Review of Current Knowledge of Toxicity of Cholinesterase Inhibitor Insecticides

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The common property of the organic phosphate–type insecticides now in commercial use is their ability to inhibit cholinesterase. The acute toxicity of these materials is a reflection of this enzyme-inhibiting action and varies appreciably with different members of the group. Subacute toxicity develops only when inhibition exceeds cholinesterase regeneration rate, particularly in the red blood cells. The hazard encountered under use conditions is a combination of the acute toxicity of the particular material and the degree of exposure by oral administration, dermal absorption, or inhalation of particulate matter. All these insecticides may be safely used if adequate precautions are taken to avoid acute exposure. Under use conditions residue hazard appears to be negligible.

LIKE SEVERAL OTHER GROUPS OF PESTICIDES, the cholinesterase-inhibiting group is of relatively recent origin. The basic work on this group of materials and their revelation as potent insecticides apparently developed with Schrader (*73*) in approximately 1937, although Grob and Harvey (*37*) cite the preparation of tetraethyl pyrophosphate (TEPP) by Clermont (*15*) in 1854. To date, most of the materials in use are either organic phosphates or organic thiophosphates. Their chemical formulas vary extensively, as do their physical properties. These variations in physical properties, principal of which are stability of solution and solubility in water, make possible the selection of suitable insecticides for various conditions of use.

In the lay press this group of materials has been classified as "nerve gases," and even the scientific writers have felt compelled to list the popular writings (*4*). Of the pesticides in use, however, few if any would classify as a gas under use conditions, as they are either readily hydrolyzable in moisture or have such low vapor pressure that their gas phase is unimportant as a hazard. As a class, the cholinesterase inhibitor pesticides are not residual under conditions of use. Decomposition occurs at various rates under exposure to field conditions and insecticidal properties are lost within a period of from a few hours to approximately 10 days to 2 weeks. They have, therefore, only two characteristics in common, the organic phosphate chemical radical, and their capacity to inhibit cholinesterase.

To evaluate the public health aspects of these pesticides, it is essential that terminology be used carefully. Unfortunately, in its relatively brief history the literature on the cholinesterase inhibitors has become highly confused in this respect. The three major aspects are pharmacodynamics, toxicity, and hazard. As used here, pharmacodynamics is the mechanism of action of the drugs or chemicals on living tissue. Toxicity is the degree of injury following a single or repeated exposure to the chemicals, while hazard is the estimate of the potential injury which might result from exposure under conditions of use. Each of these aspects has been the subject of numerous conferences and publications in the past few years, and it is the object of this presentation to summarize at least a portion of these data into an overall evaluation of the various aspects. For this purpose only the principal members of the group which have received sufficient study to contribute to our understanding are discussed here.

Pharmacodynamics

Fundamentally, the anticholinesterase agents exert their pharmacological effects through an imbalance of a biochemical enzyme system. To begin at this point, however, would be to begin in the middle of a long and fascinating story. To develop an adequate background for this story would be to review the history of the study of chemical neurotransmission. Obviously this is beyond the scope of our interest at the present time, but a few highlights would be well worth while.

About 50 years ago Hunt (*49*) and Hunt and Taveau (*50*) isolated choline esters from certain tissues and later showed that acetylcholine was about 100,000 times as potent as choline in its biological action. Experimental work continued on this problem until in 1921 Loewi (*59*) demonstrated with classic simplicity that following electrical stimulation of the frog cardiac vagus an inhibitory substance was liberated by the isolated heart into the perfusate, and that this could be transferred to a second heart and induce inhibition. This was followed later by his identification of the transferred chemical (*60*) as acetylcholine. A modern concept of neurohormonal transmission is that acetylcholine is the chemical mediator for all autonomic ganglia, the endings of the parasympathetic and somatic nervous systems, and perhaps the central nervous system. With the exception of the central nervous system these nerves, whose impulses are mediated by acetylcholine, are termed "cholinergic" and the term is frequently used to describe the effect that is produced by their function or stimulation. A counterpart of this whole system is the mediation by adrenalin or epinephrine, embracing many postganglionic nerves, particularly the sympathetic nervous system.

It was obvious to the early investigators that if a chemical so biologically potent as acetylcholine were present in the tissues following nerve function or stimulation, there must be some rapidly detoxifying mechanism to prevent its accumulation in the effector organs. Although suggested as early as 1914 (*17*), it was in the decade from approximately 1925 to 1935 that much of the basic exploratory work on this enzyme was conducted. In 1932 Stedman and coworkers (*75*) applied the name "cholin-

esterase" to the enzyme responsible for splitting acetylcholine into choline and acetic acid. In their original paper they spelled the word with a hyphen and it has later entered the literature both as a single word and as a double, unhyphenated word. Some conception of the interest engendered by this basic physiological complex of neurohormonal transmission by acetylcholine and its regulation by the hydrolyzing enzyme cholinesterase can be garnered from the fact that by 1948 Augustinsson (*2*) published a 180-page review containing 692 references on the subject of cholinesterases. In 1953 *Pharmacological Reviews* published a prize-winning manuscript by Riker (*72*) on the properties of acetylcholine and related quaternary ammonium compounds at the neuromuscular junction. Just one year later the same journal (*70*) published the proceedings of the Symposium on Neurohormonal Transmission, held in Philadelphia and attracting scientists from around the world. Certainly the end is not in sight, and at Philadelphia Sir Henry Dale, after devoting a half century to the problem, said, "I certainly do not expect that a final solution will appear in my time."

Further confusion in the literature has arisen from the naming of various types of esterases present in the body. In general, the term cholinesterase now refers to the enzyme which splits, or hydrolyzes, acetylcholine to acetic acid and choline. It has been called "true cholinesterase" and "acetylcholinesterase," the latter term obviously being the more specific. In addition to this esterase, which is primarily contained in the erythrocytes and tissues, the human also has a nonspecific esterase, sometimes referred to as "pseudo cholinesterase," which hydrolyzes not only acetylcholine but other esters. In the human this is contained primarily in the plasma. The various cholinesterases are almost universally distributed throughout the animal kingdom, but there are rather marked species differences in activity and distribution.

Pharmacologically, the specific cholinesterase is the enzyme of major interest, since it serves as a much more accurate index of activity. To oversimplify extremely, the pharmacological action of the cholinesterase inhibitors may be considered to be that which would be exhibited by an excess of acetylcholine. In relatively small doses of the inhibitors the grossly visible signs are those characteristic of the stimulation of the parasympathetic nervous system: pupillary constriction resulting in blurred vision, salivary secretion, gastric motility with nausea or cramps, and bronchial constriction causing a feeling of tightness in the chest. Other less obvious signs occur simultaneously but are largely masked by the foregoing. These are the actions which provide the pharmacological basis for therapeutic use of such cholinesterase inhibitors as physostigmine and prostigmine. It is of historical interest here that neither of these esterase inhibitors is an organic phosphate and they are not insecticidal. That this may be only a transient situation is indicated by the recent paper by Kolbezen and coworkers (*56*). Certain *N*-methylcarbamates were shown to be potent insecticides and cholinesterase inhibitors, and these may well be the next group to receive concentrated attention. Although less frequently used as a therapeutic agent, the alkaloid pilocarpine produces most of the effects of cholinesterase inhibition but is not significantly an inhibitor. It acts after nerve degeneration and is therefore assumed capable of reproducing the effects which acetylcholine exhibits on myoneural junctions.

Larger doses of physostigmine or Neostigmine are used in the treatment of myasthenia gravis. Under these conditions the parasympathetic effects described above become undesirable side actions and are effectively blocked by appropriate doses of atropine. Some of the new organic phosphates, particularly octamethyl pyrophosphoramide (OMPA) and tetraethyl pyrophosphate (TEPP), are under clinical investigation for this usage (*35*). For this class of use the parasympathetic effects, having been blocked by atropine, are no longer a consideration. The pharmacological effect involved is that of stimulation and buildup of the neurohormone, acetylcholine. Detailed discussions of the current status of this phenomenon are presented by Barron (*7*) and by Riker (*72*). It is important to remember that under these conditions the plasma and erythrocyte cholinesterase levels are reduced to approximately 10% of their normal activity before demonstrable benefits are achieved.

Toxicity

Acute Toxicity

The mechanism of pharmacological action as just outlined was known in its essential features prior to the advent of cholinesterase inhibitors as pesticides. Toxicity had not been a major problem, since the therapeutic usage was always under medical supervision and overdosage, while representing an emergency, could generally be controlled by administration of atropine and the lapse of time. The introduction of the new organic phosphate cholinesterase-inhibiting insecticides also introduced a new era of confusion, unfounded assumption, catchy phrases, and pseudo glamor, in both the scientific and the lay press. The toxicity of the new compounds was immediately associated with their anticholinesterase activity. They were described as new and forbiddingly toxic materials. In vitro cholinesterase-inhibiting activity was associated with their insecticidal potency. It was common for scientists of various training to classify these agents all together as cholinesterase inhibitors without any consideration for the differences inherent in their chemical structures. This is in spite of the earlier and well known demonstration of differences between two materials such as physostigmine and Neostigmine.

These are technical problems of interest primarily to pharmacologists, biochemists, and others directly interested in evaluating the hazard of the inhibitors under conditions of use. The acute toxicity of the more toxic members is of the same order of magnitude as that for nicotine or cyanides, both of which have found wide agricultural and/or industrial usage. There is reason to believe that as time goes on and research continues there will be an improvement in the degree of acute toxicity such as is indicated by at least one commercially important organic phosphate (malathion). The acute toxicity of the technical material is of significance only at the level of manufacture and initial formulation. Users and consumers are not in contact with the concentrated commercial material. An important aspect of the currently used inhibitor-type pesticides is that they are toxic following any route of entrance into the body. Thus, all of them are toxic following oral administration, dermal application, and inhalation of the particulate matter such as in aerosols or dusts. Inhalation of other than the particulate matter may not be hazardous, as is pointed out under a discussion of the various compounds. Except from a theoretical and research viewpoint the in vitro anticholinesterase activity is not important to the evaluation of the mammalian toxicity of the phosphate insecticides. This has been pointed out by Hazleton, Kolbezen, and others (*45*, *56*). An outstanding example of this is octamethyl pyrophosphoramide (OMPA), which has almost no in vitro anticholinesterase activity, but in either animals or insects is converted to an active inhibitor (*25*, *68*).

In vivo cholinesterase inhibition serves as a warning of impending toxicity and is useful in prophylactic industrial hygiene programs. Beyond this its reliability, either diagnostically or prognostically, is much more limited than much of the recent literature would lead the unwary reader to suspect. Several methods for evaluating cholinesterase activity have been evolved through the years. Perhaps the pioneer method was the direct titration method of Stedman (*76*), and the most fundamentally reliable method is that of Ammon (*1*) based on manometric techniques. For practical purposes the most widely used method now appears to be that of Michel (*65*) or some modification of it,

although other methods such as the colorimetric method of Metcalf (*63*), modified for whole blood by Fleisher and Pope (*29*), may eventually be equally useful. For further convenience and rapidity, particularly in the field, the Michel method has been modified to a microtechnique described by Hamblin and Golz (*40*). The establishing of human normal values has represented a major problem, to which the paper by Wolfsie and Winter (*80*) makes a basic contribution.

Measurement of plasma or erythrocyte cholinesterase activity is indicative only of the first stage of toxicity represented by excessive pharmacological action. Of the two tissues the erythrocyte activity is probably more valuable as an indication than the plasma activity, since the former represents specific acetylcholinesterase and with the phosphate undergoes the so-called "irreversible" inhibition. Once inhibited by most phosphate anticholinesterase chemicals, the erythrocyte activity is regenerated slowly and there has been speculation that this is associated with the normal replacement of the erythrocytes. Atropine or other cholinergic blocking agents have been shown to be effective in relieving the acute signs of toxicity for less than lethal doses of the inhibitors. In general, these agents are not effective in protecting against large excesses of the inhibitors.

As is true with almost any chemical, overwhelming doses defy adequate antidotal remedies. With less than overwhelmingly toxic doses the organic phosphates have what could well be termed a "built-in safety factor." The cholinesterase inhibition leading to pharmacological signs associated with excessive parasympathetic nervous system activity serves as adequate warning of excessive exposure. Adequate atropinization, administered promptly, together with other supportive measures, rapidly antagonizes these moderate and warning toxicological signs. Recovery may be expected to be complete following acute or subacute exposure, once this exposure is terminated and remedial measures are instituted. In effect, there has been no toxicity due to the compound as such at this stage. The abnormal signs observed are those of the enzyme imbalance and are not characteristic of any particular group of chemicals except the general classification of enzyme inhibitors.

Subacute Toxicity Subacute toxicity may be a problem for manufacturing, formulating, and application personnel, unless adequate precautions are taken to avoid exposure. These precautions are known and have been well publicized by the manufacturers, the Public Health Service officials, and federal and state government regulatory bodies. Briefly summarized, the precautionary measures involve adequate ventilation to avoid inhalation of any particulate matter, provision of respirators where inhalation is difficult to control through ventilation as in field application, protective clothing to avoid dermal contact since these materials are readily absorbed through the skin, and adequate personal sanitation. They should include careful education as to washing the skin, removing contaminated clothing, and the avoidance of eating or smoking while the possibility of contamination exists.

Periodic checks on cholinesterase activity of personnel continuously exposed have been followed by various groups and appear to be a worth-while measure. Progressive decline of red cell cholinesterase levels precedes the onset of toxic signs and serves as a guide for withdrawing the subject from exposure. Personnel complaining of symptoms that may be associated with their exposure to these pesticides, such as giddiness, tightness in the chest, nausea, blurred vision, intestinal cramps, or diarrhea should be immediately removed and placed under the care of an informed physician.

Chronic Toxicity Chronic toxicity might theoretically be experienced by manufacturing, formulating, and application personnel, but would be in every respect the same as the subacute toxicity described above except in degree and time of development. Again the problem of definition and clarity arises, but if subacute toxicity is assumed to be experienced within approximately 90 days' exposure, seasonal variations would eliminate the possibility of chronic exposure for a great many of the personnel involved. Experimentally, subacute and chronic experiments have been conducted on rats and dogs with a majority of the commercially important phosphate insecticides. Again the built-in safety factor of parasympathetic stimulation serves as a limiting factor in the chronic exposure. Following oral ingestion of the inhibitors, the animals develop signs of cholinesterase inhibition, and when these become excessive refuse food and show a general toxic condition. If continued, this interference with ability to eat and consequent nutritional deficiency will result in secondary involvements which are the direct cause of mortality. If, however, the inhibitor is removed from the diet, the animals recover and there are no latent signs of pathology. Interesting aspects of this type of toxicity are that the animals will resume eating and appear normal within 1 or 2 days after the inhibitor is removed from the food, although at this time their cholinesterase activity in blood and usually brain are at extremely low levels. Also, if the level of feeding is critical, the cholinesterase may be lowered to practically nondetectable levels with no gross signs of toxicity and the animals will eat and live in an almost normal fashion.

From the public health standpoint the consumer is not exposed to the toxicity of these materials when used as pesticides. Under conditions of use the organic phosphates are not classified as residual pesticides and the wealth of residue data indicate that after periods varying from 1 to 3 weeks there are no significant residues left on crops.

Hazard

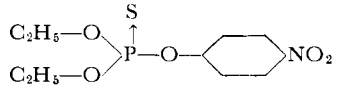
Hazard has been defined as an estimate of the potential injury which might result from exposure under conditions of use. Again different classes of population are involved at different stages of pesticide manufacture and use. It is obvious from the discussion of toxicology that plant personnel involved in the manufacture and formulation stages can be subjected to real hazard unless adequate percautions are taken. Application of the formulated materials also presents a hazard and requires careful attention to safety measures. The consuming public is not exposed to a hazard under conditions of use for, although there is no appreciable residue left on crops, extensive toxicological studies are conducted on each pesticide. These investigations are the responsibility of the manufacturer and are a requirement of federal and state laws. To understand further the safeguards against hazards to the consuming public it would be desirable to review typical research and development programs applicable to inhibitor-type pesticides. The investigational details may vary depending on the nature of the pesticide and the responsible investigator, but all such programs involve an estimate of the acute oral toxicity, acute dermal toxicity, subacute toxicity, and usually long-term chronic toxicity in one or more species of animal. Various phases of these studies are terminated with complete microscopic histopathological examination. The metabolic fate, antidotes and their efficacy, inhalation hazard, and the possibility of tissue storage are usually investigated. These data, together with residue analyses under use conditions, make possible a very careful estimate of whether a potential hazard might exist under conditions of use.

There is then a wealth of information, basic and applied, available on the organic phosphate insecticides, both as a class and as individuals. Their development as agricultural chemicals has contributed in a major degree to the extension of knowledge in the fields of enzymology, pharmacology, physiology, and entomology. It would, however, be remiss to omit mention of the role of the American economy in this development. New pesticides must justify their economic existence. To this end the manufacturer must demonstrate competitive efficacy, safety, and cost. Only

those which pass this stage are developed to the point of submission to regulatory officials for final evaluation and approval under the applicable state and federal laws. For various reasons not all the studies on each pesticide chemical are published. Hence the general literature reveals only a portion of the knowledge, but even this portion is extremely voluminous.

The following discussion highlights the characteristics of various individual compounds in the organic phosphate, cholinesterase-inhibiting class of pesticides. Data have been collected from many sources and are not always strictly analogous, since conditions and methodology may vary. There is, however, a general consensus as to order of magnitude and the values are useful for comparative purposes.

Parathion



O,O-Diethyl *O*-*p*-nitrophenyl thiophosphate

LD_{50}. 3.5 mg. per kg., female rat, oral (*44*)
12.5 mg. per kg., male rat, oral (*44*)

In_{50}. 1.5×10^{-6}, plasma, human (*34*)
1.2×10^{-5}, red blood cells, human (*34*)
1.2×10^{-6}, brain, rat (*28*)

Parathion is the generic name for *O,O*-diethyl *O*-*p*-nitrophenyl thiophosphate. Interest in it as an insecticide developed in late 1946 or early 1947 and by the spring of 1948 three reports on its pharmacology and toxicology appeared in the literature. These reports, by DuBois, and coworkers (*24*), Hagan and Woodard (*39*), and Hazleton and Godfrey (*43*), covered the essential aspects of mechanism of action, acute toxicity, and antidotes.

Also in 1948 Averell and Norris (*5*) published a sensitive method for the quantitative detection of parathion in residues. Hazleton and Holland (*44*) adapted this method to animal tissues and found that after intravenous administration, parathion could be detected universally distributed throughout the body. Subacute oral administration to rats and dogs resulted in no storage of parathion in the tissues, including fat. Small quantities were found in the urine of some of the dogs. After a 2-year feeding to male rats at levels including 100 p.p.m. of the diet there was no significant effect on growth, food consumption, or mortality (*44*). Following sacrifice at term there were no histopathological changes. Dermal toxicity of the technical material and formulations was reported. Konst and Plummer (*57*) conducted both acute and chronic experiments involving five species of animals. Of particular significance were their negative results from feeding crops with parathion residues and the absence of specific pathological changes after toxicity which produced clinical symptoms or death.

The metabolic fate of parathion has been the subject of several investigations. Mountain, Zlotolow, and O'Conor (*66*) and Gardocki and Hazleton (*32*) both discuss *p*-nitrophenol as a principal metabolite, indicating a cleavage of the parathion molecule. The latter investigators show that *p*-nitrophenol and *p*-aminophenol can be detected in the urine following doses of parathion which produce no signs of toxicity, although there was plasma cholinesterase inhibition at all doses studied. Lieben, Waldman, and Krause in a series of papers (*58*, *78*) present a rapid method for detection of *p*-nitrophenol in urine and recommend this procedure as a routine test for minimal exposures in addition to cholinesterase determination. As *p*-nitrophenol is a metabolite thus far identified only for parathion, its use in the early detection of exposure is limited to this pesticide or to others containing an aromatic nitro group. This factor appreciably limits the value of the method as a routine procedure, where exposure to various inhibitors may be involved.

Dahm and coworkers (*16*) and Pankaskie, Fountaine, and Dahm (*69*) fed parathion to cows at levels greatly in excess of normal forage residues. No parathion was found in the milk, blood, or urine.

Radioactive parathion was synthesized by Jensen and Pearce (*52*) and its fate in rabbits studied by Jensen, Durham, and Pearce (*51*). The compound was radioactively labeled with sulfur-35 and this isotope appeared in the urine promptly after dermal application or intravenous injection. There was no evidence of storage in the tissues and the excreted sulfur-35 appeared to be a metabolite, not parathion per se.

Other studies by Diggle and Gage (*21*) and by Metcalf (*64*) indicate that in vivo parathion is converted to its oxygen analog, thus freeing the sulfur. This conversion may, in fact, be essential to in vivo activity, but does not pose a practical problem. The fate of the phosphate moiety has not been adequately established but it is assumed to be associated with the enzyme kinetics, which are beyond the scope of this review.

These various metabolism studies thus present a rather complete picture of the fate of parathion in the animal body. The sulfur is replaced by oxygen and excreted in the urine. The aromatic nitro group is hydrolyzed and excreted as *p*-aminophenol and *p*-nitrophenol. The phosphate nucleus, common to all of the organic phosphate inhibitors, alters the enzyme kinetics.

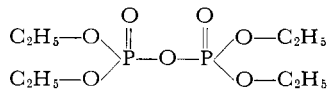
Grob and coworkers (*36*) have described the anticholinesterase response following oral administration to humans. Reports on acute parathion intoxication in humans (*42*) indicate a picture similar to that described for animals. Relatively massive doses of atropine are recommended as basic antidotal therapy (*41*, *42*). The inhalation hazard of parathion and other organic phosphates has been a common topic of discussion. The potential of such a hazard is a factor of the physical and chemical properties. In view of the extremely low vapor pressure of parathion [3.78×10^{-5} mm. of mercury (*9*)] and its chemical stability except in strong alkali, such hazard appears improbable. Despite this, however, Kay and coworkers (*54*) give analytical values for parathion in the air of treated orchards for up to 3 weeks after application. Summerford and coworkers (*77*) correlated blood cholinesterase level with symptomology in mixing plant personnel, commercial applicators, orchard workers, and other groups during and after a complete spray season. Inhalation exposure was not separated from dermal. Although some workers experienced mild to serious illness, the latter occurred only with heavy exposure and lowered cholinesterase. Fatal or near fatal illness resulted from brief, massive exposure and gross carelessness rather than from repeated or subacute exposure. Brown and Bush (*10*) concluded that continuous exposure to concentrations of parathion in the range of 2 to 8 mg. per 10 cu. meter of air is potentially hazardous. With the air sampling and analytical method employed, concentrations of this magnitude were found in a parathion manufacturing and mixing plant. Kodama and coworkers (*55*) suggest mild animal toxicity after inhalation of what was termed saturated vapor. On the other hand, Carman and coworkers (*13*) in careful studies on the physical fate of parathion under laboratory and field conditions concluded that parathion is not released to the air in the vapor or gaseous state and the effluent air from treated oranges is not toxic to flies. Other studies (*46*) indicate that air passed through technical parathion at elevated temperatures is not toxic to rats and does not affect cholinesterase levels, whereas inhaled particulate parathion, as in aerosols, is acutely and severely toxic when inhaled. Inhalation, therefore, appears to be a hazard only when particulate matter is involved, but the unpleasant odor and erratic air sampling methods have undoubtedly contributed to the mistaken impression of vapor toxicity.

Methyl Parathion

This is a common but erroneous name applied to *O,O*-dimethyl *O*-*p*-nitrophenyl thiophosphate, which differs chemically

from parathion only by the substitution of methyl groups in place of the ethyl groups of the latter. Its principal use appears to be in combination with parathion. According to DuBois and Coon (*23*), the methyl analog is approximately as toxic to rats as parathion but is a much less potent cholinesterase inhibitor. The same general principles of toxicity, enzyme inhibition, and analysis (*33*) apply, although the literature is conspicuously lacking in detailed reports compared to parathion.

Tetraethyl Pyrophosphate



Tetraethyl pyrophosphate

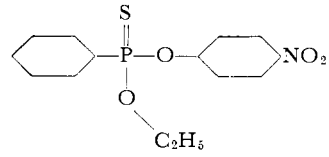
LD_{50}. 1.2 mg. per kg., female rat, oral (*30*)
2.0 mg. per kg., male rat, oral (*30*)
In_{50}. 5×10^{-9}, plasma, human (*37*)
3.5×10^{-8}, red blood cells, human (*37*)
3.2×10^{-8}, brain, human (*37*)
4×10^{-9}, brain, rat (*23*)

Tetraethyl pyrophosphate is the principal active ingredient of hexaethyl tetraphosphate (HETP) (*33*) and has been much more intensively investigated. It is extremely unstable in the presence of moisture and hence only its acute actions are of importance. As several review papers are available, the following discussion is limited to citation of aspects more directly applicable to toxicity.

In 1947 Deichmann and Witherup (*20*), Mangun and DuBois (*61*), and DuBois and Mangun (*27*) reported various aspects of acute toxicity and cholinesterase inhibition. Tetraethyl pyrophosphate appears to be the most potent cholinesterase inhibitor among the group used as insecticides, although Augustinsson (*3*) reports two other more potent inhibitors. In 1948 and 1949 pharmacological studies on hexaethyl tetraphosphate or tetraethyl pyrophosphate were reported by Dayrit, and coworkers (*18*) and by Burgen and coworkers (*12*), while Brauer (*8*) presented a detailed comparative study on enzyme inhibition. Grob and Harvey (*37*) have conducted rather extensive studies on the effects of tetraethyl pyrophosphate in man and its clinical use in myasthenia gravis. Various aspects have been compared with those of other organic phosphate insecticides by Frawley and coworkers (*30*). These include acute toxicity, inhibition from lethal doses, and rate of recovery of cholinesterase activity. Subacute administration was not practical because of its instability when exposed to air in such tests.

While tetraethyl pyrophosphate is very toxic following acute exposure, its physical and chemical properties are such that hazard can exist only under conditions of carelessness and abuse. Repeated minimal acute exposures can result in progressive inhibition of cholinesterase activity, as in myasthenia gravis therapy. The extreme instability of the compound eliminates any possibility of hazard to the public as a result of agricultural use.

Ethyl p-Nitrophenyl Thionobenzenephosphonate

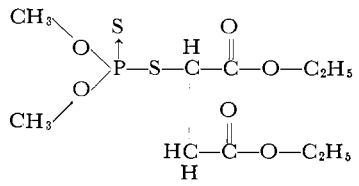


Ethyl *p*-nitrophenyl thionobenzenephosphonate

LD_{50}. 14.5 mg. per kg., female rat, oral (*30*)
91 mg. per kg., male rat, oral (*30*)

A recent comprehensive report by Hodge (*47*) indicates that ethyl *p*-nitrophenyl thionobenzenephosphonate (the *O*-ethyl *O*-*p*-nitrophenyl ester of phenyl phosphonothioic acid, EPN) fits into the general picture. The substitution of a benzene ring with a direct carbon to phosphorus bond for one ethoxy radical of parathion alters the acute toxicity somewhat, but the sex difference in rats persists, and the material remains in the acutely hazardous class. In apparent contrast to the other phosphates, Hodge reported that after prolonged administration to dogs the depressed cholinesterase level gradually returns to normal while daily administration continues. DiStefano and coworkers (*22*) report another interesting deviation from pattern with respect to antidotes. In EPN poisoned rats Coramine (nikethamide) and atropine therapy was superior to either nikethamide or atropine alone. Other analeptic agents including Metrazol, amphetamine, picrotoxin, and caffeine increased mortality. Whether this was in combination with atropine is not indicated, but nikethamide alone had no effect. The authors' caution against use of analeptics in poisoned humans is well taken. Hazleton and coworkers (*44*) have shown while Metrazol is an effective analeptic in atropinized parathion poisoned dogs, it is no more effective than artificial respiration.

Malathion



O,*O*-Dimethyl dithiophosphate of diethyl mercaptosuccinate

LD_{50}. 1156 mg. per kg., male rat, oral (*45*)
1400 mg. per kg., male and female rat, oral (*26*)
750 mg. per kg., male and female rat, intraperitoneal (*26*)
In_{50}. 8×10^{-3}, serum, rat (*26*)
2×10^{-5}, red blood cells, rat (*46*)

Malathion is the common name for *O*,*O*-dimethyl dithiophosphate of diethyl mercaptosuccinate. It is also described chemically as *S*-(1,2-dicarbethoxyethyl) *O*,*O*-dimethyl dithiophosphate and was previously identified as Experimental Insecticide 4049 or as malathon.

Chemically, the dithio nucleus of malathion is a deviation from most of the other organic phosphate insecticides. Preliminary reports on its toxicity (*48*) indicated that it was much less toxic to mammals than the materials then in use. It is a relatively weak cholinesterase inhibitor in vitro, but is relatively more toxic to insects than would be indicated by either its acute mammalian toxicity or in vitro cholinesterase inhibition. A summary of mammalian toxicity, including acute toxicity, dermal application, in vivo cholinesterase inhibition, inhalation, chronic oral ingestion, and pharmacology, has been presented by Hazleton and Holland (*45*). By comparison with previous inhibitors, malathion is relatively nontoxic by any of these routes. There were no toxic effects or anticholinesterase activity demonstrated by repeated exposure of animals to malathion vapors. Repeated exposure to malathion aerosol at 60 p.p.m. caused no toxicity other than local irritation and there was no effect on cholinesterase activity. At 5 p.p.m. in the air the aerosol produced no evidence of toxicity over a period of 4 weeks. At autopsy, microscopic examination revealed a slight irritation of the lungs.

Chronic feeding for 2 years at up to 5000 p.p.m. in the diet of male and female rats produced no gross effects except a slight growth retardation and decrease in food consumption at 5000 p.p.m. Cholinesterase levels of plasma, red cells, and brain were normal at 100 p.p.m., moderately depressed at 1000 p.p.m., and markedly depressed at 5000 p.p.m.

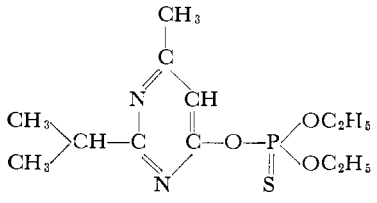
A colorimetric method for estimating malathion residues was developed by Norris, Vail, and Averell (*67*). This appears to be satisfactory for residues on all edible crops but not applicable to animal tissues, where added malathion cannot be recovered under conditions of the test (*46*).

On the basis of the relative safety of malathion under conditions of use, it became the first of the organic phosphate group to be permitted for home garden use. Recent papers by Gahan and coworkers (*31*) and by Guthrie and Baker (*38*) suggest the possibility of the use of malathion and other organic phosphates as insecticide baits, particularly for the control of DDT-resistant houseflies, and

this use may become important as sufficiently safe insecticides are developed.

Experience with both commercial and household application has to date been notably uneventful and it is to be expected that this progress will be followed by other organic phosphate insecticides exhibiting a reduced hazard all along the line.

Diazinon

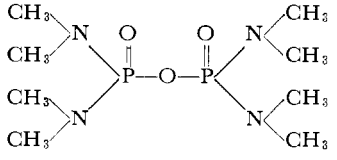


O,O-Diethyl-*O*-(2-isopropyl-4-methyl-pyrimidyl[6])thiophosphate

LD_{50}. 100 to 150 mg. per kg., rat, oral (*11*)
In_{50}. 1×10^{-5}, plasma, rat (*11*)
1×10^{-7}, red blood cells, rat (*11*)
1×10^{-6}, brain, rat (*11*)

A preliminary report on the toxicity of Diazinon has been published by Bruce, and coworkers (*11*). In acute and chronic feeding experiments Diazinon appears to be representative of the organic phosphate group but is less toxic than some of the early members. While still relatively new, it appears to offer no unusual hazard under conditions of use. Diazinon has also been found to be an effective bait for DDT-resistant flies.

Octamethyl Pyrophosphoramide



Octamethyl pyrophosphoramide

LD_{50}. 13.5 mg. per kg., male rat, oral (*30*)

Octamethyl pyrophosphoramide (OMPA or Shradan) was one of the first of the systemic insecticides. These agents enter the sap stream of plants and render the plants toxic to insects. The practical status of the systemics has recently been reviewed by Jeppson (*53*) and Wedding (*79*) as a part of a symposium on the subject.

OMPA is not a cholinesterase inhibitor in vitro, but is converted by the liver of mammals (*55*) and by various insect organs (*19*) to an active anticholinesterase agent. A very recent paper by Casida, Allen, and Stahmann (*14*) indicates that OMPA is enzymatically oxidized in vivo or chemically oxidized by permanganate at one of the amide nitrogens to produce an entirely new type of a functional group for which they proposed the name "phosphoramide *N*-oxide." In animals acutely lethal doses do not depress brain cholinesterase, but Frawley and coworkers (*30*) demonstrated more than 50% brain cholinesterase depression after subacute feeding at 100 p.p.m. Both plasma and erythrocyte activity are depressed after sublethal acute dosage, the maximum occurring in about 4 hours.

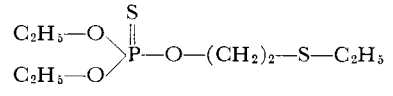
Aside from TEPP, OMPA has probably been more extensively studied in humans than any other member of the group, particularly in myasthenia gravis. Clinical evaluation is not pertinent to the present discussion, but Grob (*35*) points out longer duration and better endurance as advantages over Neostigmine, with slowness of action and weakness from overdosage being disadvantages. Schulman and coworkers (*74*) report good results with OMPA, but review the hazard of severe toxicity and death from overdosage. They arbitrarily set 30 mg. per day as the upper safe therapeutic dose.

The chronic toxicity of Shradan in rats has been studied by Barnes and Denz (*6*). At 50 p.p.m. in the diet for one year the male rats exhibited toxic signs and growth suppression during the early part, but both sexes were within normal range at term. At 10 p.p.m. there was no evidence of toxicity, and there was no pathology in either sex at either level. Cholinesterase activity of the brain was not markedly reduced, but that of the whole blood was. Additional studies confirmed the observation of Frawley and coworkers (*30*) that rat erythrocytes are especially sensitive to OMPA, showing some depression of cholinesterase activity at 1 p.p.m. in the diet.

Tusing and coworkers (*46*) found that in dogs daily doses of 1 mg. per kg. or above were severely toxic. Over a prolonged period a dose of 0.5 mg. per kg. per day inhibited red blood cell activity to practically zero and plasma to about 50%. There were no gross signs of toxicity observed, and no pathology. There is remarkable agreement between this dosage in dogs and Schulman's dosage (*74*) in humans when reduced to a milligram per kilogram basis. Repeated daily exposure of rats to concentrated vapors resulted in total inhibition of red blood cell cholinesterase, definite inhibition of plasma cholinesterase, and no inhibition in the brain.

Since federal regulations restrict the use of OMPA to cotton and ornamentals, the residue hazard is negligible. Fractional parts per million may be found in cottonseed oil, but at this level offer no hazard in view of the extensive investigations just reviewed.

Systox



At this time it is not practical to give the LD_{50} values for Systox without further qualification, as it is a mixture of isomers. This is further complicated by an apparent literature discrepancy as to the structure of the isomers. The isomer as above is referred to as Systox by Deichmann (*19*) and Martin and Miles (*62*), while Barnes and Denz (*6*) apply this term to the mixture and designate the above structure as the P = S isomer. They designate the second isomer as P = O, an exchange of S and O positions, while Deichmann (*19*) designates the second isomer as Iso Systox and replaces the O with S, in effect producing a dithio compound.

Based on the above terminology, the Barnes and Denz (*6*) data for the mixture are as follows:

LD_{50}. 4 mg. per kg., female rat, oral
10 mg. per kg., male rat, oral
In_{50}. 2.4×10^{-6}, plasma, rat
4×10^{-6}, brain, rat

In dietary feeding tests Barnes and Denz (*6*) found marked toxicity to female rats at 50 p.p.m. but no gross effects at 20 p.p.m., although cholinesterase activity in brain and whole blood was severely reduced at 16 weeks. There was no evidence of pathological change at any level.

Systox is another of the so-called "systemic" type insecticides and the evaluation of hazard is analogous to that for OMPA. Severe hazard can exist at the point of manufacture, formulation, and application, but need not if adequate industrial hygiene procedures are followed. At the consumer level control of residue level serves as protection against exposure and hence to hazard. As previously indicated, the systemics offer a great new challenge in crop protection.

While several other organic phosphate insecticides are being tested or used, those described above represent the principal classes. Two members of the chlorinated phosphate group are at present receiving considerable attention: Chlorothion, with a reported LD_{50} approximating that of malathion (*26*), and Bayer L 13/59 or Dipterex (*71*). Pending final evaluation, the near future may see a considerably greater interest in the chlorinated phosphates.

Summary

These brief reviews of the mechanism of action in general and the specific characteristics of individual members of the cholinesterase inhibitor class serve as the basis for certain justifiable generalizations.

Perhaps most important is that the manifest acute and subacute toxicity of the group is one of enzyme imbalance, from which recovery is complete with no residual tissue storage or pathology.

Protection against exposure can eliminate hazard during the handling of

the concentrated materials and formulations. Application of adequate industrial hygiene techniques has practically eliminated this hazard.

Negligence and carelessness still are responsible for occasional accidents at the point of application. Continued education appears to be the only answer to this problem, as the chemicals offer no greater hazard than other industrial and agricultural chemicals and the necessary information has been made available through research.

The nonresidual nature of the organic phosphates, together with intensive research by industry and the vigilance of regulatory authorities, combine to eliminate hazard at the consumer level.

Rarely in our history has so much been known about a class of chemicals and its individual members before they were put to constructive use. This enlightened position has been achieved through the medium of intensive research in our industrial, independent, governmental, and academic laboratories. The record is one of which all concerned may be justly proud.

Continued research promises a bright future for this class of pesticides. More specificity in pest control is to be expected; the systemically acting type opens new fields of investigation (the future of the carbamate type inhibitors remains to be determined), and it can be anticipated that as in the case of malathion a greater margin of safety will be achieved.

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ORGANOPHOSPHORUS INSECTICIDES

Dimethyl 2,2-Dichlorovinyl Phosphate (DDVP), an Organic Phosphorus Compound Highly Toxic to Insects

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The presence of traces of a highly toxic impurity in a technical grade of the new insecticide O,O-dimethyl 2,2,2-dichloro-1-hydroxyethyl phosphonate was observed. Determination of chlorine and phosphorus in isolated material indicated the impurity was a dehydrohalogenation product. It was found that O,O-dimethyl 2,2,2-dichloro-1-hydroxyethyl phosphonate could readily be dehydrohalogenated with alkali to a product having insecticidal properties equivalent to the impurity originally observed. Subsequent work has shown that dehydrohalogenation is accompanied by rearrangement to dimethyl 2,2-dichlorovinyl phosphate. The toxicity of the compound is about equivalent to parathion against houseflies but significantly less against rats.

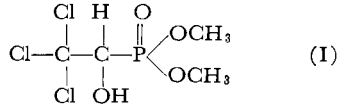
EXPERIMENTS on the toxicity of vapors of insecticidal compounds were conducted by passing air over the materials and exposing flies to the treated air. It was noticed that one of the organic phosphorus compounds under test gave unusually high fly mortality initially, but continued aeration of the sample produced no highly toxic vapors. This suggested that the initial effectiveness was due not to the compound itself but to a highly volatile impurity. These initial observations were made by W. F. Buren, V. A. Sedlak, and G. W. Pearce of this laboratory.

Analysis by a molybdenum blue colorimetric method showed a relatively high phosphorus content in the air initially, which decreased on continued aeration. The correlation between fly kill and phosphorus content, shown in Table I, indicates that the fly mortality rapidly becomes insignificant as the phosphorus concentration drops to 0.32 γ per liter of air and levels off. This behavior provided experimental evidence that a toxic impurity was present in the compound under test. The total amount of impurity present was calculated to be 0.1 to 0.2% based on the weight of material aerated and the total material in the air during the period of high mortality.

Table I. Correlation of Phosphorus Concentration in Air with Fly Mortality

Phosphorus, γ/Liter Air	Female Housefly Mortality, %
1.8	100
0.94	100
0.32	4
0.18	1.3
0.14	1.3

The material in which the highly toxic impurity was found, Bayer L 13/59 (Dipterex), is a commercial preparation of *O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate (I), whose structural formula is:



(I)

Isolation

On the basis of the foregoing evidence, efforts were made to isolate the toxic impurity in amounts large enough to identify. As no chemical method of analysis was available for tracing the impurity, bioassay techniques using houseflies were used throughout this work.

Because the unknown impurity was volatile, its recovery from air passed over relatively large quantities of *O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate appeared feasible. Accordingly, adsorption on Celite-545 and condensing in dry ice traps and solvent traps were attempted. Generally, the results were disappointing, in that the recoveries were low and the product recovered was still highly impure. However, microanalysis indicated that the material being sought probably had an atomic ratio of chlorine to phosphorus of less than 3 (*O,O*-dimethyl-2,2,2-trichloro-1-hydroxyethyl phosphonate has a theoretical ratio of 3).

Concentration of the active impurity by fractional crystallization was attempted; but although the impurity was concentrated, it could not be isolated in a sufficiently pure state.

The most successful concentration of the desired compound was accomplished by repeated washing of an ether solution with water, which removed most of the *O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate, leaving the highly