Toxicology of Organophosphate & Carbamate Compounds

Edited by

Ramesh C. Gupta

Gupta, *Toxicology of Organophosphate and Carbamate Compounds*

This book brings together the expertise of leading scientists from around the world on the complex toxicology of anticholinesterase compounds (Organophosphates and Carbamates). It provides the most up-to-date and in-depth knowledge on various aspects of OP and CM compounds, including their use, classification, mechanism-based toxicity, and prophylactic and therapeutic measurements.

Anticholinesterase compounds constitute the largest number of chemicals that are primarily used as insecticides in agriculture, industry, and around the home/garden. Some OPs (nerve agents) have been used both in chemical warfare and terrorist attacks; while other OPs and CMs have been recommended as therapeutic agents in human and veterinary medicine.

Many chemicals of both classes are extremely toxic and lack selectivity, thus their inadvertent and accidental use continues to pose a threat to human and animal health, aquatic systems, and wildlife. These are some of the contributing factors that make this class of agents so very important and will make this book a crucial reference work for every researcher dealing with these agents.

- Extensively covers pesticides, nerve agents, therapeutic drugs, and flame retardants
- Describes epidemiology of the world's major disasters involving Organophosphates and Carbamates
- Covers animal, human, aquatic, and wildlife toxicity of Anticholinesterases
- Insights into in-depth cholinergic and noncholinergic mechanisms of toxicity
- Describes recent advancements in cholinesterases, paraoxonases, carboxylesterases, oxidative stress, endocrine disruption, cardiac and pulmonary toxicity, and carcinogenesis
- Provides *in vitro* and *in vivo* models for neurotoxicity testing
- Integrates knowledge of studies in lab animals and humans
- Offers risk/safety assessment and national/international guidelines for permissible levels of pesticide residues
- Describes management of Anticholinesterase poisoning in humans

"SCIENCE IS THE GREAT ANTIDOTE TO THE POISON OF ENTHUSIASM AND SUPERSTITION"

Adam Smith (1723–1790)

TOXICOLOGY OF ORGANOPHOSPHATE AND CARBAMATE COMPOUNDS

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RAMESH C. GUPTA

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Academic Press is an imprint of Elsevier

Elsevier Academic Press
30 Corporate Drive, Suite 400, Burlington, MA 01803, USA
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
84 Theobald's Road, London WC1X 8RR, UK

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Library of Congress Cataloging-in-Publication Data
Application submitted

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

ISBN 13: 978-0-12-088523-7
ISBN 10: 0-12-088523-9

For all information on all Elsevier Academic Press publications visit our Web site at www.books.elsevier.com

Printed in the United States of America
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Dedicated to My Beloved parents, the late Chandra Gupta and Triveni Devi Gupta,
My Beloved wife, Denise, and Dear daughter, Rekha

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Foreword

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Highly toxic acetylcholinesterase (AChE)-inhibiting pesticides, organophosphates (OPs) and carbamates (CMs), are intensively used throughout the world and continue to be responsible for poisoning epidemics, especially in developing countries (e.g., Central American countries) (Wesseling *et al.*, 2005). In industrialized countries, highly toxic OPs and CMs, and other toxic pesticides, are usually much better controlled, and the likelihood of occupational and other poisonings is relatively small. However, there is a continuous need to carefully assess the risks caused by the exposure and use in the occupational environment to workers as well as generally to consumers and other exposed groups. Although the literature on OP and CM insecticides is seemingly exhaustive and systematic, this is not the case. For example, there is really no truly comprehensive and in-depth analysis available on the toxicological data of the common AChE-inhibiting compounds, OPs and CMs. This book promises a welcome improvement by providing comprehensive coverage of the toxicology of these important group of pesticides. To my knowledge, this book is the first in-depth analysis of data on toxicology, risk assessment, and management, as well as the importance of OPs and CMs to society. I believe that this book will soon be on the bookshelves of researchers in academia and industry and risk assessors and managers within regulatory authorities. However, it would also be beneficial if we could convey the information contained in this book to the attention of policymakers and political and industrial decision makers and in this way multiply its impact in society and preserve human health. In this context, there are some issues that merit special consideration and that are relevant to this book in the field of toxicology and risk assessment of AChE inhibitors.

In industrialized countries, highly toxic pesticides, including OPs and CMs, are regulated and controlled in the work environment via occupational exposure limits and by restrictions or bans on the use of the most toxic compounds. Consumers are protected by the setting of acceptable daily intake values. In the European Union, a separate community-level directive, 91/414/EC, is currently under revision for plant protection products according to which the safety of all pesticides is assessed. Similar pieces of legislation, rigorously enforced by health and other authorities, are in place in other industrialized countries. In the United States, the use of pesticides is regulated by the Federal Insecticide, Fungicide and Rodenticide Act, which originated in 1947, and the Food Quality Protection Act passed by the U.S. Congress in 1996 (Ecobichon, 2001).

In developing countries, the situation is often much worse, and the information provided in this book is badly needed. There is a clear need to resort to OP and CM insecticides use in many developing countries since they are situated in climates that in addition to favoring the growth of crops also provide optimal breeding conditions for insects capable of destroying crops or causing communicable diseases such as malaria (Ecobichon, 2001). However, because there is such a lack of knowledge and no adequate legislation and regulations, OPs and CMs and other toxic pesticides are often misused, the protective measures are largely inadequate, and safe handling of crops is often inappropriate. In developing countries, there is also a lack of adequate infrastructure, including regulatory authorities, to enforce regulations and thus protect individuals who come into contact with pesticides, even when legislation and regulations are theoretically in place and the educational system does provide the necessary knowledge base to assess risks associated with the use of these compounds (Rantanen *et al.*, 2004).

The literature on OPs and CMs is seemingly exhaustive (Ecobichon, 2001; Krieger, 2001). However, because AChE-inhibiting insecticides, as well as other pesticides, require marketing permission, most of the descriptive and much of mechanistic toxicology research has been carried out by the companies that manufacture these compounds. These data are often not publicly available, and much information

regarding toxicity of OPs and CMs is missing in the open literature. Therefore, there is a plethora of studies exploring mechanisms of acute and long-term toxicity of these compounds in experimental animals and man, but other areas are covered only vaguely, which means in effect that there is a very inconsistent database. In practice, this means that when an "old" AChE-inhibiting insecticide or another old pesticide is subjected to reevaluation for remarketing permission, the data available to the risk assessors are far from complete and additional studies are often required. Alternatively, the data may be old or scattered throughout the open literature and, hence, laborious to find. This book will improve the situation remarkably for evaluating the OP and CM pesticides.

To carry out a comprehensive analysis of the often inconsistent and incomplete database on OPs and CMs is often a highly demanding task for risk assessors and decision makers. The situation is especially difficult if one wishes to combine analytical thinking with professional risk assessment and management that also takes into consideration societal impact, perception of risks, and the significance of these compounds to society.

The editor, authors, and publisher of this book have decided to face this challenge by publishing this unique book dealing in an in-depth manner with various aspects of the toxicology of OPs and CMs. As indicated previously, there is a plethora of original papers and reviews on the toxicology of this group of compounds, especially on the mechanisms of immediate toxic actions of these compounds. Much of this information is based on studies dealing with AChE-inhibiting warfare agents, such as soman, tabun, and sarin (Savolainen, 2001). Although this information is important for protecting the general public from terrorist attacks, an even more important goal is to create a comprehensive systematic database on the toxicity of these compounds to allow a reliable and exhaustive assessment of their risks to workers and consumers and other exposed individuals. This book is unique in being able to provide a thorough assessment of all aspects of toxicology and risk assessment of OP and CM AChE-inhibiting compounds.

The list of authors of the book is impressive — the editor is to be congratulated for bringing together such a unique group of experts from various fields of OP and CM toxicology and risk assessment. The book is divided into nine sections that deal with different aspects of OP and CM toxicology and risk assessment. I am especially pleased by Section I, with its chapters on therapeutic uses, community preparedness, and epidemiology of OP and CM compounds. In Section III, noncholinesterase mechanisms of central and peripheral neurotoxicity, paraoxonase polymorphisms, and the development of tolerance are topics of special interest. The main body of the book (Section IV) discusses organ toxicity in especially interesting chapters dealing with *in vitro* testing, reproduction, placental toxicity, endocrinology, and effects on the immune system. In Section V, special-interest areas are covered, and items of personal interest to me are those dealing with oxidative stress, DNA damage and gene expression, and occupational toxicology and hygiene. Special merits of the book, covered in Section VI, are the chapters that deal with in-depth risk assessment and risk management. These issues are crucial to ensure that the research conducted on the toxicology of OPs and CMs actually has an impact on human health and society at large. Another merit of the book is found in Section VII, in which issues dealing with ecotoxicology are reviewed in the context of human toxicology. Ultimately, human toxicology and ecotoxicology of OPs and CMs are interrelated and inseparable. A novel topic is introduced in Section VIII, a discussion on biomarkers of OP exposure. This topic has direct relevance both to human exposure and to effective assessment and, therefore, it holds major potential for prevention of risks induced by OPs. This book would not be a comprehensive presentation on issues relevant to OPs and CMs without a chapter on management of OP poisonings, and fortunately this issue is well covered in Section IX. The book is an extremely welcome addition to the literature on the toxicology and risk assessment of OPs and CMs. The chapters mentioned previously are personal choices and issues that I consider to be of special interest. As a whole, the book provides a thorough and analytical coverage of issues important in the field, without omitting anything of importance, and emphasizes new issues that have not been assessed in previous textbooks or reviews. Thus, it is a credit that this book has achieved its goal, to review the toxicology of OPs and CMs, in an in-depth and comprehensive manner, including important and novel issues such as placental and reproductive toxicology and the effects of these compounds on the immune system. This book will be intensively used by not only scientists and teachers in academia, scientists in the industry, and regulators and decision makers but also students, who should be encouraged to study and learn from its wisdom.

References

Ecobichon, D. J. (2001). Toxic effect of pesticides. In *Casarett and Doull's Toxicology. The Basic Science of Poisons* (C.D. Klaassen, Ed.), 6th ed., pp. 763–810. McGraw-Hill, New York.

Krieger, R. (Ed.) (2001). *Handbook of Pesticide Toxicology. Principles*, 2nd ed. Academic Press, San Diego.

Rantanen, J., Lehtinen, S., and Savolainen, K. (2004). The opportunities and obstacles to collaboration between the developing and developed countries in the field of occupational health. *Toxicology* **198**, 63–74.

Savolainen, K. (2001). Understanding the toxic actions of organophosphates. In *Handbook of Pesticide Toxicology. Principles* (R. Krieger, Ed.), 2nd ed., pp. 1013–1042. Academic Press, San Diego.

Wesseling, C., Corriols, M., and Bravo, V. (2005). Acute pesticide poisoning and pesticide registration in Central America. *Toxicol. Appl. Pharmacol.*, **207**, S697–S705.

SECTION I

Uses, Abuses, & Epidemiology

CHAPTER 1

Introduction

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By the turn of the 21st century, the development and use of organophosphate (OP) and carbamate (CM) compounds were greater than ever before. This trend will most likely continue because scientists are discovering new applications for these compounds. Both OPs and CMs are inhibitors of acetylcholinesterase (AChE) enzyme, which terminates the action of the neurotransmitter acetylcholine (ACh). Compounds with strong AChE-inhibiting potential are used as toxicants (e.g., pesticides and nerve agents), whereas those with weak AChE-inhibiting potential are used as prophylactic agents against nerve agent poisoning or as therapeutic agents in conditions such as glaucoma, myasthenia gravis, and Alzheimer's disease. In addition, some of these compounds are used as flame retardants, whereas others are misused in intentional and malicious poisonings. Currently, AChE-inhibiting compounds constitute the largest class of pesticides used in both industrialized and developing nations.

The history and evolution of OPs and CMs is very interesting and intriguing. The earliest documentation of the synthesis of an OP compound, tetraethyl pyrophosphate, was in 1854 when Phillipe de Clermont presented a report to the French Academy of Sciences. Nearly 80 years later, in 1932, Lange and Kruger described the synthesis of dimethyl and diethyl phosphorofluoridate and noted that inhalation of their vapors produced dimness of vision and a choking sensation. Probably, these observations led Gerhard Schrader, a chemist, to the exploration of the OP class of compounds while he was engaged in the development of insecticides for I. G. Farbenindustrie. One of the earliest OP insecticides synthesized by Schrader was parathion, which is still commonly used worldwide. Prior to World War II (WWII), under the authority of the German Ministry of Defense, his priority shifted from insecticides to chemical warfare agents. The result was the development of diisopropyl phosphorofluoridate and then the development of considerably more toxic OP compounds of the G series (tabun, sarin, and soman), which were intended to be used as nerve gases/agents. In the 1950s, agent VX was synthesized in the United Kingdom with a potency manyfold greater than nerve agents of the G series. At the same time, a compound of the V series (VR), with a supertoxicity, was synthesized by the Soviet military. In 1950, two separate accidental exposures occurred at Dugway Proving Ground, wherein workers developed signs and symptoms, and laboratory evidence confirmed mild nerve agent exposure in a test area 3 days after a sarin test. Since the 1980s, these agents have been used in wars and by dictators and terrorists. For example, sarin was used in Iraq against Kurdish villages in 1988 and in the Tokyo subway attacks in Japan in 1994 and 1995. Currently, many countries possess these deadly nerve agents, and in the current world situation the possibility exists that these compounds may be misused as chemical weapons of mass destruction (CWMD). In fact, OP nerve agents have received increasing attention as concerns about chemical warfare have intensified since the terrorist attack in the United States on September 11, 2001.

After WWII, thousands of OP derivatives were synthesized worldwide in a search for compounds with species selectivity and less toxicity that could be used as insecticides more safely. In 1950, malathion was synthesized, and it has been the most popular insecticide for more than 50 years for use against certain insects, especially mosquitoes and medflies. Malathion has always been considered one of the safest OPs. However, in 1976 in Pakistan, out of 7500 spray men, 2800 became poisoned and 5 died from isomalathion that was produced during storage of formulated malathion. Many such poisoning incidences have occurred in the past with several other OPs due to their accidental or inadvertent use. Today, more than 100 OPs are in use for a variety of purposes, such as protection of crops, grains, gardens, and public health. Although OP insecticides are less toxic than the nerve agents, the illness they produce clinically resembles that produced by nerve agents.

The knowledge of autonomic pharmacology, especially the cholinergic system, enabled us to understand the mechanism of toxicity of OPs and CMs. Subsequently, CMs such as aldicarb were synthesized based on the knowledge of ACh chemistry. These compounds were found to be the most toxic insecticides of the anti-AChE class. In the early 1950s, several nucleophilic agents (hydroxylamine, hydroxamic acid, and oximes) were developed as antidotes for reactivation of inhibited AChE against OPs. With a thorough understanding of the chemistry of ACh, AChE, and OPs, pralidoxime was synthesized, which was found to have 1 million times greater potency than hydroxylamine for reactivation of inhibited

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AChE. Today, more than a dozen oximes are available, some of which are more effective against OP nerve agents and others are more effective against OP pesticides.

The history of CMs is somewhat older than the history of OPs. In 1840, the Calabar bean (ordeal poison), from a perennial plant *Physostigma venenosum*, was brought to England from a tropical part of West Africa, where it was used for witchcraft. Approximately 25 years later, physostigmine (eserine alkaloid) was isolated by several investigators and used to treat glaucoma. Almost 50 years later, an aromatic ester of carbamic acid, neostigmine, was synthesized and used in the treatment of myasthenia gravis. It was not until the 1960s and 1970s that carbamates (esters of carbamic acid) were synthesized for pesticidal use. Today, CMs are preferred for pesticide use over OPs because some OPs have been found to be extremely toxic, whereas others induce delayed neuropathy in humans and animals. Carbaryl was the first CM compound used as an insecticide. Whereas OPs are irreversible AChE inhibitors and extremely toxic, CMs are reversible AChE inhibitors and therefore considered relatively less toxic. Although based on acute toxicity, some of the CMs, such as aldicarb, carbofuran, and many others, are extremely toxic. In 1984, an estimated 400,000 people were exposed to a toxic methyl isocyanate gas (used in the production of CM pesticides) that leaked from the Union Carbide plant in Bhopal, India. From this catastrophic incident, approximately 8000 humans and 4000 animals died. In 1985, an unprecedented outbreak of aldicarb poisoning occurred in which approximately 2000 California residents became sick due to consumption of contaminated melons. Since the early 1980s, both OPs and CMs have been used for multiple purposes, such as pesticides (crop and grain protection, indoors and around homes) and in veterinary (ectoparasiticides and endoparasiticides) and human medicine (in neurodegenerative diseases such as Alzheimer's disease). In human medicine, some of these compounds are also prescribed in myasthenia gravis and glaucoma and as prophylaxis to combat anticipated nerve agent poisoning. Like OPs, thousands of CMs have been synthesized, but not more than two dozen compounds have been used practically. In terms of volume, currently the use of CMs exceeds the use of OPs.

OPs and CMs are the most commonly used pesticides throughout the world. This is partly due to their lack of residue persistence in the environment and in exposed individuals and also due to lesser resistance development in insects compared to the organochlorine pesticides. From the public health standpoint, in today's world the use of pesticides is a must rather than an option. For example, sporadic incidences of West Nile virus are reported in many countries, whereas malaria is still a major problem in developing countries. In both cases, the common vector is the mosquito. Without the use of pesticides against vectors of diseases, the impact on human and animal health would be devastating and the economic loss would be enormous. On the one hand, the world is greatly benefited from the use of pesticides; on the other hand, pesticides are major contributors to environmental pollution.

Many OPs and CMs are extremely toxic, and the majority of them lacks species selectivity and so, because of their global use, they constantly pose a threat to the environment, human and animal health, aquatic systems, and wildlife. It is important to note that OP and CM pesticides are encountered in intentional poisonings in humans and malicious poisonings in animals. Today, carbofuran is the pesticide most often associated with accidental and malicious poisoning in companion and domestic animals because of its widespread availability and extreme toxicity. Depending on the magnitude, frequency, and length of exposure, these compounds can produce minor health effects, such as mild discomfort or chest pain, or effects as severe as paralysis, coma, and death. The World Health Organization estimates that approximately 3 million people worldwide suffer from acute pesticide poisoning annually. By employing *in vivo* and *in vitro* models, these compounds are known to produce a variety of toxicological effects on the central nervous system, peripheral nervous system, cardiovascular, ocular, neurobehavioral, immunological, reproductive, placental, cutaneous, and other body systems, in addition to endocrine disruption, oxidative stress, and carcinogenesis. With the advent of sophisticated technologies, highly sensitive potentiometric and amperometric biosensors have been developed for qualitative and quantitative detection and monitoring of chemical warfare agents and OP and CM pesticides. Essentially, these biosensors aid in chemical and food safety, environmental monitoring, and agricultural production.

During the past few years, investigators in the field of anticholinesterases have realized the need for a comprehensive compendium that can provide in-depth knowledge on various aspects of these compounds, including their use, toxicity, safety, regulations, and prophylactic and therapeutic measurements. This reference book, which is a collective work of approximately 100 subject experts from many countries, offers a plethora of cutting-edge knowledge on various aspects of OPs and CMs. The book is organized into nine sections with a total of 49 chapters. The editor and authors have made every effort to cite every important work in the field and avoid any duplication, but the possibility of some omissions and duplications certainly exists. Since OPs and CMs are used worldwide in agriculture, in gardens, in and around homes and offices, in therapeutic applications, in intentional and malicious poisonings, and possibly as CWMD, the book is intended for students and teachers; toxicologists; physicians; public health personnel and administrators; risk and safety assessors; local, state, federal, and international pesticide regulators and policy makers; industrial and agricultural watchdog groups; and medical, veterinary, and environmental advocacy groups.

The editor truly appreciates the hard work and sincere efforts of each author, without which this book would not have been possible.

CHAPTER 2

Classification and Uses of Organophosphates and Carbamates

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I. INTRODUCTION

Organophosphates (OPs) are a large class of chemicals. Since World War II, an estimated several thousand OPs have been synthesized for various purposes. The majority of these compounds are used as pesticides, whereas others are used as nerve agents, flame retardants, and parasiticides in veterinary medicine. Different OP compounds have structural similarities within classes. All OPs definitely share one thing in common: They all have a phosphorus atom and a characteristic phosphoryl bond (P═O) or thiophosphoryl bond (P═S). Essentially, OPs are esters of phosphoric acid with varying combinations of oxygen, carbon, sulfur, or nitrogen attached. Of course, the chemistry of these compounds is much more complex and classification is somewhat confusing. In fact, complexity in classification of OPs arises due to different side chains attached to the phosphorus atom and the position at which the side chains are attached. More than 50 years ago, the Anglo-American system reached an agreement to adopt an "international nomenclature" instead of individual systems from four countries (British, Swedish, German, or American). However, none of the systems has ever been universally accepted. Compared to OPs, carbamate (CM) pesticides are of relatively recent origin and constitute another important group of pesticides. In addition to their use as pesticides, CMs are used as drugs of choice in human medicine against Alzheimer's disease, myasthenia gravis, and glaucoma and in veterinary medicine as parasiticides. Classification of CMs is simpler than classifying OPs. Some CMs have structural similarity with the neurotransmitter acetylcholine (ACh), and therefore they cause direct stimulation of ACh receptors, in addition to acetylcholinesterase (AChE) inactivation. Although thousands of CMs have been synthesized, only a few dozen have practical utility. The classification of OPs and CMs presented in this chapter is based on their chemical structures and intended use or any syndrome they produce.

II. ORGANOPHOSPHATES

Currently, there are hundreds of OP compounds in use, which are derivatives of phosphoric, phosphonic, or phosphinic acid. Throughout this chapter and the book, the term *organophosphate* is used as a generic term to include all the organic compounds containing phosphorus. These compounds are classified based on side chains and other elements attached to the phosphorus atom.

A. Types of Organophosphates

There are at least 13 types of OPs, which are briefly presented in Table 1. The OPs that are derivatives of phosphoric or phosphonic acid possess anticholinesterase activity, unlike those that are derivatives of phosphinic acid. There are some OP compounds that do not conform to the structural requirement as shown in Table 1, but they possess anti-AChE activity. Usually, OP compounds have two alkyl substituents and an additional substituent group (leaving group), which is more labile to hydrolysis than the alkyl groups (Marrs, 1993). It is important to note that phosphorothioates (P═S) possess minimal or no anticholinesterase (anti-AChE) activity and require desulfuration to the analogous oxon before acquiring anti-AChE activity. Also, not all OPs exert anti-AChE activity, and therefore they are of low toxicity. For example, *S,S,S*-tributyl phosphorotrithioate and *S,S,S*-tributyl phosphorotrithioite (merphos), which are used as defoliants, and glyphosate and gluphosinate, which are used as herbicides, are of low mammalian toxicity.

B. OP Pesticides

The majority of OP compounds are used as pesticides, and chemical descriptions for commonly used compounds are given in Table 2.

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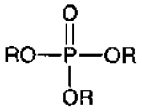
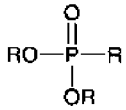
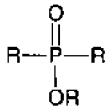
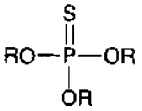
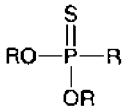
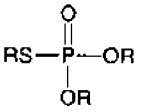
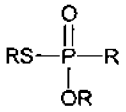
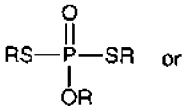
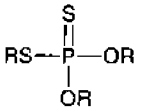
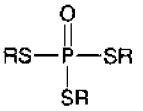
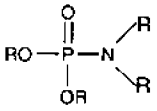
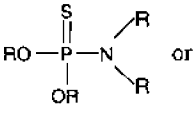
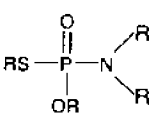
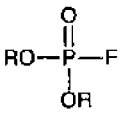
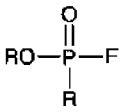
TABLE 1. Types of Organophosphates[a]

Type	Chemical structure	Examples
Phosphates		Chlorfenvinphos Dichlorvos Monocrotophos Tri-*o*-cresyl phosphate
Phosphonates		Trichlorfon
Phosphinates		Glufosinate
Phosphorothioates (S═)		Bromophos Diazinon Fenthion Parathion Pirimiphos-methyl
Phosphonothioates (S═)		EPN Leptophos
Phosphorothioates (*S*-substituted)		Demeton-*S*-methyl Echothiophate
Phosphonothioates (*S*-substituted)		VX
Phosphorodithioates	 	Azinphos-ethyl Azinphos-methyl Dimethoate Disulfoton Malathion Methidathion
Phosphorotrithioates		DEF (tribufos)
Phosphoramidates		Fenamiphos
Phosphoramidothioates	 	Methamidophos Isofenphos

(continues)

TABLE 1. *(continued)*

Type	Chemical structure	Examples
Phosphorofluoridates		Diisopropyl phosphorofluoridate (DFP)
Phosphonofluoridates		Cyclosarin Sarin Soman

[a]Adapted from Marrs (1993).

C. OP Nerve Agents/Gases

Nerve agents of the OP group include tabun (GA), sarin (GB), soman (GD), cyclosarin (GF), and VX. Soman, sarin, and cyclosarin are phosphonofluoridates, and VX is a phosphonothioate. Whereas soman has four isomers (C+ P−, C− P−, C+ P+, and C− P+), sarin and VX each have two isomers. VX is a mixture of two enantiomers resulting from the chiral center at the phosphorus atom, designated as P(+) and P(−). There are significant differences in the reported toxicity and AChE inhibition rates of these isomers of nerve agents. Toxicological significance of stereoisomerism for other OPs, which have the potential of inhibiting AChE, has been described (Battershill *et al.*, 2004). This makes the ability to distinguish between them desirable for toxicological studies or the development of antidotal therapies (Benschop and De Jong, 1988; Smith, 2004; Kuča and Kassa, 2004). Based on acute toxicity, VX is the most toxic compound among all the nerve agents. OP nerve agents are extremely toxic and have been used in wars and by terrorists on several occasions. They irreversibly inhibit the enzyme AChE at its active site. People who work at military sites where these nerve agents are stored may potentially be exposed. Soldiers and military personnel can be exposed to these compounds during war, and the general population can be exposed by accidental release from a military storage facility and during their transportation or destruction. For further details about OP nerve agents, see Chapter 5.

D. OPs Causing Delayed Neurotoxicity/ Neuropathy

OP compounds that produce delayed neurotoxic effects are esters of phosphorus-containing acids. More than 35 years ago, tri-*o*-cresyl phosphate (TOCP) was known to produce delayed neurotoxic effects in humans and chickens characterized by ataxia and weakness of the limbs, developing 10–14 days after exposure (Johnson, 1969). This syndrome is called OP-induced delayed neuropathy (OPIDN). TOCP and certain other compounds have minimal or no anti-AChE properties; however, they cause phosphorylation and aging (dealkylation) of a protein in neurons called neuropathy target esterase, and subsequently lead to OPIDN. Today, many compounds, such as diisopropyl phosphorofluoridate, *N,N'*-diisopropylphosphorodiamidic fluoride (mipafox), tetraethyl pyrophosphate, paraoxon, parathion, *o*-cresyl saligenin phosphate, and haloxon, are known to produce this syndrome. For details of OPIDN syndrome, see Chapter 25.

E. OPs Causing Intermediate Syndrome

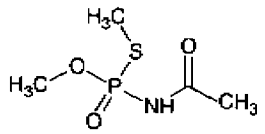
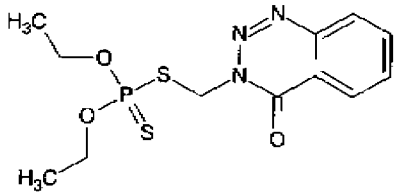
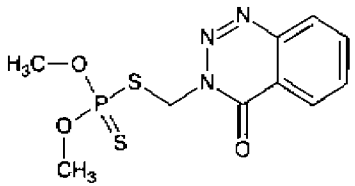
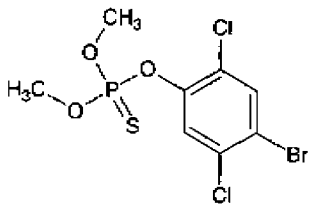
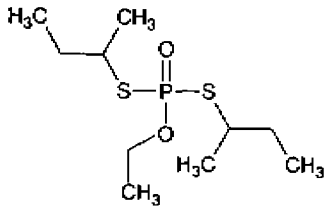
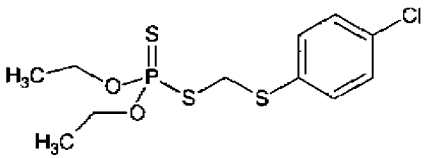
OP insecticide-induced intermediate syndrome (IMS) was reported for the first time in human patients in Sri Lanka in 1987 (Senanayake and Karalliede, 1987). Since then, this syndrome has been diagnosed in OP-poisoned patients in South Africa (1989), Turkey (1990), Belgium (1992), the United States (1992), Venezuela (1998), France (2000), and elsewhere. IMS is usually observed in individuals who have ingested a massive dose of an OP insecticide either accidentally or in a suicide attempt. IMS is clearly a separate clinical entity from acute toxicity and delayed neuropathy. A similar syndrome has also been observed in dogs and cats poisoned maliciously or accidentally with massive doses of certain OPs. OPs that are known to cause IMS include bromophos, chlorpyrifos, diazinon, dicrotophos, dimethoate, fenthion, malathion, merphos, methamidophos, methyl parathion, monocrotophos, omethoate, parathion, phosmet, and trichlorfon. These compounds and IMS are discussed further in Chapter 26.

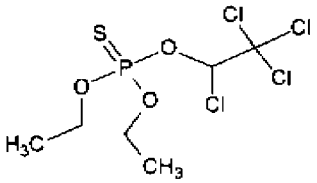
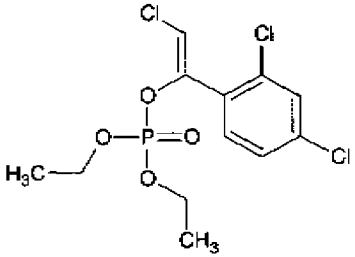
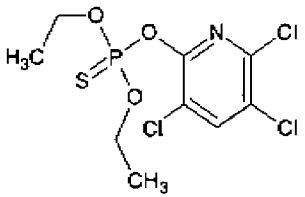
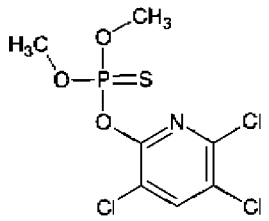
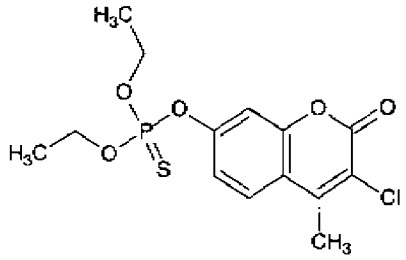
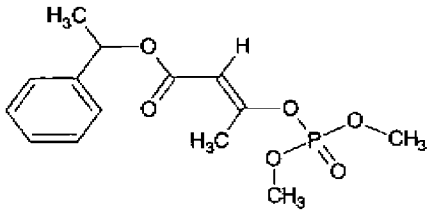
F. OPs Used as Flame Retardants

Several OPs are used as fire retardants. Chemical structures of three commonly used compounds are as follows:

1. Tris (2-chloroethyl) phosphate (TCEP)
   $O = P - (OCH_2CH_2Cl)_3$
2. Tris (2-chloropropyl) phosphate (TCPP)
   $O = P - (OCH_2CHClCH_3)_3$
3. Tris (1,3-dichloroisopropyl) phosphate (TDCPP)
   $O = P - (OCH(CH_2Cl)_2)_3$

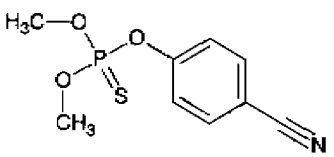
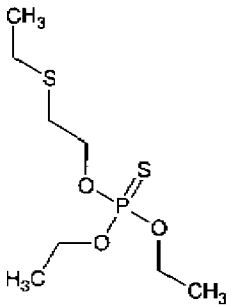
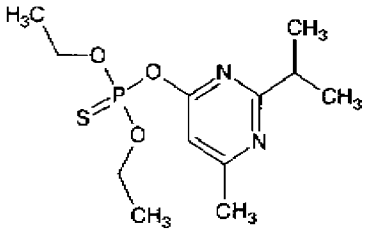
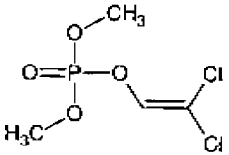
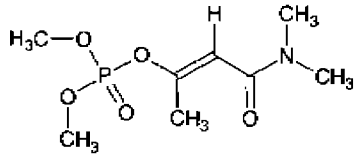
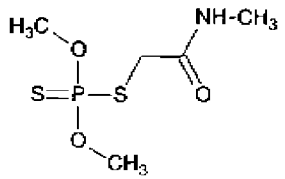
TABLE 2. A Brief Chemical Description of Commonly Used OP Pesticides

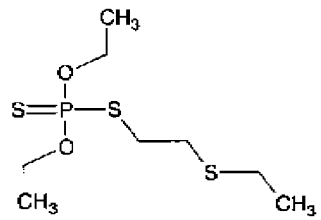
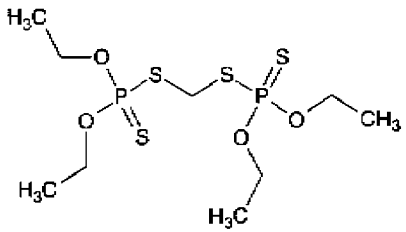
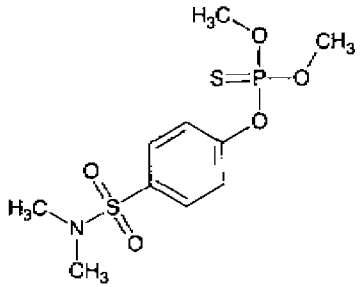
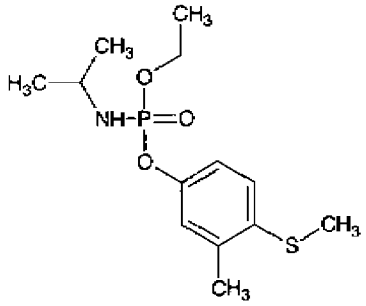
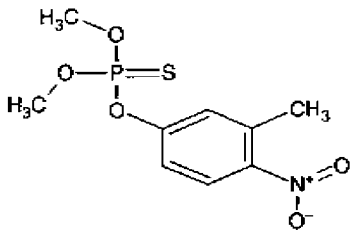
Chemical (CAS No.)	Chemical structure	Chemical name/ empirical formula	Molecular weight	Oral LD_{50} in rat (mg/kg)	Dermal LD_{50} in rabbit (mg/kg)
Acephate (30560–19–1)		*O,S*-dimethyl acetylamidothiophosphate $C_4H_{10}NO_3PS$	183.17	866	>2000
Azinphos-ethyl (2642–71–9)		*O,O*-diethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl) methyl] dithiophosphate $C_{12}H_{16}N_3O_3PS_2$	345.38	13	250
Azinphos-methyl (86–50–0)		*O,O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl) methyl] dithiophosphate $C_{10}H_{12}N_3O_3PS_2$	317.32	5	220
Bromophos (2104–96–3)		*O*-(4-bromo-2, 5-dichlorophenyl) *O,O*-dimethyl thiophosphate $C_8H_8BrCl_2O_3PS$	366.00	1600	2188
Cadusaphos (95465–99–9)		*S,S*-di-*sec*-butyl *O*-ethyl dithiophosphate $C_{10}H_{23}O_2PS_2$	270.40	391	143
Carbophenothion (786–19–6)		*S*-{[(4-chlorophenyl)thio] methyl} *O,O*-diethyl dithiophosphate $C_{11}H_{16}ClO_2PS_3$	342.87	6	22

Chlorethoxyphos (54593–83–8)		*O,O*-diethyl *O*-(1,2,2, 2-tetrachloroethyl) thiophosphate $C_6H_{11}Cl_4O_3PS$	336.00	1.8	12.5
Chlorfenvinphos (470–90–6)		2-chloro-1-(2,4-dichloro-phenyl) vinyl diethyl phosphate $C_{12}H_{14}Cl_3O_4P$	359.57	12	3200
Chlorpyrifos (2921–88–2)		*O,O*-diethyl *O*-(3,5,6-trichloropyridin-2-yl) thiophosphate $C_9H_{11}Cl_3NO_3PS$	350.59	135	2000
Chlorpyriphos-methyl (5598–13–0)		*O,O*-dimethyl *O*-(3,5,6-trichloropyridin-2-yl) thiophosphate $C_7H_7Cl_3NO_3PS$	322.53	941	2000
Coumaphos (56–72–4)		*O*-(3-chloro-4-methyl-2-oxo-2*H*-chromen-7-yl) *O*, *O*-diethyl thiophosphate $C_{14}H_{16}ClO_5PS$	362.77	13	—
Crotoxyphos (7700–17–6)		1-phenylethyl (2*E*)-3-[(dimethoxyphos-phoryl)oxy]but-2-enoate $C_{14}H_{19}O_6P$	314.27	125	385

(continues)

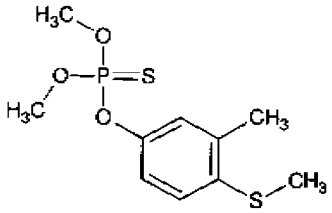
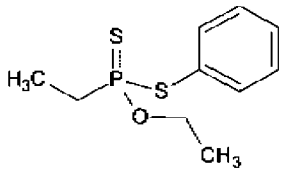
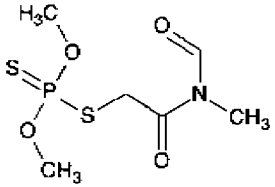
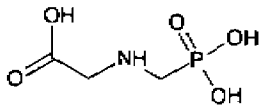
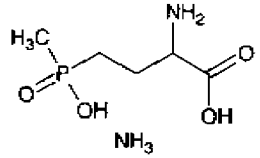
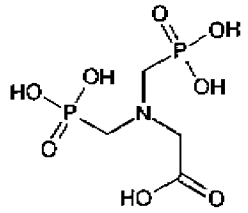
TABLE 2. *(continued)*

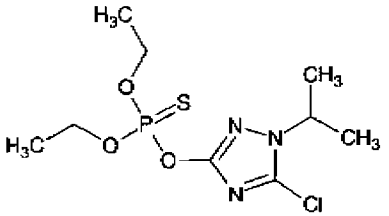
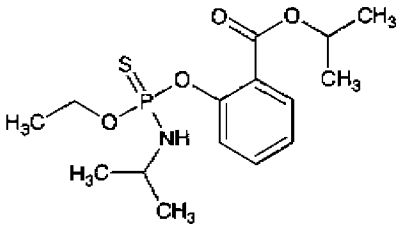
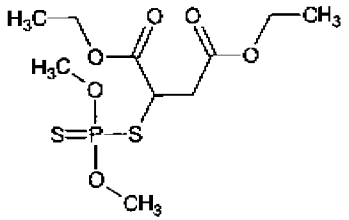
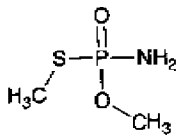
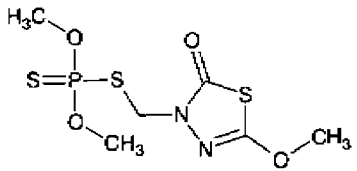
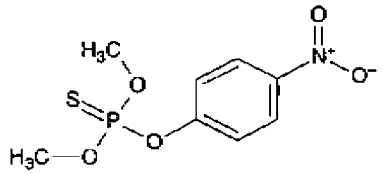
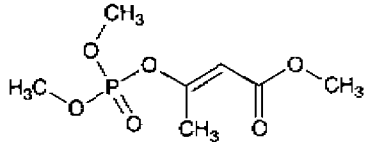
Chemical (CAS No.)	Chemical structure	Chemical name/ empirical formula	Molecular weight	Oral LD_{50} in rat (mg/kg)	Dermal LD_{50} in rabbit (mg/kg)
Cyanophos (2636–26–2)		*O*-(4-cyanophenyl) *O,O*-dimethyl thiophosphate $C_9H_{10}NO_3PS$	243.22	610	800
Demeton-O (8065–48–3)		*O,O*-diethyl *O*-[2-(ethylthio)ethyl] thiophosphate $(C_2H_5O)_2PSOC_2H_4SC_2H_5$	258.34	2.5	8
Diazinon (333–41–5)		*O,O*-diethyl *O*-(2-isopropyl-6-methylpyrimidin-4-yl) thiophosphate $C_{12}H_{21}N_2O_3PS$	304.35	300	379
Dichlorvos (62–73–7)		2,2-dichlorovinyl dimethyl phosphate $C_4H_7Cl_2O_4P$	220.98	25	59
Dicrotophos (141–66–2)		(1*E*)-3-(dimethylamino)-1-methyl-3-oxoprop-1-en-1-yl dimethyl phosphate $C_8H_{16}NO_5P$	237.19	22	223
Dimethoate (60–51–5)		*O,O*-dimethyl *S*-[2-(methylamino)-2-oxoethyl] dithiophosphate $C_5H_{12}NO_3PS_2$	229.26	250	400

Disulfoton (298–04–4)		*O,O*-diethyl *S*-[2-(ethylthio)ethyl] dithiophosphate $C_8H_{19}O_2PS_3$	274.40	2	6
Ethion (563–12–2)		*O,O,O',O'*-tetraethyl *S,S'*-methylene bis(dithiophosphate) $C_9H_{22}O_4P_2S_4$	384.48	27	915
Famphur (52–85–7)		*O*-{4-[(dimethylamino) sulfonyl]phenyl} *O,O*-dimethyl thiophosphate $C_{10}H_{16}NO_5PS_2$	325.34	35	2730
Fenamiphos (22224–92–6)		Ethyl 3-methyl-4-(methylthio) phenyl isopropylamidophosphate $C_{13}H_{22}NO_3PS$	303.36	15.3	—
Fenitrothion (122–14–5)		*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) thiophosphate $C_9H_{12}NO_5PS$	277.23	250	1300

(continues)

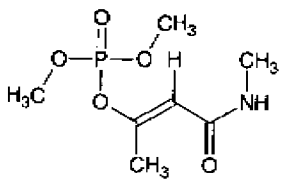
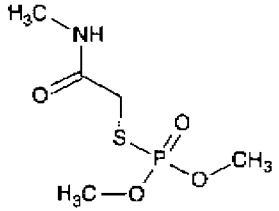
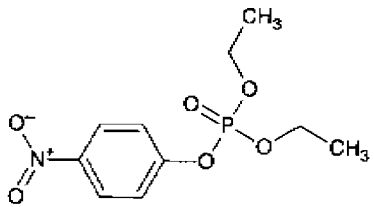
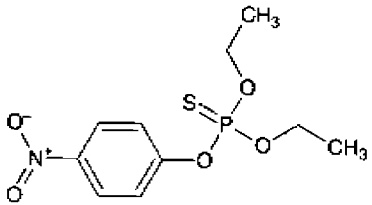
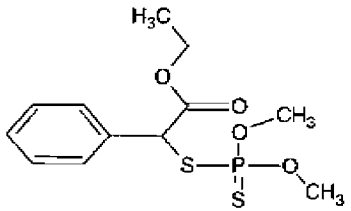
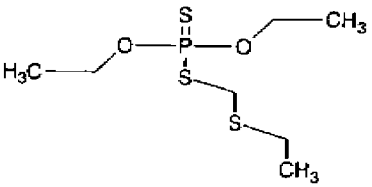
TABLE 2. *(continued)*

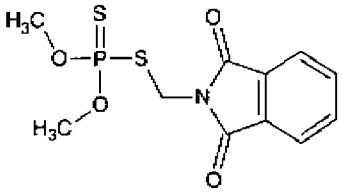
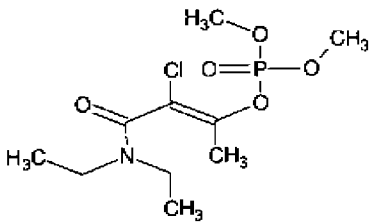
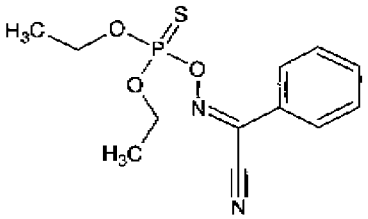
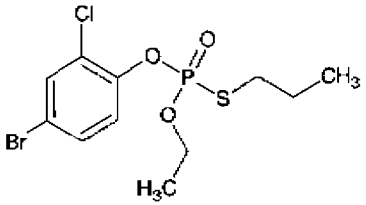
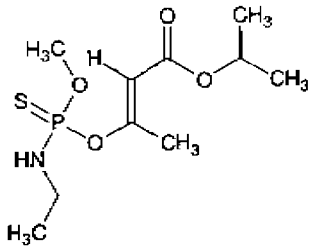
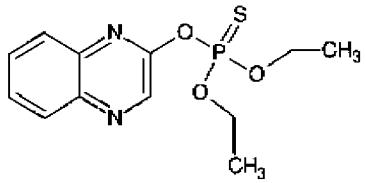
Chemical (CAS No.)	Chemical structure	Chemical name/ empirical formula	Molecular weight	Oral LD_{50} in rat (mg/kg)	Dermal LD_{50} in rabbit (mg/kg)
Fenthion (55–38–9)		*O,O*-dimethyl *O*-[3-methyl-4-(methylthio) phenyl] thiophosphate $C_{10}H_{15}O_3PS_2$	278.33	255	330
Fonofos (944–22–9)		*O*-ethyl *S*-phenyl ethylphosphonodithioate $C_{10}H_{15}OPS_2$	246.33	8	25
Formothion (2540–82–1)		*S*-{2-[formyl (methyl) amino]-2-oxoethyl} *O,O*-dimethyl dithiophosphate $C_6H_{12}NO_4PS_2$	257.27	365	>1000
Glyphosate (1071–83–6)		*N*-(phosphonomethyl)-glycine $C_3H_8NO_5P$	169.07	4300	>5000
Glufosinate ammonium (77182–82–2)		2-amino-4-[hydroxy(methyl) phosphoryl]butanoic acid ammoniate $C_5H_{15}N_2O_4P$	198.16	2000	>4000
Glyphosine (2439–99–8)		*N,N*-bis(phosphonomethyl) glycine $C_4H_{11}NO_8P_2$	263.08	3925	>5010

Isazophos (42509–80–8)		*O*-(5-chloro-1-isopropyl-1*H*-1,2,4-triazol-3-yl) *O,O*-diethyl thiophosphate $C_9H_{17}ClN_3O_3PS$	313.75	40	>3100
Isofenphos (25311–71–1)		Isopropyl 2-{[ethoxy (isopropylamino)phosphorothioyl] oxy}benzoate $C_{15}H_{24}NO_4PS$	345.40	32	162
Malathion (121–75–5)		Diethyl 2-[(dimethoxyphosphorothioyl) thio]succinate $C_{10}H_{19}O_6PS_2$	330.36	885	4000
Methamidophos (10265–92–6)		*O,S*-dimethyl amidothiophosphate $C_2H_8NO_2PS$	141.13	13	110
Methidathion (950–37–8)		*S*-[(5-methoxy-2-oxo-1,3,4-thiadiazol-3(2*H*)-yl)methyl] *O,O*-dimethyl dithiophosphate $C_6H_{11}N_2O_4PS_3$	302.33	25	200
Methyl parathion (298–00–0)		*O,O*-dimethyl *O*-(4-nitrophenyl) thiophosphate $C_8H_{10}NO_5PS$	263.21	9	63
Mevinphos (7786–34–7)		Methyl (2*E*)-3-[(dimethoxyphosphoryl)oxy]but-2-enoate $C_7H_{13}O_6P$	224.15	3	16

(continues)

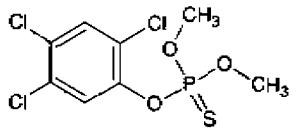
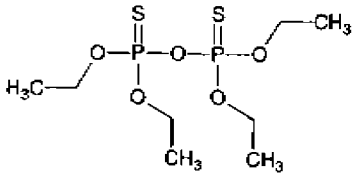
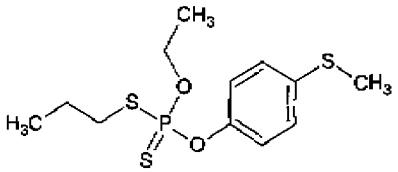
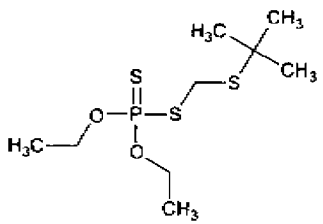
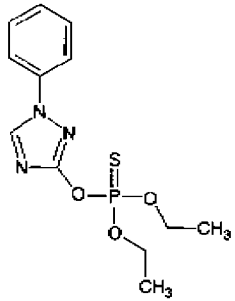
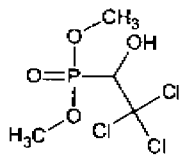
TABLE 2. *(continued)*

Chemical (CAS No.)	Chemical structure	Chemical name/ empirical formula	Molecular weight	Oral LD_{50} in rat (mg/kg)	Dermal LD_{50} in rabbit (mg/kg)
Monocrotophos (6923–22–4)		Dimethyl (1*E*)-1-methyl-3-(methylamino)-3-oxoprop-1-en-1-yl phosphate $C_7H_{14}NO_5P$	223.16	8	354
Omethoate (1113–02–6)		*O,O*-dimethyl *S*-[2-(methylamino)-2-oxoethyl] thiophosphate $C_5H_{12}NO_4PS$	213.19	50	1400
Paraoxon (311–45–5)		Diethyl 4-nitrophenyl phosphate $C_{10}H_{14}NO_6P$	275.19	1.8	—
Parathion (56–38–2)		*O,O*-diethyl *O*-(4-nitrophenyl) thiophosphate $C_{10}H_{14}NO_5PS$	291.26	3	6.8
Phenthoate (2597–03–7)		Ethyl [(dimeth-oxyphosphorothioyl)thio] (phenyl)acetate $C_{12}H_{17}O_4PS_2$	320.36	200	4000
Phorate (298–02–2)		*O,O*-diethyl *S*-[(ethylthio)methyl] dithiophosphate $C_7H_{17}O_2PS_3$	260.38	1.6	2.5

Phosmet (732–11–6)		*S*-[(1,3-dioxo-1,3-dihydro-2*H*-iso-indol-2-yl)methyl] *O,O*-dimethyl dithiophosphate $C_{11}H_{12}NO_4PS_2$	317.32	147	3160
Phosphamidon (13171–21–6)		(1*Z*)-2-chloro-3-(diethylamino)-1-methyl-3-oxoprop-1-en-1-yl dimethyl phosphate $C_{10}H_{19}ClNO_5P$	299.69	15	125
Phoxim (14816–18–3)		Phenylglyoxylo-nitrile oxime, *O,O*-diethyl phosphothioate $C_{12}H_{15}N_2O_3PS$	289.30	1845	1126
Profenofos (41198–08–7)		*O*-(4-bromo-2-chlorophenyl) *O*-ethyl *S*-propyl thiophosphate $C_{11}H_{15}BrClO_3PS$	373.63	400	472
Propetamphos (31218–83–4)		Isopropyl (2*E*)-3-{[(ethylamino)(methoxy) phosphorothioyl]oxy}but-2-enoate $C_{10}H_{20}NO_4PS$	281.31	82	2300
Quinalphos (13593–03–8)		*O,O*-diethyl *O*-quinoxalin-2-yl thiophosphate $C_{12}H_{15}N_2O_3PS$	298.30	65	340

(continues)

TABLE 2. *(continued)*

Chemical (CAS No.)	Chemical structure	Chemical name/ empirical formula	Molecular weight	Oral LD_{50} in rat (mg/kg)	Dermal LD_{50} in rabbit (mg/kg)
Ronnel (299–84–3)		*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) thiophosphate $C_8H_8Cl_3O_3PS$	321.55	1250	2000
Sulfotepp (3689–24–5)		*O,O,O,O*-tetraethyl dithiodiphosphate $C_8H_{20}O_5P_2S_2$	322.32	5	—
Sulprofos (35400–43–2)		*O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl dithiophosphate $C_{12}H_{19}O_2PS_3$	322.45	107	820
Terbufos (13071–79–9)		*S*-[(*tert*-butylthio)methyl] *O,O*-diethyl dithiophosphate $C_9H_{21}O_2PS_3$	288.43	1.6	1
Triazophos (24017–47–8)		*O,O*-diethyl *O*-(1-phenyl-1*H*-1,2, 4-triazol-3-yl) thiophosphate $C_{12}H_{16}N_3O_3PS$	313.32	83	280
Trichlorfon (52–68–6)		Dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate $C_4H_8Cl_3O_4P$	257.44	630	>2100

These compounds are haloalkyl phosphates and act as flame retardants when added to polymers. They are used extensively in important commercial products, such as textiles, building materials, and packaging materials (Aston *et al.*, 1996). TCEP is used as a flame retardant additive in such products as polyurethane and polyisocyanurate foams, carpet backing, flame retardant paints, lacquers, coatings, resins, and adhesives (U.S. Environmental Protection Agency, 1988; Green, 1993). TCPP and TDCPP are also used as flame retardant additives in flexible plastic products such as polyurethane foam. These compounds are used worldwide; as a result, contamination of river and lake water has been noted in several countries, including Japan, Canada, and the United States, and in Europe. Toxicity tests have shown these compounds to be toxic to aquatic organisms, and they are a concern in terrestrial ecosystems following chronic exposure. Mammalian toxicity is of less concern since these compounds do not possess anti-AChE activity.

III. CARBAMATES

The CM compounds are esters of carbamic acid. Unlike OPs, CM compounds are not structurally complex. CMs are used as pesticides in agricultural crops and gardens, as therapeutic drugs in human medicine (Alzheimer's disease, myasthenia gravis, glaucoma, and in prophylaxis of OP nerve gas poisoning), and in veterinary medicine (as parasiticides).

A. CM Pesticides

The volume of CMs used exceeds that of OPs because they are considered to be safer than OPs. Some of the commonly used *N*-methyl carbamate insecticides are shown in Table 3. For other CMs, readers are referred to previous publications (Kidd and James, 1991; Tomlin, 1997).

IV. THIOCARBAMATES

The thiocarbamates include a wide variety of fungicides, such as ferbam, mancozeb, maneb, and thiram. The thiocarbamates are also used as herbicides and include butylate, *S*-ethyl dipropylthiocarbamate, pebulate, metham, molinate, cycloate, and vernolate. Their acute toxicity to humans is generally considered to be low, but they can be irritating to the skin and eyes. Inhalation of spray mist or dust from these pesticides may cause throat irritation, sneezing, and coughing.

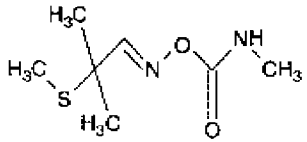
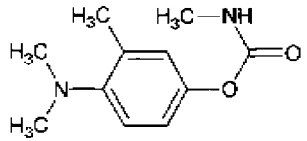
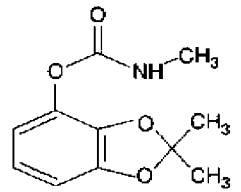
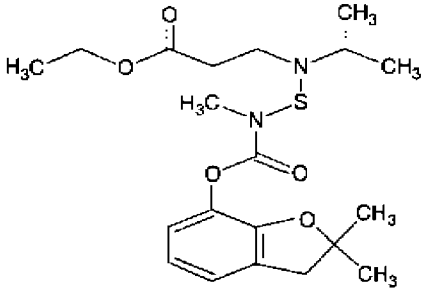
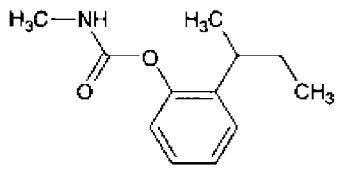
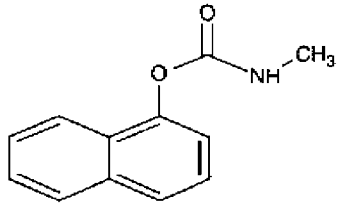
V. AChE INHIBITORS IN HUMAN MEDICINE

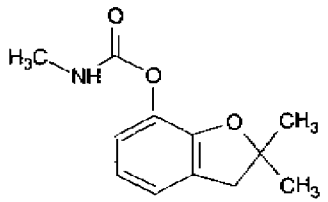
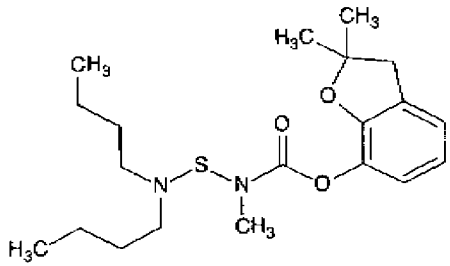
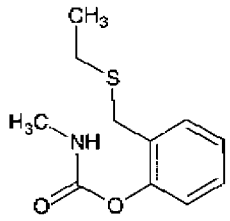
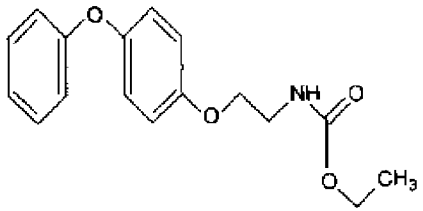
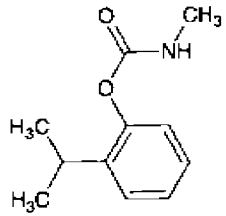
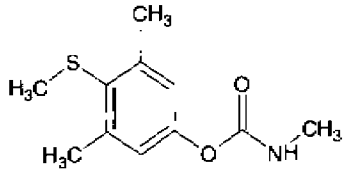
A. Alzheimer's Disease

Acetylcholinesterase inhibitors (AChEIs) have been widely recognized as an effective treatment for Alzheimer's disease (AD). These compounds prevent the breakdown of ACh by inhibiting AChE in the brain regions (cortex and hippocampus) relevant to AD, thereby slowing the development of cognitive impairments and neurodegeneration and alleviating the symptoms of AD. The most widely studied AChEIs are carbamates, such as physostigmine and tacrine. Physostigmine was isolated from the Calabar bean and its structure was elucidated by Stedman and coworkers in 1925. Tacrine belongs to the first generation of AChEIs because it was the first drug approved for the treatment of AD. However, physostigmine's very short duration of action and tacrine's frequent dosing along with the need for monitoring liver enzymes (hepatotoxicity) have made the use of these compounds obsolete. One of the many tacrine derivatives, ameridine, is still under clinical trial study in Japan (Sugimoto *et al.*, 2002). For many years, the OP compound trichlorfon (metrifonate), a slow-release reservoir of dichlorvos, which has a racemic mixture of two isomers, was used in the treatment of mild to moderate AD. However, metrifonate was found to cause serious respiratory complications and therefore was discontinued for treatment of AD. Currently, it is labeled as a mutagen, carcinogen, and possibly teratogen, and therefore it is used only as an investigational drug.

The use of second-generation AChEIs in the treatment of AD has been found to be promising for rectifying cholinergic deficits. It is well established that the cholinesterases, particularly butyrylcholinesterase (BuChE), are associated with the pathogenesis and progression of AD (Guillozet *et al.*, 1997; Darvesh *et al.*, 2003). Therefore, CMs such as rivastigmine and eptastigmine, which are pseudo-irreversible AChEIs and interact with the catalytic as well as regulatory anionic sites of the enzyme, have received a great deal of attention (Cuadra *et al.*, 1994). Rivastigmine is derived from physostigmine and overcomes the deficiencies of physostigmine (Sugimoto *et al.*, 2002). A list of these compounds also includes a novel carbamate compound TV3326 (*N*-propargyl-(3R) aminoindan-5-yl)-ethyl methyl carbamate), which is not only an AChE inhibitor but also an MAO inhibitor (Weinstock *et al.*, 2002). Another derivative of physostigmine is phenserine (a phenylcarbamate compound), which is a potent and highly selective AChE inhibitor with a >50-fold activity compared to BuChE. Phenserine has been tested in clinical trials for the treatment of AD, although no clinical data have been released. Ganstigmine (CHF 2819) is another novel orally active AChEI developed for the treatment of AD. It is a selective inhibitor of AChE (>115 times greater than against BuChE). Ganstigmine is also more selective for inhibition

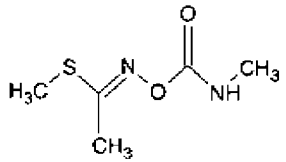
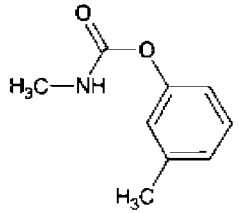
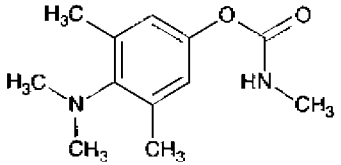
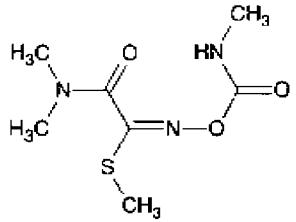
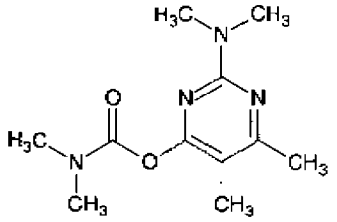
TABLE 3. A Brief Chemical Description of Commonly Used CM Pesticides

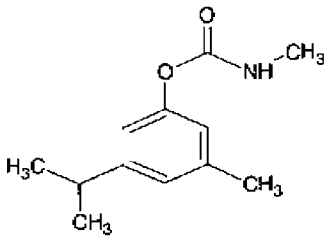
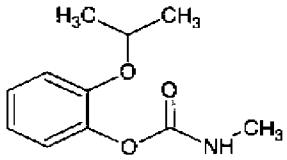
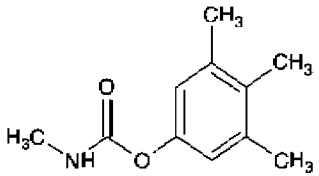
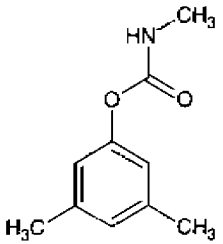
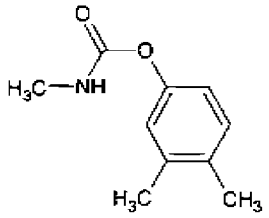
Chemical (CAS No.)	Chemical structure/ empirical formula	Chemical name	Molecular weight	Oral LD_{50} in rat (mg/kg)	Dermal LD_{50} in rabbit (mg/kg)
Aldicarb (116–06–3)		(1*E*)-2-methyl-2-(methylthio)propanal *O*-[(methyl-amino)carbonyl] oxime $C_7H_{14}N_2O_2S$	190.26	0.9	5
Aminocarb (2032–59–9)		4-(Dimethylamino)-3-methylphenyl methylcarbamate $C_{11}H_{16}N_2O_2$	208.26	30	275
Bendiocarb (22781–23–3)		2,2-Dimethyl-1,3-benzodioxol-4-yl methylcarbamate $C_{11}H_{13}NO_4$	223.23	34	566
Benfuracarb (82560–54–1)		2,3-Dihydro-2, 2-dimethyl-7-benzofuranyl *N*-[*n*[2-(ethylcarbonyl) ethyl]-*N*-isopropyl sulfenamoyl]-N-methylcarbamate $C_{20}H_{30}N_2O_5S$	410.53	138	>2000
BPMC (3766–81–2)		2-*sec*-Butylphenyl *N*-methylcarbamate $C_{12}H_{17}NO_2$	422.87	340	4200
Carbaryl (63–25–2)		1-Naphthyl methylcarbamate $C_{12}H_{11}NO_2$	201.22	307	2000

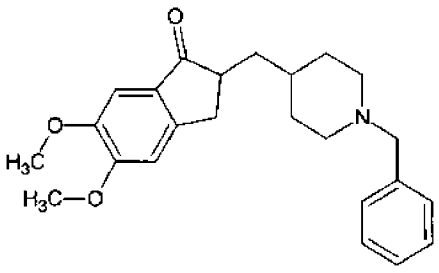
Carbofuran (1563–66–2)		2,2-Dimethyl-2,3-dihydro-1-benzofuran-7-yl methylcarbamate $C_{12}H_{15}NO_3$	221.25	8	2550
Carbosulfan (55285–14–8)		2,3-Dihydro-2,2-dimethyl-7-benzofuranyl-[(di-butylamino)thio] methyl carbamate $C_{20}H_{32}N_2O_3S$	380.55	209	>2000
Croneton (29973–13–5)		2-[(Ethylthio)methyl]phenyl methylcarbamate $C_{11}H_{15}NO_2S$	225.31	200	1000
Fenoxycarb (72490–01–8)		Ethyl [2-(4-phenoxyphenoxy)ethyl] carbamate $C_{17}H_{19}NO_4$	301.34	10,000	2000
Isoprocarb (2631–40–5)		2-Isopropylphenyl methylcarbamate $C_{11}H_{15}NO_3$	193.24	450	—
Methiocarb (2032–65–7)		3,5-Dimethyl-4-(methylthio)phenyl methylcarbamate $C_{11}H_{15}NO_2S$	225.31	15	2000

(continues)

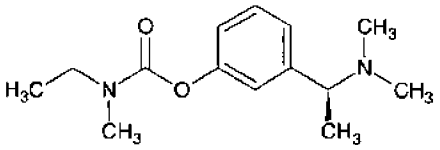
TABLE 3. *(continued)*

Chemical (CAS No.)	Chemical structure/ empirical formula	Chemical name	Molecular weight	Oral LD_{50} in rat (mg/kg)	Dermal LD_{50} in rabbit (mg/kg)
Methomyl (16752–77–5)		Methyl (1*E*)-*N*-{[(methylamino) carbonyl]oxy} ethanimidothioate $C_5H_{10}N_2O_2S$	162.21	17	5000
Metolcarb (1129–41–5)		3-Methylphenyl methylcarbamate $C_9H_{11}NO_2$	165	268	—
Mexacarbate (315–18–4)		4-(Dimethylamino)-3, 5-dimethylphenyl methylcarbamate $C_{12}H_{18}N_2O_2$	222.28	15	5000
Oxamyl (23135–22–0)		Methyl 2-(dimethyl-amino)-*N*-{[(methyl-amino)carbonyl]oxy}-2-oxoethan-imidothioate $C_7H_{13}N_3O_3S$	219.26	5	710
Pirimicarb (23103–98–2)		2-(Dimethylamino)-5, 6-dimethyl-pyrimidin-4-yl dimethylcarbamate $C_{11}H_{18}N_4O_2$	238.29	147	>500

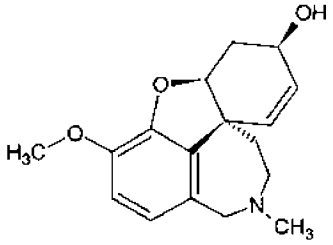
Promecarb (2631–37–0)		3-Isopropyl-5-methylphenyl methylcarbamate $C_{12}H_{17}NO_2$	207.27	61	>1000
Propoxur (114–26–1)		2-Isopropoxyphenyl methylcarbamate $C_{11}H_{15}NO_3$	209.24	95	>1000
Trimethacarb (12407–86–2)		3,4,5-Trimethylphenyl methylcarbamate $C_{11}H_{17}NO_2$	193.24	125	>2000
XMC (2655–14–3)		3,5-Dimethylphenyl methylcarbamate $C_{10}H_{13}NO_2$	179.22	542	—
Xylylcarb (2425–10–7)		3,4-Dimethylphenyl methylcarbamate $C_8H_{10}N_4O_2$	179.22	384	—



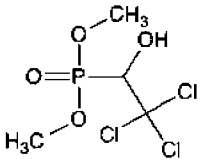
DONEPEZIL



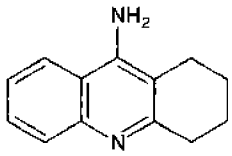
RIVASTIGMINE



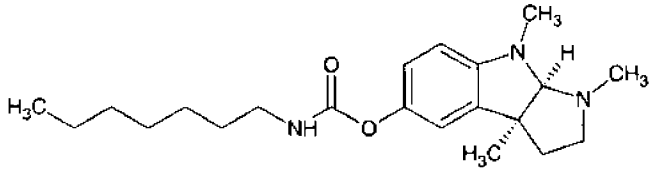
GALANTAMINE



TRICHLORFON



TACRINE



EPTASTIGMINE

FIG. 1. Chemical structures of compounds indicated in Alzheimer's disease.

of central (brain) AChE than peripheral (heart) AChE (Racchi *et al.*, 2004). Chemical structures of some of the compounds that have been used in the past or are currently in use are shown in Fig. 1. These compounds are discussed in detail in Chapters 3 and 4.

B. Myasthenia Gravis

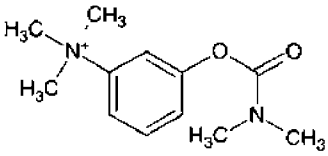
From a pharmacological standpoint, postsynaptic disorders are treated with cholinesterase inhibitors (AChEIs), such as neostigmine, physostigmine, and pyridostigmine. However, AChEIs represent only symptomatic therapy, and they are of little aid in most cases of moderate to severe or progressive myasthenia gravis (MG), particularly if there is oropharyngeal or respiratory muscle involvement. Therefore, use of AChEIs as the mainstay of therapy for MG has been deemphasized. In the past, three commonly used AChEIs were physostigmine, pyridostigmine, and galantamine. Currently neostigmine, pyridostigmine, and ambenonium are the standard anti-AChE compounds used in the symptomatic treatment of MG for cholinergic crisis (Fig. 2). These compounds presumably counteract MG by compensating for lost ACh receptors through elevation of neurotransmitter levels, resulting in increased neuromuscular transmission and improved muscular strength.

C. Glaucoma

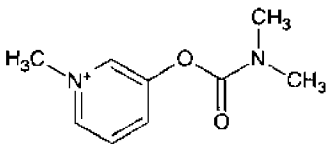
Physostigmine (eserine) and echothiophate (phospholine) are the two AChE-inhibiting compounds indicated for glaucoma. Both compounds are known to exert ocular side effects.

D. Urine Voiding Dysfunction

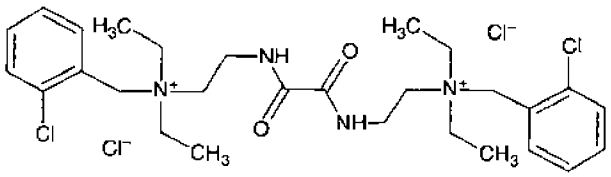
Impairment of destrusor muscle contractibility appears to be one of the causes of voiding dysfunction in both men and women. The destrusor muscle becomes weak due to many factors, including aging, prostate hypertrophy, diabetes mellitus, and multiple sclerosis. AChE-inhibiting carbamates, such as physostigmine, distigmine bromide, and neostigmine bromide, seem to have the potential for correcting the problem. A study conducted on guinea pigs suggested that a novel anti-AChE compound, TAK-802, may be useful in the treatment of voiding dysfunction associated with impaired destrusor contractility (Nagabukuro *et al.*, 2004).



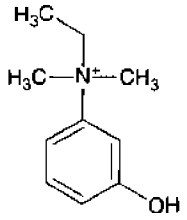
NEOSTIGMINE



PYRIDOSTIGMINE



AMBENONIUM



EDROPHONIUM

FIG. 2. Chemical structures of anti-AChE compounds indicated in myasthenia gravis.

E. OP Nerve Agent Poisoning

During the Persian Gulf War (Operation Desert Shield/Storm) in 1990, military personnel received a reversible AChE inhibitor, pyridostigmine bromide, as a prophylactic measure to combat anticipated deadly OP nerve agent exposure. Due to severe side effects, the drug has been discontinued for application in the setting of such military action (Keeler *et al.*, 1991).

VI. ACHE INHIBITORS IN VETERINARY MEDICINE

A. Anthelmintics

Six OP compounds have been used as anthelmintics in domestic animals: dichlorvos, trichlorfon, haloxon, coumaphos, naphthalophos, and crufomate. The first two were used in horses and the latter four in ruminants. These compounds affect parasites by ACh accumulation attributed to AChE inhibition, leading to interference with neuromuscular transmission and subsequently paralysis, followed by expulsion of parasites from the animal's body (Reinemeyer and Courtney, 2001).

B. Ectoparasiticides

Currently, more than a dozen OP compounds are used as ectoparasiticides in veterinary medicine. These compounds are known to cause paralysis and death of insects by virtue of irreversible AChE inhibition and subsequent accumulation of ACh. These compounds include chlorfenvinphos, chlorpyrifos, coumaphos, cythioate, diazinon, dichlorvos, ethion, famphur, fenthion, malathion, phosmet, pirimiphos-methyl, ronnel, tetrachlorvinphos, and trichlorfon.

Unlike OPs, only two CMs (carbaryl and propoxur) are recommended for the control of ectoparasites. The mechanism of action of CMs is similar to that of OPs, except CMs reversibly inhibit AChE.

VII. CONCLUSIONS

Both OPs and CMs are synthesized compounds. Within each class, the chemicals have some similarities and some differences. Because of the differences, these compounds produce varying degrees of cholinergic and noncholinergic effects, and as a result, they have different applications. Although the majority of these chemicals are used as pesticides, some are used as chemical weapons of mass destruction. In addition, many of these compounds are used as therapeutic drugs in human and veterinary medicine. It is expected that in the future many more new OPs and CMs will be synthesized and novel applications will be discovered.

Acknowledgments

I thank Mrs. Debra A. Britton and Mrs. Denise M. Gupta for their assistance and support in the preparation of this chapter.

References

Aston, L. S., Noda, J., Seiber, J. N., and Reece, C. A. (1996). Organophosphate flame retardants in needles of *Pinus ponderosa* in the Sierra Nevada Foothills. *Bull. Environ. Contam. Toxicol.* **57**, 859–866.

Battershill, J. M., Edwards, P. M., and Johnson, M. K. (2004). Toxicological assessment of isomeric pesticides: A strategy for

testing of chiral organophosphorus (OP) compounds for delayed polyneuropathy in a regulatory setting. *Food Chem. Toxicol.* **42,** 1279–1285.

Benschop, H. P., and De Jong, L. P. A. (1988). Nerve agent stereoisomers: Analysis, isolation and toxicology. *Acc. Chem. Res.* **21,** 366–374.

Cuadra, G., Summers, K., and Giacobini, E. (1994). Cholinesterase inhibitor effects on neurotransmitters in rat cortex *in vivo*. *J. Pharmacol. Exp. Ther.* **270,** 277–284.

Darvesh, S., Walsh, R., Kumar, R., *et al.* (2003). Kinetic properties of human acetylcholinesterase and butyrylcholinesterase in the presence of drugs for Alzheimer's disease. *Alzheimer Dis. Assoc. Disord.* **17,** 117–126.

Green, J. (1993). A review of phosphorus-containing flame retardants. *J. Fire Sci.* **10,** 470–487.

Guillozet, A. L., Smiley, J. F., Mash, D. C., and Mesulam, M. M. (1997). Butyrylcholinesterase in the life cycle of amyloid plaques. *Ann. Neurol.* **42,** 909–918.

Johnson, M. K. (1969). Delayed neurotoxic action of some organophosphorus compounds. *Br. Med. Bull.* **25,** 231–235.

Keeler, J. R., Hurst, C. G., and Dunn, M. A. (1991). Pyridostigmine used as a nerve agent pretreatment under wartime conditions. *J. Am. Med. Assoc.* **266,** 693–695.

Kidd, H., and James, D. R. (1991). *The Agrochemicals Handbook,* 3rd ed. Royal Society of Chemistry Information Services, Cambridge, UK.

Kuča, K., and Kassa, J. (2004). Oximes-induced reactivation of rat brain acetylcholinesterase inhibition by VX agent. *Hum. Exp. Toxicol.* **23,** 167–171.

Marrs, T. C. (1993). Organophosphate poisoning. *Pharmacol. Ther.* **58,** 51–66.

Nagabukuro, H., Okanishi, S., and Doi, T. (2004). Effects of TAK-802, a novel acetylcholinesterase inhibitor, and various cholinomimetics on the urodynamic characteristics in anesthetized guinea pigs. *Eur. J. Pharmacol.* **494,** 225–232.

Racchi, M., Mazzucchelli, M., Porrello E., Lanni, C., and Govoni, S. (2004). Acetylcholinesterase inhibitors: Novel activities of old molecules. *Pharmacol. Res.* **50,** 441–451.

Reinemeyer, C. R., and Courtney, C. H. (2001). Chemotherapy of parasitic diseases. In *Veterinary Pharmacology and Therapeutics* 8th ed., (H. R. Adams, Ed.), pp. 947–979. Blackwell, Ames, IA.

Senanayke, N., and Karalliede, L. (1987). Neurotoxic effects of organophosphorus insecticides: An intermediate syndrome. *N. Engl. J. Med.* **316,** 761–763.

Smith, J. R. (2004). Analysis of the enantiomers of VX using normal-phase chiral liquid chromatography with atmospheric pressure chemical ionization-mass spectrometry. *J. Anal. Toxicol.* **28,** 390–392.

Sugimoto, H., Iimura, Y., and Yamanishi, Y. (2002). The new generation of acetylcholinesterase inhibitors: In *Mapping the Progress of Alzheimer's and Parkinson's Disease* (Y. Mizuno, A. Fisher, and I. Hanin, Eds.), pp. 193–198. Kluwer/Plenum, New York.

Tomlin, C. D. S. (1997). *A World Compendium. The Pesticide Manual,* 11th ed. British Crop Protection Council, Farnham, Surrey, UK.

U.S. Environmental Protection Agency (1988). Twenty-third report of the interagency testing committee to the administrator: Receipt of report and request for comments regarding priority list of chemicals. *Fed. Reg.* **53,** 46244–46272.

Weinstock, M., Poltyrer, T., Bejar, C., Sagi, Y., and Youdin, M. B. H. (2002). TV3326, a novel cholinesterase and MAO inhibitor and Parkinson's disease. In *Mapping the Progress of Alzheimer's and Parkinson's Disease* (Y. Mizuno, A. Fisher, and I. Hanin, Eds.), pp. 193–198. Kluwer/Plenum, New York.

CHAPTER 3

Therapeutic Uses of Cholinesterase Inhibitors in Neurodegenerative Diseases

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I. INTRODUCTION

The most common form of age-related neurodegenerative disease in the United States is Alzheimer's disease (AD), with approximately 4 million Americans afflicted; in the European Union, approximately 3 million people suffer from the disease. Furthermore, as the average life span continues to increase, it is anticipated that AD and other age-related neurodegenerative diseases will become increasingly major public health concerns. Already, the annual cost of disease (including paid and unpaid caregiver costs, as well as losses in productivity due to illness and premature mortality) of patients with AD in the United States alone has been estimated to be $100 billion (Leifer, 2003), and the prevalence of AD is expected to increase by approximately three-fold by the middle of this century. This represents a potentially staggering cost, both monetarily and in terms of lost human potential, to developed societies.

A detailed understanding of the pathogenesis of AD, which might be useful in the rational development of strategies to prevent or treat disease, is not yet available, although substantial progress has been made in this area, especially in the past decade. Also, there is reason for considerable optimism, particularly regarding the possibility of treatments that may prevent or at least delay the onset of AD. However, AD is a complex disorder, with both genetic and environmental factors that affect the risk of disease, cognitive and neuropsychiatric manifestations that may vary between patients and over time, and, as is becoming increasingly apparent, only a part of a spectrum of age-related neurodegenerative disease that is still in the process of clinical, pathologic, and biochemical definition. The historical lack of an ideal animal model for AD has necessitated that at least some of this definition has derived from empirical observations of the effects of putative therapeutic agents, such as the acetycholinesterase inhibitors (AChEIs). Because the major nongenetic risk factor for AD is the passage of time, which is currently inconveniently recapitulated in the laboratory setting, it is likely that we will continue to learn more about these diseases through the further characterization of clinical responses of AD and, increasingly, other age-related dementias to such therapies. This chapter describes the rationale for such therapies in AD, aspects of their application to clinical disease, and what we have learned and may continue to learn from the effects of these drugs in neurodegenerative disease.

II. THE CHOLINERGIC HYPOTHESIS

Early investigations on postmortem tissue demonstrated a reduction of choline acetyltransferase (ChAT) activity and of cholinergic neurons in the basal forebrain of patients affected by AD (Davies and Maloney, 1976; Perry *et al.*, 1977; Whitehouse *et al.*, 1981). Decreases in presynaptic cholinergic neurons are also observed in the cerebral cortex and hippocampus as AD progresses (Bartus *et al.*, 1982; Coyle *et al.*, 1983). These observations led to the formulation, approximately 25 years, ago, of the "cholinergic hypothesis," which states that loss of cholinergic function in the cerebrum contributes significantly to cognitive dysfunction in AD (Bartus, 2000). In addition to ChAT, cholinesterases, particularly butyrylcholinesterase (BuChE), have been associated with the pathogenesis and progression of AD (Guillozet *et al.*, 1997; Darvesh *et al.*, 2003). Based on these findings, it has been hypothesized that cholinesterase inhibitors that inhibit both AChE and BuChE stabilize disease progression better than those that inhibit only AChE in AD patients (Ballard, 2002; Giacobini, 2000). Importantly, the cholinergic hypothesis does not necessarily stipulate that cholinergic deficiency initiates or contributes to the progression of disease, although we deal with this possibility later. However, the goal of any medical hypothesis is to produce new, effective therapies for

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disease; in this light, the cholinergic hypothesis, which has brought several compounds with significant efficacy to the clinical treatment of AD, has been regarded as one of the successes of modern neuropharmacology (Bartus, 2000). We will next discuss the origins of the cholinergic hypothesis, the uses of AChEIs in the therapy of AD, determinations of their clinical efficacy, and what the use of these compounds has taught us about the disease.

III. CHOLINERGIC FAILURE, NEUROPATHOLOGICAL CORRELATES, AND THE SYMPTOMS OF AD

Impairments in AD occur in both memory and other areas of cognition, such as language or visuospatial awareness. In addition, neuropsychiatric symptoms including psychosis and mood alterations such as depression, apathy, and agitation may present during the course of dementia, (Lyketsos *et al.*, 2001). These perturbations reflect structural and neurochemical alterations in brain regions such as the hippocampus and cerebral cortex that house normal functions of memory and cognition. Hippocampal and cortical neurons are innervated by cholinergic afferents from the basal forebrain, the site of the nucleus basalis of Meynert, which contains approximately 80% of the cholinergic neurons of the central nervous system and is characterized by marked atrophy in advanced AD (Cummings and Back, 1998). In experimental rodent models, lesions of cholinergic pathways result in impairments in the performance of memory tasks (Dunnett *et al.*, 1987). Taken together, these observations are consistent with the idea that cholinergic failure may contribute strongly to symptoms of AD. The cholinergic hypothesis has also been strengthened by observations of correlations between cholinergic failure and the degree of clinical dementia.

Interestingly, however, cholinergic failure and dementia have also been found to correlate with the extent of extracellular senile plaques in brain tissue (Arendt *et al.*, 1985; Etienne *et al.*, 1986; Perry *et al.*, 1978). Senile plaques are one of the pathologic hallmarks of AD; another characteristic lesion, the intracellular neurofibrillary tangle, is found in a topographically organized pattern in the gray matter of the limbic system and progressively in the neocortex in AD. Although this association has invited speculation that cholinergic failure may contribute substantially to the pathogenesis of AD, historically this has not been widely believed to be the case based on several observations. The first involves studies of patients with amnestic mild cognitive impairment (MCI), a condition of memory loss that is widely considered a paradigm of preclinical AD (Petersen, 2000; Petersen *et al.*, 2001). In MCI, levels of ChAT have been reported to be increased, with a subsequent decrease to normal levels with the onset of clinical dementia (DeKosky *et al.*, 2002; Frölich, 2002). In advanced AD, the loss of cholinergic neurons results in up to a 90% reduction in the activity of ChAT, which is needed for the synthesis of the neurotransmitter ACh, and ACh levels decrease by 90%, especially in the cerebral cortex and hippocampus (Murphy *et al.*, 1998). Furthermore, although cholinergic failure is most pronounced late in the course of AD, anticholinesterase therapies (as described later) have been best characterized and appear most efficacious in mild to moderate dementia, although studies on the use of AChEIs for longer term disease are ongoing. Finally, the pattern of memory loss in AD, in which more recent memories are lost first, with loss of older memories later, is not well recapitulated by models that invoke only neuronal loss, but it can be accounted for if changes in cholinergic activity that occur in MCI are viewed as responses to other instigators of brain dysfunction in AD (Small *et al.*, 2001; Small, 2004).

IV. DETERMINATION OF THE EFFECTS OF AChEIs ON SYMPTOMS IN AD

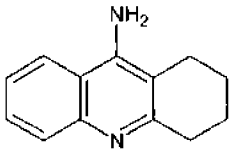
The effects of AChEI therapy on AD have proven to be modest, and the determination of the significance of effects of this magnitude required the development of standardized tools of measurement that encompass important aspects of AD symptomatology. Commonly used instruments include the Mini-Mental Status Examination (MMSE) (Folstein *et al.*, 1975), which assays cognition with the use of 11 questions that produce a single score ranging from 0 (severe impairment) to 30 (no impairment). The second tool is the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-cog) (Rosen *et al.*, 1984), designed to determine cognitive function in AD. This test also consists of 11 items that assess cognitive functions that are typically impaired specifically in AD, with an output score ranging from 0 (no impairment) to 70 (marked impairment); the average rate of score increase in patients with AD is 7–11 points per year (Kramer-Ginsberg *et al.*, 1988; Stern *et al.*, 1994). Neuropsychiatric symptoms described in Section III can likewise be quantified with the use of the Neuropsychiatric Inventory (Cummings *et al.*, 1994) or a noncognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-noncog). Recently, there has been an enlargement of the scope of assessment to include effects on so-called functional symptoms. Functional decline in AD involves the loss of ability to perform activities of daily living, such as the simple arithmetic of basic finances, driving, or using household tools such as the telephone, with progression of losses to the point that patients in a terminal state are no longer able to feed or bathe themselves. These losses increase the burden on patient caregivers, are highly correlated with the decision to place patients in institutional facilities, and contribute significantly to the financial burden of disease. Functional losses are also quantifiable through such tools as the

Alzheimer's Disease Functional Assessment and Change Scale and the Progressive Deterioration Scale (Reisberg *et al.*, 1986).

V. A BRIEF HISTORY OF THE USE OF AChEIs IN AD

AChEIs initially came to the attention of investigators concerned with dementias with the recognition of their capacity to enhance cognition in animals with scopolamine-induced amnesia (Bartus, 1978). With the discovery that ACh was depleted in the hippocampus (Smith and Swash, 1978) and initial reports of effects of physostigmine on cognition in both normal subjects and AD patients (Davis *et al.*, 1978; Muramoto *et al.*, 1979; Peters and Levin, 1979; Smith and Swash, 1979), the stage was set for trials of a variety of AChEIs as potential therapeutic agents in AD. Some drugs, such as galantamine, had long been used for other indications. Two compounds, velnacrine and eptastigmine, were halted in development due to the association of blood dyscrasias with their use. More typical limitations of a variety of agents center on drug tolerability, the response rate to a tolerated dose, and intersubject variability. Overall, the response of AD patients to AChEI therapy has been characterized as modest, with 3 or 4 point decreases in ADAS-cog scores compared with the yearly rate of cognitive decline in untreated controls. The U.S. Food and Drug Administration (FDA) uses a 4-point improvement in ADAS-cog scores as a criterion for a clinically significant response to therapy — an improvement also used by many investigators to define rate of response, which is typically 30–40% of AD patients undergoing treatment. The FDA has approved four AChEIs for the treatment of AD; in fact, these are currently the clinical mainstays of AD therapy.

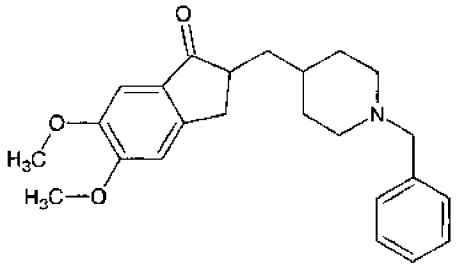
A. Tacrine



Tacrine

In 1993, tacrine (aminoacridine, competitive unselective reversible inhibitor of cholinesterases) was the first anticholinesterase drug to receive FDA approval for the treatment of mild to moderate AD. The benefits of treatment were evident in end points that used ADAS as well as general clinical impressions, with 10–26% of recipients of high doses showing measurable improvement over the course of 30 weeks (Knapp *et al.*, 1994). However, only a minority of patients were able to tolerate the maximally effective dose (160 mg/day) due to hepatotoxicity manifested as asymptomatic transaminase elevations, as well as nausea and vomiting, diarrhea, and anorexia. Other limitations of tacrine, such as a relatively short half-life (2–3 hr) and significant interindividual variation in clearance rates that necessitated drug titration and plasma determinations, motivated the search for novel agents. It is available under the trade name Cognex, but is rarely prescribed.

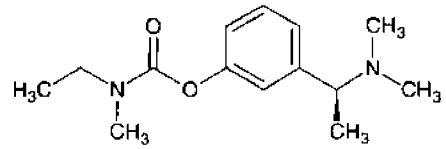
B. Donepezil



Donepezil

Donepezil, a piperidine-based rapidly reversible noncompetitive AChEI, was approved by the FDA for treatment of cognitive dysfunction in AD in 1996. The drug was developed specifically for pharmacotherapy of AD (Bryson and Benfield, 1997; Barner and Gray, 1998) and has been found to have selectivity for brain AChE over peripheral forms of the enzyme (Kosasa *et al.*, 2000). Donepezil is highly selective for AChE, with significantly lower affinity for BuChE. Major advantages over tacrine are its long half-life (70 hr) and uniform dosing in patients with renal or hepatic impairment. In the course of a study of the effects of 24 weeks of treatment with ADAS-cog, MMSE, and other end point determinations, cognitive function was found to be significantly improved by 12 weeks. The drug was generally well tolerated at low (5 mg/day) doses, with a slight but statistically significant increased incidence of diarrhea and vomiting at 10 mg/day that nevertheless resolved spontaneously without reduction in dosage (Rogers and Friedhoff, 1998; Rogers *et al.*, 1998). The drug is marketed under the trade name Aricept.

C. Rivastigmine

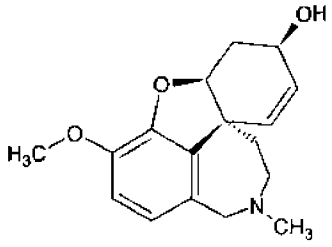


Rivastigmine

Rivastigmine, approved by the FDA in 2000, is a noncompetitive pseudo-irreversible carbamate AChEI that was

selected for study based on its high affinity for brain AChE compared to peripheral forms of the enzyme (Weinstock *et al.*, 1992, 2004; Enz *et al.*, 1993). Rivastigmine selectively inhibits monomeric AChE, especially in the cortex and hippocampus, and is thereby thought to facilitate cholinergic neurotransmission by slowing the degradation of ACh released by functionally intact cholinergic neurons (Polinsky, 1998; Ibach and Haen, 2004). The mechanism of action of rivastigmine differs from that of donepezil because donepezil is an AChE-selective inhibitor, whereas rivastigmine is a dual inhibitor of both AChE and BuChE. Furthermore, unlike donepezil, rivastigmine is bound more tightly to the active center of AChE than a naturally occurring choline ester. Rivastigmine was shown to improve cognition as determined by ADAS scores, participation in activities of daily living, and global evaluation scores in patients with mild to moderate AD in a multicenter trial of 28 weeks' duration (Rosler *et al.*, 1999). As with donepezil, most side effects were found to be gastrointestinal and transient in nature. The drug, titrated up to 12 mg/day with two or three times daily dosing, yielded ADAS improvements in 24% of treated patients versus 16% for placebo controls. It is marketed under the trade name Exelon.

D. Galantamine



Galantamine

Galantamine is a phenanthrene alkaloid that was initially isolated from the common snowdrop, *Galanthus niralus*. Galantamine acts as a rapidly reversible, competitive AChEI. The agent has been used for approximately 40 years in the treatment of myasthenia gravis and in reversal of pharmacologic neuromuscular blockade; hence, considerable familiarity with its pharmacokinetic and toxicologic properties existed prior to its approval by the FDA for treatment of symptoms of AD in 2001. Several trials have demonstrated efficacy in the treatment of cognitive symptoms in AD at a dose of up to 32 mg/day, as determined by ADAS scores and other end points (Rainer, 1997; Raskind *et al.*, 2000; Tariot *et al.*, 2000). Nausea appears to be the most common side effect. Stimulation of nicotinic receptors has been proposed to be an additional mechanism of action of galantamine, and there is evidence that such an effect may be relevant to AD (Dajas-Bailador *et al.*, 2003). Galantamine is marketed under the trade name Reminyl.

VI. THE PERSISTENCE OF AChEI EFFICACIES

The initial results for the previously discussed agents were generally based on trials of 6 months' duration or less. These and subsequent studies have indicated that treatment of AD with AChEIs tends to lead to an improvement in cognition that is maintained for up to approximately 1 year. This is typically followed by declines in cognition, but to levels that still improved relative to those of untreated controls. Several studies, typically continuations of trials under open-label conditions, demonstrate benefit of up to several years' duration with FDA-approved AChEIs (Knopman *et al.*, 1996; Lilienfeld and Parys, 2000; Rogers *et al.*, 2000; Doody *et al.*, 2001; Rockwood *et al.*, 2001; Tariot, 2001). The efficacy of a variety of agents with widely varying structures but that all share anticholinesterase properties, as well as the continuation of clinical efficacy of these drugs into the stage of AD that is characterized neurochemically by cholinergic failure, and in some instances initiated in more severely affected AD patients (Feldman *et al.*, 2001), is widely seen to support the cholinergic hypothesis of AD.

VII. PUTATIVE EFFECTS OF AChEIs ON AD PATHOGENETIC MECHANISMS

Although the clinical indication of AChEIs is limited to symptomatic therapy for AD, increasing experience with their use, and the persistence of drug efficacy in particular, has led some to suggest that these agents may alter the natural history of disease (Farlow *et al.*, 2000, 2003; Doraiswamy *et al.*, 2002; Erkinjuntti *et al.*, 2003; Stefanova *et al.*, 2003). It has been hypothesized that normal activation of neurons in aging and during the course of AD may lead to preservation of neuronal function and/or promotion of survival of remaining neurons; this "cognitive reserve" or "use it or lose it" hypothesis has been proposed to account for the effects of education and other socioeconomic factors on the risk of development of cognitive impairment in general and AD in particular in a variety of populations (Yu *et al.*, 1989; Brayne and Calloway, 1990; Moritz and Petitti, 1993; Stern *et al.*, 1995; Evans *et al.*, 1997; Hall *et al.*, 2000; Qiu *et al.*, 2001; Salemi *et al.*, 2002; Karp *et al.*, 2004). Additional proposed interactions of AChEIs with specific aspects of steps that are widely believed to be important in the pathogenesis of AD have been reviewed (Lane *et al.*, 2004) and are discussed next.

A. Effects of AChEIs on Amyloid β Peptide

Approximately 5% of cases of AD are attributable to mutations in known genes that act in an autosomal dominant fashion. Each of these is related to the metabolism of the amyloid precursor protein (APP) and its cleavage products, most prominently amyloid β (Aβ). The remaining 95% of

cases appear as sporadic disease with a relatively late onset and a complex etiology attributable to interactions between aging, environmental, and other genetic factors (Munoz and Feldman, 2000; Lahiri *et al.*, 2004). The pathologic similarities between inherited and sporadic forms of AD, however, have led to the "amyloid hypothesis" that increased production or accumulation of Aβ, with its subsequent aggregation and accumulation in cerebrum as senile plaques, provides the pathogenetic foundation for all forms of AD (Hardy and Selkoe, 2002).

Efforts to understand the effects of Aβ have focused on its aggregation and reorganization to form progressively insoluble structures. This has been attributed to the presence in Aβ of an unstable domain that can readily adopt multiple conformations, some of which are prone to form aggregates that may propagate as filamentous structures with decreased solubility that are deposited in cerebrum as amyloid. This propensity appears to be key to the pathologic actions of Aβ because the uniform effect of genetic mutations in familial forms of AD is the promotion of Aβ aggregation and insolubility, either via alterations in the structure of Aβ (in the case of mutations in APP) or by increasing the relative amount of the more amyloidogenic 42-residue species relative to the 40-residue species (in the case of mutations in presenilin 1 or 2, which determine the carboxy-terminal cleavage of the Aβ peptide) (Marjaux *et al.*, 2004). One effect of AChEIs may be mediated by ACh receptor-mediated activation of protein kinase C and mitogen-activated protein kinase pathways (Haring *et al.*, 1998; Beach *et al.*, 2001; Beach, 2002). Such activation promotes an alternate cleavage of APP that produces nonamyloidogenic forms of the peptide. Conceivably, the persistence of ACh in the context of AChEI treatment may promote this process.

In addition to Aβ, senile plaques contain a host of other proteins (Liao *et al.*, 2004), including AChE, which has been proposed to serve as a nucleating factor in the deposition of Aβ with reduced solubility, or otherwise promote the toxicity or aggregation state of Aβ *in vivo* (Inestrosa *et al.*, 1996; Alvarez *et al.*, 1997; De Ferrari *et al.*, 2001; Rees *et al.*, 2003). Other studies indicate that Aβ complexed with AChE is more toxic than Aβ species alone (Alvarez *et al.*, 1998). A number of AChEIs have been reported to inhibit at least partially Aβ aggregation in the presence of AChE (Bartolini *et al.*, 2003; Piazzi *et al.*, 2003); the significance of this observation and its validity in the case of clinically widely used AChEIs remain unknown.

B. Effects of AChEIs on Tau

According to the amyloid hypothesis, abnormalities involving Aβ peptides also lead to changes in the organization of tau to produce neurofibrillary tangles, the lesion in brain tissue that is more closely associated with the presence of clinical AD. Deposition of tau in tangles is enhanced by tau hyperphosphorylation, which can be accomplished by a variety of kinases, especially glycogen synthase kinase-3 (GSK3). Kinase activities described previously that are promoted by ACh not only affect APP metabolism but also are associated with decreases in the activity of GSK3, leading to decreased tau phosphorylation and conceivably to a reduction in tangle generation (Forlenza *et al.*, 2000). Consistent with this mechanism, a rivastigmine-treated AD group showed no change in cerebrospinal fluid (CSF) levels of tau after 1 year, whereas significant increases were observed in untreated patients (Stefanova *et al.*, 2003). However, the CSF tau content of patients treated with tacrine resembled that of controls more closely, making interpretation of these results difficult.

C. Effects of AChEIs on Cerebrovascular Parameters

Recently, the coexistence of vascular lesions and those of AD in cerebrum of patients with so-called mixed dementia have received increasing attention, along with the possibility of heretofore unanticipated interactions between pathophysiological factors (Langa *et al.*, 2004). Some authors have suggested that factors that lead to the development of senile plaques and neurofibrillary tangles may be promoted by cerebrovascular disease (de la Torre, 2002; Honig *et al.*, 2003; Casserly and Topol, 2004); conversely, deposition of amyloid in vessel walls compromises cerebrovascular function and, in the extreme, promotes the risk of hemorrhagic stroke. Interestingly, AChEI therapy for mixed dementia yields favorable, but modest, clinical results similar to those observed in the treatment of AD (Kumar *et al.*, 2000; Erkinjuntti *et al.*, 2002) and in some studies demonstrates efficacy in the treatment of dementia associated with cerebrovascular disease alone (Erkinjuntti *et al.*, 2004). As in the case of AD, cholinergic deficiency has been demonstrated in vascular dementia and has been attributed to ischemic injury to cholinergic neurons, as supported by rodent models of ischemic injury (Togashi *et al.*, 1994). However, effects of ACh on the vascular endothelium may account for a portion of both the interaction of AD and vascular dementia and the efficacy of AChEIs in both of these conditions. ACh at this site mediates the release of nitric oxide, a vasodilator that may account for increases in cerebral blood flow and glucose metabolism upon treatment with AChEIs (Minthon *et al.*, 1993; Harkins *et al.*, 1997; Lojkowska *et al.*, 2003). Conceivably, such improvements could influence the natural history of vascular dementia and AD.

VIII. AChEIs IN THE TREATMENT OF OTHER DEMENTIAS

Dementia associated with Parkinson's disease (PDD) and dementia with Lewy bodies (DLB) together comprise the second most common form of age-related dementia after

AD. A recent body of literature suggests that AChEIs may be of utility in the treatment of these disorders, which have also been reported to be associated with cholinergic deficit (Aarsland *et al.*, 2004). This deficit, as in the case of AD, has been attributed to basal forebrain degeneration, and this may be sufficient to account for any observed clinical efficacy of AChEIs. However, the interactions of AChE with Aβ in senile plaques described previously suggest other possibilities as well. Many neurodegenerative diseases are characterized by the presence of a variety of proteinaceous aggregates that are associated with neurotoxicity. As in the case of amyloid plaques, upon further examination these aggregates have been found characteristically to be constituted of numerous proteins that may or may not be predicted based on sequence structure analysis (Yoon and Welsh, 2004). Although the presence of AChE has not been reported in Lewy bodies of PDD or DLB, its association with these lesions would suggest the possibility of a more generalized role of AChE in the pathogenesis of age-related dementias. Whether treatments with AChEIs alter the course of these dementias, and, if so, by what mechanism, awaits further clinical studies and the further molecular characterization of these disorders.

IX. CONCLUSIONS

Effective preventive and therapeutic strategies for AD and other age-related neurodegenerative diseases are the most pressing need in modern clinical neurological practice. Remarkable advances in our insight into the pathogenesis of AD in particular hold forth the prospect that manipulation of pathways of amyloid peptide synthesis, posttranslational processing, aggregation, degradation, interactions with other macromolecules, or the relation of Aβ to oxidative, inflammatory, or neuroexcitatory processes that may promote or be promoted by its presence in the cerebrum may profoundly alter the incidence or progression of AD in the future. The multifactorial nature of the etiology of sporadic AD seems to imply a likelihood that the most effective treatment strategies will target several or many of these processes. Indeed, many models of the progression of AD invoke self-reinforcing cycles of cerebral damage that may be checked in part at any one of a number of steps but that may be best approached by therapeutic strategies that target multiple aspects of disease pathogenesis. Definitive experimental evidence for a role of ACh in the pathogenesis of human neurodegenerative disease may prove elusive and perhaps become unambiguously manifest only in the context of cotreatments with AChEIs and agents that target other aspects of disease. In the meantime, the symptomatic effects alone of AChEIs dictate that they will remain important treatments for AD and, in all likelihood, for an expanding list of age-related dementing illnesses.

References

Aarsland, D., Mosimann, U. P., and McKeith, I. G. (2004). Role of cholinesterase inhibitors in Parkinson's disease and dementia with Lewy bodies. *J. Geriatr. Psychiatr. Neurol.* **17,** 164–171.

Alvarez, A., Opazo, C., Alarcon, R., Garrido, J., and Inestrosa, N. C. (1997). Acetylcholinesterase promotes the aggregation of amyloid-beta-peptide fragments by forming a complex with the growing fibrils. *J. Mol. Biol.* **272,** 348–361.

Alvarez, A., Alarcon, R., Opazo, C., Campos, E. O., Munoz, F. J., Calderon, F. H., Dajas, F., Gentry, M. K., Doctor, B. P., De Mello, F. G., and Inestrosa, N. C. (1998). Stable complexes involving acetylcholinesterase and amyloid-beta peptide change the biochemical properties of the enzyme and increase the neurotoxicity of Alzheimer's fibrils. *J. Neurosci.* **18,** 3213–3223.

Arendt, T., Bigl, V., Tennstedt, A., and Arendt, A. (1985). Neuronal loss in different parts of the nucleus basalis is related to neuritic plaque formation in cortical target areas in Alzheimer's disease. *Neuroscience* **14,** 1–14.

Ballard, C. G. (2002). Advances in the treatment of Alzheimer's disease: Benefits of dual cholinesterase inhibition. *Eur. Neurol.* **47,** 64–70.

Barner, E. L., and Gray, S.L. (1998). Donepezil use in Alzheimer disease. *Ann. Pharmacother.* **32,** 70–77.

Bartolini, M., Bertucci, C., Cavrini, V., and Andrisano, V. (2003). Beta-amyloid aggregation induced by human acetylcholinesterase: Inhibition studies. *Biochem. Pharmacol.* **65,** 407–416.

Bartus, R. T. (1978). Evidence for a direct cholinergic involvement in the scopolamine-induced amnesia in monkeys: Effects of concurrent administration of physostigmine and methylphenidate with scopolamine. *Pharmacol. Biochem. Behav.* **9,** 833–836.

Bartus, R. T. (2000). On neurodegenerative diseases, models, and treatment strategies: Lessons learned and lessons forgotten a generation following the cholinergic hypothesis. *Exp. Neurol.* **163,** 495–529.

Bartus, R. T., Dean, R. L., 3rd, Beer, B., and Lippa, A. S. (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217,** 408–414.

Beach, T. G. (2002). Muscarinic agonists as preventative therapy for Alzheimer's disease. *Curr. Opin. Invest. Drugs* **3,** 1633–1636.

Beach, T. G., Walker, D. G., Potter, P. E., Sue, L. I., and Fisher, A. (2001). Reduction of cerebrospinal fluid amyloid beta after systemic administration of M1 muscarinic agonists. *Brain Res.* **905,** 220–223.

Beach, T. G., Walker, D. G., Potter, P. E., Sue, L. I., and Fisher, A. (2001). Reduction of cerebrospinal fluid amyloid beta after systemic administration of M1 muscarinic agonists. *Brain Res.* **905,** 220–223.

Brayne, C., and Calloway, P. (1990). The association of education and socioeconomic status with the Mini Mental State Examination and the clinical diagnosis of dementia in elderly people. *Age Ageing* **19,** 91–96.

Bryson, H. M., and Benfield, P. (1997). Donepezil. *Drugs Aging* **10,** 234–241.

Casserly, I., and Topol, E. (2004). Convergence of atherosclerosis and Alzheimer's disease: Inflammation, cholesterol, and misfolded proteins. *Lancet* **363,** 1139–1146.

Coyle, J. T., Price, D. L., and DeLong, M. R. (1983). Alzheimer's disease: A disorder of cortical cholinergic innervation. *Science* **219,** 1184–1190.

Cummings, J. L., and Back, C. (1998). The cholinergic hypothesis of neuropsychiatric symptoms in Alzheimer's disease. *Am. J. Geriatr. Psychiatr.* **6,** S64–S78.

Cummings, J. L., Mega, M., Gray, K., Rosenberg-Thompson, S., Carusi, D. A., and Gornbein, J. (1994). The Neuropsychiatric Inventory: Comprehensive assessment of psychopathology in dementia. *Neurology* **144,** 2308–2314.

Dajas-Bailador, F. A., Heimala, K., and Wonnacott, S. (2003). The allosteric potentiation of nicotinic acetylcholine receptors by galantamine is transduced into cellular responses in neurons: Ca^{2+} signals and neurotransmitter release. *Mol. Pharmacol.* **64,** 1217–1226.

Darvesh, S., Walsh, R., Kumar, R., *et al.* (2003). Kinetic properties of human acetylcholinesterase and butyrylcholinesterase in the presence of drugs for Alzheimer's disease. *Alzheimer Dis. Assoc. Disord.* **17,** 117–126.

Davies, P., and Maloney, A. J. (1976). Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* **2,** 1403.

Davis, K. L., Mohs, R. C., Tinklenberg, J. R., Pfefferbaum, A., Hollister, L. E., and Kopell, B. S. (1978). Physostigmine: Improvement of long-term memory processes in normal humans. *Science* **201,** 272–274.

De Ferrari, G. V., Canales, M. A., Shin, I., Weiner, L. M., Silman, I., and Inestrosa, N. C. (2001). A structural motif of acetylcholinesterase that promotes amyloid beta-peptide fibril formation. *Biochemistry* **40,** 10447–10457.

DeKosky, S. T., Ikonomovic, M. D., Styren, S. D., Beckett, L., Wisniewski, S., Bennett, D. A., Cochran, E. J., Kordower, J. H., and Mufson, E. J. (2002). Upregulation of choline acetyltransferase activity in hippocampus and frontal cortex of elderly subjects with mild cognitive impairment. *Ann. Neurol.* **51,** 145–155.

de la Torre, J. C. (2002). Vascular basis of Alzheimer's pathogenesis. *Ann. N. Y. Acad. Sci.* **977,** 196–215.

Doody, R. S., Geldmacher, D. S., Gordon, B., Perdomo, C. A., and Pratt, R. D. (2001). Donepezil Study Group. Open-label, multicenter, phase 3 extension study of the safety and efficacy of donepezil in patients with Alzheimer disease. *Arch. Neurol.* **58,** 427–433.

Doraiswamy, P. M., Krishnan, K. R., Anand, R., Sohn, H., Danyluk, J., Hartman, R. D., and Veach, J. (2002). Long-term effects of rivastigmine in moderately severe Alzheimer's disease: Does early initiation of therapy offer sustained benefits? *Prog. Neuropsychopharmacol. Biol. Psychiatr.* **26,** 705–712.

Dunnett, S. B., Whishaw, I. Q., Jones, G. H., and Bunch, S. T. (1987). Behavioural, biochemical and histochemical effects of different neurotoxic amino acids injected into nucleus basalis magnocellularis of rats. *Neuroscience* **20,** 653–669.

Enz, A., Amstutz, R., Boddeke, H., Gmelin, G., and Malanowski, J. (1993). Brain selective inhibition of acetylcholinesterase: A novel approach to therapy for Alzheimer's disease. *Prog. Brain Res.* **98,** 431–438.

Erkinjuntti, T., Kurz, A., Gauthier, S., Bullock, R., Lilienfeld, S., and Damaraju, C. V. (2002). Efficacy of galantamine in probable vascular dementia and Alzheimer's disease combined with cerebrovascular disease: A randomised trial. *Lancet* **359,** 1283–1290.

Erkinjuntti, T., Skoog, I., Lane, R., and Andrews, C. (2003). Potential long-term effects of rivastigmine on disease progression may be linked to drug effects on vascular changes in Alzheimer brains. *Int. J. Clin. Pract.* **57,** 756–760.

Erkinjuntti, T., Roman, G., and Gauthier, S. (2004). Treatment of vascular dementia—Evidence from clinical trials with cholinesterase inhibitors. *J. Neurol. Sci.* **226,** 63–66.

Etienne, P., Robitaille, Y., Wood, P., Gauthier, S., Nair, N. P., and Quirion, R. (1986). Nucleus basalis neuronal loss, neuritic plaques and choline acetyltransferase activity in advanced Alzheimer's disease. *Neuroscience* **19,** 1279–1291.

Evans, D. A., Hebert, L. E., Beckett, L. A., Scherr, P. A., Albert, M. S., Chown, M. J., Pilgrim, D. M., and Taylor, J. O. (1997). Education and other measures of socioeconomic status and risk of incident Alzheimer disease in a defined population of older persons. *Arch. Neurol.* **54,** 1399–1405.

Farlow, M., Anand, R., Messina, J., Jr., Hartman, R., and Veach, J. (2000). A 52-week study of the efficacy of rivastigmine in patients with mild to moderately severe Alzheimer's disease. *Eur. Neurol.* **44,** 236–241.

Farlow, M., Potkin, S., Koumaras, B., Veach, J., and Mirski, D. (2003). Analysis of outcome in retrieved dropout patients in a rivastigmine vs placebo, 26-week, Alzheimer disease trial. *Arch. Neurol.* **60,** 843–848.

Feldman, H., Gauthier, S., Hecker, J., Vellas, B., Subbiah, P., and Whalen, E. (2001). Donepezil MSAD Study Investigators Group. A 24-week, randomized, double-blind study of donepezil in moderate to severe Alzheimer's disease. *Neurology* **57,** 613–620.

Folstein, M. F., Folstein, S. E., and McHugh, P. R. (1975). "Mini-mental state." A practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* **12,** 189–198.

Forlenza, O. V., Spink, J. M., Dayanandan, R., Anderton, B. H., Olesen, O. F., and Lovestone, S. (2000). Muscarinic agonists reduce tau phosphorylation in non-neuronal cells via GSK-3beta inhibition and in neurons. *J. Neurol. Transm.* **107,** 1201–1212.

Frölich, L. (2002). The cholinergic pathology in Alzheimer's disease—Discrepancies between clinical experience and pathophysiological findings. *J. Neural. Transm.* **109,** 1003–1014.

Giacobini, E. (2000). Cholinesterase inhibitors: From the Calabar bean to Alzheimer therapy. In *Cholinesterases and Cholinesterase Inhibitors* (E. Giacobini, Ed.), pp. 181–226. Martin Dunits, London.

Guillozet, A. L., Smiley, J. F., Mash, D. C., and Mesulam, M. M. (1997). Butyrylcholinesterase in the life cycle of amyloid plaques. *Ann. Neurol.* **42,** 909–918.

Hall, K. S., Gao, S., Unverzagt, F. W., and Hendrie, H. C. (2000). Low education and childhood rural residence: Risk for Alzheimer's disease in African Americans. *Neurology* **54,** 95–99.

Hardy, J., and Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science* **297,** 353–356.

Haring, R., Fisher, A., Marciano, D., Pittel, Z., Kloog, Y., Zuckerman, A., Eshhar, N., and Heldman, E. (1998). Mitogen-activated protein kinase-dependent and protein kinase C-dependent pathways link the m1 muscarinic receptor to beta-amyloid precursor protein secretion. *J. Neurochem.* **71,** 2094–2103.

Harkins, S. W., Taylor, J. R., Mattay, V., and Regelson, W. (1997). Tacrine treatment in Alzheimer's disease enhances cerebral

blood flow and mental status and decreases caregiver suffering. *Ann. N. Y. Acad. Sci.* **826,** 472–474.

Honig, L. S., Tang, M. X., Albert, S., Costa, R., Luchsinger, J., Manly, J., Stern, Y., and Mayeux, R. (2003). Stroke and the risk of Alzheimer disease. *Arch. Neurol.* **60,** 1707–1712.

Ibach, B., and Haen, E. (2004). Acetylcholinesterase inhibition in Alzheimer's disease. *Curr. Pharm. Design* **10,** 231–251.

Inestrosa, N. C., Alvarez, A., Perez, C. A., Moreno, R. D., Vicente, M., Linker, C., Casanueva, O. I., Soto, C., and Garrido, J. (1996). Acetylcholinesterase accelerates assembly of amyloid-beta-peptides into Alzheimer's fibrils: Possible role of the peripheral site of the enzyme. *Neuron* **16,** 881–891.

Karp, A., Kareholt, I., Qiu, C., Bellander, T., Winblad, B., and Fratiglioni, L. (2004). Relation of education and occupation-based socioeconomic status to incident Alzheimer's disease. *Am. J. Epidemiol.* **159,** 175–183.

Knapp, M. J., Knopman, D. S., Solomon, P. R., Pendlebury, W. W., Davis, C. S., and Gracon, S. I. (1994). A 30-week randomized controlled trial of high-dose tacrine in patients with Alzheimer's disease. The Tacrine Study Group. *J. Am. Med. Assoc.* **271,** 985–991.

Knopman, D., Schneider, L., Davis, K., Talwalker, S., Smith, F., Hoover, T., and Gracon, S. (1996). Long-term tacrine (Cognex) treatment: Effects on nursing home placement and mortality. Tacrine Study Group. *Neurology* **47,** 166–177.

Kosasa, T., Kuriya, Y., Matsui, K., and Yamanishi, Y. (2000). Inhibitory effect of orally administered donepezil hydrochloride (E2020), a novel treatment for Alzheimer's disease, on cholinesterase activity in rats. *Eur. J. Pharmacol.* **389,** 173–179.

Kramer-Ginsberg, E., Mohs, R. C., Aryan, M., Lobel, D., Silverman, J., Davidson, M., and Davis K. L. (1988). Clinical predictors of course for Alzheimer patients in a longitudinal study: A preliminary report. *Psychopharmacol. Bull.* **24,** 458–462.

Kumar, V., Anand, R., Messina, J., Hartman, R., and Veach, J. (2000). An efficacy and safety analysis of Exelon in Alzheimer's disease patients with concurrent vascular risk factors. *Eur. J. Neurol.* **7,** 159–169.

Lahiri, D. K., Sambamurti, K., and Bennett, D. A. (2004). Apolipoprotein gene and its interaction with the environmentally driven risk factors: Molecular, genetic and epidemiological studies of Alzheimer's disease. *Neurobiol. Aging* **25,** 651–660.

Lane, R. M., Kivipelto, M., and Greig, N. H. (2004). Acetylcholinesterase and its inhibition in Alzheimer disease. *Clin. Neuropharmacol.* **27,** 141–149.

Langa, K. M., Foster, N. L., and Larson, E. B. (2004). Mixed dementia: Emerging concepts and therapeutic implications. *J. Am. Med. Assoc.* **292,** 2901–2908.

Leifer, B. P. (2003). Early diagnosis of Alzheimer's disease: Clinical and economic benefits. *J. Am. Geriatr. Soc.* **51,** S281–S288.

Liao, L., Cheng, D., Wang, J., Duong, D. M., Losik, T. G., Gearing, M., Rees, H. D., Lah, J. J., Levey, A. I., and Peng, J. (2004). Proteomic characterization of postmortem amyloid plaques isolated by laser capture microdissection. *J. Biol. Chem.* **279,** 37061–37068.

Lilienfeld, S., and Parys, W. (2000). Galantamine: Additional benefits to patients with Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.* **11**(Suppl. 1), 19–27.

Lojkowska, W., Ryglewicz, D., Jedrzejczak, T., Minc, S., Jakubowska, T., Jarosz, H., and Bochynska, A. (2003). The effect of cholinesterase inhibitors on the regional blood flow in patients with Alzheimer's disease and vascular dementia. *J. Neurol. Sci.* **216,** 119–126.

Lyketsos, C. G., Breitner, J. C., and Rabins, P. V. (2001). An evidence-based proposal for the classification of neuropsychiatric disturbance in Alzheimer's disease. *Int. J. Geriatr. Psychiatr.* **16,** 1037–1042.

Marjaux, E., Hartmann, D., and De Strooper, B. (2004). Presenilins in memory, Alzheimer's disease, and therapy. *Neuron* **42,** 189–192.

Minthon, L., Gustafson, L., Dalfelt, G., Hagberg, B., Nilsson, K., Risberg, J., Rosen, I., Seiving, B., and Wendt, P. E. (1993). Oral tetrahydroaminoacridine treatment of Alzheimer's disease evaluated clinically and by regional cerebral blood flow and EEG. *Dementia* **4,** 32–42.

Moritz, D. J., and Petitti, D. B. (1993). Association of education with reported age of onset and severity of Alzheimer's disease at presentation: Implications for the use of clinical samples. *Am. J. Epidemiol.* **137,** 456–462.

Munoz, D. G., and Feldman, H. (2000). Causes of Alzheimer's disease. *Can. Med. Assoc. J.* **162,** 65–72.

Muramoto, O., Sugishita, M., Sugita, H., and Toyokura, Y. (1979). Effect of physostigmine on constructional and memory tasks in Alzheimer's disease. *Arch. Neurol.* **36,** 501–503.

Murphy, M. F., Sramek, J. J., Kurtz, M. M., *et al.* (1998). *Optimizing the Development of the Next Generation of Compounds for Alzheimer's Disease.* Greenwich Medical Media, London.

Perry, E. K., Gibson, P. H., Blessed, G., Perry, R. H., and Tomlinson, B. E. (1977). Neurotransmitter enzyme abnormalities in senile dementia. Choline acetyltransferase and glutamic acid decarboxylase activities in necropsy brain tissue. *J. Neurol. Sci.* **34,** 247–265.

Perry, E. K., Tomlinson, B. E., Blessed, G., Bergmann, K., Gibson, P. H., and Perry, R. H. (1978). Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *Br. Med. J.* **2,** 1457–1459.

Peters, B. H., and Levin, H. S. (1979). Effects of physostigmine and lecithin on memory in Alzheimer disease. *Ann. Neurol.* **6,** 219–221.

Petersen, R. C. (2000). Mild cognitive impairment: Transition between aging and Alzheimer's disease. *Neurologia* **15,** 93–101.

Petersen, R. C., Doody, R., Kurz, A., Mohs, R. C., Morris, J. C., Rabins, P. V., Ritchie, K., Rossor, M., Thal, L., and Winblad, B. (2001). Current concepts in mild cognitive impairment. *Arch. Neurol.* **58,** 1985–1992.

Piazzi, L., Rampa, A., Bisi, A., Gobbi, S., Belluti, F., Cavalli, A., Bartolini, M., Andrisano, V., Valenti, P., and Recanatini, M. (2003). 3-(4-[[Benzyl(methyl)amino]methyl] phenyl)-6,7-dimethoxy-2H-2-chromenone (AP2238) inhibits both acetylcholinesterase and acetylcholinesterase-induced beta-amyloid aggregation: A dual function lead for Alzheimer's disease therapy. *J. Med. Chem.* **46,** 2279–2282.

Poliasky, R.J. (1998). Clinical pharmacology of rivastigmine: A new generation acetylcholinesterase inhibitor for the treatment of Alzheimer's disease. *Clin. Ther.* **20,** 634–647.

Qiu, C., Backman, L., Winblad, B., Aguero-Torres, H., and Fratiglioni, L. (2001). The influence of education on clinically