PESTICIDE CHEMISTRY

Human Welfare and the Environment

Volume 3

Mode of Action, Metabolism and Toxicology

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

---

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 Nóyaku Gakkai. VI. Nihon Shokubutsu Bóeki Kyókai. VII. Title. VIII. Series. [DNLM: 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. Keaney, 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

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

Metabolism and Degradation of Pesticides and Xenobiotics

Bioactivation of Pesticides and Xenobiotics

Novel Aspects of the Metabolism of Pesticides and Xenobiotics

Photochemical and Non-Biological Transformation of Pesticides and Xenobiotics

Distribution and Fate of Xenobiotics in Soils and Sediments

Metabolism of Pesticides and Xenobiotics in Terrestrial and Aquatic Organisms

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^{++} sequesteration 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 member 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.

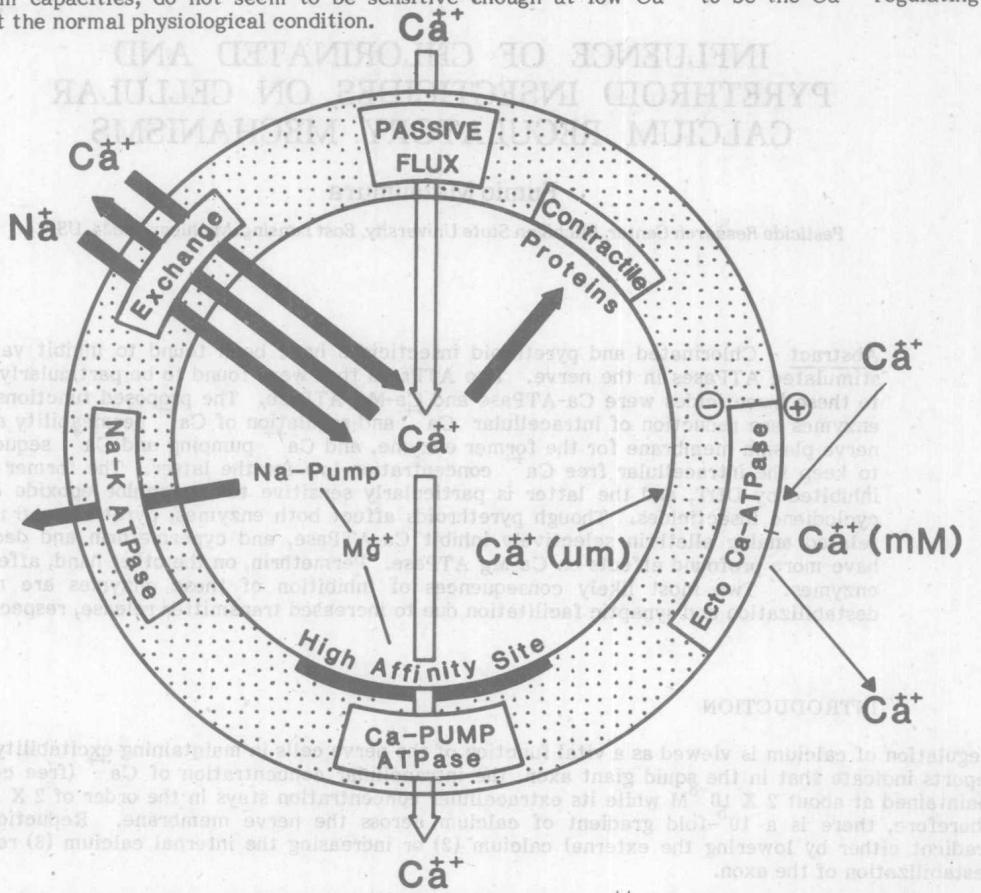

Fig. 1. Diagramatic sketch showing the regulation of Ca^{++} and the interrelationships among the levels of extra- and intra-cellular Ca^{++}, the Ca-pump and Na-pump of nerves. Ca^{++} may enter the nerve cells and axons via passive influx or Na^{+} - Ca^{++} exchange diffusion. The concentration of Ca^{++} bound to the outer surface of the membrane is probably controlled by ecto-ATPase and its optimum concentration is in mM range (10^{-2} - 10^{-3}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^{-7}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").

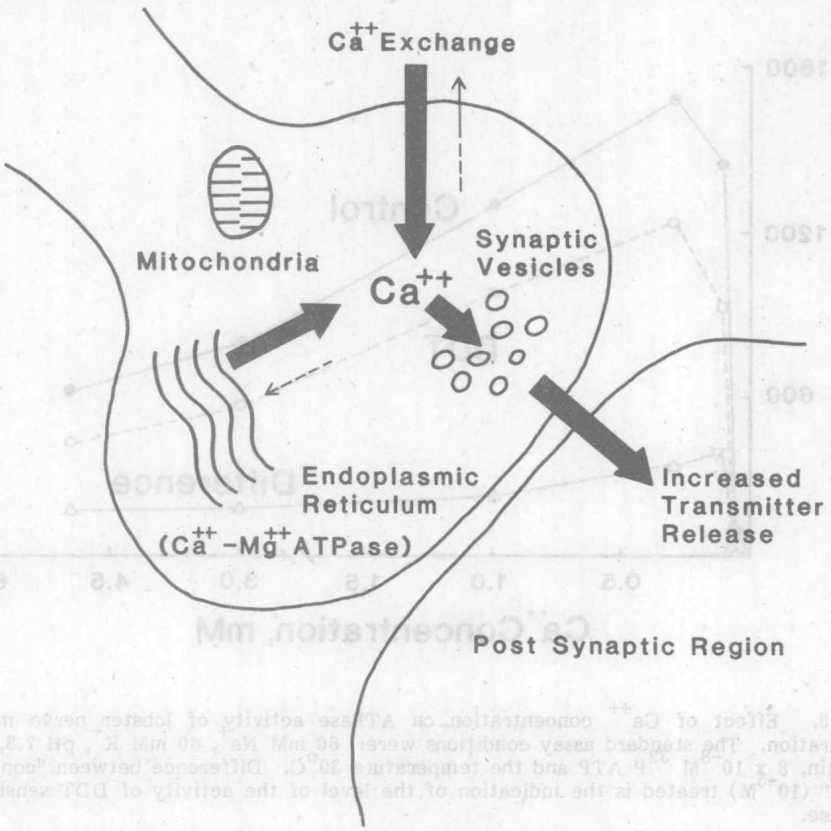

Fig. 2. Schematic illustration of the overall effects of heptachlor epoxide on the processes of transmitter release. Arrows represented by solid lines show that the process is stimulated by heptachlor epoxide. Those represented by dotted lines show its inhibitory effects. The overall effect of heptachlor 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_{90}=1 mM) and Ruthenium red (I_{50} = 10 μM). 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-allethrin 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