Esterase Inhibitors as Pesticides

Because of favorable biological properties they are displacing other types of established compounds.

John E. Casida

Entomologists have relatively few types of chemical weapons for combatting the host of insect pests which continually compete with man for his food and fiber supply or directly endanger his health. Use of the chlorinated hydrocarbon insecticides such as DDT, lindane, and dieldrin has led to a realization of the great benefits that can result from efficient insect control. However, increasing concern over persisting residues which might effect slow but cumulative contamination of our environment and over the genetic selection of resistant insect strains which has somewhat restricted the areas of effectiveness of the chlorinated hydrocarbons has resulted in a serious reconsideration of alternative approaches to insect control. Tremendous progress has been made in utilizing predators, parasites, and pathogens of insects in control programs. The induction of dominant lethal mutations in insects by radiation or chemicals is also playing an ever more important role in keeping certain insects in check. Attractants, repellents, and other means to stimulate insects to approach or avoid a specific area are getting increased attention. Inorganic insecticides and insect toxicants of botanical origin are widely used, and intensive research is still under way on synthetic pyrethroids. Many other new types of synthetic organic insecticides have been found, but only the organophosphates and carbamates have distinct advantages over the chlorinated hydrocarbons in many areas of insect control.

Organophosphate insecticides, like the chlorinated hydrocarbons, have now been in use for about 25 years, and carbamates for half that period. The balance of usage has slowly been shifting in the direction of the organophosphates and carbamates, and if this trend continues these compounds will soon be the major chemical weapons for insect control. The organophosphates and carbamates are frequently referred to as the "anticholinesterase insecticides" since they inhibit acetylcholinesterase and hence lead to disruption of nerve function. Hundreds of millions of pounds of esterase inhibitors are used as pesticides every year, and the use includes about 120 different active ingredients in thousands of formulations. A brief status report on problems and prospects related to "esterase inhibitors as pesticides" appears timely. More complete considerations can be found in recent reviews and books cited (*1*).

Recognition of Biological Activity

Calabar beans, the seeds from the vine *Physostigma venenosum* Balfour, were used for centuries as ordeal poisons in West African witchcraft trials. If guilty, the accused person presumably ate the bean slowly, and typical symptoms developed. The innocent person, unafraid of the trial, supposedly ate the poison rapidly, and vomiting from gastric irritation afforded automatic protection. The active ingredient, physostigmine or eserine, was isolated 100 years ago and in 1925 was identified as the *N*-methylcarbamate of eseroline. Its miotic activity and antagonism by atropine were recognized as early as 1863, and its mode of action as a cholinesterase inhibitor, resulting in accumulation of acetylcholine, was found by Loewi and Navratil in 1926. Many synthetic analogs are used in clinical medicine as parasympathomimetic agents. The first carbamates active as contact insecticides were fluoro compounds prepared by Schrader in 1936–1944, but it was not until the 1947–1952 studies of Gysin that *N,N*-dimethylcarbamates were developed for practical use as insecticides. Insecticidal *N*-methylcarbamates were prepared by Kolbezen, Metcalf, and Fukuto and by Union Carbide Corporation starting in 1953, the latter group reporting the potency of the important 1-naphthyl ester in 1957. Thousands of new methylcarbamates have been tested as potential insecticides in the last 10 years.

Lange and Krueger, working in Berlin, noted in a 1932 paper dealing with synthesis of dialkylphosphorofluoridates certain toxic effects of the vapors on themselves. Biological and chemical studies on organophosphate toxicants during the following decade were largely restricted to German laboratories. Studies by Schrader (I. G. Farbenindustrie) progressed rapidly from their initiation in 1936, resulting in the use of several organophosphate insecticides in Germany during World War II. Tetraethyl pyrophosphate, originally prepared and noted to have a "burning taste" by de Clermont in 1854, was recognized as an extremely potent insecticide by Schrader in 1938 and as a cholinesterase inhibitor by Gross in 1939. German scientists during this period also noted the parasympathomimetic effects and found that atropine is an antidote for the organophosphates. Schrader prepared around 2000 organophosphates by the end of the war. Tens of thousands have been subsequently prepared and tested for pesticidal activity.

The action of cholinesterase inhibitors on man was quite well understood prior to use of agents of this type in pest control. The pharmacological action of physostigmine had been investigated for almost a century, and of synthetic analogs for 25 years before carbamate insecticides were first employed. Emphasis in the development of organophosphates as insecticides was placed on finding compounds of high insecticidal activity and low mammalian toxicity. Other laboratories evaluated related compounds as potential chemical warfare agents. A wealth of

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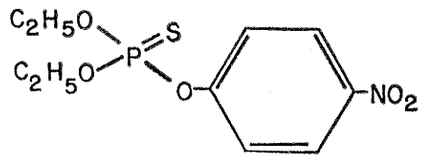
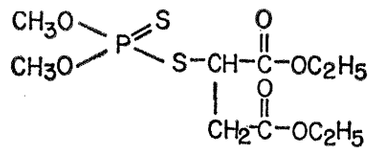
background information on the action of the "organophosphorus nerve poisons" on man resulted from extensive studies by many agencies in several countries during and after World War II. The mode of action of cholinesterase inhibitors is more clearly understood than that of any other type of pesticide or even of any other type of pharmacologically active agent.

Structure-Activity Relationships

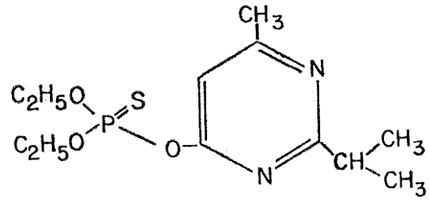
Schrader proposed a type formula for insecticidal organophosphates in 1937, and his structural model has held for over a quarter of a century without major modification. Active compounds are almost entirely of the type R_1R_2P(O or S)-acyl. A pentavalent phosphorus with sulfur or oxygen directly bonded is required; R_1 and R_2 may be alkoxy, alkyl, aryloxy, aryl, or amino groups; the acyl group can be extremely varied—for example, fluoride, cyanide, pyrophosphate, substituted-phenoxy, thio alcohols, aliphatic and heterocyclic enols, and others. The early organophosphate insecticides were mostly phosphate and phosphorothionate esters [(alkoxyl)$_2$P(O or S)-acyl], but recently increased attention has been given to testing phosphonates and phosphonothioates [(alkoxy)(alkyl)-P(O or S)-acyl] and phosphinates and phosphinothioates [(alkyl)$_2$P(O or S)-acyl]. Slight structural changes greatly alter the spectrum of biological activity. The groupings about the phosphorus atom are chosen on the basis of the selective toxicity conferred on the overall molecule and the stability or residual persistence of the compound on or in the organism or other substrate. Their reactivity is determined by the magnitude of electrophilic character of the phosphorus atom, the strength of the P-acyl bond, and the steric effects of the substituents. Most, but not all, of the insecticides are thionate, =P(S)—, compounds which are more resistant to hydrolysis and usually less toxic to mammals than their oxygen, =P(O)—, analogs. A few of the more important contact insecticides are *O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate (parathion), its methyl homolog (methyl parathion), and *O,O*-dimethyl *S*-[1,2-bis(ethoxycarbonyl)ethyl] phosphorodithioate (malathion). Some of the other contact organophosphates are (*2*) carbophenothion, Chlorthion, Ciodrin, diazinon, dicapthon, dichlorvos, dioxathion, endothion, EPN, ethion, Ethyl Guthion, fenthion, Guthion, Methyl Trithion, naled, sulfotepp, Sumithion, tepp, and trichlorfon.



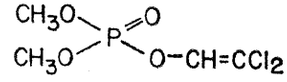
Parathion



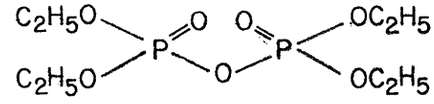
Malathion



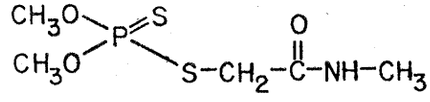
Diazinon



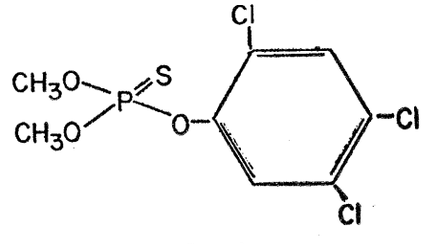
Dichlorvos



Tepp



Dimethoate



Ronnel

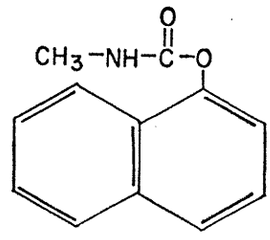
Schrader and Kükenthal noted in 1941 that certain organophosphates were absorbed and translocated by plants in concentrations sufficient to kill insects feeding distant from the point of application. A few years earlier they had noted similar properties for the methylal of β-fluoroethoxy-β'-hydroxyethyl ether. Among the current plant systemics are the mixture of *O,O*-diethyl *S*(or *O*)-2-(ethylthio)ethyl phosphorothioates (demeton), *O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate (dimethoate), Bidrin, Di-Syston, menazon, Meta-Systox-R, methyl demeton, mevinphos, phorate, and phosphamidon. Some of these may be coated on seeds, applied to soil, or sprayed on foliage to act as long-lasting plant chemotherapeutic agents. As compounds of greater selective toxicity became available they were tested for control of endo- and ectoparasites of mammals. By 1954 it was recognized that organophosphates were active systemically for control of larvae of the cattle grub, *Hypoderma lineatum* (de Villers). Ronnel, *O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate, was established in 1955 as the first practical animal systemic by investigators of the Dow Chemical Company and by Bushland, Eddy, Lindquist, and co-workers in the U.S. Department of Agriculture. Among the other animal systemics subsequently developed are coumaphos and Ruelene. These compounds can be fed to or sprayed on the animal to yield sufficient absorbed insecticide to serve as a chemotherapeutic agent for arthropod pests.

The use of organophosphates as agricultural chemicals is not restricted to insecticides and acaricides. Some acaricidal compounds, such as dioxathion, also have fungicidal activity. Selected examples of other uses are as follows: *O,O*-dimethyl (2,2,2-trichloro-1-hydroxyethyl)phosphonate (Neguvon) and *O,O*-di-(2-chloroethyl) *O*-(3-chloro-4-methylcoumarin-7-yl) phosphate (Haloxon) as anthelmintics; *O,O*-diethyl *O*-(2,4-dichlorophenyl) phosphorothioate (Nemacide), *O,O*-diethyl *O*-(2-pyrazinyl) phosphorothioate (Zinophos), and phenyl *N,N'*-dimethylphosphorodiamidate (Nellite) as nemato-

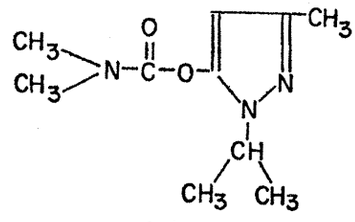
cides; *S,S,S*-tributyl phosphorotrithioate (DEF) and its phosphorotrithioite analog (Merphos) as cotton defoliants; and *O*-methyl *O*-(2,4-dichlorophenyl) isopropylphosphoramidothioate (Zytron) as a crabgrass control agent.

The insecticidal carbamates are mostly of the type formula (CH_3) (R_1) NC (O)OR_2, where R_1 is methyl or hydrogen and R_2 is a substituted phenyl or heterocyclic enol radical. Compounds of this type which contain groupings that are fully ionized at physiological *p*H are almost inactive as insecticides even if they are extremely potent as cholinesterase inhibitors. Such compounds have gained considerable importance in clinical medicine. Examples of highly insecticidal *N*-methylcarbamates are 1-naphthyl methylcarbamate (carbaryl) and the substituted phenyl analogs (*2*) Zectran, Banol, Baygon, and others. Potent *N,N*-dimethylcarbamate insecticides are 1-isopropyl-3-methyl-5-pyrazolyl dimethylcarbamate (Isolan) and the related compounds dimetan, dimetilan, Pyramat, and Pyrolan. Isolan and some of the other carbamates show systemic activity in plants.

A few pesticidal phosphates and carbamates do not conform to these type formulas. Programs for industrial synthesis of new pesticides are obviously seeking such exceptions. Several other types of esterase inhibitors are known, but the compounds reported are much less potent as insecticides than the organophosphates and carbamates.



Carbaryl



Isolan

Mode of Action or Inaction on Insects

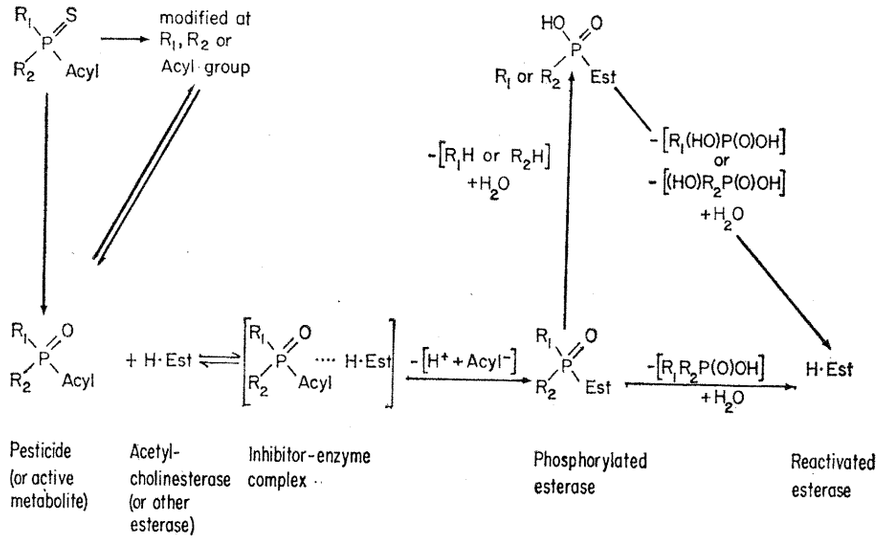
Organophosphates and carbamates are used primarily as contact insecticides and acaricides. Some, however, are more potent on ingestion or as fumigants. Lethal doses for susceptible insects are usually in the range of 0.2 to 20 μg of insecticide per gram of insect regardless of the site of entry. The usual insecticidal dosage on field crops ranges from 0.2 to 2 pounds per acre. Phosphates were first used as substitutes for the botanical insecticides nicotine and pyrethrum. They are now used for almost all types of insect and mite control problems. Symptoms elicited in a poisoned insect include tremors, hyperactivity, convulsions, paralysis, and death. Recovery from early symptoms of poisoning is more frequent with the carbamate than with the phosphate insecticides.

Insecticidal action of organophosphates and carbamates appears to result primarily from inhibition of the acetylcholinesterase in the central nervous system. The carbamates may also have a direct effect at the cholinergic receptor sites. Insects lack cholinergic systems for synaptic transmission at neuromuscular junctions, and the cholinergic synapses of their central nervous system are more highly protected from penetration of ionized materials than those of mammals. Accordingly, ionized carbamates and phosphates are usually noninsecticidal even when injected through the cuticle, and the antidotes effective in mammals are ineffective with insects. Acetylcholine and choline acetylase have been demonstrated in many insect and mite species. Other cholinesters may also be present. Considerable variation occurs in the insect cholinesterases in their specificity for reaction with cholinesters, organophosphates, and carbamates. This selectivity probably contributes to the species specificity in poisoning. Nerve cholinesterases from the house fly, honey bee, and German cockroach have been highly purified. Insect aliesterases and certain other insect esterases are also susceptible to inhibition by these compounds, but the physiological significance of inhibition of these esterases has not been established. Dozens of esterases sensitive to inhibition have been demonstrated in insects, but acetylcholinesterase inhibition appears to be the primary biochemical lesion in poisoning.

Phosphorothionate insecticides generally must be metabolized to the oxygen analogs prior to cholinesterase inhibition in vivo. Other sites of oxidative attack that may be necessary to yield the actual in vivo cholinesterase inhibitor include the oxidation of thioethers to sulfoxides and sulfones, and of the *N,N*-dialkylamino groups, eventually leading to *N*-dealkylation. A variety of insect esterases hydrolyze phosphate and phosphorothionate insecticides at the *R*-P or P-acyl sites, or at ester groupings within the acyl site such as the ethoxycarbonyl group of malathion. The hydroxylation or oxidation reactions usually increase the potency of the organophosphates as cholinesterase inhibitors, while the hydrolyses almost totally destroy the inhibitory activity. The carbamates may be hydroxylated at the *N*-methyl group or at a site within the enolic ring structure, or they may be hydrolyzed for detoxification. Insect enzymes carrying out these oxidation, hydroxylation, and hydrolysis reactions have been studied; some have been purified.

Piperonyl butoxide and a variety of other synergists increase the insecticidal activity of those organophosphates and particularly carbamates that are detoxified largely by hydroxylation processes. Considerable specificity of synergist action may occur because of differential blockage of the multiple sites and types of detoxification involved with certain esterase-inhibiting insecticides. It appears possible that the methylene grouping within methylenedioxyphenyl synergists may alkylate the enzymatic site active in such hydroxylations, but no direct experimental evidence is available on this point. Certain organophosphates inhibit the hydrolysis of other organophosphates at the phosphoric and carboxylic ester groupings and thus serve as synergists. These same organophosphates usually potentiate the mammalian toxicity of the insecticides as well. The potency of the synergists is most dramatic in those insect strains such as flies and mosquitoes that have been selected for resistance mechanisms dependent on increased rates of insecticide detoxification.

Repeated use of insecticides tends to single out as survivors those insects which have the appropriate gene constitution and biochemical mechanisms to resist the toxicants. The rapid selection of resistant strains poses a major threat to the continued efficient use of



Organophosphate pesticides or active metabolites thereof phosphorylate the esteratic site of acetylcholinesterase or other esterases. Dephosphorylation or reactivation may be rapid or very slow, depending on the groupings about the phosphorus, the nature of the esterase, and other reactive materials present. The relative rates of the indicated reactions contribute greatly to pesticidal potency; selective toxicity; resistance mechanisms in selected pest strains; efficiency of antidotes, synergists, and potentiators; and residual persistence.

organophosphate and carbamate insecticides, even though the problem is not yet as serious as it is with the chlorinated hydrocarbons. This problem is of greatest importance with, but is not restricted to, flies, mosquitoes, and phytophagous mites and aphids. It is of great concern to agencies involved in malaria and yellow fever control programs. Resistant strains of more than 30 arthropod species have been reported for organophosphates; several of these for carbamates also. A major contributing factor in resistance appears to be the increased ability of the resistant strains to detoxify the esterase inhibitor. In at least one resistant mite strain the cholinesterases appear to differ in certain characteristics from those in a susceptible strain. Resistance does not always extend to closely related compounds. Hence an analog involving only a slight structural modification frequently will still be active on the resistant strain. In other cases exposure to one class of insecticides may yield strains resistant to another class of insecticides of entirely different structure and mode of action. The level of resistance may be greater to a compound to which the population has never been exposed than to the compound used in the selection. Genetic studies have established that resistance is usually but not always monofactorial. Variations in the ecology and method of reproduction of the pest greatly influence the rate at which resistant strains are selected. The method and thoroughness of insecticide application are also important factors.

In the control of agricultural pests it is essential to use compounds that will spare the insect predators, parasites, and pollinators. Resurgences of pest populations have frequently occurred after insecticidal applications had killed entomophagous insects. Systemic organophosphates and compounds of short-lived residual action have been used very successfully in conjunction with parasites in programs of integrated pest control. Most of the organophosphate and carbamate insecticides must be carefully employed to avoid mortality in highly susceptible honey bee populations. Failure to select properly the insecticide and time of application may result in greater pest problems or lower yields than are obtained without application of insecticide.

Residues and Tolerances

Use of pesticides on agricultural crops and livestock creates a potential hazard to consumers from residues persisting as food contaminants. There are no known cases of illness in the United States or the United Kingdom due to consumption of residues on food when the pesticide was used as directed. However, residues in food crops treated with parathion have resulted in poisoning of agricultural workers from occupational exposure in the United States, and of consumers in other countries with less strict use regulations. Tolerances, or the maximum residue levels legally allowable in the United States in the raw agricultural commodity, are derived by considering the benefits from and conditions for use of the compound in food production, the fate of the chemical, and possible toxic effects produced by the pesticide on long-term ingestion by mammals. The sensitivity of cholinesterases and other esterases in vivo and in vitro to inhibition by organophosphates and carbamates has greatly facilitated the gathering of this information. With rats and dogs, the dietary level of organophosphate pesticide resulting in significant depression of erythrocyte or plasma cholinesterase activity over a 90-day to 2-year feeding period ranges from less than 0.1 to more than 1000 parts per million (ppm). The threshold level of organophosphate insecticide for detectable blood cholinesterase inhibition is usually less than one-tenth that giving any other indications of toxic effects on the animal. By these criteria the maximum no-effect level is about 0.5 to 1.0 ppm for parathion, 50 to 100 ppm for malathion, and 200 to 400 ppm for carbaryl. Tolerance values for apples, cherries, and grapes for fresh consumption are 1.0, 8.0, and 10 ppm, respectively, for these three insecticides. The intervals considered safe for the consumer between the last application of insecticidal dosages and harvest are 14, 3, and 0 to 1 days, respectively.

The insecticide when applied to insect control is subjected to various physical, chemical, and biochemical processes. Deposits of some chemicals and formulations are readily lost by the action of wind and rain. Chemical instability or volatility accounts for the very short residual action in insect control of such compounds as dichlorvos, mevinphos, naled, and tepp. Exposure to ultraviolet light results in oxidation or isomerization of phosphorothionates and oxidation of aliphatic and aromatic thioether groups and certain aromatic dimethylamino groupings in phosphate and carbamate insecticides. Most compounds penetrate in part into the

waxy covering of fruit or leaves after foliar application, or roots after soil application, and are then decomposed largely by enzyme action. Dissipation of absorbed residues, as with plant and animal systemics, is at least as certain and predictable as it is when the compound remains only on the surface of the organism, and it may be more certain and predictable. Even though the pesticidal type of organophosphates and carbamates are foreign or unnatural to the organism, biochemical systems capable of degrading them appear to be omnipresent. The biochemical fate of the compound cannot always be deduced from knowledge of chemical conditions destroying the compound, nor by analogy with the biological fate of similar molecules, for a single substituent on the molecule may greatly alter the site and rate of enzymatic degradation of the pesticide. The rate of biochemical dissipation of residues varies with the organism, the site within the organism, and the physiological condition of the organism. For example, parathion is destroyed within a few minutes in the rumen of a cow, or within a few hours after ingestion by man, but it may persist for several weeks after application to the fruit of an orange tree.

Hydrolysis, oxidation, reduction, and conjugation are the major biochemical mechanisms involved in metabolism of esterase inhibitors. The metabolites are largely excreted in the urine by mammals but may be stored or incorporated into normal constituents in plants. All available chemical tools must be utilized to establish the fate of each fragment of the molecule. The potential toxicity of each metabolite or other degradation product formed must then be determined. Radiotracers, a wide variety of chromatographic techniques, critical spectrophotometric analysis, and enzymatic and bioassay indicators are usually employed. Fortunately, the incorporation of radioactive elements (phosphorus-32, carbon-14, tritium, and others) into the pesticide molecule can usually be achieved with relative ease. Hydrolysis of both phosphates and carbamates almost always serves to detoxify the molecule. With malathion, enzymatic hydrolysis may result initially at any one of five sites on the molecule. Hydrolysis or hydroxylation and subsequent conjugation serves to detoxify most of the carbamates. Oxidation of certain organophosphorus insecticides may result in products thousands of times more potent as cholinesterase inhibitors than their precursors, but not necessarily any more toxic to mammals if separately administered. Oxidation of phosphorothionates to phosphates, and of sulfides to sulfoxides and sulfones, yields toxic metabolites which are then further degraded and detoxified. *N*-Alkyl groups may be altered or removed from the molecule. These oxidative processes frequently yield toxic metabolites which persist long after the virtual disappearance of the compound administered. Residue analysis is greatly complicated by these metabolic reactions. A dozen metabolites may form, four of them being toxic products additional to the compound originally applied. An analytical procedure sensitive to trace amounts must be devised for the toxic products, without interference from nontoxic metabolites. This extremely difficult challenge has generally been met with success, but not without causing a delay of a few months to years before the compound could be used commercially.

Milk constitutes a special residue problem because of the necessity to keep it free from contamination. The organophosphates and carbamates are generally safer in this respect than the chlorinated hydrocarbons and thus may in certain cases be applied directly to lactating animals, or to their forage shortly before pasturing or harvest. Metabolites of the organophosphates and carbamates appear in the milk but are generally considered to be harmless. Exceptions appear with animals treated with systemics, for example in cattle grub control, where the more stable and fat-soluble compounds may appear in the milk in large amounts; several days or weeks after treatment may be necessary to free the milk of residue.

Toxicity to Vertebrates

People involved in manufacturing, formulating, and applying phosphate and carbamate pesticides may accidentally contact one or more of these materials. They should be aware of the nature and the potency of the compounds they are using. Improper conditions of storage may yield degradation products from phosphorothionates that are more hazardous than the original compound. Critical quality control must also be maintained in manufacturing, for traces of toxic impurities may greatly increase the hazardous nature of the technical grade pesticide. It must be clearly understood that even though organophosphates used as insecticides and those developed as potential chemical warfare agents are of similar type and mode of action, they are very different in their hazard to man. The amounts of esterase-inhibiting insecticides that are poisonous to man range from several drops to many grams. Some must be handled with extreme care and others, equally effective in insect control, can be used with only minor safety precautions. Sixteen deaths in the United States in 1956 were attributed to esterase-inhibiting pesticides. It has been estimated that there are about 100 significant cases of poisoning by pesticides for each death from that cause. These accidents could generally be avoided, despite the millions of pounds of organophosphates and carbamates used, by attention to the precautions on the pesticide container label, particularly if the compounds are stored and used in such a way that children or others unfamiliar with the hazard are not exposed. The more toxic organophosphates have also been frequently employed in suicide attempts, usually with success, to complicate the interpretation of the statistics. For example, in Japan there were about 5000 cases of nonfatal poisonings and 2000 deaths during the 4-year period 1953–56 from parathion alone. Over 85 percent of the deaths were known to be suicides.

The effects of organophosphates on mammals are more clearly understood than for any other type of synthetic organic toxicant. Studies on phosphate chemical warfare agents and carbamates for use in clinical medicine have contributed greatly to this fundamental information which is so essential to the interpretation of conditions for safe use of similar compounds such as insecticides. Cholinergic stimulation by these toxicants results from inhibition of acetylcholinesterase, the only biochemical or biophysical lesion implicated in almost all signs of poisoning. Organs innervated by the parasympathetic system are potentiated in response to acetylcholine by these agents, resulting in excessive salivation, lacrimation, myosis, vomiting, and other signs. Fibrillations, fasciculations, weakness,

ataxia, and paralysis appear in the involuntary muscles. Effects on the central nervous system directly or as reflex responses to peripheral action lead to asphyxia or, occasionally, to cardiac failure. Therapy consists of atropinization, dosing with a cholinesterase reactivator such as 2-pyridine aldoxime methiodide (2-PAM) or certain related and even more effective compounds, artificial respiration in severe cases, decontamination of the skin, stomach, and eyes, and maybe symptomatic treatment. The aldoximes are not beneficial with certain carbamates such as carbaryl. The prognosis is more favorable the sooner the treatment is begun and the slower the onset of poisoning symptoms.

The mechanism of acetylcholinesterase inhibition by organophosphates and carbamates has been more extensively investigated than that of any other enzymic inhibition reaction. In the hydrolysis of the normal substrate, acetylcholine, an extremely unstable acetyl derivative of cholinesterase, is formed. On reaction with carbamates a more stable carbamoyl esterase results, and reaction with phosphates yields an even more stable phosphoryl esterase. The enzyme-inhibitor complex formed prior to carbamoylation or phosphorylation may also be important in poisoning, particularly with the carbamates. The stability of the phosphoryl and carbamoyl esterases depends on the substituents remaining with the phosphoryl or carbamoyl group attached to the esterase. The more stable the phosphorylated esterase formed, the slower the reactivation or dephosphorylation of the inhibited esterase and the more prolonged the physiological disruption. Reactivation of the more stable phosphorylated esterases can frequently be accelerated by aldoximes such as 2-PAM. Spontaneous loss of one alkyl group from a dialkyl phosphoryl esterase makes the reactivation much more difficult. The active site of sensitive esterases generally consists of the imidazole group of histidine and a serine with the amino-nitrogen linked to the α-carboxyl of a dibasic amino acid residue. Phosphorylation as by an insecticide yields the following amino acid sequence at the esteratic site: -glycyl-aspartyl or glutamyl-phosphoryl seryl-glycyl or alanyl-.

The toxicity of the esterase-inhibiting pesticides to mammals varies considerably with species, sex, age, route and frequency of administration, and previous history of exposure to other chemicals. One species may be 10 or more times more sensitive than another, and one sex of the same species may be 10 times more sensitive than the other sex. Most of the compounds are more toxic by ingestion or inhalation than by skin exposure, but some are equally or even more toxic through the skin. With most of the carbamates and with a few phosphate insecticides, the mammal may survive many daily doses just below the acute lethal level, while other compounds are much more cumulative in their action. The esterase-inhibiting pesticides generally differ from the chlorinated hydrocarbons in not being stored for prolonged periods in fatty tissues and in failing to yield significant gross or microscopic pathology, except that associated with pulmonary or cerebral congestion or changes secondary to convulsions in near lethal doses. Sufficient information is available on human exposures to organophosphate and carbamate pesticides to interpret the results from laboratory mammals in relation to the hazard for man with reasonable confidence.

Mixtures of pesticides are frequently employed to keep a pest infestation in check. Selected combinations of organophosphate insecticides are more toxic to mammals than anticipated from the individual potencies of the components. All possible paired combinations of the major organophosphate insecticides in use have been examined. This potentiation is restricted to a combination of malathion and EPN, and a few other pairs. Some insecticide synergists, industrial chemicals, certain tranquilizers, and other drug types may sensitize animals to organophosphates. Examples are piperonyl butoxide, tri-*o*-cresyl phosphate, 3-methylcholanthrene, thiopental, and such phenothiazine-substituted drugs as promazine and chloropromazine. Organophosphates may also sensitize individuals to certain ester-containing drugs by inhibiting enzymes involved in their hydrolysis. Most of these potential multiple exposure problems are based only on laboratory studies with experimental animals, and their applicability to human hazards is not fully established.

Certain responses of the organism not explicable on the basis of cholinesterase inhibition must also be considered. A delayed and prolonged neurotoxicity with associated demyelination and muscular weakness results from some organophosphates in several species, including man, but careful attention is given to avoiding the use of such compounds as insecticides. About 200 organophosphates are known to have this effect on hens. Over 10,000 cases of ataxia in humans have resulted from tri-*o*-cresyl phosphate, a noninsecticidal phosphate important as an industrial chemical, but only two human cases have been attributed to an insecticide. This insecticide is no longer used. Some of the organophosphate insecticides at high doses do, however, produce a prolonged muscular weakness in hens which bears some resemblance to the typical neurotoxic syndrome. The biochemical lesion leading to the typical delayed neurotoxicity has not been defined. Some organophosphates and carbamates induce teratogenic effects or embryonic abnormalities when injected into hen eggs. Again, the precise biochemical lesion involved is not known, although certain nicotinamide analogs including the coenzymes containing this vitamin will relieve or mitigate this effect. The reproductive potential of animals is generally not affected by organophosphate and carbamate insecticides, but little information has been reported on the effect of dietary exposure through several generations. Several such studies are now in progress. Some organophosphates induce a narcosis unassociated with cholinesterase inhibition. At very high levels for prolonged periods, certain organophosphates have been reported to alter the behavioral pattern of experimental animals. Small quantities of organophosphorus pesticides which only slightly lower the cholinesterase activity have been found, in other situations, to result in changes in the strengths of conditioned reflexes. Isolated cases of skin sensitization to certain organophosphates are known. Despite intensive investigations, no evidence is available of carcinogenic or tumorigenic tendencies among the insecticidal organophosphate and carbamate compounds. Since related compounds are known to be carcinogenic, careful evaluation must be made of each new candidate pesticide.

Birds, fish, and wild mammals are also exposed to pesticides. With esterase-inhibiting insecticides, the hazard from acute toxicity to wildlife is usually greater than that from chronic poison-

ing, while with the chlorinated hydrocarbon insecticides the long-term or chronic effects have usually been more critical. Organophosphates and carbamates are being considered as alternatives to chlorinated hydrocarbons whenever insecticide application is absolutely necessary in areas where extensive direct or indirect exposure of wildlife may be involved. This shift has resulted from the greater selectivity for the pest, particularly where high levels of resistance to chlorinated hydrocarbons are involved, and from the lower persistence of the esterase inhibitors, resulting in less drastic or cumulative effects on the wildlife population.

Other Types of Pesticidal Activity

Acetylcholinesterase and acetylcholine are present and appear to be physiologically important in all animals with an organized nervous system. Industrial testing of candidate organophosphates has been largely for insecticidal activity. Compounds potent as cholinesterase inhibitors or precursors for the formation of such inhibitors in insects were therefore selected for development. Nematocidal and anthelmintic activity appear much less frequently than potent insecticidal activity among the organophosphates, but the search for such compounds is a very active field of current endeavor. Soil nematodes are effectively controlled by certain organophosphates at doses equal to or considerably below those of other nematocidal types of chemicals. Helminths in pets, livestock, and man have been controlled with organophosphates of relatively low mammalian toxicity with slight or no symptoms of poisoning in the host. Organophosphates and carbamates have been used as snail and slug poisons. Certain organophosphates might even be used for selective control of pest birds.

The organophosphates and carbamates applied to plants for insect control have been carefully checked for possible deleterious effects on plant growth. Use recommendations indicate any potentially susceptible plant species. None of the esterase-inhibiting pesticides have yielded off-flavors in foods harvested from treated plants, a problem which is occasionally encountered with chlorinated hydrocarbon insecticides.

Certain organophosphates have been developed as defoliants, particularly for cotton, and as herbicides, such as for crabgrass control. Fungicidal, fungistatic, bactericidal, and bacteriostatic organophosphates are also known. The mode of action on plant life is obviously different from that on the nervous system of animals. This difference allowed the development and very effective use of plant systemics that did not harm the plant but killed insects feeding on the plants. But slight structural modification allows disruption of additional and as yet undefined systems important to plants, with great selectivity in the resulting phytotoxicity. The organophosphates active on plant life are generally phosphorylating agents of varying reactivity, but it is not known to what extent the phosphorylation of enzymically active sites is involved in this type of action. On exposure to these agents vertebrates frequently display symptoms and patterns of esterase inhibition that are different from those they display on exposure to insecticides. Insecticidal carbamates may initiate fruit drop (carbaryl does so on apples) or rarely produce other abnormal symptoms in plants; these are pointed out in the use recommendations. Fungicidal and herbicidal carbamates generally, however, are not of the same chemical type and mode of action as the insecticidal carbamate esterase inhibitors.

Summary

Thirty years of testing has yielded over 120 esterase inhibitors in current use for pest control. Several hundred million pounds of these organophosphates and carbamates are employed each year as insecticides and acaricides and, to a much lesser extent, as anthelmintic agents, nematocides, and herbicides. Systemics or chemotherapeutic agents for control of insect pests of plants and animals first became practical with the organophosphates. Compounds of lower mammalian toxicity and other favorable biological properties continue to appear and displace established compounds and broaden the use areas. Problems of resistance and residues in certain areas of insect control by chlorinated hydrocarbons will result in a further shift to esterase-inhibitors for pest control. Interpretation of the potential hazards of pesticides to man is dependent on the availability of fundamental information on their modes of action combined with use experience; this knowledge is available for the organophosphates and carbamates that act as acetylcholinesterase inhibitors.

References and Notes

1. Several recent reviews and books provide a more comprehensive summary of some aspects considered, for example: A. W. A. Brown, *Bull. Entomol. Soc. Am.* **7**, 6 (1961); R. C. Bushland, R. D. Radeleff, R. O. Drummond, *Ann. Rev. Entomol.* **8**, 215 (1963); J. E. Casida, *ibid* **8**, 39 (1963); E. H. Colhoun, *Advan. Insect Physiol.* **1**, 1 (1963); W. A. L. David, *Ann. Rev. Entomol.* **3**, 377 (1958); W. F. Durham, *Residue Rev.* **4**, 33 (1963); W. J. Hayes, Jr., "Clinical handbook on economic poisons. Emergency information for treating poisoning," *U.S. Public Health Serv. Publ. 476* (1963); D. F. Heath, *Organophosphorus Poisons. Anticholinesterases and Related Compounds* (Pergamon, New York, 1961); G. B. Koelle, Ed., *Cholinesterases and Anticholinesterase Agents* (Springer, Berlin, 1963); L. I. Medved', E. I. Spynu, I. S. Kagan, *Residue Rev.* **6**, 42 (1964); R. L. Metcalf, *Bull. Entomol. Soc. Am.* **5**, 3 (1959); *Agr. Chem.* **16**, No. 6, 20 (1961); J. W. Mitchell, B. C. Smale, R. L. Metcalf, *Advan. Pest Control Res.* **3**, 359 (1960); W. O. Negherbon, *Insecticides*, vol. 3 of "Handbook of Toxicology" (Saunders, Philadelphia, 1959); R. D. O'Brien, *Toxic Phosphorus Esters. Chemistry, Metabolism and Biological Effects* (Academic Press, New York, 1960); R. D. O'Brien and J. G. Matthysee, *Agr. Chem.* **16**, No. 10, 16 (1961); ———, *ibid.* **16**, No. 11, 27 (1961); W. E. Ripper, *Advan. Pest Control Res.* **1**, 305 (1957); L. Rosival, L. Vrbovský, Fr. V. Selecký, *Toxikologie und Pharmakodynamik der phosphororganischen Verbindungen* (Pressburg, Czechoslovakia, 1959); B. C. Saunders, *Some Aspects of the Chemistry and Toxic Action of Organic Compounds Containing Phosphorus and Fluorine* (University Press, Cambridge, 1957); G. Schrader, "The development of new insecticides," *B.I.O.S. Final Rept. No. 714* (1947); *Die Entwicklung neuer insektizider Phosphorsäure-Ester* (Verlag Chemie, Weinheim/Bergstr., Germany, 1963); A. Stempel and J. A. Aeschlimann, "Synthetic analogs of physostigmine," in *Medicinal Chemistry*, F. F. Blicke and R. H. Cox, Eds. (Wiley, New York, 1956), vol. 3, chap. 4, pp. 238–339.
2. Chemical designations and synonomy for most of the compounds indicated only by common name can be found in E. E. Kenaga, *Bull. Entomol. Soc. Am.* **9**, 67 (1963), and S. C. Billings, *ibid.* **9**, 189 (1963).
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