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PESTICIDES AND NEUROLOGICAL DISEASES

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PREFACE

To capture the interest of a reader and to encourage him/her to delve beyond the first few pages, it is, in my opinion, essential to explain why a book is written. In 1974, one of us (D.J.E.) was involved in the treatment and monitoring of a patient accidentally exposed to an unknown but rather substantial amount of an organophosphorus insecticide. The duration of the subtle central and neuromuscular effects of this particular agent was a suprise, "toxicity" being observed long after the patient was considered to have recovered on the basis of normal values for erythrocytic and plasma cholinesterase analysis. Psychiatric sequelae were observed for at least three months after the so-called "recovery". Episodes of fatigue and muscular weakness persisted for several months, becoming less frequent and latterly occurring only after excess exertion. Continual observation of the patient along with discussions with her physicians were never conclusive but did suggest that something was still amiss. Being intrigued that such effects could occur following exposure to a widely used, seemingly "safe" insecticide, a search of the literature was begun only to find that, in general, such signs were either unobserved, overlooked, or dismissed as having no relationship to the poisoning. In many cases, once the inhibited enzymatic activities approached normal values, observation of the patients ceased and no extended follow-up of the individuals' well being occurred. There was, however, sufficient published literature mentioning long-term physical and behavioral effects to whet the curiosity.

An examination of the literature revealed that such signs and symptoms were not restricted to acute and chronic poisoning by organophosphorus agents but were observed following exposure to chlorinated hydrocarbon insecticides, carbamate esters, fungicides, etc. This book, then, is our attempt to bring together the literature dealing with the covert toxicity of pesticides, particularly that involving the nervous systems. The aim of the monograph is to provide the reader with a relatively complete, critical, yet readable survey of what is known. We have considered animal toxicity studies in relation to human poisonings as a means of determining mechanisms of action of the various agents. We have attempted to sift good evidence from bad or inconclusive data and hope that it is clear to the reader when we are stating facts, conjecture and/or opinion.

AUTHORS

Donald Ecobichon received his undergraduate training in Pharmacy at the University of Toronto, obtaining his B. Sc. in 1960 and proceeding directly to graduate studies in Pharmacology at Toronto where he worked under Dr. Werner Kalow, using organophosphorus esters to characterize and identify electrophoretically separated human tissue esterases thereby beginning a long association with pesticides. Following his doctoral thesis in 1964 and a year as a postdoctoral fellow in Ottawa at the National Research Council of Canada, he joined the faculty of the Ontario Veterinary College, University of Guelph, and initiated studies on mechanisms of action of chlorinated hydrocarbon insecticides, then current problems in the field of veterinary medicine. In 1969, he joined the Faculty of Medicine, Dalhousie University, Halifax, Canada and, with his graduate students, initiated a number of toxicological studies involving the hepatotoxicity and structure-activity relationships of polychlorinated and polybrominated biphenyls, the biotransformation of organophosphorus ester insecticides, and the transplacental and milk transfer of chemicals in pregnant and lactating animals. In 1977, he moved to the Faculty of Medicine, McGill University, Montreal, Canada where he has continued research on the acquisition of chemicals by perinatal animals transplacentally and via milk and has become deeply involved in toxicological aspects associated with the aerial spraying of insecticides in eastern Canadian forests for spruce budworm abatement.

Robert M. Joy received his B.S. degree in Pharmacy from Oregon State University and his Ph.D. degree in Pharmacology from Stanford University. After postdoctoral training in Neuropharmacology at the University of California, Davis, Dr. Joy joined the faculty in the School of Veterinary Medicine in 1970. He is presently the co-director of the Health Sciences Neurotoxicity Unit, Associate Professor of Pharmacology and Toxicology, School of Veterinary Medicine, and Associate Professor of Pharmacology, School of Medicine at that Institution.

Dr. Joy has been active in research into mechanisms of neurotoxicity, particularly for the chlorinated hydrocarbon insecticides. He has been the recipient of numerous grants for research relating to DDT, dieldrin, and related compounds. He has authored over 30 papers in this area.

Dr. Joy remains active in the area of research and education in neuropharmacology and neurotoxicology. He is a member of many scientific societies, including the American Society of Pharmacology and Experimental Therapeutics, the Society of Toxicology, and the Society for Neurosciences. Dr. Joy is also a Diplomate of the American Board of Toxicology.

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INTRODUCTION

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I. INTRODUCTION

The agricultural chemicals commonly labeled as pesticides are perhaps the largest group of poisonous substances being disseminated throughout our environment. The term "pesticide" denotes any agent intended for preventing, destroying, repelling, or mitigating any pest and this classification may be subdivided into groups such as insecticides, acaricides, nematocides, herbicides, avicides, rodenticides, molluscicides, etc. depending upon the species of pest. Historically, pesticides were developed by the age-old empirical method of trial-and-error screening of innumerable inorganic and organic chemicals which might selectively kill a species of pest. In the last 50 years, there has been a change in the approach to pesticide development, considerable attention being focussed on the physical and chemical properties of the agent and, following experimentation, selection of the most potent analogs of a particular chemical structure for further development. From such an approach, we have achieved chemical mastery over a wide range of pests, but only at a cost which even now has not been fully appreciated. It would be reasonable to state that neither could we have achieved nor can we maintain the standard of living which we enjoy today without the use of these chemicals. There is no question of stopping the annual application of these agents on our gardens and crops, our forests and fields, or on our animals and ourselves. The pesticide dilemma is not whether to use or not use — but the choice of agent, when it must be used, and how much should be applied. As has been stated recently, there is little evidence that we have really developed a philosophy on pesticides which will ensure the kind of discretionary use that the nature of these materials requires.[1]

Grain treated with organomercurial or chlorinated hydrocarbon fungicides has been responsible for some spectacular poisonings of epidemic-sized proportions. In 1956, 1960, and again in 1971 to 1972, ethylmercuric and methylmercuric chloride-treated seed grain was consumed by rural people in Iraq, resulting in severe poisoning. In the last and most serious episode, the government of Iraq acknowledged that some 6530 individuals were hospitalized and 459 died.[2,3] An outbreak of poisoning of 100 people occurred in West Pakistan in 1961 following the ingestion of seed wheat with a mixture of phenylmercuric acetate and ethylmercuric chloride.[4] The ingestion of hexachlorobenzene, a chlorinated aromatic hydrocarbon fungicide, in treated grain was responsible for the induction of several thousand cases of acquired toxic cutaneous porphyria in southeastern Turkey between 1955 and 1959.[5,6] Large scale poisonings have been caused by the insecticides DDT,[7] endrin,[8,9] parathion,[10] and malathion.[11] Parathion was also implicated as the causative agent associated with the poisoning of orchard workers in California.[12] More recently, Kepone® (1,1a,3,3a,4,4a,5b,6-decachloro-octahydro 1,3,4-methano-2H-cyclobuta [c,d] pentalen-2-one) has been implicated in the serious poisoning of chemical workers involved in its manufacture.[13]

These are only a few of the many incidents in which agricultural chemicals have been involved in widespread poisonings, but they serve to at least highlight the toxicological problems arising from these agents. It should be noted that many of these incidents were a consequence of abuse, misuse, or ignorance of the chemicals involved. The U.S. Environmental Protection Agency has estimated that each year in the U.S., some 45,000 individuals are poisoned by pesticides, some 3000 serious cases which

require hospitalization, and an estimated 200 deaths occur. The fatalities primarily occur among people handling pesticides: farmers, crop-dusters, and factory workers involved in the manufacture of these chemicals.[14]

II. HISTORICAL DEVELOPMENT

As long as man has been combating pests ravaging his crops, he has been interested in anything which would selectively eliminate them. In early times, sulfur was used as a fumigant and insecticide. Inorganic salts including copper sulfate, lime sulfur (mixture of sulfur and lime), copper arsenite (Paris Green), lead arsenate, and copper sulfate mixed with lime (Bordeaux Mixture) all were found to be useful insecticides and fungicides. Extracts from tobacco leaves which contained nicotine were used as the first naturally occurring insecticides. A brief visit to any "garden" shop will confirm that many of these old preparations are still in use today. In the mid-1800s, two economically important and effective natural insecticides were introduced. Derris, the most active component of which is the structurally complex compound, rotenone (Figure 1, Structure I), was isolated from the powdered roots of *Derris ellipticus* (Malaya and East Indies), and from a species of South American plant, *Lonchocarpus*. When diluted with an adsorbant clay and dusted on plants, cattle, and sheep, the rotenoids have proven to be useful insecticides for exoparasites as well as being environmentally safe since they are readily degraded by sunlight and air to inactive oxidized products. The second natural insecticide is pyrethrum, obtained from the flowers of *Chrysanthemum cinerarinefolium* and grown commercially in several regions of the world (Kenya, Ecuador, New Guinea, Iran, Japan). The complex mixtures of esters collectively known as pyrethrins, the structure of pyrethrin I (Figure 1, Structure II) being one example, are extracted with ethylene dichloride or kerosene from dried and ground chrysanthemum flowers. While a major disadvantage of pyrethrum is a lack of persistence associated with instability in light and air, it has been and still is used successfully, alone or in combination with other insecticides or synergists, in aerosolized dispensers for the control of insects in the home. In contrast to the rotenoids which were uneconomical to synthesize, considerable success has been achieved recently in the synthesis of pyrethroid esters with subsequent modification of the chemical structure and improvement of insecticidal potency.[15]

A. Chlorinated Hydrocarbon Insecticides

The first chlorinated hydrocarbon insecticide, hexachlorocyclohexane (HCH) (Figure 1, Structure III), incorrectly called benzene hexachloride (BHC), was synthesized by Michael Faraday in 1825, though the significant insecticidal properties were not recognized until over 100 years later. Prepared by the chlorination of benzene, the product loses the aromatic characteristics of the benzene ring. While its chemical properties were studied extensively because of the interesting isomeric forms (α,β,γ,δ, and ε isomers) depending upon the positions of the chlorines and the "chair" (*trans*) and "boat" (*cis*) forms of the cyclohexane ring, it was not until World War I (1914 to 1918) that the insecticidal properties were considered. While it was never used at that time, related compounds such dichlorobenzene were used as fumigants.[16] While protective patents were issued in the 1930s, no real attempt was made to develop HCH until World War II when sources of commonly used derris and pyrethrum were shut off. HCH was rediscovered by both France and England between 1940 and 1942. The gamma isomer, γ-HCH (γ—1,2,3,4,5,6—hexachlorocyclohexane), commonly known as lindane, was recognized as a highly potent insecticide and was marketed in France by Solvay et Cie as "Isogam" and in England by Imperial Chemical Industries Ltd. as "Gammexane®".[17] An interesting narrative on the development and testing is pre-

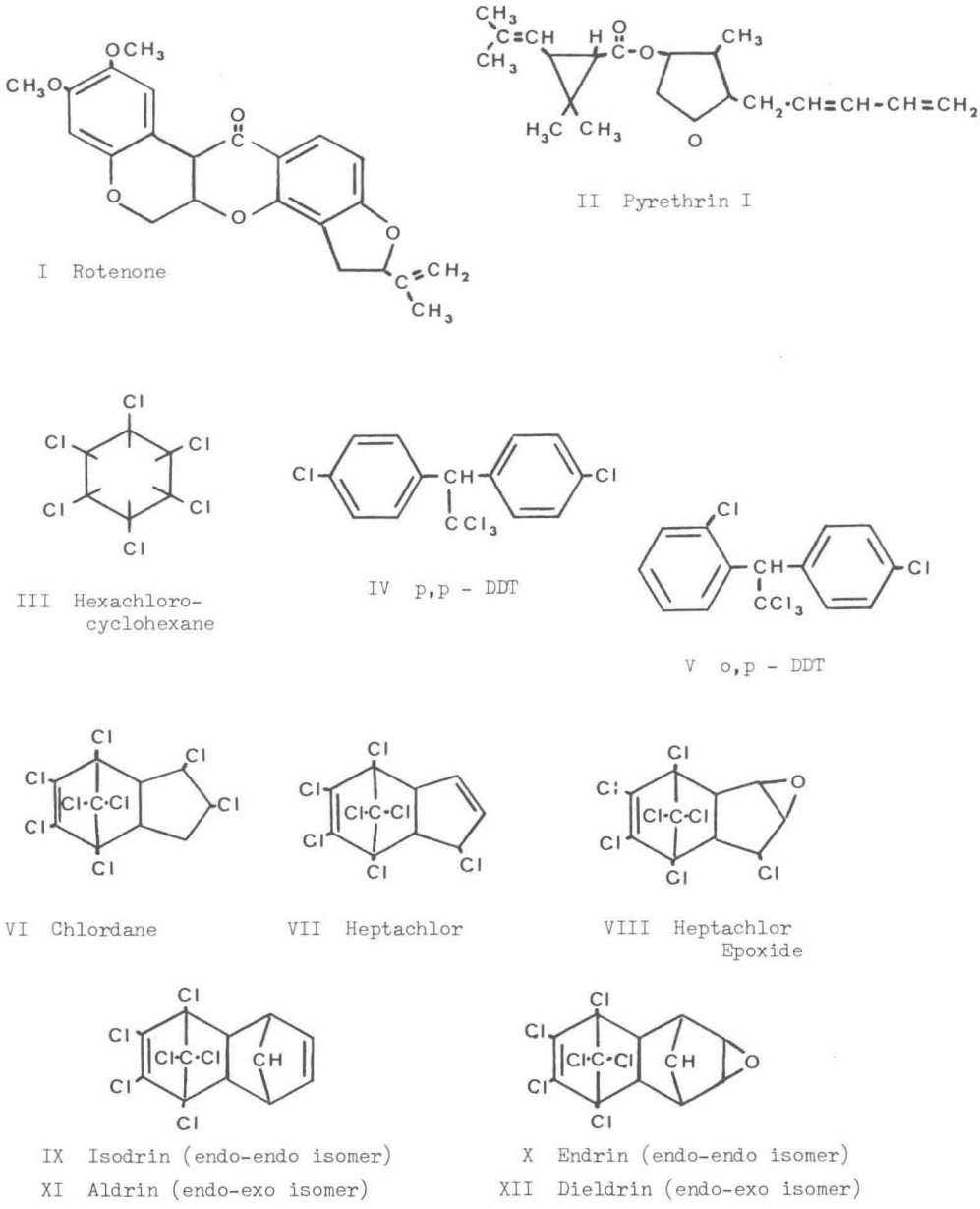


FIGURE 1. Structures of commonly-used "natural" and chlorinated hydrocarbon insecticides.

sented by Brooks.[17] This chemical has been used extensively in controlling insects of importance to public health, having greater acute toxicity toward a wide variety of insects than DDT while having comparable acute toxicity to that of DDT in most mammalian species.

Dichlorodiphenyltrichloroethane or DDT (o,p′- and p,p′-isomers, Figure 1, Structures IV and V) was first synthesized in 1874 by Ziedler but the insecticidal potency was not appreciated until 1939 when Dr. Paul H. Muller of the Geigy Company of Switzerland began experimenting with this molecule and some analogs.[18] His discoveries earned him a Nobel Prize in Medicine in 1948 and his work ushered in a new era

in pesticide chemistry, one in which attention was drawn to the chemical structure of the agent and to the influence which substituent groups had on lipid solubility, chemical stability, and insecticidal potency of the molecule. The story of the development of this chemical is well known but, for the uninitiated, post-DDT generation, bears repeating in part.

The search which led to the discovery of DDT began in 1932 when P. Lauger of the Geigy Company, searching for new moth-proofing agents, was experimenting with triphenylmethane dyes. The synthesis of some chlorinated benzene carbinols led P. H. Muller to synthesize 1,1,1-trichloro-2,2-(p-chlorophenyl) ethane (p,p'-DDT) and a number of analogs, testing their potency on a number of insect species. While his first paper on DDT was published in 1946, it is obvious from various records that Dr. Muller knew that he had discovered an important insecticide since patent applications were made in Switzerland in March 1940. The agent, marketed as Gesarol®, Gesaron®, and Neocid®, was tested against houseflies, cockroaches, mosquitoes and agricultural pests (potato beetle, European corn borer, alfalfa weevils, codling moth, fruit moth, etc.). Field trials for the control of typhus carried by body lice were conducted in the Balkans in 1942 in collaboration with the international Red Cross. The toxicological assessment was well in hand when news of DDT came to the attention of the Allied governments in 1942, then faced with the problems of maintaining the health of military personnel in regions where insect-carried diseases were endemic. By mid-1943, supplies of DDT were available to soldiers but the first extensive test of its effectiveness came in Naples in December 1943 where typhus threatened to become epidemic among the civilian population. Some 1,300,000 people were "dusted" at two "delousing" stations during January 1944 and medical history was made since this was the first time that a typhus epidemic was halted in mid-winter before it even started. The negligible human toxicity observed in individuals exposed to what now seem to be massive amounts of this agent greatly aided in heralding DDT as the boon of mankind. As can be observed from relevant production data, the world-wide use of this agent in the post-war years expanded apace until the mid 1960s when concerns about its environmental impact began to be expressed.[17]

With all of the fanfare which accompanied the introduction of DDT into widespread agricultural and human health use, the cyclodiene-type chlorinated hydrocarbons emerged on the scene with little attention being paid to them. This unique group of highly stable insecticides is prepared from hexachlorocyclopentadiene with cyclopentadiene by the Diels-Alder reaction giving rise to chlordene which, after further chlorination, gives rise to the potent insecticides chlordane and heptachlor (Figure 1, Structures VI and VII). The insecticidal properties of these chemicals were first described in 1945.[19] Chlordane, in particular, was a chemist's delight since it existed in two stereoisomeric, *endo* and *exo* forms and could have the chlorines on the five-membered ring on the same side or on the opposite side, giving use to *cis* and *trans* isomers, respectively. Technical chlordane, a complex mixture of compounds including heptachlor and hexachlor, was a potent insecticide. This led to the isolation and characterization of heptachlor, improvement upon the synthesis procedure to provide greater yields of this agent which was introduced for agricultural use in 1948 by the Velsicol Corporation. Heptachlor is converted to an epoxide, heptachlor epoxide (Figure 1, Structure VIII) in vivo and is stored in that form. The epoxide is much more potent than the parent compound.[20]

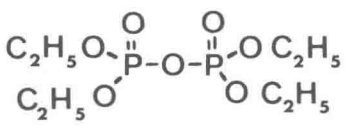
The most important cyclodiene insecticides are those containing four fused five-membered rings prepared by the Diels-Alder reaction between hexachlorocyclopentadiene with vinyl chloride with a subsequent reaction with cyclopentadiene to produce isodrin (Figure 1, Structure IX). When isodrin is peroxidated chemically or via metabolic pathways found in insects and various animals, endrin (Figure 1, Structure X), a commonly used and highly potent insecticide, is the product. The synthesis of these two chemicals and the ease with which the Diels-Alder reaction could be manipulated led to the synthesis of aldrin (Figure 1, Structure XI) (adduct of vinyl chloride with cyclopentadiene which is subsequently dehydrochlorinated and subjected to a second Diels-Alder reaction with hexachlorocyclopentadiene). The epoxide of aldrin, dieldrin (Figure 1, Structure XII), may be produced by chemical reaction in vitro or via meta-

bolic pathways in vivo. Dieldrin and aldrin, named after Diels and Alder who discovered the process of diene synthesis, are the best known of the cyclodiene insecticides, being chemically stable, highly lipophilic, environmentally persistent agents which are excellent contact insecticides but possess very little, systemic action. They were introduced into the market in little over 5 to 7 years and, in spite of their wide application, little had been written about their chemistry or mode of action by 1955.[21]

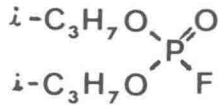
Soloway stated that, to be biologically active, cyclodienes must contain two correctly separated electronegative centers which might associate with the biological site of action.[22] The molecular topogaphy of the agents was also important and, in spite of their appearance when drawn as flat structures, they do have similar shapes. The marked toxicity of the epoxides appears to be due to the orientation of the oxygen to the rings (dieldrin and heptachlor epoxide). Tragically, there are no better insecticides than the entire group of chlorinated hydrocarbons but their downfall was due primarily to the properties of (1) physical and chemical stability, (2) low rate of biodegradability, (3) lipophilicity, (4) persistence, and (5) bioaccumulation which gave them such distinct advantages as insecticides.[23]

B. Organophosphorus Esters

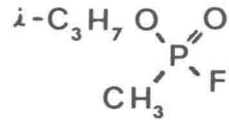
The story of organophosphorus esters began in the early 1800s when Lassaigne prepared these compounds by reacting alcohol and phosphoric acid.[24] Moschinin is said to have first synthesized tetraethylpyrophosphate (TEPP, Figure 2, Structure I) by heating the silver salt of pyrophosphoric acid with ethyl chloride while he was working in the laboratory of A. Wurtz. It was, however, Ph. De Clermont, in 1854, who synthesized, described, and even tasted TEPP, albeit without noting any of its toxic effects.[25] At the beginning of the 1900s, Michaelis in Rostock, Germany and Arbusov in Kazan, Russia were important figures in the field of organophosphorus chemistry, elucidating the fundamental reactions involved in the synthesis of many different derivatives. Nylen in Uppsala, Sweden reported the synthesis of TEPP by two different pathways, but was completely ignorant of the toxicity of this agent.[26] It has been noted that the yield of TEPP is quite low during synthesis unless great care is taken, and it may be this feature which (1) protected early chemists who inhaled or tasted the reaction product and (2) delayed the recognition of its toxicity for almost 80 years. In 1932, Lange in Berlin synthesized some compounds containing a phosphorus-fluorine bond (esters of monofluorophosphoric acid from silver salts and alkyl halides). During the synthesis of dimethyl- and diethylphosphorofluoridate, Lange and his graduate student, Gerda von Krueger, noted toxic effects of the vapors on themselves, the pertinent observations being included in a published chemical paper.[27] Lange was unable to convince the chemical industry, and I. G. Farbenindustrie in particular, that the alkyl esters synthesized might be useful insecticides.[26] In 1934, Gerhard Schrader was appointed by Otto Bayer to pursue the development of synthetic insecticides for I. G. Farbenindustrie, but it was not until 1936 that Schrader began working on phosphorus and sulfur acid fluorides in search of aphicidal and acaricidal compounds, initially discovering methane sulfonyl fluoride which was used as a fumigant. From 1938 to 1944, Schrader developed a series of fluorine-containing esters including DFP (di-isopropylfluorophosphate) and Sarin (1-methylethyl methylphosphonofluoridate), pyrophosphate esters including TEPP and OMPA (octamethylpyrophosphortetramide) and thio- and thionophosphorus esters including parathion (O,O-diethyl-O-[4-nitrophenyl] phosphorothioate) and its oxygen analog paraoxon (O,O-diethyl-O-(4-nitrophenyl) phosphate (Figure 2, Structures II to VI). He was aware of the toxic signs produced by these esters and, while the potency of some of these chemicals prevented their development and use as insecticides, they were of immediate interest to the German Ministry of Defense which recognized their value as chemical warfare agents. Production



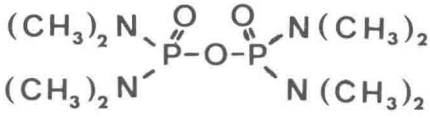
I Tetraethylpyrophosphate (TEPP)



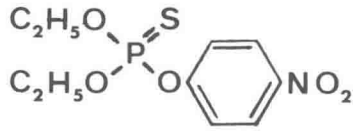
II Di-isopropyl-fluorophosphate



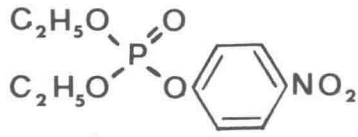
III Sarin



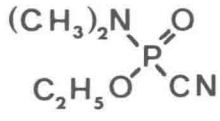
IV Octamethylpyrophosphoramide (OMPA)



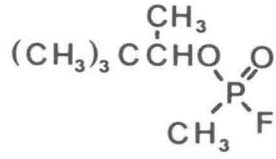
V Parathion



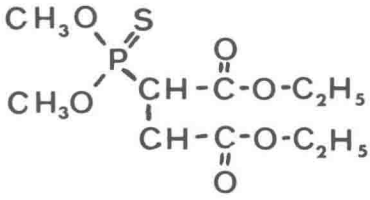
VI Paraoxon



VII Tabun

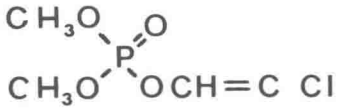


VIII Soman

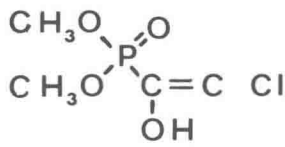


IX Malathion

X Systox (mercaptophos)



XI Dichlorvos



XII Trichlorfon

FIGURE 2. Structures of organophosphorus esters, showing the basic structure and the variations introduced to modify the physicochemical properties and the insecticidal potency.

of stocks of Tabun (ethyl-N, N-dimethyl phosphoramidocyanidate) (Figure 2, Structure VII) and Sarin was carried out in a factory outside of Dühernfurt, near Breslau. Soman (1,2,2-trimethylpropyl methylphosphonofluoridate) (Figure 2, Structure VIII), another "nerve gas", was also synthesized at this factory. The pharmacological and toxicological studies of these compounds were carried out in a number of industrial and military laboratories.[26]

British scientists had taken note of the comments of Lange and Krueger concerning the toxicity of acyl phosphorofluoridates, and during World War II, they were paying

particular attention to fluorine-containing compounds. With this lead, it is interesting to note that studies conducted by these two protagonists were almost parallel, DFP and other alkyl phosphorofluoridates being the prime test chemicals.[28-31] A similar line of investigation was being followed at Edgewood Arsenal in the U.S., again DFP being a compound of choice in such studies.[32] Scientists on both sides of the Atlantic were well aware of the potent, irreversible, anticholinesterase properties of these esters,[33,34] When the structures and properties of the German nerve gases Tabun and Soman became known, it was realized that they were more potent than DFP by an order of two of magnitude.[33,35]

With the cessation of hostilities and the exchange of information in the post-war period, the chemistry of organophosphorus insecticides developed at a rapid rate. The decade from 1950 to 1960 can well be said to have been the era of the organophosphates. Malathion [diethyl(dimethoxyphosphinothioyl)thiobutanedioate] was introduced by the American Cyanamid Company in 1950, this ester (Figure 2, Structure IX) contains carboxy ester groups. In 1951, G. Schrader continued developing new insecticides including Systox® (demeton or mercaptophos, a mixture of the thiono- and thioloisomers of O,O-diethyl-2-ethylmercaptoethyl phosphorothioate) (Figure 2, Structure X), thereby introducing a new class of insecticides having a thioether group. In 1952, the Perkow reaction was first described in which alpha-halogen carbonyl compounds were reacted with triethyl phosphite, resulting in the synthesis of a number of new dialkylvinyl phosphate esters such as dichlorvos (2,2-dichlorovinyl dimethyl phosphate) (Figure 2, Structure XI) and trichlorfon (O,O-dimethyl[2,2,2-trichloro-1-hydroxyethyl]phosphate, Figure 2, Structure XII).[36] The thio- and thionophosphorus esters arising from parathion and containing substituted aryl and heterocyclic groups have also been synthesized. Today, a wide range of organophosphorus esters having a variety of biological properties are available for such equally diversified range of uses as insecticides, nematocides, acaricides, fungicides, etc.

Before the myriad of organophosphorus ester insecticides were introduced into agricultural practice, another class of phosphoric acid esters was known to be highly toxic. These chemicals, triaryl esters of phosphoric acid (the most common being tricresyl phosphates), have been used industrially as plasticizing agents, as additives to extreme-pressure lubricants in hydraulic systems, and as lead scavengers in gasoline. The earliest reference to the toxicity of these compounds was found to have been made in 1899, when severe polyneuritis was found among patients with pulmonary tuberculosis who were being treated with phospho-creosote.[37] Prohibition in the U.S. contributed greatly to our knowledge of these esters after an estimated 20,000 individuals who had imbibed a certain brand of alcoholic extract of Jamaica ginger suffered a debilitating peripheral neuropathy commonly known as "Ginger Jake Paralysis".[38,39] As a consequence of this epidemic, the extract was found to be adulterated with cresyl phosphate esters and the potent neurotoxin was identified as being one isomer: tri-ortho-cresyl phosphate or TOCP.[40,41] Despite our awareness of the biological effects of this chemical since 1931, epidemic-proportioned outbreaks of neuropathy continued to appear sporadically over the next 40 years, the source of TOCP usually being a contaminated cooking or salid oil. The most recent outbreak occurred in Viet Nam between 1970 and 1971, when a "black market" cooking oil was identified as a TOCP-containing military aviation lubricant supplied to South Vietnamese helicopter units.[42] TOCP will be discussed at length in a later chapter.

C. Carbamic Acid Esters

The story of "trial by ordeal", a crude form of justice practiced in West Africa during which the person suspected of being guilty was required to ingest a milky slurry of ground beans from the Calabar plant *(Physostigma venenosum)*, is well known.[43]