclic sulfur systems .....	118f
4-8	Bacterial assays, mutagenic properties of captan in .....
animal sources of .....	86
plant sources of .....	112-113
4-6	Bacterial ferredoxins .....
Adrenal cortex mitochondria, role of adrenodoxin in mixed-function oxidation system of .....	116t
114f	Benzphetamine by a reconstituted monooxygenase oxidase enzyme system from rabbit liver, linearity of the metabolism of parathion and .....
Adrenodoxin .....	29f
113	S-Benzyl thiocarbamate sulfoxides, MCPBA oxidation of .....
in mixed-function oxidation system of adrenal cortex mitochondria, role of .....	69
114f	Bio-accumulation constant .....
(Alkoxy carbonyl)(alkylamino)sulfenylphosphoramidothioates .....	108
46-47	
S-Alkyl thiocarbamate sulfoxides, cis-elimination mechanism of .....	C
68	<sup>13</sup> C-labeled mephosfolan structures ....
S-Alkyl thiocarbamate sulfoxides, MCPBA oxidation of .....	106
69	<sup>14</sup> C and <sup>36</sup> Cl-toxaphene by washed <i>P. putida</i> cells, metabolism of .....
Alkylsulfenyl methylcarbamates .....	121t
37-39	captan radioactivity after soil application followed by spinach culture under closed conditions, balance account of .....
N-Alkylsulfenyl derivatives of insecticidal methylcarbamates, toxicological properties of .....	92t
37t-38t	dichlofluaniid with cysteine, in vitro reactions of radioactivity after spray application on strawberry plants under closed conditions, balance account of .....
Alkylthiosulfenylmethylcarbamates ....	88t
44	radioactivity after spray application on strawberry plants under open conditions, balance account of .....
Alkylthiotriazines and thiocarbamates .....	89t
53-55	in strawberries, proposed metabolism of .....
Allium species, organosulfur compounds from .....	91f
5f	-mephosfolan distribution of radioactivity in cotton plants after foliar application of .....
Aminosulfenyl derivatives of methomyl .....	105t
44	
Arylsulfenyl methylcarbamates .....	
37-39	
N-Arylsulfenyl derivatives of insecticidal methylcarbamates, toxicological properties of .....	
37t-38t	
N-Arylsulfenyl derivatives of propoxur to the honey bee and house fly, toxicity of .....	
39t	
Arylsulfenylated methylcarbamates and types of selectivity .....	
38	
Arylthiosulfenylmethylcarbamates .....	
44	
Atrazine in sorghum, metabolic pathway of .....	
134f	

<sup>14</sup> C (continued)	<sup>14</sup> C (continued)
-mephosfolan (continued)	-phospholan (continued)
at 2 mg/kg, excretion of radioactivity via respiration gases, urine and feces by rats treated with .....	radioactivity levels in tissues of rats following a single oral dose of 2 mg/kg .....
radioactivity levels in tissues of rats following a single oral dose of 5 mg/kg .....	in rat, pathways of metabolism of .....
at 0.75 kg/ha, stimulated rice paddy treated with .....	in plant tissues as a function of solubility, distribution of .....
-treated paddy, residual radioactivity in rice plants grown in .....	-ring cyanatryl, elimination of radioactivity from the blood of rats dosed orally with .....
-mexacarbate in the cell-free extracts of <i>B. megaterium</i> , effect of cofactors on the degradation of .....	-toxaphene by washed <i>P. putida</i> cells incubated aerobically, effects of added cofactors on metabolic fate of .....
-mexacarbate by ferredoxin in the presence and absence of a flavin cofactor, degradation of .....	C-S lyase(s)
PCNB	catabolism utilizing microfloral .....
ether-soluble metabolites isolated from the roots of peanut plants treated with .....	cleavage, <i>S</i> -methylation of .....
formation of 80% methanol-insoluble residues in peanut plants treated with .....	and microfloral <i>S</i> -glucuronidases, utilizing tissue .....
HPLCs of water-soluble extracts from peanut cell cultures treated with .....	CI(CH) <sub>4</sub> MS of cyclic <i>S,S</i> -propylene dithiocarbonate from <sup>13</sup> C imido-labeled and <sup>13</sup> C ethyl-labeled mephosfolan photolysates .....
metabolism, experimental methods .....	<sup>36</sup> Cl-toxaphene, AgNO <sub>3</sub> complexation of aqueous buffer phase from washed <i>P. putida</i> cell incubations with .....
methylene chloride residues isolated from plant tissues treated with .....	<sup>36</sup> Cl-toxaphene by washed <i>P. putida</i> cells, metabolism of <sup>14</sup> C- and .....
-treated peanut plants, from the roots of	Camphor, role of putidaredoxin in methylene hydroxylation system for .....
ether-soluble fractions .....	Captan .....
insoluble residue .....	in bacterial assays, mutagenic properties of .....
methylene chloride-soluble residues .....	radioactivity after soil application followed by spinach culture under closed conditions, balance account of <sup>14</sup> C .....
water-soluble fractions .....	in spinach and soil, metabolism of (trichloro- <sup>14</sup> C-methyl) .....
-treated plant tissues, nonpolar methylene chloride-soluble residues from .....	and <i>bis</i> -(trichloromethyl) disulfide assayed with <i>S. typhimurium</i> TA 100, mutagenic activity of .....
-treated plant tissues, polar methylene chloride-soluble residues from .....	Carbamate proinsecticides .....
structures of chloroform-soluble residues isolated from peanut roots treated with .....	Carbamates, derivatized .....
water-soluble metabolites isolated from the roots of peanut plants treated with .....	Carbofuran .....
-phosfolan	derivatives of .....
excretion of radioactivity via respiration gases, urine, and feces by rats treated with a single oral dose of .....	toxicological properties of the <i>N</i> -sulfinyl .....
	Catabolism
	of glutathione conjugates of pesticides in higher plants .....
	of MAP metabolites, pathways for the in vivo .....
	utilizing microfloral C-L lyases .....
	utilizing tissue C-S lyases and microfloral <i>S</i> -glucuronidases .....

- S*-Chloroallyl thio- and dithiocarbamate herbicides, toxicological significance of oxidation and rearrangement reactions of ..... 65-82
- S*-Chloroallyl thiocarbamates,  $^1\text{H}$  chemical shift data for ..... 67*t*
- S*-(2-Chloroallyl) *N,N*-diethylthiocarbamate and its oxidation products,  $^1\text{H}$  chemical shift data for ..... 70*t*
- S*-(3-Chloroallyl) thiocarbamate sulf-oxides rearrangement ..... 69
- Chloroform-soluble residues isolated from peanut roots treated with  $^{14}\text{C}$  PCNB, structures of ..... 150*f*
- m*-Chloroperoxybenzoic acid (MCPBA), oxidation of ..... 66
- of *S*-alkyl thiocarbamate sulfoxides of *S*-benzyl thiocarbamate sulf-oxides ..... 69
- of *S*-methallyl thiocarbamate ..... 68
- Chloroplast ferredoxins, biological functions of ..... 112
- Chloro-sulfallate, their sulfine derivatives and further oxidation products,  $^1\text{H}$  chemical shift data for sulfallate ..... 72*t*
- Cotton plants after foliar application of  $^{14}\text{C}$ -mephosfolan, distribution of radioactivity in ..... 105*t*
- Cotton plants, mephosfolan metabolism in ..... 101
- Cultures containing NADH-FAD-FMN ..... 124
- Cyanatryn ..... 53, 54*f*
- de-ethyl ..... 56*f*
- glutathione conjugate ..... 57*f*
- via liver microsomes, *S*-oxygenation of ..... 59
- metabolism, in vitro studies on the *S*-oxygenating enzyme involved in ..... 59-60
- S*-oxidation of ..... 60
- S*-oxide ..... 55, 57*f*
- and 3,4-dichlorothiophenol, reaction between ..... 57*f*
- and DNA, reactions between ..... 63
- in/with rat(s)
- elimination of radioactivity from the blood of, dosed orally with  $^{14}\text{C}$ -ring ..... 62*f*
- liver microsomes and NADPH, TLC of the products derived from the incubation of  $^{14}\text{C}$ -mercapturic acids derived from the metabolism of ..... 54*f*
- in three species in vitro, *N*-de-ethylation ..... 59*t*
- in three species in vitro, *S*-oxygenation ..... 59*t*
- Cyolane phospholan ..... 97
- Cysteine, in vitro reactions of  $^{14}\text{C}$  dichlofluamide with ..... 93*f*
- Cytochrome P-450
- containing monooxygenase system, metabolism of parathion to paraoxon by ..... 23*f*
- containing monooxygenase system, metabolism of parathion to diethyl phosphorothionate and diethyl phosphate by ..... 25*f*
- iron-sulfur proteins as an electron transfer component to ..... 113-115
- moleculé, formation of the hydrodisulfide linkage in ..... 32*f*
- monooxygenase-catalyzed metabolism of phosphorothionate triesters, chemical mechanisms of ..... 19-34
- monooxygenase-catalyzed metabolism of parathion, products of ..... 24
- Cytrolane mephosfolan ..... 97
- D**
- De-ethyl cyanatryn ..... 56*f*
- N*-De-ethylation of cyanatryn in three species in vitro ..... 59*t*
- Degradation
- of  $^{14}\text{C}$ -mexacarbate in the cell-free extracts of *B. megaterium*, effect of cofactors on ..... 126*t*
- of  $^{14}\text{C}$ -mexacarbate by ferredoxin in the presence and absence of flavin cofactor ..... 119*t*
- effects of aerobic and anaerobic culture conditions on toxaphene ..... 120
- of mexacarbate in cell-free extracts of *B. megaterium* ..... 124-127
- of mexacarbate by ferredoxin ..... 117
- of pesticidal chemicals by microorganism, roles of iron-sulfur proteins in ..... 111-129
- of toxaphene by *P. putida*, metabolic ..... 117-120
- Delay factor ..... 35-49
- in methylcarbamate insecticides ..... 37
- Desmethylmexacarbate, structures of mexacarbate and ..... 118*f*
- Desulfuration with *p*-toluenesulfonic acid ..... 73
- Dialkylaminosulfenylmethylcarbamates ..... 43
- Diallate
- mutagenic properties of ..... 76
- sulfoxide ..... 74
- and triallate, oxidation of ..... 77*f*



Dichlofluamid .....	85	<b>E</b>	
<sup>14</sup> C			
radioactivity after spray applica- tion on strawberry plants under closed conditions, balance account of .....	88t		<i>cis</i> -Elimination mechanism of <i>S</i> -alkyl thiocarbamate sulfoxides .....
radioactivity after spray applica- tion on strawberry plants under open conditions, balance account of .....	89t		Elution profile of protein, radio- activity, and thiocyanate from a Sephadex G-25 column of recon- stituted monooxygenase from rat liver .....
in strawberries, proposed metab- olism of .....	91f		Enrichment in paraoxon following incubation of parathion with rabbit liver microsomes, <sup>18</sup> O .....
in comparison to its trichloro analogue assayed with <i>S. typhi-</i> <i>murium</i> TA 100, negative response of .....	95f		Enzyme involved in cyanatryn metab- olism in vitro studies of the <i>S</i> -oxygenating .....
with cysteine, in vitro reactions of ..	93f		Ether-soluble fractions from the roots of <sup>14</sup> C PCNB-treated peanut plants .....
metabolism in plants .....	87		Ether-soluble metabolites isolated from the roots of peanut plants with <sup>14</sup> C PCNB .....
photolysis of .....	87f		Ethyl acetate phase by GLC analysis, determination of functional groups of metabolites in .....
in strawberries, metabolism of (flurodichloro <sup>14</sup> C-methyl)- ..	88		<i>S</i> -Ethyl <i>N,N</i> -dipropylthiocarbamate via <i>S</i> -oxygenation and gluta- thione conjugation, metabolism of ..
3,4-Dichlorothiophenol reaction between cyanatryn <i>S</i> -oxide and ..	57f		
Diethyl phosphate <i>p</i> -nitrophenol formation in the mammalian metabolism of parathion .....	24		<b>F</b>
Diethyl phosphorothionate and diethyl phosphate by the Cytochrome P-450-containing monooxygenase system, metabolism of parathion to .....	25f		Ferredoxin(s) .....
<i>O,S</i> -Dimethyl <i>N</i> -( <i>N'</i> -Alkoxy carbonyl- <i>N'</i> -alkylaminosulfonyl)phosphoram- idothioates to houseflies and mice, toxicity of .....	47t		bacterial .....
<i>N</i> -Dimethoxyphosphinothioyl deriva- tives of methylcarbamate esters ..	36		molecular weight of .....
<i>N,N</i> -Dipropylthiocarbamate via <i>S</i> - oxygenation and glutathione con- jugation metabolism of <i>S</i> -ethyl ..	58f		biological functions of chloroplast ..
Disulfide, <i>bis</i> -(trichloromethyl) .....	94		degradation of mexacarbate by .....
Dithietane insecticide .....	104f		plant-type .....
Dithietane pesticides, role of gluta- thione conjugation in .....	104f		in the presence and absence of a flavin cofactor, degradation of <sup>14</sup> C-mexacarbate by .....
Dithiocarbamate(s)			Fish in the rice paddy environment, metabolism in .....
herbicides, toxicological significance of oxidation and rearrange- ment reactions of <i>S</i> -chloro- allyl thio- and .....	65-82		Flavin cofactor, degradation of <sup>14</sup> C- mexacarbate by ferredoxin in the presence and absence of .....
metabolism of .....	74-75		Flavin cofactor-ferredoxin system, degradation pathway of mexa- carbate through .....
sulfines .....	71-74		Flavodoxin(s)
oxidations of .....	73		as iron-sulfur protein mimics .....
rearrangements .....	71-73		isolation, microbial .....
spectral features of .....	71		molecular weight of selective .....
synthesis of .....	71		Folpet .....
Dithiolane insecticides in plants, animals, and the environment, comparative metabolism of .....	97-109		Fungicides, biological and chemical behavior of perhalogenmethyl- mercapto .....
DNA, reactions between cyanatryn <i>S</i> -oxide and .....	63		<b>G</b>
			<i>S</i> -glucuronidases, catabolism utilizing tissue C-S lyases and micro- floral .....

- Glutathione conjugate(s)  
of cyanatryn ..... 57f  
of pesticides in higher plants,  
catabolism of ..... 133-164  
of xenobiotics, role of GUI micro-  
flora in metabolism of ..... 165-178
- Glutathione conjugation  
in dithietane pesticides, role of ..... 104f  
in the mephosfolan metabolism in  
plants, role of ..... 103  
metabolism of *S*-ethyl *N,N*-dipro-  
pylthiocarbamate via *S*-oxy-  
genation and ..... 58f  
of xenobiotics with ..... 165
- Goldfish reared in rice paddy environ-  
ment, recovery of mephosfolan-  
derived radioactivity from ..... 108t
- GUI microflora in metabolism of  
glutathione conjugates of xeno-  
biotics, role of ..... 165-178
- H**
- <sup>1</sup>H chemical shift data  
for *S*-chloroallyl thiocarbamates .... 67t  
*S*-(2-chloroallyl) *N,N*-diethylthio-  
carbamate and its oxidation  
products ..... 70t  
for sulfallate, chloro-sulfallate, their  
sulfine derivatives and further  
oxidation products ..... 72t  
thiocarbamate sulfoxides ..... 67t
- Herbicide(s)  
metabolism in mammals, *S*-oxy-  
genation in ..... 53-64  
thiocarbamate ..... 55  
toxicological significance of oxida-  
tion and rearrangement reac-  
tions of *S*-chloroallyl thio- and  
dithiocarbamate ..... 65-82
- Heterocyclic sulfur systems ..... 8-10  
animal sources of ..... 10  
plant sources of ..... 8-10
- Honey bee and house fly, toxicity of  
*N*-arylsulfinyl derivatives of  
propoxur to ..... 39t
- House flies and mice, toxicity of *O,S*-  
dimethyl *N*-(*N'*-alkoxycarbonyl-  
*N'*-alkylaminosulfinyl)-phos-  
phoramidothioates to ..... 47t
- Hydrocarbon-oxidizing enzyme system 112
- Hydrodisulfide linkage in the Cyto-  
chrome P-450 molecule, forma-  
tion of ..... 32f
- I**
- Insecticidal methylcarbamates, *N*-  
alkylsulfinyl derivatives of ..... 45
- Insecticidal methylcarbamates, toxico-  
logical properties of  
*N*-alkylsulfinyl derivatives of ..... 37t-38t  
*N*-arylsulfinyl derivatives of ..... 37t-38t  
and the corresponding *N,N'*-thio-  
biscarbamates ..... 40t
- Insecticide(s)  
delay factor in methylcarbamate .... 37  
dithiethane ..... 104f  
Larvin—a broad-spectrum ..... 41  
in plants, animals, and the environ-  
ment, comparative metabolism  
of dithiolane ..... 97-109
- Iron-sulfur protein(s)  
clusters in ..... 114f  
in degradation of pesticidal chem-  
icals by microorganisms, roles  
of ..... 111-129  
as an electron transfer component  
to Cytochrome P-450 ..... 113-115  
mimics, flavodoxins as ..... 115  
in pesticide degradation, involve-  
ment of ..... 115-117  
properties of ..... 111-113
- Isopropyl *N*-chlorosulfinyl-*N*-methyl-  
carbamate ..... 45
- L**
- Larvin—a broad-spectrum insecticide 41  
Leukotriene C-1 ..... 5f  
Liver microsomes, the *S*-oxygenation  
of cyanatryn via ..... 59
- Low-molecular-weight organosulfur  
compounds in nature ..... 3-16
- M**
- MAP (see Mercapturic acid pathway)
- MCPBA (see *m*-Chloroperoxybenzoic  
acid)
- N*-Malonyl conjugates, formation of .. 143
- N*-Malonylcysteine conjugate(s)  
formation ..... 139  
in plants ..... 142f  
in soybean root, metabolism of  
propachlor to ..... 147f
- Mammalian metabolism of parathion,  
diethyl phosphate *p*-nitrophenol  
formation in ..... 24
- Mammals, *S*-oxygenation in herbicide  
metabolism in ..... 53-64
- Megaredoxin ..... 113-115  
in steroid hydroxylase system of  
*B. megaterium*, role of ..... 118f
- Mephosfolan  
-derived radioactivity from goldfish  
reared in rice paddy environ-  
ment, recovery of ..... 108t

Mephosfolan ( <i>continued</i> )	
metabolism	
pathways of	100f
in plants	101-103
in cotton	101
in rice, study of	101
role of glutathione conjugation	
in	103
in rats	99-101
photolysates, $\text{Cl}(\text{CH}_2)_4$ mass spectrum of cyclic <i>S,S</i> -propylene dithiocarbonate from $^{13}\text{C}$ imido-labeled and $^{13}\text{C}$ ethyl-labeled	107f
structures, $^{13}\text{C}$ -labeled	106
Mercapturic acid pathway (MAP)	165
metabolites, pathways for the in vivo catabolism of	176
Mercapturic acids derived from the metabolism of cyanatryn in the rat	54f
Metabolic	
degradation of toxaphene by <i>P. putida</i>	117-120
fate of $^{14}\text{C}$ -toxaphene by washed <i>P. putida</i> cells incubated aerobically, effects of added cofactors on	123t
mechanism for PCMTB in rats	173
pathway	
for 2-acetamido-4-chloromethylthiazole in rats	175f
of atrazine in sorghum	134f
for propachlor in rats	166f
Metabolism	
of $^{14}\text{C}$ - and $^{36}\text{Cl}$ -toxaphene by washed <i>P. putida</i> cells	121t
of $^{14}\text{C}$ dichlofluanid in strawberries, proposed	91f
of dithiocarbamates	74-75
carbamylation reactions of	75
oxidations and rearrangements of	74-75
of dithiolane insecticides in plants, animals, and the environment, comparative	97-109
in fish in the rice paddy environment	106-109
of (fluorodichloro $^{14}\text{C}$ -methyl)dichlofluanid in strawberries	88
in mammals, <i>S</i> -oxygenation in herbicide	53-64
of mephosfolan, pathways of	100f
of parathion	
diethyl phosphate <i>p</i> -nitrophenol formation in the mammalian	24
to paraoxon, chemical mechanism of the microsomal	21
products	
of the Cytochrome P-450 monooxygenase-catalyzed	24
Metabolism ( <i>continued</i> )	
pentachloroaniline	151
of PCMTB in conventional and germfree rats, comparison of	172t
of phosphorothionate triesters, chemical mechanisms of the Cytochrome P-450 monooxygenase-catalyzed	19-34
in plants, dichlofluanid	87
in plants, mephosfolan	101-103
cotton	101
in rice	101
role of glutathione conjugation in	103
of propachlor	
by conventional, germfree and antibiotic-treated rats, comparisons of	168t
species differences in	170
in various species and antibiotic-treated pigs	171t
in/of rats	
$^{14}\text{C}$ phosfolan, pathways of	99f
mephosfolan	99-101
phosfolan	98
of thiothiocarbamates	74-75
carbamylation reactions of	75
oxidations and rearrangements of	74-75
of (trichloro- $^{14}\text{C}$ -methyl) captan in spinach and soil	91-92
in vitro studies on the <i>S</i> -oxygenating enzyme involved in cyanatryn	59-60
by washed cells, effects of added cofactors on toxaphene	120-124
Metabolites	
in ethyl acetate phase by GLC analysis, determination of functional groups of	125t
isolated from the roots of peanut plants treated with $^{14}\text{C}$ PCNB, ether-soluble	140f
isolated from the roots of peanut plants treated with $^{14}\text{C}$ PCNB, water-soluble	138f
S-Methylly thiocarbamate, MCPBA oxidation of	68
Methamidophos	46
Methanol-insoluble residues in peanut plants treated with $^{14}\text{C}$ PCNB, formation of 80%	148f
Methimazole, <i>S</i> -oxygenation of	61f
Methomyl, aminosulfonyl derivatives of	44
Methyl transferase system from onion root, activity of substrates for	159t
S-Methylation of the C-S lyase cleavage	169
Methylcarbamates	
alkylsulfonyl	37-39
arylsulfonyl	37-39

- Methylcarbamates (*continued*)  
 and the corresponding *N,N'*-thiobis-  
 carbamates, toxicological  
 properties of insecticidal ..... 40*t*  
 derivatives of insecticidal  
*N*-alkylsulfinyl ..... 45  
 toxicological properties of ..... 37*t*-38*t*  
*N*-arylsulfinyl ..... 45  
 toxicological properties of ..... 37*t*-38*t*  
 esters, *N*-dimethoxyphosphinothioyl  
 insecticides, delay factor in ..... 37  
 and types of selectivity arylsul-  
 fenylated ..... 38  
*N*-Methylcarbamate, isopropyl  
*N*-chlorosulfinyl- ..... 45  
 Methylene chloride-soluble residues  
 from <sup>14</sup>C PCNB-treated plant  
 tissues, nonpolar ..... 151-157  
 from <sup>14</sup>C PCNB-treated plant  
 tissues, polar ..... 151-157  
 isolated from plant tissues with  
<sup>14</sup>C PCNB ..... 152*f*  
 in plants, nonpolar ..... 154  
 from the roots of <sup>14</sup>C PCNB-treated  
 peanut plants ..... 151  
 Methylene hydroxylation system for  
 camphor, role of putidaredoxin in ..... 118*f*  
*bis*-(Methylthio)tetrachlorobenzene  
 (*bis*-MTTCB) ..... 170  
 Mexacarbate, degradation of  
 in cell-free extracts of *B.*  
*megaterium* ..... 124-127  
 by ferredoxin ..... 117  
 through flavin cofactor-ferredoxin  
 system, pathway ..... 118*f*  
 Mexacarbate and desmethylmexacar-  
 bate, structures of ..... 118*f*  
 Mice, toxicity of *O,S*-dimethyl *N*-(*N'*-  
 alkoxy-carbonyl-*N'*-alkaminosul-  
 fenyl)phosphoramidothioates to  
 house flies ..... 47*t*  
 Microbial flavodoxin isolation ..... 115  
 Microflora in metabolism of gluta-  
 thione conjugates of xenobiotics,  
 role of GUI ..... 165-178  
 Microfloral C-S lyases, catabolism  
 utilizing ..... 167-174  
 Microfloral *S*-glucuronidases, catabo-  
 lism utilizing tissue C-S lyases  
 and ..... 174-176  
 Microorganism, roles of iron-sulfur  
 proteins in degradation of pesti-  
 cidal chemicals by ..... 111-129  
 Microsomal metabolism of parathion  
 to paraoxon, chemical mecha-  
 nism of ..... 21  
 Microsomes  
 following incubation with parathion  
 and its metabolites, <sup>35</sup>S and <sup>32</sup>P  
 bound to ..... 26*t*  
 Microsomes (*continued*)  
 metabolism of parathion by  
 mammalian ..... 20*f*  
<sup>18</sup>O enrichment in paraoxon follow-  
 ing incubation of parathion  
 with rabbit liver ..... 21*t*  
*S*-oxygenation of cyanatryn via liver  
 ..... 59  
 Mixed-function oxidase system of  
*B. megaterium* ..... 124  
 Mixed-function oxidation system of  
 adrenal cortex mitochondria, role  
 of adrenodoxin in ..... 114*f*  
 Molinate initiated by *S*-oxygenation,  
 metabolism of ..... 61*f*  
 Monoxygenase  
 -catalyzed metabolism of parathion,  
 products of the Cytochrome  
 P-450 ..... 24  
 -catalyzed metabolism of phos-  
 phorothionate triesters, chemi-  
 cal mechanisms of the Cyto-  
 chrome P-450 ..... 19-34  
 oxidase enzyme system from rabbit  
 liver, linearity of the metabo-  
 lism of parathion and benz-  
 phetamine by a  
 reconstituted ..... 29*f*  
 system  
 metabolism of parathion to  
 diethyl phosphorothionate  
 and diethyl phosphate by  
 the Cytochrome P-450-  
 containing ..... 25  
 metabolism of parathion to para-  
 oxon by the Cytochrome  
 P-450-containing ..... 23*f*  
 reconstituted  
 from rabbit liver, parathion  
 metabolism by ..... 27*t*  
 from rat liver, elution profile  
 of protein, radioactivity,  
 and thiocyanate from a  
 Sephadex G-25 column  
 of ..... 31*f*  
 from rat liver, SDS-PAGE  
 of ..... 31*f*  
*bis*-MTTCB (*bis*-methylthio)tetra-  
 chlorobenzene) ..... 170  
 Mutagenic  
 activities of the promutagens ..... 79*f*  
 activity of captan- and *bis*-(tri-  
 chloromethyl) disulfide assayed  
 with *S. typhimurium*  
 TA 100 ..... 95*f*  
 properties  
 of captan in bacterial assays ..... 86  
 of diallate ..... 76-80  
 of sulfallate ..... 80  
 of trillate ..... 80  
 Mutagenicity studies ..... 94

N	P
NADH-FAD-FMN, cultures containing .....	<sup>32</sup> P bound to microsomes following incubation with parathion and its metabolites, <sup>35</sup> S and .....
124	26 <i>t</i>
<i>p</i> -Nitrophenol formation in the mammalian metabolism of parathion, diethyl phosphate .....	<i>P. putida</i> (see <i>Pseudomonas putida</i> )
24	PCB (polychlorinated biphenyls) .....
4-Nitrothiophenol at 25°C, reaction rate constants of perhalogenated methylmercapto derivatives in the reaction with .....	PCMTB (see Pentachloromethylthio-benzene)
86 <i>f</i>	<i>S</i> -(PCP)
Nonpolar methylene chloride-soluble residues from <sup>14</sup> C PCNB-treated plant tissues .....	GSH, peptide inhibition of pentachlorothiophenol formation from .....
151-157	156
O	GSH in the presence of the enzyme from onion, breakdown of .....
Onion	155 <i>f</i>
breakdown of <i>S</i> -(PCP)GSH in the presence of the enzyme from ..	thioacetate in peanut roots, origin of .....
155 <i>f</i>	153 <i>f</i>
enzyme system .....	thiolactate in peanut roots, origin of .....
156	153 <i>f</i>
assayed for pentachlorothiobenzene synthesis .....	Paraoxon
156	chemical mechanism of the microsomal metabolism of parathion to .....
in vitro synthesis of pentachlorothiobenzene from PCNB, GSH, and <sup>14</sup> C SAM in the presence of .....	21
156	by the Cytochrome P-450-containing monooxygenase system, metabolism of parathion to .....
root, activity of substrates for the methyl transferase system from ..	23 <i>f</i>
159 <i>t</i>	following incubation of parathion with rabbit liver microsomes, <sup>18</sup> O enrichment in .....
Organosulfur compounds from allium species .....	21 <i>t</i>
5 <i>f</i>	from parathion, formation of .....
Organosulfur compounds in nature, low-molecular-weight .....	22
3-16	Parathion .....
Oxidation	19-34
of diallate and triallate .....	elution from a Sephadex-25 column .....
77 <i>f</i>	30
and rearrangement reactions, toxicological significance of .....	formation of paraoxon from .....
75-80	22
of <i>S</i> -chloroallyl thio- and dithiocarbamate herbicides .....	metabolism of
65-82	and benzphetamine by a reconstituted monooxygenase oxidase enzyme system from rabbit liver, linearity of .....
of proherbicides .....	29 <i>f</i>
75-76	diethyl phosphate <i>p</i> -nitrophenol formation in the mammalian .....
of sulfallate .....	24
78 <i>f</i>	diethyl phosphorothionate and diethyl phosphate by the .....
<i>S</i> -Oxidation of cyanatryn .....	Cytochrome P-450-containing monooxygenase system .....
60	25 <i>f</i>
<i>S</i> -Oxide(s)	mammalian microsomes .....
cyanatryn .....	20 <i>f</i>
55, 57 <i>f</i>	to paraoxon, chemical mechanism of the microsomal .....
and DNA, reactions between ..	21
63	to paraoxon by the Cytochrome P-450-containing monooxygenase system .....
of parathion .....	23 <i>f</i>
24	products of .....
reactions of .....	19-34
60-63	the Cytochrome P-450 monooxygenase-catalyzed .....
<i>S</i> -Oxidized mercapturic acid of PCMTB .....	24
174	by a reconstituted monooxygenase system from rabbit liver .....
<i>S</i> -Oxygenating enzyme involved in cyanatryn metabolism, in vitro studies on .....	27 <i>t</i>
59-60	and its metabolites, <sup>35</sup> S and <sup>32</sup> P bound to microsomes following incubation with .....
<i>S</i> -Oxygenation	26 <i>t</i>
of cyanatryn via liver microsomes ..	<i>S</i> -oxides of .....
59	24
of cyanatryn in three species in vitro ..	
59 <i>t</i>	
in herbicide metabolism in mammals .....	
53-64	
metabolism of molinate initiated by ..	
61 <i>f</i>	
of methimazole .....	
61 <i>f</i>	

- Parathion (*continued*)  
 with rabbit liver microsomes,  $^{18}\text{O}$ -  
 enrichment in paraoxon fol-  
 lowing incubation of ..... 21*t*
- Peanut  
 cell cultures treated with  $^{14}\text{C}$  PCNB,  
 formation and stability of  
*S*-(PCP)MalCys and  
*S*-(TCNP)MalCys in ..... 146*f*  
 cell cultures treated with  $^{14}\text{C}$  PCNB,  
 HPLCs of water-soluble  
 extracts from ..... 144*f*-145*f*  
 plants treated with  $^{14}\text{C}$  PCNB, in  
 the roots of  
 ether-soluble fractions from ..... 139-149  
 ether-soluble metabolites isolated  
 from ..... 140*f*  
 formation of 80% methanol-in-  
 soluble residues in ..... 148*f*  
 insoluble residue in ..... 143  
 methylene chloride-soluble resi-  
 dues from ..... 149-151  
 water-soluble fractions from ..... 136-139  
 water-soluble metabolites  
 isolated ..... 138*f*
- roots  
 origin of *S*-(PCP) thioacetate in  
 treated with  $^{14}\text{C}$  PCNB, struc-  
 tures chloroform-soluble  
 residues isolated from ..... 150*f*
- Pentachloroaniline metabolism ..... 151
- Pentachloromethylthiobenzene  
 (PCMTB) ..... 170  
 in conventional and germfree rats,  
 comparison of the metabolism  
 of ..... 171*t*  
*S*-oxidized mercapturic acid of ..... 174  
 in rats, metabolic mechanism for ..... 173
- Pentachloronitrobenzene (PCNB) ..... 137*f*  
 GSH, and  $^{14}\text{C}$  SAM in the presence  
 of the onion enzyme system, in  
 vitro synthesis of pentachloro-  
 thioanisole from ..... 156  
 in higher plants, metabolic path-  
 way of ..... 158*f*
- Pentachlorothioanisole from PCNB,  
 GSH, and  $^{14}\text{C}$  SAM in the pres-  
 ence of the onion enzyme system,  
 in vitro synthesis of ..... 156
- Pentachlorothioanisole synthesis,  
 onion enzyme system assayed for ..... 156
- Pentachlorothiophenol formation from  
*S*-(PCP) GSH, peptide inhibition  
 of ..... 156
- Pentachlorothiophenol, in vitro forma-  
 tion of ..... 155*f*
- Peptide inhibition of pentachlorothiophenol  
 formation from *S*-(PCP)  
 GSH ..... 156
- Perhalogenmethylmercapto fungicides,  
 biological and chemical behavior  
 of ..... 85-96
- Perhalogenated methylmercapto  
 derivatives in the reactions with  
 4-nitrothiophenol at 25°C, reac-  
 tion rate constants ..... 86*f*
- Pesticidal chemicals by microorgan-  
 isms, roles of iron-sulfur proteins  
 in degradation of ..... 111-129
- Pesticide(s)  
 based on bioactive natural product  
 models ..... 11*f*  
 degradation, involvement of iron-  
 sulfur proteins in ..... 115-117  
 in higher plants, catabolism of  
 glutathione conjugates of ..... 133-164  
 role of glutathione conjugation in  
 dithietane ..... 104*f*  
 the search for new ..... 3-16  
 types of sulfur-containing ..... 11*f*
- Phosfolan metabolism in rats ..... 98
- Phosphinoaminothiomethylcarbamates ..... 43
- Phosphorothionate triesters, chemical  
 mechanisms of the Cytochrome  
 P-450 monooxygenase-catalyzed  
 metabolism of ..... 19-34
- Photodegradation studies ..... 103-106
- Photolysis of dichlofluanid ..... 87*f*
- Pigs, metabolism of propachlor in  
 various species and antibiotic ..... 171*t*
- Plant(s)  
 catabolism of glutathione conjugates  
 of pesticides in higher ..... 133-164  
 metabolic pathway of PCNB in  
 higher, ..... 158*f*  
 metabolism in  
 dichlofluanid ..... 87  
 mephosfolan ..... 101-109  
 role of glutathione conjugation in  
 the mephosfolan ..... 103  
*N*-malonylcysteine conjugates in ..... 142*f*  
 nonpolar methylene chloride-  
 soluble residues in ..... 154  
 tissues as a function of solubility,  
 distribution of  $^{14}\text{C}$  in ..... 137*f*  
 tissues, methylene chloride-soluble  
 residues from  $^{14}\text{C}$  PCNB-treated  
 isolated ..... 152*f*  
 nonpolar ..... 151-157  
 polar ..... 151-157  
 -type ferredoxins ..... 112
- Polar methylene chloride-soluble  
 residues from  $^{14}\text{C}$  PCNB-treated  
 plant tissues ..... 151-157
- Polychlorinated biphenyls (PCB) ..... 173
- Proherbicides, toxicological signifi-  
 cance of oxidation and rearrange-  
 ment reactions of ..... 75-76

Proinsecticides, carbamate .....	37	Rabbit liver ( <i>continued</i> )	
Promutagens, mutagenic activities of	79f	parathion metabolism by a reconstituted monooxygenase system from .....	27t
Propachlor .....	167	Radioactivity	
metabolism (of)		in cotton plants after foliar application of <sup>14</sup> C-mephosfolan .....	105t
by conventional, germfree, and antibiotic-treated rats, comparisons of .....	168t	from goldfish reared in rice paddy environment, recovery of mephosfolan-derived .....	108t
to an <i>N</i> -malonylcysteine conjugate in soybean root .....	147f	levels in tissues of rats following a single oral dose of 2 mg/kg <sup>14</sup> C-phosfolan .....	99t
species differences in .....	170	levels in tissues of rats following a single oral dose of 5 mg/kg <sup>14</sup> C-mephosfolan .....	101t
in various species and antibiotic-treated pigs .....	171t	via respiration gases, urine and feces by rats treated with <sup>14</sup> C-mephosfolan at 2 mg/kg, excretion of .....	101t
in rats .....	166f	via respiration gases, urine and feces by rats treated with a single oral dose of <sup>14</sup> C-phosfolan, excretion of .....	98t
Propesticide action sulfur in .....	35-49	in rice plants grown in <sup>14</sup> C-mephosfolan-treated paddy, residual ..	105t
Propoxur .....	38	Rat(s)	
to the honey bee and house fly, toxicity of <i>N</i> -arylsulfonyl derivatives of .....	39t	comparison of the metabolism of PCMTB in conventional and germfree .....	171t
<i>S,S</i> -Propylene dithiocarbonate from <sup>14</sup> C imido-labeled mephosfolan photolysates, CI(CH <sub>3</sub> ) mass spectrum of cyclic .....	107f	comparisons of the metabolism of propachlor by conventional, germfree, and antibiotic-treated ..	168t
Proteins, iron-sulfur		dosed orally with <sup>14</sup> C-ring cyanatryn following a single oral dose of 2 mg/kg <sup>14</sup> C-phosfolan, radioactivity levels in tissues of .....	62f
in degradation of pesticidal chemicals by microorganisms, roles of .....	111-129	following a single oral dose of 5 mg/kg <sup>14</sup> C-mephosfolan, radioactivity levels in tissues of ..	101t
as an electron transfer component to Cytochrome P-450 .....	113	liver	
in pesticide degradation involvement of .....	115-117	elution profile of protein, radioactivity, and thiocyanate from a Sephadex G-25 column of reconstituted monooxygenase system from ..	31f, 32f
properties of .....	111-113	microsomes and NADPH, TLC of the products derived from the incubation of <sup>14</sup> C cyanatryn with .....	56f
<i>Pseudomonas oleovorans</i> , role of rubredoxin in $\omega$ -hydroxylation of alkanes of .....	114f	microsomes, sulfur bound to .....	30
<i>Pseudomonas putida</i> ( <i>P. putida</i> ) cell(s), washed		SDS-PAGE of a reconstituted monooxygenase system from ..	31f
incubated aerobically, effects of added cofactors on metabolic fate of toxaphene by .....	123t	mephosfolan metabolism in .....	99-101
incubations with <sup>36</sup> Cl-toxaphene, AgNO <sub>3</sub> complexation of aqueous buffer phase from ..	122t	mercapturic acids derived from the metabolism of cyanatryn in ..	54f
metabolic degradation of toxaphene by .....	117-120	metabolic pathway for 2-acetamido-4-chloromethylthiazole in .....	175f
metabolism of <sup>14</sup> C- and <sup>36</sup> Cl-toxaphene by .....	121t		
Putidaredoxin .....	113		
in methylene hydroxylation systems for camphor, role of .....	118f		
<b>R</b>			
Rabbit liver			
linearity of the metabolism of parathion and benzphetamine by a reconstituted monooxygenase oxidase enzyme system from ..	29f		
microsomes, <sup>18</sup> O enrichment in paraoxon following incubation of parathion with .....	21t		

- Rat(s) (*continued*)  
 metabolic pathway for propachlor... 166f  
 pathways of metabolism of <sup>14</sup>C  
 phospholan in ..... 99f  
 phospholan metabolism in ..... 98  
 treated with <sup>14</sup>C-mephosfolan at 2  
 mg/kg, excretion of radio-  
 activity via respiration gases,  
 urine and feces by ..... 101t  
 treated with a single oral dose of  
<sup>14</sup>C-phosfolan, excretion of  
 radioactivity via respiration  
 gases, urine and feces by ..... 98t
- Rice  
 environment, metabolism in fish  
 in ..... 106-109  
 paddy environment, recovery in  
 mephosfolan-derived radio-  
 activity from goldfish reared in 108t  
 paddy treated with <sup>14</sup>C-mephosfolan  
 at 0.75 kg/ha, stimulated ..... 102f  
 plants grown in a <sup>14</sup>C-mephosfolan-  
 treated paddy, residual radio-  
 activity in ..... 105t  
 plants, metabolism study of  
 mephosfolan in ..... 101
- Rubredoxin(s) ..... 111-112  
 in  $\omega$ -hydroxylation of alkanes of  
*P. oleovorans*, role of ..... 114f
- S**
- <sup>35</sup>S and <sup>32</sup>P bound to microsomes fol-  
 lowing incubation with parathion  
 and its metabolites ..... 26t
- Salmonella typhimurium* (*S. typhi-*  
*murium*) ..... 79f  
 TA 100 ..... 77f, 78f, 94  
 mutagenic activity of captan and  
*bis*-(trichloromethyl) disul-  
 fide assayed with ..... 95f  
 negative response of dichlofuanid  
 in comparison to its tri-  
 chloro analogue assayed  
 with ..... 95f
- Selectivity, arylsulfenylated methyl-  
 carbamates and types of  
 selectivity ..... 38
- Sephadex-25 column, parathion  
 elution from ..... 30
- Soil application followed by spinach  
 culture under closed conditions,  
 balance account of <sup>14</sup>C captan  
 radioactivity after ..... 92t
- Soil, metabolism of (trichloro-<sup>14</sup>C-  
 methyl) captan in spinach and ..... 91-92
- Solubility, distribution of <sup>14</sup>C in plant  
 tissues as a function of ..... 137f
- Sorghum, metabolic pathway of atra-  
 zine in ..... 134f
- Soybean root, metabolism of propa-  
 chlor to an *N*-malonylcysteine  
 conjugate in ..... 147f
- Spinach culture under closed condi-  
 tions, balance account of <sup>14</sup>C  
 captan radioactivity after soil  
 application followed by ..... 92t
- Spinach and soil, metabolism of  
 (trichloro-<sup>14</sup>C-methyl) captan in 91-92
- Strawberry(ies)  
 metabolism of (fluorodichloro <sup>14</sup>C-  
 methyl) dichlofuanid in ..... 88  
 plants under closed conditions,  
 balance account of <sup>14</sup>C dichlo-  
 fuanid radioactivity after spray  
 application on ..... 88t  
 plants under open conditions, bal-  
 ance account of <sup>14</sup>C dichlo-  
 fuanid radioactivity after spray  
 application on ..... 89t  
 proposed metabolism of <sup>14</sup>C dichlo-  
 fuanid in ..... 91f
- Sulfallate  
 chloro-sulfallate, their sulfine deriv-  
 atives and further oxidation  
 products, <sup>1</sup>H chemical shift  
 data for ..... 72t  
 mutagenic properties of ..... 80  
 oxidation of ..... 78f
- Sulfines, dithiocarbamate ..... 71-74  
*N*-Sulfinyl derivatives of carbofuran  
 toxicological properties of ..... 46t  
*N*-Sulfinylmethylcarbamates ..... 44-46  
 Sulfonamidothiomethylcarbamates ..... 43  
 Sulfoxide, diallate ..... 74  
 Sulfoxides thiocarbamate ..... 55, 66
- Sulfur  
 acyclic compounds containing ..... 7f  
 bound to rat liver microsomes ..... 30  
 -containing pesticides, some com-  
 mon types of ..... 11f  
 in propesticide action ..... 35-49  
 systems, acyclic ..... 4-8  
 systems, heterocyclic ..... 8-10
- T**
- Thiaheterocycles, naturally occurring ..... 9f  
 Thienamycin ..... 5f  
*N,N'*-Thiobiscarbamates ..... 40  
 toxicological properties of insecti-  
 cidal methylcarbamates and  
 the corresponding ..... 40t  
*N,N'*-thiobiscarbofuran ..... 40
- Thiocarbamate(s)  
 alkylthiotriazines and ..... 53-55  
 herbicides ..... 55



