

PESTICIDE CHEMISTRY

Human Welfare and the Environment

Volume 3

Mode of Action, Metabolism and Toxicology

018618

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY
(Applied Chemistry Division)

PESTICIDE CHEMISTRY: HUMAN WELFARE AND THE ENVIRONMENT

Proceedings of the 5th International Congress of Pesticide Chemistry,
Kyoto, Japan, 29 August - 4 September 1982

Editors-in-Chief

J. MIYAMOTO

Pesticides Division, Sumitomo Chemical Co., Ltd.
Takarazuka, Hyogo, Japan

and

P. C. KEARNEY

Agricultural Environmental Quality Institute, US Department of Agriculture,
Beltsville, Maryland, USA

Volume 3

MODE OF ACTION, METABOLISM AND TOXICOLOGY

Volume Editors

S. MATSUNAKA

Department of Plant Protection, University of Kobe, Japan

D. H. HUTSON

Shell Research Ltd., Sittingbourne, Kent, UK

and

S. D. MURPHY

University of Texas Medical School, Houston, Texas, USA



PERGAMON PRESS

OXFORD · NEW YORK · TORONTO · SYDNEY · PARIS · FRANKFURT

U.K.	Pergamon Press Ltd., Headington Hill Hall, Oxford OX3 0BW, England
U.S.A.	Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, New York 10523, U.S.A.
CANADA	Pergamon Press Canada Ltd., Suite 104, 150 Consumers Road, Willowdale, Ontario M2J 1P9, Canada
AUSTRALIA	Pergamon Press (Aust.) Pty. Ltd., P.O. Box 544, Potts Point, N.S.W. 2011, Australia
FRANCE	Pergamon Press SARL, 24 rue des Ecoles, 75240 Paris, Cedex 05, France
FEDERAL REPUBLIC OF GERMANY	Pergamon Press GmbH, Hammerweg 6, D-6242 Kronberg-Taunus, Federal Republic of Germany

Copyright © 1983 International Union of Pure and
Applied Chemistry

All Rights Reserved. No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means: electronic, electrostatic, magnetic tape, mechanical, photocopying, recording or otherwise, without permission in writing from the copyright holders.

First edition 1983

Library of Congress Cataloging in Publication Data

International IUPAC Congress of Pesticide Chemistry
(5th: 1982: Kyoto, Japan)

Pesticide chemistry, human welfare and the environment.

(IUPAC symposium series)

At head of title: International Union of Pure and Applied Chemistry. Applied Chemistry Division. Congress organized by The Science Council of Japan, Pesticide Science Society of Japan, and the Japan Plant Protection Association.

Includes bibliographical references.

1. Pesticides—Congresses. I. Miyamoto, J. (Junshi) II. Kearney, P. C. (Philip C.), 1932- III. International Union of Pure and Applied Chemistry. Applied Chemistry Division. IV. Nihon Gakujutsu Kaigi. V. Nihon Nōyaku Gakkai. VI. Nihon Shokubutsu Bōeki Kyōkai. VII. Title. VIII. Series. [DNLN: 1. Pesticides—Congresses. 2. Pesticide—Residues—Congresses. 3. Toxicology—Congresses. 4. Pest control—Congresses. WA 240 P4738] SB950.93.I57 . 1982 632'.95 82-24602

British Library Cataloguing in Publication Data

International Congress of Pesticide Chemistry: (5th: 1982: Kyoto) Pesticide chemistry.

1. Pesticides. Environmental aspects—Congresses

I. Title II. Miyamoto, J. III. Kearney, P. C.

IV. International Union of Pure and Applied Chemistry. Applied Chemistry Division

632'.95042 QH545.P4

ISBN 0 08 029219 4 (4 vol set)

ISBN 0 08 029224 0 (volume 3)

In order to make this volume available as economically and as rapidly as possible the authors' typescripts have been reproduced in their original forms. This method unfortunately has its typographical limitations but it is hoped that they in no way distract the reader.

Printed in Great Britain by A. Wheaton & Co. Ltd., Exeter

018618

PESTICIDE CHEMISTRY: HUMAN WELFARE AND THE ENVIRONMENT

Volume 3

MODE OF ACTION, METABOLISM AND TOXICOLOGY

Some Other IUPAC Titles of Interest from Pergamon Press

IUPAC Symposium Series

BENOIT & REMPP: Macromolecules

BRITTON & GOODWIN: Carotenoid Chemistry and Biochemistry

BROWN & DAVIES: Organ-Directed Toxicity — Chemical Indices and Mechanisms

CIARDELLI & GIUSTI: Structural Order in Polymers

FREIDLINA & SKOROVA: Organic Sulfur Chemistry

LAIDLER: Frontiers of Chemistry (Proceedings of the 28th IUPAC Congress)

NOZAKI: Current Trends in Organic Synthesis

ST-PIERRE & BROWN: Future Sources of Organic Raw Materials (CHEMRAWN I)

SHEMILT: Chemistry & World Food Supplies (CHEMRAWN II)

STEC: Phosphorus Chemistry Directed Towards Biology

TROST & HUTCHINSON: Organic Synthesis — Today and Tomorrow

IUPAC Nomenclature Guides

IRVING, FREISER & WEST: Compendium of Analytical Nomenclature

IUPAC: Nomenclature of Inorganic Chemistry & How to Name an Inorganic Substance (2-part set)

RIGAUDY & KLESNEY: Nomenclature of Organic Chemistry

WHIFFEN: Manual of Symbols & Terminology for Physicochemical Quantities and Units

Journals

CHEMISTRY INTERNATIONAL — IUPAC's international news magazine.

PURE AND APPLIED CHEMISTRY — IUPAC's official journal, featuring proceedings of IUPAC conferences, nomenclature rules and technical reports.

Organizing Committee

Chairman: M. Nakajima

Vice Chairman: H. Fukami

Secretary General: T. Misato

Members: T. Endo, M. Eto, T. Fujita, S. Goto, T. Hosotsuji, W. Iida, T. Iwata,
A. Kawana, K. Koshimizu, N. Kurihara, S. Kuwatsuka, S. Matsunaka, T. Mitsui,
J. Miyamoto, J. Mizutani, K. Munakata, M. Saito, T. Saito, N. Takahashi,
S. Takahashi, T. Tanaka, M. Uchiyama, K. Ueki, T. Ueno, I. Yamamoto,
K. Yamashita, K. Yasutomi

Scientific Programme Committee

Chairman: J. Miyamoto

Vice Chairman: P. C. Kearney

Members: P. Doyle, N. Drescher, T. Fujita, S. Goto, R. Greenhalgh, D. H. Hutson,
S. Kuwatsuka, S. Matsunaka, S. D. Murphy, N. Takahashi

Congress Organizers

The Science Council of Japan
Pesticide Science Society of Japan
Japan Plant Protection Association

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

IUPAC Secretariat: Bank Court Chambers, 2-3 Pound Way,
Cowley Centre, Oxford OX4 3YF, UK

PREFACE

The Fifth International Congress of Pesticide Chemistry, sponsored by the International Union of Pure and Applied Chemistry, and organized jointly by the National Science Council of Japan, Pesticide Science Society of Japan and Japan Plant Protection Association, was held at Kyoto International Conference Hall in Kyoto, Japan, 29 August - 4 September 1982. The opening of the Congress culminated four years of intensive planning by the Scientific Programme Committee, the Organizing Committee, and a host of internationally recognized scientists dedicated to pesticide chemistry. The main theme of the Congress, **Human Welfare -- Environment -- Pesticides**, was intended to encompass current research topics in pesticide chemistry, not only for increased agricultural production, but also for public health purposes. Xenobiotics other than pesticides were also included. One thousand, six hundred scientists from 55 countries attended the Congress.

Two distinguished scientists, Professor Dr. K. H. Büchel, Bayer AG, Leverkusen, FRG, and Dr. I. J. Graham-Bryce, East Malling Research Station, UK, presented plenary lectures dealing with political, economic and philosophical aspects of pesticide use, as well as future pesticide research for improving human welfare. A number of distinguished invitees also addressed the Congress participants, including the President of IUPAC, Professor S. Nagakura.

Eight main topics were selected as the subjects of the Congress, either because of their timely nature or because the area needs critical review. They included: Synthesis of Pesticides and Growth Regulators; Chemical Structure and Biological Activity; Bioactive Natural Products: Chemistry, Biochemistry and Physiology; Biochemistry of Pests and Mode of Action of Pesticides (including Mechanism of Resistance and Phytotoxicity); Metabolism and Degradation of Pesticides and Xenobiotics; Toxicology of Pesticides and Xenobiotics; Pesticide Residues and Methodology; and Formulation Chemistry. Each main topic included one symposium and several poster-discussion sessions.

Each symposium consisted of several invited presentations, providing the participants with current and provocative overviews on important aspects of the respective topics. Poster-discussion sessions constituted the main body of presentations to the Congress and were intended to cover a wide variety of areas. Each included a few invited papers in addition to the contributed papers. Invited scientists served as leaders during the follow-up discussion after the poster presentations. Overall the Congress was organized into 49 sessions under the eight main topics with 694 submitted papers, including 236 invited papers.

In addition to the eight main topics encompassed by the Congress, three complementary symposia dealing with related subjects were held simultaneously, with 31 invited presentations: Pyrethroid Insecticides -- Biological Activity, Mode of Action, Metabolism and Toxicology; Antibiotics for Agricultural Use; and Herbicides and Plant Growth Regulators for Rice Culture.

The proceedings of the Congress, entitled **Pesticide Chemistry: Human Welfare and the Environment**, comprise four volumes containing over 250 invited papers presented at the symposia, complementary symposia, and poster-discussion sessions. Specifically, the contents are: Volume 1: plenary lectures, synthesis, structure-activity; Volume 2: natural products, complementary symposia; Volume 3: mode of action, metabolism and degradation, toxicology; and Volume 4: residue analysis, formulation chemistry. It is sincerely hoped, by this overview of the present status of chemical and biochemical pest control, that readers gain an appreciation of how pesticide science continues to contribute to human welfare.

Junshi Miyamoto

Philip C. Kearney

CONTENTS

Organizing Committee	x
Preface	xi
Biochemistry of Pests and Mode of Action of Pesticides (including Mechanism of Resistance and Phytotoxicity)	
<i>Biochemical Systems as Possible Targets for Pesticides</i>	
Influence of Chlorinated and Pyrethroid Insecticides on Cellular Calcium Regulatory Mechanisms	3
F. MATSUMURA	
Behavioral and Lethal Actions of Amidines on Invertebrates	15
R. M. HOLLINGWORTH and A. E. LUND	
Insect Chitin Synthetase as a Biochemical Probe for Insecticidal Compounds	25
E. COHEN and J. E. CASIDA	
Biosynthetic Processes of Ergosterol as the Target of Fungicides	33
T. KATO	
Resistance to Pyrimidine Fungicides which Inhibit Ergosterol Biosynthesis	43
M. A. de WAARD and J. DEKKER	
Molecular Characterization of the Target Site(s) for Herbicides which Affect Photosynthetic Electron Transport	51
C. J. ARNTZEN, K. E. STEINBACK, W. VERMAAS and I. OHAD	
Toxic Oxygen Species and Herbicide Action	59
A. D. DODGE	
<i>Advances in Research for Mode of Pesticide Action</i>	
Diagnostic Techniques for Nerve Poison Mode of Action	67
T. A. MILLER	
Pharmacokinetic Approaches to the Action of Pyrethroids in Insects	69
D. M. SODERLUND	
Pyrethroid- and DDT-evoked Release of GABA from the Nervous System in vitro	75
R. A. NICHOLSON, R. G. WILSON, C. POTTER and M. H. BLACK	
Mode of Action of Herbicides that Affect Cell Division	79
F. D. HESS	
Mode of Action and MO Calculation of Two Classes of Herbicides Interacting with the Reducing Side of Photosystem II	85
A. TREBST, W. DRABER and W. T. DONNER	
Evidence for Two Different Herbicide Binding Proteins at the Reducing Side of Photosystem II	91
W. OETTMEIER, K. MASSON, C. FEDTKE, J. KONZE and R. R. SCHMIDT	
Mode of Action of Nitrodiphenylethers Affecting Pigments and Membrane Integrity	97
R. LAMBERT, G. SANDMANN and P. BÖGER	
<i>Interaction of Pesticides with Membranes</i>	
Site of Action of Pyrethroid Insecticides in Neuronal Membranes as Revealed by the kdr Resistance Factor	103
M. P. OSBORNE and A. SMALLCOMBE	
Nerve Membrane Sodium Channels as the Major Target Site of Pyrethroids and DDT	109
T. NARAHASHI	

Interaction of Pyrethroids and DDT-like Compounds with the Sodium Channels in the Nerve Membrane	115
J. van den BERCKEN and H. P. M. VIJVERBERG	
Mode of Action of Tridemorph and Related Compounds	123
A. KERKENAAR	
Ergosterol Biosynthesis: A Target of Fungitoxic Action	129
H. D. SISLER, R. C. WALSH and B. N. ZIOGAS	
Kitazin P and Edifenphos, Possible Inhibitors of Phosphatidylcholine Biosynthesis	135
O. KODAMA and T. AKATSUKA	
Mode of Action of Disease Protectants	
Site of Action of Carboxamides in Mitochondrial Complex II	141
G. A. WHITE, G. D. THORN, B. A. C. ACKRELL, E. B. KEARNEY, R. R. RAMSEY and T. P. SINGER	
Mode of Action of Rice Blast Protectant, Probenazole	147
Y. SEKIZAWA	
Mode of Antifungal Action of a New Fungicide, Tolclofos-Methyl	153
T. KATO	
Biochemical Mechanism Causing Tolerance, Resistance and Selectivity	
Use of Heterozygous Diploid Strains of <i>Aspergillus nidulans</i> for the Recognition of Genetically Active Pesticides	159
S. G. GEORGOPOULOS	
Metabolism of a Phosphorothiolate Fungicide IBP by Strains of <i>Pyricularia oryzae</i> with Varied Sensitivity	165
Y. UESUGI and M. KATAGIRI	
Characterization of a 32 Kilodalton Herbicide-binding Polypeptide	171
H. Y. NAKATANI, K. SATOH, K. E. STEINBACK and C. J. ARNTZEN	
Behaviour of Metribuzin in Tolerant and Susceptible Soybean Varieties	177
C. FEDTKE and R. R. SCHMIDT	
Effect of Insecticide Rotations on Evolution of Resistance	183
G. P. GEORGHIOU, A. LAGUNES and J. D. BAKER	
The Biochemistry of Insecticide Resistance in the Peach-Potato Aphid, <i>Myzus persicae</i>	191
A. L. DEVONSHIRE, G. D. MOORES and C. L. CHIANG	
The Presence of Two Forms of Glutathione S-Transferases with Distinct Substrate Specificity in OP-resistant and -susceptible Housefly Strains	197
N. MOTOYAMA, A. HAYASHI and W. C. DAUTERMAN	
Changed Acetylcholinesterase and Resistance in Leaf- and Planthoppers	203
H. HAMA	
The Biochemical Aspects of Herbicide Antidotes in Plants	
Discovery and Development of Antidotes to Improve Herbicide Selectivity	209
G. R. STEPHENSON and F. M. PALLOS	
On the Mode of Action of EPTC and its Antidotes	213
F. DUTKA and T. KÖMIVES	
Influence of Herbicides and Antidotes on the Glutathione Levels of Maize Seedlings	219
G. R. STEPHENSON, A. ALI and F. M. ASHTON	
Rapid Multilevel Interactions of a Thiocarbamate Herbicide and its Protectant in Maize Cell Cultures	225
G. EZRA, H. M. FLOWERS and J. GRESSEL	
EPTC Inhibition of Gibberellin Precursor Biosynthesis and Reversal of the Inhibition by N,N-Diallyl-2,2-Dichloroacetamide	233
R. E. WILKINSON	

Metabolism and Degradation of Pesticides and Xenobiotics*Bioactivation of Pesticides and Xenobiotics*

- Propesticides: Bioactivation in Pesticide Design and Toxicological Evaluation 239

J. E. CASIDA

- Selective Toxicity Conferred by Activation 247

W. C. DAUTERMAN

- Comparative Biochemistry of Animal, Plant, and Microorganism Oxidases 255

T. OMURA, N. HARADA and H. YOSHIOKA

- Bioactivation Involving Chemically Reactive Oxygenated Carbon 263

D. H. HUTSON

- Bioactivations Involving Halogen-containing Substituents 275

N. KURIHARA

- Bioactivation Involving Nitrogen-containing Substituents 279

K. TATSUMI, H. YAMADA and S. KITAMURA

- Metabolism and Degradation of Pesticides and Xenobiotics: Bioactivations Involving Sulfur-containing Substituents 287

I. SCHUPHAN and J. E. CASIDA

Novel Aspects of the Metabolism of Pesticides and Xenobiotics

- Malonylcysteine Conjugates as End-products of Glutathione Conjugate Metabolism in Plants 295

G. L. LAMOUREUX and D. G. RUSNESS

- Conjugation Reactions of Pesticide Metabolites with Lipids in Animals 301

D. A. SCHOOLEY and G. B. QUISTAD

- Pyrethroid Detoxification and Synergism in Insects 307

I. ISHAAYA and J. E. CASIDA

Photochemical and Non-Biological Transformation of Pesticides and Xenobiotics

- Photodegradation of Pesticides in Solution: Isomerization, Dehalogenation and Ester Cleavage Reactions of the Pyrethroid Insecticides 311

L. O. RUZO

- Thermal Degradation of Pesticides and Xenobiotics: Formation of Polychlorinated Dioxins and Dibenzofurans 317

C. RAPPE and S. MARKLUND

- Light-induced Transformations of Pesticides on Silica Gel as a Model System for Photodegradation on Soil 323

H. HULPKE, R. STEGH and R. WILMES

- Atmospheric Reactions of Pesticides 327

D. G. CROSBY

- Assessment of Abiotic Transformation 333

S. GÄB, F. KORTE, W. MERZ and H.-J. NEU

Distribution and Fate of Xenobiotics in Soils and Sediments

- Behavior and Fate of Pesticides in Paddy Ecosystems 339

Y.-L. CHEN

- Humus-bound Residues of Phenylamide Herbicides: their Nature, Persistence and Monitoring 345

R. BARTHA, I.-S. YOU and A. SAXENA

- Anaerobic Microsites in Soils and their Possible Effect on Pesticide Degradation 351

K. HAIDER

Metabolism of Pesticides and Xenobiotics in Terrestrial and Aquatic Organisms

- Bound Pesticide Residues in Plants 357

R. HUBER and S. OTTO

The Capabilities of Fish and other Aquatic Organisms for Xenobiotic Metabolism G. L. FOUREMAN and J. R. BEND	363
The Fate of Pesticide Plant Metabolites in Vertebrates J. HARVEY, Jr.	369
Comparative Metabolism of Sulfamethazine [4-Amino-N-(4,6-Dimethyl-2-Pyrimidinyl)- Benzenesulfonamide] in the Rat, Chicken, Pig and Sheep G. PAULSON, C. STRUBLE and A. MITCHELL	375
Metabolism of Pesticides and Xenobiotics in Man in Comparison with other Mammalian Species	
The Use of Non-Human Primates as Models for the Metabolism of Pesticides and Xenobiotics in Man D. R. HAWKINS	381
Ethical Considerations Involving Studies of Pesticides and other Xenobiotics in Man W. J. HAYES, Jr.	387
New Approaches to Xenobiotic Metabolism Studies	
Metabolism Studies with Liver Homogenate, Hepatocyte Suspension and Perfused Liver T. NAKATSUGAWA and S. TSUDA	395
Deuteration as a Tool in the Study of Xenobiotic Metabolism J. PORTIG	401
Toxicology of Pesticides and Xenobiotics	
Assessing the Toxicity of Pesticides and Related Compounds in Non-Target Species	
The Role of Biochemical Studies in Modern Toxicological Assessment of Pesticides W. N. ALDRIDGE	409
Neurophysiological and Behavioral Assessment of Pesticide Toxicity A. M. REVZIN	419
Genetic Toxicology Applied to Assessment of Mutagenic, Carcinogenic and Teratogenic Action of Pesticides and Related Compounds I. F. H. PURCHASE	425
Assessing Pesticide Toxicity in Man and Correlations with Laboratory Animal Studies J. DOULL	433
An Assessment of the Hazard of Synthetic Pyrethroid Insecticides to Fish and Fish Habitat N. Y. KHAN	437
Biochemical Toxicology of Pesticides and Xenobiotics in Non-Target Species	
Absorption and Transport of Insecticides in Vertebrates F. E. GUTHRIE, S. M. AHDAYA, P. V. SHAH and B. P. MALIWAL	451
Oxygen Activation and Lipoperoxidative Mechanisms of Toxicity of Pesticides and other Xenobiotics J. S. BUS	457
Mechanistic Studies on the Inhibition of Cytochrome P-450-Mediated Mixed Function Oxidation C. F. WILKINSON, M. MURRAY, C. MARCUS and C. DUBÉ	463
Chromatographic Translobular Migration of Xenobiotics T. NAKATSUGAWA, S. TSUDA and W. K. SHERMAN	469
Assessment of Mutagenesis, Carcinogenesis and Teratogenesis of Pesticides and other Xenobiotics	
Organochlorine Pesticides and Inhibition of Intercellular Communication as the Mechanism for their Liver Tumor Production G. M. WILLIAMS	475

Assessment of Teratogenicity of Ethylenethiourea S. TERAMOTO, M. KANEDA, R. SUZUKI, H. AOYAMA, H. KOBAYASHI and Y. SHIRASU	479
Neurotoxicity and other Organ System Effects of Pesticides and other Xenobiotics Toxicology of Pyrethroids W. N. ALDRIDGE	485
Neurotoxic Esterase: Characterization and Potential for a Predictive Screen for Exposure to Neuropathic Organophosphates R. J. RICHARDSON and B. R. DUDEK	491
Methods for Testing Immune Effects of Toxic Chemicals: Evaluation of the Immunotoxicity of Various Pesticides in the Rat J. G. VOS, E. I. KRAJNC, P. K. BEEKHOF and M. J. van LOGTEN	497
Functional, Morphologic and Biochemical Correlates of Pulmonary Toxicity of Paraquat L. L. SMITH	505
Toxic Actions and Interactions of Acute and Chronic Exposure to Pesticides Limited in vivo Liver Bioassays for Identifying Long-term Biological Effects G. M. WILLIAMS	511
Interactions between Pesticides and other Xenobiotics in the Kidney J. B. HOOK and V. C. SERBIA	515
Dietary Factors Affecting Pesticides Toxicity S. M. CHARBONNEAU and I. C. MUNRO	521
Effect of Protein Malnutrition on the Toxicity of Pesticides F. KALOYANOVA and M. TASHEVA	527
Mechanisms of Tolerance to Anticholinesterase Insecticide Toxicity S. D. MURPHY, L. G. COSTA and B. SCHWAB	531
Test Procedures for Assessing Hazards of Pesticides and other Xenobiotics in Ecosystems Consecutive System of Tests for Assessment of the Effects of Chemical Agents in the Aquatic Environment D. M. M. ADEMA, J. KUIPER, A. O. HANSTVEIT and H. H. CANTON	537
Standardization of Test Procedures and Species in Ecotoxicity Assessments: An Algal Test to Observe the Photosynthetic Activity of <i>Porphyra</i> Sp. using Radio Isotope Techniques T. YOSHIDA, T. MARUYAMA, H. KOJIMA and H. SAITO	545
Fate and Biological Effects of Insecticides in Ponds N. O. CROSSLAND and K. E. ELGAR	551
Author Index to Volume 3	557
Subject Index to Volume 3	559

BIOCHEMISTRY OF PESTS AND MODE OF ACTION
OF PESTICIDES

INFLUENCE OF CHLORINATED AND PYRETHROID INSECTICIDES ON CELLULAR CALCIUM REGULATORY MECHANISMS

Fumio Matsumura

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

Abstract - Chlorinated and pyrethroid insecticides have been found to inhibit various Ca-stimulated ATPases in the nerve. Two ATPases that were found to be particularly sensitive to these insecticides were Ca-ATPase and Ca-Mg ATPase. The proposed functions of these enzymes are reduction of intracellular Ca^{++} and regulation of Ca^{++} permeability across the nerve plasma membrane for the former enzyme, and Ca^{++} pumping and Ca^{++} sequestration to keep the intracellular free Ca^{++} concentration low for the latter. The former is mainly inhibited by DDT, and the latter is particularly sensitive to heptachlor epoxide and other cyclodiene insecticides. Though pyrethroids affect both enzymes, pyrethrin and its closely related analog allethrin selectively inhibit Ca-ATPase, and cypermethrin and decamethrin have more profound effects on Ca-Mg ATPase. Permethrin, on the other hand, affected both enzymes. Two most likely consequences of inhibition of these enzymes are membrane destabilization and synaptic facilitation due to increased transmitter release, respectively.

INTRODUCTION

Regulation of calcium is viewed as a vital function of the nerve cells in maintaining excitability. Recent reports indicate that in the squid giant axon, the intracellular concentration of Ca^{++} (free calcium) is maintained at about 2×10^{-8} M while its extracellular concentration stays in the order of 2×10^{-3} M (1). Therefore, there is a 10^5 -fold gradient of calcium across the nerve membrane. Reduction of this gradient either by lowering the external calcium (2) or increasing the internal calcium (3) results in a destabilization of the axon.

In 1969 it was found by two groups of scientists that nerve ATPases are sensitive to chlorinated hydrocarbon insecticides (4,5). Subsequent research works have shown that two types of ATPases, mitochondrial (6) and Ca-stimulated ATPases (7), are particularly sensitive to these insecticides. The function of the former ATPase is known to be coupled to energy production, and indeed *in vivo* the system works to synthesize ATP from ADP and inorganic phosphate. The functions of the latter ATPases have not been known until recently. However, there has been a tremendous progress made in this regard recently, and we know now much more about the meaning of the presence of these ATPases in the nervous system.

In this paper I have made an attempt to summarize our work in the past 10 years on the inhibitory action mechanisms of chlorinated and pyrethroid insecticides on nerve ATPases. As will be shown, we have found that these insecticides have profound effects on the enzymes involved in calcium regulation. Such actions could explain at least some of the symptoms these chemicals induce.

GENERAL MECHANISMS OF CALCIUM REGULATION

The general scheme of calcium regulation in a cell is illustrated in Fig. 1. The major machineries eliminating intracellular calcium and thereby maintaining a low intracellular concentration are (a) Ca-Mg ATPase which is generally considered to be responsible for Ca-pump and (b) Na/Ca exchange which will be explained later. Contractile proteins usually constitute an inner cell membrane network which acts as a supporting layer to the cell membrane such as axolemma. They consist of actin-myosin type proteins which bind with calcium though it is not a calcium-regulating component *per se*. In addition there is an *ecto* ATPase which is activated by Ca^{++} and Mn^{++} (8) at the surface of the cell. This overall scheme is generally applicable to the axonic system.

In the case of synaptic system (Fig. 2), however, there are some specific modifications of this general scheme. The major function of the synaptic organization is to regulate synthesis, loading and release of transmitters. Though there are a number of theories as to how this is done, there appears to be general agreement on the role of calcium being the vital link to all synaptic transmitter releasing processes. Here an increase in the intracellular concentration of Ca^{++} at the presynaptic terminal triggers the process leading to the release of transmitter. It is known that influx of calcium in the presynaptic region is stimulated by depolarization. Within the synapse, however, Ca^{++} is sequestered by two distinctly different organelles, endoplasmic reticulum and by mitochondria. There have been heated debates as to

which of these organelles seem to be more important in controlling Ca^{++} . Recent studies, however, clearly show that the former is much more important in this regard (10). Mitochondria, though they have high calcium capacities, do not seem to be sensitive enough at low Ca^{++} to be the Ca^{++} regulating organelle at the normal physiological condition.

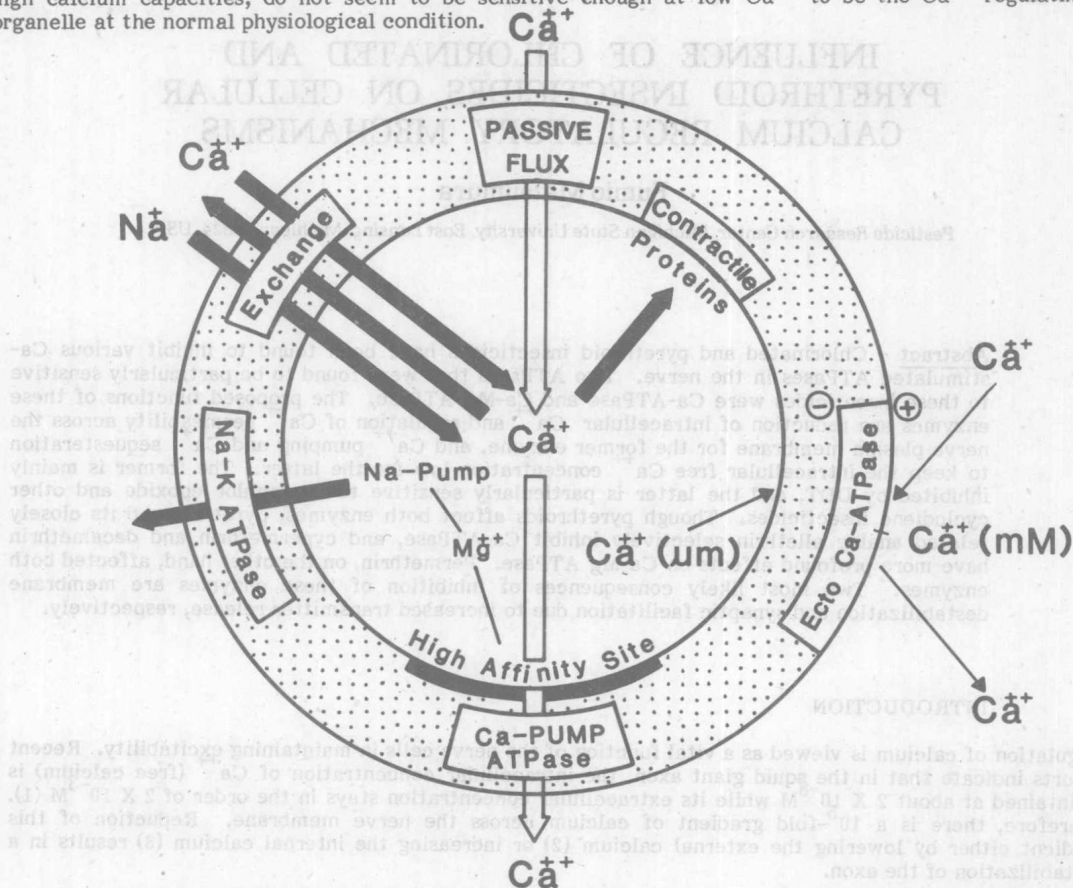


Fig. 1. Diagrammatic sketch showing the regulation of Ca^{++} and the interrelationships among the levels of extra- and intra-cellular Ca^{++} , the Ca-pump and Na-pump of nerves. Ca^{++} may enter the nerve cells and axons via passive influx or Na^+ - Ca^{++} exchange diffusion. The concentration of Ca^{++} bound to the outer surface of the membrane is probably controlled by ecto-ATPase and its optimum concentration is in mM range (10^{-6} - 10^{-5} M). The levels of free intracellular Ca^{++} coming to the inner surface of the membrane is in the range of μM levels (10^{-6} - 10^{-5} M). The levels of free intracellular Ca^{++} are dependent on the balance of influx and efflux, and equilibrium reached by the interaction of Ca^{++} binding proteins. (The role of mitochondria is omitted from this figure for simplification.) The Ca-pump and Na-pump are the function of the membrane-bound ATPase while the ecto-Ca-ATPase work to keep the level of Ca^{++} at the outer surface of the membrane. Inhibition of this enzyme leads to a decrease in the level of surface bound Ca^{++} and results in destabilization of the nerve cell.

[Sketch modified from Vincenzi and Hinds (10).]

Regulation of calcium within the synaptosome is viewed as homologous to the one found in the muscle, where calcium is stored in the intracellular lumen, sarcoplasmic reticulum of which lining is loaded with Ca-Mg.ATPase. The function of this Ca-Mg ATPase is apparently to pump Ca^{++} from the intracellular sites into the lumen where the binding proteins (e.g., calsequestrin) can tie up the bulk of calcium.

DDT INHIBITION OF Na-Ca ATPASE

Figure 3 shows the effect of different concentrations of Ca^{++} on lobster nerve ATPase activity under the standard assay condition.

It can be seen that Ca^{++} stimulates the total ATPase activity in lobster nerves both in control and nerves treated with DDT. The optimum concentration of Ca^{++} which produces maximum stimulation in the ATPase activity was found to be 0.3 mM. The total percent increase at this concentration over the base value was found to be around 94%.

It must be noted here that not all of the Ca-stimulated ATPase activity was inhibited by DDT under this experimental condition. For the sake of clarity here Ca-stimulated ATPase activities are divided into two groups: DDT-sensitive and DDT-insensitive Ca-ATPases. The activity due to the DDT-sensitive ATPase may be obtained by subtracting the value for control by that for DDT-treated (i.e., "difference").

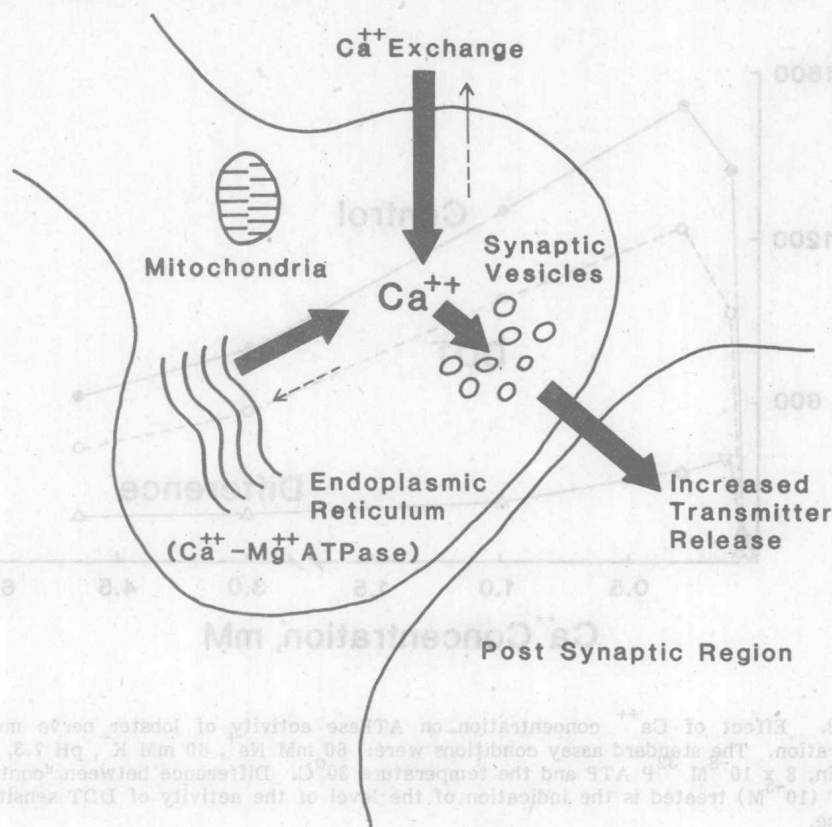


Fig. 2. Schematic illustration of the overall effects of heptachlor epoxide on the processes of transmitter release. Arrows represented by solid lines show that the process is stimulated by heptachlor epoxide. Those represented by dotted lines show its inhibitory effects. The overall effect of heptachlor epoxide is the increase of internal free Ca^{++} which triggers the release of the transmitter. The role of mitochondria in Ca^{++} regulation was not examined in this work.

The percent DDT inhibition, shown as the "difference" between control and control + DDT, was found to be between 30-40% under the present conditions.

To study the nature of this enzyme, using the standard assay condition, the effects of monovalent cations (Na^+ and K^+) were studied. The optimum concentrations of Na^+ and K^+ for DDT-sensitive Ca-ATPases were found to be 60 mM. The values were lower than those for DDT-insensitive Ca-ATPases. In another set of experiments, Na^+ was replaced with Li^+ . Under such conditions, the DDT sensitivity of the ATPase activity was still observed, suggesting thereby that, unlike Na-K ATPase, Na ions could be partially replaced by Li^+ for the maintenance of the activity of this enzyme.

The effect of incubation temperature on the levels of DDT inhibition also was studied. It was found that DDT inhibition was more pronounced at low temperature. The DDT-sensitive ATPase has a lower temperature quotient ($Q_{10}=1.26$) than the insensitive one ($Q_{10}=1.70$).

To study the nature of this DDT-sensitive ATPase, we have also examined the effect of various inhibitors and neuroactive agents (Table 1). It was found to be sensitive to lanthanum ($I_{50}=1 \text{ mM}$) and Ruthenium red ($I_{50}=10 \text{ }\mu\text{M}$). The high potency and specificity of Ruthenium red as an inhibitor of Ca-ATPase as opposed to Mg-ATPase is well documented by Watson et al. (11). Also it is clear that the concentration of Ruthenium red needed to inhibit Ca-Mg ATPase was much higher than that known to inhibit mitochondrial Ca-ATPase which has been documented to be in the order of 10^{-7} M .

The sensitivities to other neuroactive agents which have similar mode of action as DDT were: veratrine 37%, inhibition at 10^{-4} g/ml and D-trans-allevethrin 41% inhibition at 10^{-4} M . Cyanide ion had little effect up to 10 mM. Likewise iodide ion had little or no effect. However, fluoride and mersalyl acid (12) seem