III. BIOLOGIC EFFECTS OF EXPOSURE

Parathion (O,O-diethyl O-p-nitrophenyl phosphorothioate) belongs to a family of organophosphorus (OP) compounds, members of which act directly or indirectly as cholinesterase (ChE) inhibitors. Direct inhibitors, such as paraoxon, are capable of reacting directly with the ChE enzymes thereby inactivating them. Parathion is called an indirect inhibitor because it must be converted in the environment or in vivo to the oxon (ie, active form) before it can effectively inhibit ChE's. Organophosphorus insecticide usage is increasing today as a result of the present restrictions against the use of DDT and related, persistent, chlorinated hydrocarbon insecticides.

Parathion is converted in the body in part to paraoxon, a strong inhibitor of the enzyme acetylcholinesterase.[1] Upon inhibition of this enzyme in the tissues, acetylcholine, the substance responsible for transmission of nerve impulses in much of the nervous system, accumulates, producing an initial overstimulation and subsequent blockage of nerve stimuli.[1]

Extent of Exposure

In the United States during 1970, approximately 15,259,000 pounds of parathion were produced.[2] Technical grade material is formulated for insecticidal application usually as 15% and 25% wettable powders, dust concentrates (20% to 25%) and ready-to-use dry dust mixtures (1% to 10%), granules (2% to 25%), or emulsifiable concentrates (2-8 pounds/gallon).[3,4] Parathion may be encountered as a relatively pure substance in the form of technical grade material, as a less-concentrated component of various formulations, such as described above, or as dilute sprays and dusts during field application. Even when parathion is contained in enclosed systems, potential exposures may occur from transfer of liquid, spillage, or from leaking equipment. Exposure may also occur during formulation, bagging operations, mixing , and application or by accidental or intentional contact with pesticidal preparations or incompletely emptied containers of formulations. Such accidental exposures have been particularly frequent among children less than 3 years old.

A number of occupations with potential exposure to parathion are listed in Table XVI-3.

Besides parathion, there are a number of other organophosphorus insecticides in use. In most cases, the newer compounds have a lower mammalian acute toxicity than parathion.[5] Partly as a consequence of the increasing use of competing insecticides, the amount of parathion manufactured in this country dropped from a high figure of 20,000,000 pounds (estimated) in 1968 to 15,259,000 in 1970.[2]

Koelle[1] stated that parathion has probably been responsible for more cases of accidental poisoning and death than any other OP pesticide compound.

The magnitude of poisoning by parathion from occupational exposures is more difficult to assess than that from accidental poisonings. A relationship between occupational poisonings due to pesticides and all other poisonings for the State of California can be obtained from California Department of Public Health Reports.[6,7] Under California law,[6] each physician who attends an injured employee must file a report, the Doctors' First Report of Work Injury, with the Division of Labor Statistics and Research in the California Department of Industrial Relations (State of California Labor Code, 1967, Section 6407). The employer also files a report, the Employer's Report of Industrial Injury. By definition, work injury includes occupational disease. The physicians' reports of occupational disease are reviewed and subsequently published by the California Department of Public Health in statistical report form. Agricultural workers, exclusive of self-employed persons and unpaid family labor, are covered by the California Workmen's Compensation Law and thus come under the reporting system.

In California during 1970, a total of 33,085 cases of occupationally-related diseases from all causes were reported.[7] Of these, 207 cases of systemic poisoning, or 0.63%, were due to OP pesticides and 55 (27%) were attributed to parathion.[6] Sprayers, pickers, and truck and tractor drivers were the occupational groups most affected. The report does not identify formulators as a category of occupational exposure. Irrigators and loaders were also frequently involved in poisoning by agricultural chemicals. The data from California essentially agree with those reported by Hatcher and Wiseman[8] and Tabershaw and Cooper,[9] indicating that the greatest opportunities for occupational exposure to parathion exist during formulation and packaging operations, application equipment loading operations, and aerial and ground applications. In addition to the 55 reported cases of systemic parathion poisoning, 3 cases

complained of respiratory conditions, 3 of skin conditions, 3 of eye conditions, and 31 unspecified, for a total of 95 cases of occupational illness due to formulations of parathion.[6] Of these 95 cases, 77 were in agriculture, 8 in manufacturing, 5 in transportation, communication, and utilities, 4 occurred in state and local government operations, and 1 in trade.

The report of Hatcher and Wiseman[8] is an example of an effort to identify parathion poisoning in a heavily agricultural area and the resulting documentation of a large number of cases. In the lower Rio Grande Valley in Texas during 1968, 118 cases of OP pesticide poisoning were reported. Ninety of these, or 78.2%, involved exposure to parathion. Of the 118 cases of OP pesticide poisoning, 97 were the result of dermal exposure, 20 occurred by the respiratory route, and only one case, a suicide attempt, was by ingestion. These cases occurred during a year when parathion was used more extensively than before in an effort to overcome resistance by some of the major cotton pests. The authors concluded that the workmen occupationally exposed either did not know or failed to appreciate the increased hazards associated with dermal exposure to parathion, and used only the precautions they had used in previous years when handling methyl parathion alone. The majority of acute organophosphorus poisonings occurred in men employed in aerial application, almost 89.5% of those affected being ground personnel who assisted in loading the planes with spray materials and fuel, row flagging, loading, unloading and opening drums of undiluted material, or unloading and cleaning aircraft. No deaths resulted from the acute OP pesticide intoxications documented in this study.

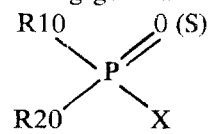
Exposure of field workers to pesticide residues on the foliage of crops has recently become a matter of national concern (*Federal Register* 38:10715-17, May 1, 1973). During the years 1959-1963 in California, 369 cases of poisoning attributed to parathion residues were reported[10,11] in field laborers involved in picking oranges, grapefruit, olives, and peaches. At least 3 severe episodes of OP insecticide residue poisoning in orchard workers occurred in California during 1970.[12] Each of the 3 orchards involved had been treated about 2 weeks prior to crew entry with one or more of a combination of ethion, Guthion, Delnav, or parathion. Fourteen of 35 crew members were hospitalized after picking oranges in a grove treated with parathion and Delnav nearly 5 weeks and 4 months earlier, respectively. In another episode, 10 workers experienced nausea, vomiting, and diarrhea while working in an orange grove previously treated (5 weeks) with a mixture of parathion and malathion. Quinby and Lemmon[13] reported parathion residue poisonings in California, Washington, and British Columbia.

Summaries of pertinent state pesticide regulations are presented in Appendices VI and VII.

NIOSH estimates that approximately 250,000 workers (including field workers exposed to residues) in the US are potentially exposed to parathion.

Historical Reports

According to Holmstedt[14], the first indications that OP compounds might be highly toxic appeared during the early 1930's when Willy Lange and Gerda von Krueger experienced symptoms while synthesizing dimethyl and diethyl phosphorofluoridate. As early as 1934, Otto Bayer of the I. G. Farbenindustrie appointed Gerhard Schrader to investigate synthetic insecticides. By 1936, Schrader had turned his attention to organophosphorus compounds and, in 1937, he patented the following general formula for contact



insecticides.[14] Parathion (E605) and paraoxon (E600) came out of Schrader's laboratory in 1944.[14] Much of the information about Schrader's work was disseminated outside Germany at the end of World War II when British Intelligence published information about parathion in 1947 in the BIOS reports, as cited by Metcalf and March.[15] Limited production for agricultural use was begun in this country in 1948.[16] Cases of parathion poisoning were reported soon thereafter. In 1949, Grob et al[17] reported that a man employed as a mixer of liquid parathion and clay powder had died after repeated exposures to the insecticide. The following year, Grob et al[18] described the toxic effects of parathion in 32 men and 8 women following accidental exposures. Since that time, parathion has been responsible for many occupational poisonings as documented, for example, by the California Department of Public Health [6,10,12] during the ensuing years.

Effects on Humans

Table III-3 summarizes the experimental and epidemiologic effects noted in humans exposed to

TABLE III-1

SIGNS AND SYMPTOMS ASSOCIATED WITH ACUTE AND SUBACUTE EXPOSURES TO PARATHION

Effector Organ	Sign or Symptom
1. MUSCARINIC manifestations	
(a) Gastrointestinal	Anorexia; nausea; vomiting; abdominal cramps; diarrhea; tenesmus; involuntary defecation; eructation; "heartburn"; substernal pressure
(b) Sweat glands	Increased sweating
(c) Salivary glands	Increased salivation
(d) Lacrimal (tear) glands	Increased lacrimation
(e) Cardiovascular system	Bradycardia, fall in blood pressure
(f) Bronchial tree	Tightness in chest; wheezing suggestive of broncho-constriction; dyspnea; cough; increased bronchial secretion; pulmonary edema
(g) Pupils	Pinpoint (miosis) and nonreactive
(h) Ciliary body	Blurring of vision
(i) Bladder	Increased urinary frequency; involuntary urination
2. NICOTINIC manifestations	
(a) Striated muscle	Muscular twitching; fasciculation; cramping; weakness (including muscles of respiration)
(b) Sympathetic ganglia and adrenals	Pallor; tachycardia; elevation of blood pressure
3. CENTRAL NERVOUS SYSTEM manifestations	Uneasiness; restlessness; anxiety; tremulousness; tension; apathy; giddiness; withdrawal and depression; headache; sensation of "floating"; insomnia with excessive dreaming (nightmares); ataxia; slurred, slow speech with repetition; drowsiness; difficulty in concentrating; confusion; emotional lability; coma with absence of reflexes; Cheyne-Stokes respirations; convulsions; hyperpyrexia; depression of respiratory and circulatory centers (with dyspnea and fall in blood pressure)

Derived from 18,52

parathion. Evaluative or qualifying information on each study is included in the more complete description of the study as reported in the text.

(a) Physiology

The actions of such organophosphorus compounds as parathion depend upon the enzymes which they inhibit and the physiologic effects of such enzyme inhibition. These enzymes catalyze the hydrolysis of acetylcholine and other choline esters. In 1932, Stedman and his coworkers[19] suggested the term "choline-esterase" (sic) for the enzyme which is present in serum. Of the choline esters, the only one with demonstrated physiologic importance to man is acetylcholine, the substance which mediates the transmission of nerve impulses to the heart and to other parasympathetically innervated structures, including the iris, the salivary glands, the stomach and small intestine, the urinary bladder, the bronchial glands, and a few postganglionic sympathetic fibers, such as those to the eccrine sweat glands.[1] Acetylcholine has been shown to have a transmitter function also in 3 additional classes of nerves: the preganglionic fibers

of both the sympathetic and parasympathetic systems, motor nerves to skeletal muscles, and certain neurons within the central nervous system.[1]

In man, there are two principal types of enzymes which hydrolyze choline esters: (1) acetylcholinesterase (AChE), or true cholinesterase, and (2) butyrocholinesterase (BuChE), frequently called plasma cholinesterase, serum cholinesterase, or pseudocholinesterase.[1] Acetylcholinesterase occurs in neurons, at the neuromuscular junction, in erythrocytes, and in certain other tissues.[1] Practically all of the pharmacologic effects of the anti-cholinesterase agents, including those of parathion, are due to the inhibition of AChE, with the subsequent accumulation of endogenous acetylcholine.[1]

Throughout the remainder of the document, the designation ChE is used interchangeably with the word cholinesterase. In all instances where ChE appears, it will be preceded or followed by either RBC, designating the erythrocyte enzyme, or plasma (or both), or blood, or whole blood in order to clearly indicate which cholinesterase(s) is/are being referred to. In a similar vein, the designation RBC is used interchangeably with erythrocyte.

BuChE is present in various types of glial or satellite cells of the central and peripheral nervous systems, as well as in the plasma, liver, and other organs.[1] It has no known physiologic function; inhibition of the plasma enzyme at most sites produces no apparent functional derangement.[1] Lehmann and Liddell[20] speculated that the plasma ChE may hydrolyze those cholinesters which inhibit acetylcholinesterase. These include propionylcholine and butyrylcholine, which can be formed in vitro by enzyme systems responsible for the synthesis of acetylcholine and may be produced also by bacterial action in the gut. There are a number of atypical plasma ChE's, discovered through an investigation of abnormal responses to the muscle relaxant, succinylcholine, which appear to be genetically controlled variants with differing abilities to hydrolyze acetylcholine and related compounds.[20,21]

Parathion has only a slight direct inhibitory action on plasma and RBC ChE's but its active metabolite, paraoxon, is a potent inhibitor of these enzymes.[22-25] The resultant phosphorylated enzyme is stable, so that hydrolysis leading to reactivation of the enzyme occurs slowly. Hydrolysis is limited, however, by another spontaneous reaction, aging, which leads to a stable phosphorylated RBC ChE, refractory to spontaneous or induced hydrolysis.[1] Aging of the phosphorylated enzyme has been attributed to a mono dealkylation of the phosphoryl or phosphonyl moiety, resulting in a change in the electronic charge of the phosphorus atom such that it can no longer be approached by the hydroxyl ion of water.[26]

Regeneration of non-aged phosphorylated AChE is accelerated by nucleophilic reactivators, such as choline, pyridine, hydroxylamine, hydroxamic acids, and oximes.[27] In 1951, Wilson[28] reported that choline and hydroxylamine reactivated diethyl phosphorylated-AChE considerably faster than water alone. Childs et al[29] found that the oximes were generally superior to the hydroxamic acids in reactivating OP-inhibited ChE. Wilson[30-32] synthesized and tested several monoquaternary pyridine aldoximes. Subsequently,[33] pyridine-2-aldoxime (2-PAM; pralidoxime) methochloride was found to be highly effective in the reactivation of non-aged, inhibited RBC and neuroeffector ChE's.

(b) Absorption

Parathion is absorbed through the gastrointestinal tract,[34-36] the respiratory tract,[37,38] and the skin, the mucous membranes, and the eyes.[11,16,39-41] Gleason et al[42] pointed out that the response sequence and the interval between exposure and response are partly dependent upon the portal of entry. Respiratory tract symptoms usually appear first during a respiratory exposure [43] whereas the presenting symptoms are more likely to be gastrointestinal following ingestion.[16] As shown by experimental results,[5] parathion (equivalent dose basis) is less toxic by the dermal route than by ingestion. Holmstedt[43] speculated that this may be due to enzymes in the skin causing hydrolysis of parathion, much in the same way that paraoxon is detoxified partially during passage through the skin in man, rabbit, and cat as reported by Fredriksson et al.[44] However, the latter investigators[44] were unable to show that significant metabolism of parathion takes place in dermal tissue; it was absorbed in essentially unchanged form. Passage of parathion through human skin was shown to be relatively slow (0.001 μg/min/sq cm in vitro), [45] and consequently a dermal exposure may result in a substantial as well as a prolonged period of absorption. However, although dermal absorption of parathion is a relatively slow process, the compound is neither irritant nor caustic in nature[39] and therefore provides no warning to the individual that his skin has been contaminated with parathion. Thus, dermal absorption leading to signs and symptoms of poisoning may occur without any awareness on the part of the exposed

individual. In actuality, dermal absorption has been shown to be a potentially greater hazard than respiratory absorption for parathion applicators.[46]

In controlled studies, Durham et al[46] evaluated the comparative dermal and respiratory exposure of workers subjected to the mist from an airblast spray machine during parathion application in orchards. Three test subjects were about equally exposed to the parathion spray drift by transport in a vehicle following the spray machine at such a distance that the spray mist that came into contact with their bodies had to be fine enough to remain suspended in the air for a comparatively long period. The fine mist encountered approximated that to which the operator of the spray rig was exposed. One individual was completely covered with rubber and plastic clothing to prevent skin contamination. He wore no respirator and thus had a respiratory exposure. A second worker wore a respirator and breathed only noncontaminated air; however, he wore ordinary clothing and thus his exposure was dermal only. The third person had neither respiratory nor dermal protection, other than ordinary clothing. All three subjects wore absorbent pads to provide a measure of their surface exposures to airborne parathion. Analysis of the absorption pads worn by the subjects confirmed that all were subjected to parathion of the same order of magnitude (approximately 0.03 mg/sq in. for the 2 "protected" workers). However, total p-nitrophenol (PNP) excretion for the man wearing the rubber and plastic clothing (respiratory exposure only) was 0.088 mg; for the individual using the pure air supply (dermal exposure only), 0.666 mg; and for the man using no special protective equipment (both respiratory and dermal exposure), 0.433 mg. Since parathion has been shown by Fredriksson[44] not to be hydrolyzed or transformed into paraoxon by the skin of man, the use of urinary PNP excretion appears to be a valid procedure for the relative measurement of parathion absorption. The results show that dermal absorption exceeded that by the respiratory route by several times, clearly indicating the potential danger from dermal exposure to parathion.

Dermal absorption of parathion may be increased by the solvent used. Absorption of parathion from the skin of the forearm was approximately tripled by its application in the known irritant xylol above that measured when it was applied in acetone.[47]

(c) Metabolism

The observation by Diggle and Gage[22] that parathion in a pure state was a poor inhibitor of rat brain ChE in vitro led to the conclusion that metabolic conversion was necessary. Incubation of parathion with liver slices did produce the active inhibitor, O,O-diethyl-O-p-nitrophenyl phosphate (paraoxon).[24,48] Kubistova[24] demonstrated in vitro that enzymatic oxidation takes place also in the gut, lungs, kidneys, and suprarenal glands. Gardocki and Hazleton[49] found PNP to be the major nonphosphorus-containing end product of parathion metabolism in dogs, although traces of p-aminophenol were also found. PNP has been shown to be a major urinary metabolite of parathion in man.[50,51]

(d) Acute Effects

The acute effects of parathion are due largely to its ability to inhibit ChE's throughout the body.[1] As indicated in the preceding section, inhibition of these enzymes in animals (including man) leads to the accumulation of endogenously produced acetylcholine, with the resultant signs and symptoms in man set forth in Table III-1.[18,52] This table classifies man's response according to muscarinic, nicotinic, and central nervous system (CNS) responses. Muscarinic effects refer to the action of parathion on autonomic effector cells of the eyes, heart, lungs, stomach, blood vessels, and other organs.[1] Varying proportions of muscarinic receptors are also present on autonomic ganglion cells and on certain cortical and subcortical neurons.[1] The nicotinic actions of parathion refer to its effects (initial stimulation in high doses leading to subsequent blockade) on autonomic ganglion cells and the neuromuscular junction, actions comparable to those of nicotine.[1] Such a classification scheme is of some use in the rationale for treatment and diagnosis, since atropine, in the dosages normally used to treat parathion poisoning, blocks the muscarinic and CNS effects, but not the nicotinic effects.[1]

The frequently observed delay in onset of symptoms of poisoning after exposure to parathion is attributed to the requirement that parathion be metabolized in the body to paraoxon.[25] However, commercial preparations of parathion have been reported[22] to contain small amounts of the S-ethyl isomer which can produce localized effects at the site of contact. The sequence of symptoms depends on the route of entry of parathion preparations into the body and on their composition.[16,43]

Appearance (signs of) of poisoning with dermal exposure to parathion is delayed, the onset being insidious after a latent period of one or more hours.[9,17,52] Delayed absorption of parathion from material deposited on the skin or clothing can also occur over a period of several weeks or months. Kazen et al[53] found parathion on the hands of one

man 2 months after his last known contact with the insecticide. The authors found 38.8μg of parathion on the hands of another pesticide applicator 31 days after spraying. The delay of occurrence of systemic effects following dermal exposure to parathion may be attributable to two factors:

(1) the slow rate of absorption through the skin[45];

(2) contaminating paraoxon may be partially detoxified during passage through the skin.[44]

Although the acute lethal dose for man is unknown, the results of animal experiments, as presented in the Animal Toxicity section, demonstrate the extreme toxicity of parathion to mammals.

Many occupational accidents involving parathion have occurred through the dermal route.[6,8,9,18] Tabershaw and Cooper[9] presented the case histories of several workers who developed signs and symptoms (eg, nausea, vomiting, weakness, blurring of vision) of OP insecticide poisoning while picking citrus fruits in orchards previously sprayed with parathion. In some cases, the insecticide had been applied as long as 25 days before worker entry, thus implicating the dermal route of exposure. However, it is difficult to rule out completely the possibility of concurrent respiratory and oral intake.

Hartwell et al[37] exposed human volunteers to vapor or particulates of parathion generated by either heating parathion dust or technical grade parathion or by spraying technical grade parathion into a chamber. No air sampling was performed. Urinary PNP excretion was determined from immediately prior to exposure until 40 hours after exposure. Both RBC and plasma ChE activities were determined. Neither the 2% dust heated to 82°F and 120°F nor the technical grade heated to 82°F and 105°F produced depressions exceeding 30% of preexposure values in either RBC or plasma ChE activity levels. Also, urinary PNP excretion was minimal in these exposures. However, a subject exposed to parathion accidentally heated to 150°F experienced signs of poisoning, a decline in RBC and plasma ChE activities to 2% and 12% of normal, respectively, and the excretion of large quantities of PNP (approximately 6 mg in 7 days).

In one Texas episode,[8] a group of field workers entered a cotton field containing plants about 3½ feet tall approximately 12 hours after an aerial application of a mixture containing parathion and methyl parathion. After working around the dew-laden plants for 2½-3 hours, 23 of the workers became ill, exhibiting signs and symptoms of organophosphorus insecticide poisoning. No indication of the dosage received was provided. Because of the extremely low vapor pressures of the pesticides,[54-56] the time of entry into the treated field, the dew-laden nature of the foliage, and the necessity of worker-plant surface contact, these acute poisoning cases were probably due primarily to dermal absorption of the insecticides.

(e) Chronic Effects

Another hazard to workers relates to repeated exposures to small quantities of parathion, such that during a period of time the cumulative effect on tissue AChE may lead to toxic effects. Parathion itself, or its metabolic oxidation product paraoxon, does not cumulate to a significant degree in the body as evidenced by urinary PNP excretion.[50,51] However, the effects of repeated small doses do become cumulative if replacement of AChE at its sites in tissues does not keep pace with the extent of inhibition of the enzyme.[57]

In an effort to determine, on the basis of daily ingestion, the amount of parathion capable of producing minimal toxicity, Rider et al[34] exposed groups of five human volunteers to daily oral doses of either 3.0 mg, 4.5 mg, 6.0 mg, or 7.5 mg for periods approximating 30 consecutive days. Each group contained 2 control subjects who received only corn oil. The groups exposed to 3.0 mg and 4.5 mg showed no changes from baseline ChE levels. The group at the 6.0-mg level sustained a slight (unspecified) depression of plasma ChE. The effects of 7.5 mg/day were such that by day 16 the plasma ChE levels of 2 subjects had decreased to 50% and 52% of pretest levels, respectively, at which point administration of parathion was discontinued; by day 23, the plasma ChE activity of another test subject had dropped to 54% of his pretest level, at which point his participation in the study was ended. The remaining 2 subjects continued throughout the 35-day test period. Their lowest plasma ChE values were 86% and 78% of pretest values, respectively. The lowest RBC ChE activity levels obtained during the study for the 3 subjects to whom the administration of parathion was discontinued were 63%, 78%, and 86% of pretest levels, respectively. The 2 subjects who completed the test period experienced no significant reduction of RBC ChE activity. The plasma ChE was affected to a greater extent than the RBC ChE. The investigators considered that average depressions of the ChE activities in blood of 20-25% below control values were significant. The results demonstrated that the daily ingestion of 7.5 mg of parathion by man during 35 days led to a

significant reduction in blood ChE activities and thus constitutes an unsafe ingestion level. Because of the incompleteness of the data on the 6.0-mg daily dose, NIOSH concludes that only the 4.5-mg daily dose can be regarded as the maximum safe dose for parathion tested.

In another study, Edson[35] found that an oral dose of 7.2 mg parathion/day, 5 days/week, for 6 weeks produced a 33% decrease in whole blood ChE activity (16% and 37% for RBC and plasma ChE, respectively) in 4 adult female volunteers. This corresponded to a daily oral intake of 0.078 mg/kg. No significant effects on the activities of ChE's in blood were observed as a result of the daily oral ingestion by groups of 4 subjects of either sex of 0.6, 1.2, 2.4, or 4.8 mg of parathion for periods ranging from 25 to 70 days. The results showed that a safe no-effect daily oral dose of parathion in humans was smaller than 0.078 mg/kg and greater than 0.058 mg/kg.

In 1958, Rider et al[36] published the results of similar studies which demonstrated that the daily ingestion of 0.05 mg/kg/day of parathion by humans produced no significant decrease in plasma or RBC ChE activities and thus constituted a safe ingestion level.

Kay et al[58] studied the effects of exposure to parathion sprays on the ChE activities of the blood of Quebec apple-growers. Airborne parathion concentrations were determined, using impingers and fritted glass bubblers in series, both during and after spraying of 15% wettable powder (W/w) in concentrations of 0.75 to 1.5 lb/100 gal of water and dispersed at the rate of 300-400 gallons/acre. The orchards were sprayed from early May through June. Measurements were made in 4 orchards during approximately 4 weeks, beginning on May 28th. Both hand-held and mechanical sprayers were used. Air samples taken from the breathing zones of the operators ranged from 2 mg to 15 mg/m^3 of air, reflecting downwind versus upwind (with heavy "blow back" and resultant high exposure) spraying. The sprayers were exposed for approximately 2 days at 10-day intervals during a 2-month period. Personal protective measures, such as coveralls, rubber boots, caps, and fabric mitts, were used by some workers. Respirators were indifferently used by approximately one-third of the group. Both RBC and plasma ChE activities were determined by a modified Michel technique. Blood samples were taken thrice during the spraying period and singly during the first and fourth months following the termination of exposure, for use as control values. Depression of RBC ChE activity at the end of exposure averaged 21% for all sprayers, 27% for those reporting symptoms, and 17% for those reporting no symptoms, the difference betweeen the depressions in the symptomatic and the symptom-free groups being insignificant. The results for plasma ChE, however, presented a different picture. In the group reporting symptoms, the plasma ChE activity was 20% lower than in the group reporting no symptoms. Using the average control value for all subjects determined approximately 3 months following exposure, the plasma enzyme activities in the groups reporting symptoms and no symptoms were depressed 22% and 3.5%, respectively. The authors reported that approximately one-half the exposed workers reported no symptoms of ill-health. The symptoms mentioned by the remaining half included nausea, headaches, and other non-specific symptoms on one or more occasions, with a few cases of confining illness of short duration. Two major points must be emphasized concerning these results. Firstly, the exposure to parathion was intermittent allowing time for partial return of blood ChE activity. Regeneration and replacement of enzyme activity between exposures undoubtedly account for the fact that depressions were not more severe in light of the magnitude of the airborne exposure to parathion. Secondly, little emphasis can be placed on the association between the occurrence of the reported non-specific symptoms and the levels of depression of RBC and plasma ChE activities due to the inability to calculate incidence rates from the data. Hayes et al[59] found that part-time OP insecticide applicators experienced nausea no more frequently than did controls (5% vs 4%) and headache less often (5% vs 19%).

CNS effects of chronic exposure to parathion and similar compounds have been reported.[60-63] Gershon and Shaw[61] reported the effects on the CNS of 16 workers "chronically exposed" to OP insecticides, including parathion. Of the 16 cases mentioned, only 4 case reports were presented by the authors. In one case involving parathion exposure, the individual exhibited severe depression, nightmares, impairment of concentration and memory, headache, and irritability. A schizophrenic reaction including auditory hallucinations was observed in a second parathion-exposed worker. Nausea and vomiting, muscle cramps, and dizziness also occurred in both. Parathion exposure levels were not reported. In both of these cases, after cessation of exposure for several months, these effects disappeared and the patients were feeling well. However, it must be emphasized that both of these cases involved expo-

sure to parathion and other ChE-inhibiting insecticides. Other investigators[64-66] have presented reasons, and data, to doubt the conclusion of Gershon and Shaw that the psychoses studied by them were consequences of exposure to OP compounds.

Mixed exposures to pesticides are common in agriculture, a situation which complicates distinguishing definite, specific biologic responses to exposures to individual compounds. In a similar vein, Davignon et al[62] reported on the chronic effects of insecticides, including parathion, in man. Signs of possible chronic intoxication due to insecticides were sought among 441 apple-growers. Because the growers had been exposed at various times to undetermined quantities of malathion, parathion, azinphosmethyl, carbophenothion, DDT, endrin, carbaryl, and other insecticides, the observed increased incidences of leukopenia and neurologic abnormalities, including weakening or loss of reflexes and disturbances in equilibrium, could not be attributed securely to parathion.

Durham et al,[63] in a study of 53 persons exposed to OP insecticides to determine whether or not mental effects may precede or take place in the complete absence of other more disabling signs or symptoms of OP poisoning, found no mental effects at levels where other clinical symptoms were not also present. In one case, an aircraft loader was exposed to undetermined amounts of parathion, tetraethylpyrophosphate, demeton, and mevinphos at various times for approximately 6 weeks. Sometime after the third week, he frequently became dizzy, noticed a slowing of his driving reactions, and complained of a loss of sense of timing. Prior to the appearance of these effects, his blood ChE activity was shown to be severely depressed (RBC and plasma ChE activities were reported as 0.10 and 0.27ΔpH/hr, respectively). In addition, other more common signs of poisoning, such as muscular twitching (eyelids), nausea, and anorexia, had preceded the mental effects. Mental confusion and hallucinations occurred in another worker after 6 weeks of spraying trees with parathion using a hand-held sprayer. Headache, nausea, and paresthesia preceded the mental effects, which included confusion, hallucinations, and amnesia. None of the so-called mental effects persisted after termination of exposure.

There is an indication that electromyography (EMG) can detect changes in neuromuscular function in parathion-exposed workers who show no evident effect from their exposure.[67,68] These changes were present in test subjects exposed to a variety of pesticides even when there was no measurable decrease in blood ChE activity or adverse changes as indicated by routine physical examination.[67] EMG may prove to be a sensitive method to provide an early warning of exposure to parathion as well as other OP pesticides.

Some attention has been given to the possibility that parathion may produce the type of paralysis reported as a consequence of exposure to TOCP (tri-o-cresyl phosphate), DFP, and mipafox (N,N'-diisopropylphosphorodiamidic fluoride).[69] Bidstrup et al[69] stated that according to their observations the noted effects of mipafox closely resembled those of "ginger" paralysis caused by the drinking of extract of Jamaica ginger contaminated with TOCP. Although a case report[70] of suspected parathion-produced delayed paralysis was published in 1950, it does not clearly implicate parathion as a cause of permanent neuromuscular damage. Petry[70] reported that delayed polyneuritis developed in a worker who had sprayed a dilute solution of parathion within closed hothouses during a 4-week period. Subsequent to each of 10-12 applications, the man experienced nausea, an urge to vomit, and anorexia. Approximately 50 days following the last spraying, during which time he became ill with severe gastritis and was hospitalized, he noticed a feeling of tingling, numbness, and weakness in his legs which ultimately progressed to a permanent paralysis of the peroneus muscles. However, this appears to be an isolated human case and delayed adverse neuromuscular effects, such as paralysis, have not been shown to be a usual effect of parathion exposure, either acute or chronic.

The question of variability of individual susceptibility to parathion poisoning has not been studied fully. Experimental data[71] indicate strongly that both plasma and RBC ChE may serve as "buffers", protecting functional AChE from inhibition by DFP. Although few in number, there are in the general population people who have a genetic plasma ChE variant[20,21] which, in the heterozygous or abnormal homozygous state, may serve less well as a buffer than the normal enzyme. However, Tabershaw and Cooper[9] in studying the case histories of 108 individuals poisoned by parathion or other OP pesticides found that 5 persons with the heterozygous variant were not more severely poisoned than those with the normal enzyme. People heterozygous for this variant occur in the normal population to the extent of 37/1,000, while the frequency of the atypical homozygote has been

estimated at 1/2820 of the population.[21] Depression of liver function by hepatotoxic materials can result in lower plasma ChE.[72] Low plasma levels are also seen in malnutrition, chronic debilitating disease, acute infectious disease, and anemia.[72-74] Drugs which have been shown to decrease plasma ChE activity include physostigmine and related compounds,[75] Vitamin K,[72] folic acid,[72] tetraethylammonium chloride,[72] quinine and a number of other antimalarial drugs,[76] morphine, codeine, and related analgesics.[77]

The rate of return of plasma ChE activity to its normal value in man following depression by parathion has been shown to be approximately 3-4%/day.[18] In a study of 18 subjects whose plasma and RBC ChE had been depressed by parathion, the plasma enzyme increased at an average rate of approximately 9% of normal activity during each of the first 3 days. This rate decreased to 5% by the fourth day and to 3% by the tenth day, after which it remained steady. During the first 3 days following depression, the RBC enzyme activity increased at an average rate of approximately 3.3% of normal activity. This rate diminished to between 1-2%/day by the fourth day and remained relatively constant thereafter (RBC ChE activity increased about 90% in 70 days).

Epidemiologic Studies

Epidemiologic studies of parathion-exposed worker populations have been directed at identifying the types of workers at greatest risk, the environmental conditions associated most frequently with poisoning, and the biologic results of prolonged exposure in an effort to determine whether or not chronic effects occur. One study[50] involving exposure to parathion indicated that mixing-plant personnel, commercial ground applicators, aircraft application workers, and orchard workers were the groups at greatest risk, while field men and warehousemen were at lesser risk.

In 1976, Maddy and Peoples[78] reported on occupational illnesses due to exposure to pesticides or their residues in California during 1973-75. Under the category of systemic illness the 5 occupations experiencing the greatest problems in those years were: (1) ground applicators—96 cases; (2) mixers and/or loaders—74 cases; (3) indoor workers exposed to pesticides—50 cases; (4) formulation plant workers—41 cases; and (5) firemen exposed to pesticide fires—37 cases. Field workers exposed to pesticide residues was the number 6 category, with 28 reported systemic illnesses.

The remaining 18 categories accounted for only 39% of the reported occupational illnesses due to pesticides. These data indicate the more hazardous occupations in the pesticide "industry." This report by Maddy and Peoples[78] encompasses all pesticides and is not specific to parathion.

In 1950, Brown and Bush[79] reported a study of workers in an industrial plant manufacturing both concentrated parathion and a dust formulation containing parathion. Air samples were taken at 8 locations throughout the plant; 2 samples were taken in the breathing zone of workers and the others were general atmosphere samples. Blood ChE (RBC and plasma) activity measurements were performed on 12 workers engaged in various jobs during a 6-month period. The concentration of parathion in the air varied from 0.1 to 0.8 mg/m^3, with a mean exposure concentration of about 0.2-0.3 mg/m^3. Details of times of sampling were not given. A greater than 30% depression in either or both plasma and RBC ChE activity was observed in 5 exposed workers from whom successive blood samples were taken during a 6-month production period. The authors reported that the plasma enzyme activity was depressed more than that of the RBC's. However, based on blood ChE activity levels measured 5 months following the cessation of parathion manufacture (ie, using these values as control levels) RBC enzyme activity was depressed to a greater extent than that of the plasma in these 5 workers during the exposure phase. Since the data do not allow for correlation of the ChE activity of whole blood with airborne concentrations of parathion on a precise basis, the only reasonable conclusion from the results is that continuous exposure to an airborne concentration of parathion above 0.2 mg/m^3 can result in a reduction in blood ChE activity. The data do not identify a safe exposure level. No mention was made of percutaneous parathion absorption.

Arterberry and associates[50] used measurements of both blood ChE and urinary excretion of PNP to identify occupations with high exposures to parathion. These biologic indicators were used to determine the extent of exposure to parathion by workers performing a variety of jobs. The study groups included mixing plant personnel, commercial ground applicators, part-time ground applicators, aircraft application workers, workers in orchards (especially thinners), field men, warehousemen, miscellaneous workers, and residents living near orchards. Only the mixing plant workers showed a definite decrease in ChE activity within the blood, limited to ChE activity in the RBC's (36% decline). PNP excretion was greatest

in the commercial ground applicator group and decreased in the following order: part-time ground applicators, mixing plant personnel, aircraft application workers, and workers in orchards (especially thinners). The PNP excretion of the field men and warehousemen was less than half that of the orchard workers. The study demonstrated that mixing plant personnel, ground applicators (commercial and part-time), aircraft application workers, and orchard workers were the groups at greatest risk, while field men and warehousemen were at lesser risk.

Quinby and Lemmon[13] called attention to the residue of parathion on the foliage of trees and vines as a source of poisoning. They investigated 11 episodes of group poisoning involving more than 70 persons from pesticide residues on the surfaces of plants. Illness was confirmed by low blood ChE values and relief of symptoms by atropine. One episode involving 16 cases occurred 33 days after spraying. Two days after the outbreak of poisoning, residue analysis showed that the leaves contained 8 ppm of parathion.

Milby and coworkers[11] studied an outbreak of parathion poisoning in 186 peach orchard workers. After elimination of the spraying-picking interval by matching workers in the various orchards on the basis of similar mean intervals, illness developed in orchards that received an average of 7.14 pounds/acre of parathion but did not develop in those that received an average of 4.99 pounds/acre. However, Milby et al,[11] utilizing breathing zone air samples and skin washes to measure the potential inhalation and dermal exposures, calculated that the maximal daily dose of parathion with which a worker could have come into contact was not in excess of 4 mg. This value was derived in the following manner: the ingestion of 4 peaches/day with parathion residue levels of 125μg/peach; inhalation of 350μg parathion/day based on the highest breathing zone concentration found in the study, 35μg/m^3, and a breathing rate of 10 m^3/day; and a calculated daily dermal exposure of around 3,000μg based on multiplying the results of skin-rinse analyses by skin surface areas. They concluded that insufficient parathion was present on the surface of trees to produce illness in the orchard workers, and that some unmeasured, yet active anticholinesterase agent, probably paraoxon, also must have been present. Supporting this conclusion was the fact that one sample of leaves analyzed for paraoxon indicated the presence of 3.0 ppm paraoxon and 2.8 ppm parathion.

Animal Toxicity

Table III-4 summarizes animal data on the toxicity of parathion by various routes of administration. Evaluative or qualifying information on each study is included in the more complete description of the study as reported in the text.

(a) Acute Effects

LD50 data for various species are presented in Table III-2.

Because there is a significant sex difference in response to parathion in rats,[5,80] separate values are provided for males and females. Such marked sex differences were not seen in other species.[5,80,81]

(b) Subacute Studies

A cumulative toxic action of parathion in female rats was shown by DuBois et al.[80] After daily intraperitoneal doses of 3 mg/kg of parathion, none of 15 test animals survived more than five doses of the insecticide. Daily intraperitoneal doses of 1 and 2 mg/kg of parathion for 10 days resulted in 46% and 87% mortality, respectively. None of 5

TABLE III-2

LD50 VALUES OF PARATHION
(mg/kg)

	IV [80]	IP [80]	Oral [5]	Dermal [5]
Rats				
Male	—	7	13	21
Female	—	4	3.6	6.8
Mice	—	9-10	—	—
Cats	3-5	—	—	—
Dogs	12-20	—	—	—

TABLE III-3

EFFECTS ON HUMANS FROM PARATHION EXPOSURE

Route(s) of Exposure	Number of Subjects Exposed	Exposure Concentration and Duration	Effects	Reference
Respiratory	1	2% parathion dust (PD) heated to 82°F for 30 min. on day 1; 2% PD heated to 120°F for 30 min. on days 2 and 3; 1 ml tech parathion (TP) heated to 82°F for 30 min. on day 6; 1 ml TP heated to 105°F for 30 min. on day 8; 1 ml TP heated to 120°F for 30 min. on days 10-12 (in all exposures breathing zone concentrations of parathion were unknown).	No signs or symptoms of parathion poisoning observed. At 82°F, lowest RBC ChE activity was 80% of pre-exposure value. Insignificant plasma ChE inhibition. No depression of ChE activities at 105°F. At 120°F, lowest RBC and plasma blood ChE activities were 63% and 66% of pre-exposure values, respectively.	[37]
"	1	First 3 exposures listed for above subject.	No signs or symptoms of parathion poisoning observed. At 82°F, lowest RBC ChE activity was 85% of pre-exposure value; insignificant inhibition of plasma ChE. At 120°F, lowest RBC and plasma ChE activities were 78% and 89% of preexposure values, respectively.	[37]
"	1	1 ml TP heated to 105-115°F for 30 min. on 4 consecutive days; 5 ml TP heated to 150°F for 10 min. on 5th day.	No clinical signs of poisoning either during or following the first 4 exposures; after 10 min. at 150°F (accidental), the subject developed unspecified signs of parathion poisoning.	[37]
Dermal and respiratory	1 female and 32 male adults (39 nonexposed controls)	A "few days" (unspecified) exposure during each 10-day spraying interval over a 2-month period to airborne concentrations of parathion ranging from 2 to 15 mg/cu m (during orchard-spraying operations).	Signs and symptoms, including headaches and nausea, were reported by about one-half of the exposed group. Average RBC and plasma ChE activities were 21% and 13% lower at the end of the spray period than they were about 4 months later. The plasma ChE activity in the group with symptoms was 20% lower than in the symptom-free group.	[58]
"	1 male	Exposed to a 1:10,000 spray of parathion 10-12 times during a 1-month period (during hothouse spraying operations).	Subject experienced nausea and vomiting several hours after each spraying; was hospitalized about 2 months later with gastritis. Ultimately developed polyneuritis in the legs.	[70]
"	12, plus 1 listed as being "not exposed"	Intermittent exposures to parathion at concentrations of 0.1-0.8 mg/cu m.	No signs or symptoms of parathion poisoning. Apparently significant depressions of both RBC and plasma ChE activities in some employees.	[79]
"	115*	Varied, but unknown (no air sampling performed).	36% decline (mean) in the RBC ChE activity in the blood of mixing-plant employees. No significant decline in the plasma ChE activity of either mixing-plant employees or any of the other exposure groups.	[50]

TABLE III-3 (CONTINUED)

Route(s) of Exposure	Number of Subjects Exposed	Exposure Concentration and Duration	Effects	Reference
Dermal	Not stated (cotton-field workers)	Entered the field about 12 hours after the aerial application of a mixture of methyl parathion and parathion; worked for 2.5-3 hours.	23 workers exhibited signs/symptoms of OP poisoning; 13 required hospitalization while 10 were successfully treated as outpatients.	[8]
Oral	5, plus 2 nonexposed controls	Subjects orally ingested capsules containing 3.0, 4.5, 6.0, or 7.5 mg parathion/day for approximately 30 days. Controls received corn oil only.	No significant inhibition of RBC or plasma ChE activities at 3.0 and 4.5 mg/day doses. "Slight" (unstated %) inhibition of plasma ChE at the 6.0 mg parathion/day dose. Plasma ChE activities were inhibited to 50% and 52% of pretest levels in 2 of 5 subjects receiving 7.5 mg parathion/day for 16 days. After 23 daily doses of 7.5 mg parathion, a third subject's plasma ChE activity was 54% of his normal value. Effects were less on the RBC activities of the exposed subjects. No signs/symptoms of poisoning were reported by the authors.	[34]
"	4 (males and females)	Subjects orally ingested 0.6, 1.2, 2.4, 4.8, or 7.2 mg parathion/day, 5 days/week for 25-70 days (4 adult females received the 7.2 mg/day doses).	Effects on blood ChE activities were observed only at the 7.2 mg/day dose; whole blood ChE activity declined to 67% of control activity after 6 weeks (RBC and plasma ChE activities at this time were 84% and 63% of control activities, respectively). No signs/symptoms of parathion poisoning were reported by the author.	[35]
"	8 (4 males and 4 females)	Subjects orally ingested 4 dose-levels of parathion for 12 weeks at 3 weeks/dose-level. The successive doses (capsules containing parathion in corn oil) were 0.003, 0.010, 0.025, and 0.050 mg/kg. 2 subjects received corn oil only.	No effects on RBC or plasma ChE at any dose level. No signs/symptoms of parathion poisoning were reported by the authors.	[36]

* 35 mixing-plant personnel, 2 commercial ground applicators, 44 part-time ground applicators, 4 aircraft application workers, 7 orchard workers, 3 fieldmen, warehousemen, and miscellaneous workers, and 20 residents near orchards.

TABLE III-4

RESULTS OF ANIMAL TOXICITY STUDIES OF PARATHION

Route of Administration	Species	Number	Results	Reference
Intraperitoneal injection:	Rats			[80]
a) 3 mg/kg/day	(female)	a) 15	a) None survived more than 5 doses	
b) 2 mg/kg/day		b) 15	b) 87% mortality after 10 days	
c) 1 mg/kg/day		c) 24	c) 46% mortality after 10 days	
d) 0.5 mg/kg/day		d) 5	d) No deaths after 20 days of dosing	
Oral: 1 mg/kg for 9 days, followed by 2 mg/kg for 2 days, followed by 5 mg/kg for 4 days	Rats (sex unstated)	Unreported	No alteration of conditioned behavior	[82]
Oral:	Mice	20/group for behavioral tests; 6/group for ChE determinations		[83]
a) 12 mg/kg			a) Impaired performance on a passive-avoidance task; killed 40% of test mice	
b) 8 mg/kg			b) Impaired performance; killed 19% of test mice	
c) 6 mg/kg			c) Impaired performance; killed 10% of test mice	
d) 4 mg/kg			d) Impaired performance	
Subcutaneous injection: 1-4 mg/kg for 6 days	"	"	No effect on passive-avoidance learning; marked effects on brain AChE	[83]
Intraperitoneal injection: 4.5 mg/kg	Rats (female)			[86]
a) injected into animals fed a casein-free diet for 30 days		a) 12	100% mortality	
or				
b) into animals fed a 15% casein diet for 30 days		b) 12	67% mortality	
Subcutaneous injection: (administered in 3 successive doses "adequate to produce a severe cholinergic response")	Chickens	Unreported	No muscular weakness or paralysis observed	[88]
Intraperitoneal injection:	Rats			[89]
a) 1 mg/kg/day	(female)	a) 13	All treated rats exhibited signs of acute parathion poisoning (tremors, respiratory difficulty, cyanosis). A progressive myopathy developed in rats receiving 1 and 1.3 mg/kg/day. Less than 5% of muscle fibers studied were affected.	
and				
b) 1.3 mg/kg/day		b) 13		
Intraperitoneal injection: 1.0 and 1.5 mg/kg (animals killed at 2 or 5 hours after parathion injection)	Rats (male)	"4 or more"/dose	Both doses produced similar depressions of brain and RBC ChE: 20-25% and slightly less than 50% inhibition, respectively. No gross signs of parathion poisoning were observed. At 2 hours after parathion injection, plasma levels of free corticosterone increased by 75%.	[91]
Oral: 1.3, 2.6, or 5.3 mg/kg/day for 5 days	Mice (male)	Unreported	No significant alteration in the metabolism of androgens.	[92]

TABLE III-4 (CONTINUED)

Route of Administration	Species	Number	Results	Reference
Inhalation:				
a) 0.04-230.0 mg/cu m for 4 hours	a) Rats (male)	a) 34/group	a) LC50 = 84.0 mg/cu m RBC ChE50 = 5.4 mg/cu m Plasma ChE50 = 7.3 mg/cu m	[Edgewood Arsenal study]
b) 0.015-37.1 mg/cu m for 4 hours	b) Dogs (male)	b) 4/group	b) LC50 = greater than 37.1 mg/cu m	
c) 0.01 mg/cu m 7 hours/day, 5 days/week, for 6 weeks	c) Rats (male)	c) 80/group	c) 1 rat died on the 1st day. RBC ChE activity decreased to 69% of normal during week 4.	
d) 0.10 mg/cu m 7 hours/day, 5 days/week, for 6 weeks	d) "	d) 80/group	d) No toxic signs were seen. RBC ChE activity decreased to 57% of normal after 1 week of exposure.	
e) 0.74 mg/cu m 7 hours/day, 5 days/week, for 6 weeks	e) "	e) 80/group	e) Animals developed congestion of the lungs. RBC ChE activity decreased to 58% of normal after 1 week of exposure. RBC ChE activity decreased to 16% of normal after 5 weeks of exposure.	
f) 0.001 mg/cu m 7 hours/day, 5 days/week, for 6 weeks	f) Dogs (male)	f) 6/group	f) No significant inhibition of either RBC or plasma ChE activities.	
g) 0.01 mg/cu m 7 hours/day, 5 days/week, for 6 weeks	g) "	g) "	g) RBC ChE activity decreased to 79% of normal after 2 weeks of exposure; 101% of normal at the end of the 6-week exposure period. After 6-weeks exposure, the plasma ChE activity was depressed to 58% of normal.	
h) 0.20 mg/cu m 7 hours/day, 5 days/week, for 6 weeks	h) "	h) "	h) RBC Che activity decreased to 54% of normal after 2 weeks of exposure; plasma ChE activity at this time was 26% of normal.	
Oral:				
a) 0.18-7.0 mg/kg (single doses)	a) Rats (male)	a) 10/group	a) RBC ChE50 = 2.6 mg/kg plasma ChE50 = 2.5 mg/kg	[Edgewood Arsenal study]
b) 0.50, 1.26, 2.5, and 10.0 mg/kg (single doses)	b) Dogs (male)	b) 4/group	b) RBC ChE50 = 1.5 mg/kg plasma ChE50 = 1.7 mg/kg	
c) 0.25 mg/kg, 5 days/week, for 6 weeks	c) Rats (male)	c) 80/group	c) RBC and plasma ChE activities were inhibited to 46% and 52% of normal after 6 weeks of exposure.	
d) 0.10 mg/kg, 5 days/week, for 6 weeks	d) "	d) "	d) Lowest RBC ChE activity observed was 78% of normal after 4 weeks of exposure. Plasma ChE activity inhibited to 20% of normal after 2 weeks of exposure.	
e) 0.05 mg/kg, 5 days/week, for 6 weeks	e) "	e) "	e) No significant inhibition of RBC or plasma ChE activities.	
f) 0.50 mg/kg, 5 days/week, for 6 weeks	f) Dogs (male)	f) 6/group	f) RBC and plasma ChE activities were 42% and 15% of normal, respectively, after 6 weeks of exposure.	
g) 0.10 mg/kg, 5 days/week, for 6 weeks	g) "	g) "	g) RBC and plasma ChE activities were 80% and 61% of normal, respectively, after 6 weeks of exposure.	
h) 0.05 mg/kg, 5 days/week, for 6 weeks	h) "	h) "	h) RBC and plasma ChE activities were 83% and 54% of normal, respectively, after 6 weeks of exposure.	

TABLE III-4 (CONTINUED)

Route of Administration	Species	Number	Results	Reference
Intraperitoneal injection: a) 3.0 mg/kg, given as a single ip injection to pregnant rats on the 11th day after insemination	Rats (female)	a) 5	a) Symptoms of poisoning in the dams. High incidence of resorptions and reduced fetal and placental weights. One edematous fetus out of 28.	[97]
b) 3.5 mg/kg, given as a single ip injection to pregnant rats on the 11th day after insemination		b) 5	b) Symptoms of poisoning in the dams. High incidence of resorptions and reduced fetal and placental weights. No fetal malformations reported.	
Subcutaneous injection: a) 2 mg/kg/day for 4 days	Rats	a) 28	a) Signs of parathion poisoning, including salivation, lacrimation, diarrhea, and tremors observed in test animals. 2 of 28 rats died after the 2nd injection. Severe depression of blood and brain AChE's.	[98]
b) 1.5 mg/kg/day for 4 days beginning on days 1, 7, or 13 of gestation		52 rats used in experiments b) and c)	b) Signs of parathion poisoning in dams. Brain AChE activity of pups was normal but was depressed in the dams. No fetal resorptions were observed.	
c) 2.0 mg/kg/day for 4 days beginning on days 1, 7, or 13 of gestation.			c) Signs of parathion poisoning in dams. 4 rats died, 3 during the 3rd trimester. Brain AChE activity of pups was normal but was depressed in the dams. No fetal resorptions were observed.	
Intraperitoneal injection: injected ip with single doses of 4, 8, 10, 11, or 12 mg/kg at varying times during gestation. Laparotomy performed on mice on the 19th day of gestation.	Mice (female)	Unreported	12 mg/kg administered on gestational days 12, 13, and 14 produced 90% incidence of deaths in utero. 12 mg/kg administered on gestational days 8, 9, and 10 produced 27% incidence of deaths in utero. Parathion significantly reduced fetal body weight.	[99]

female rats injected with 0.5 mg/kg/day died after 20 days of continuous dosing. The symptoms preceding death of the animals were similar to those observed in acutely poisoned rats. From these results the investigators concluded that ". . . continued exposure to sublethal doses of parathion results in subacute poisoning in rats and suggests the possibility of a cumulative action by parathion in other animals after continued exposure to the insecticide."

The effect of parathion on avoidance behavior in the rat was reported by Bignami and Gatti.[82] Parathion was given orally to rats previously trained to give avoidance responses in fully automated shuttle-boxes. No modifications of behavior were observed during daily doses of 1 mg/kg of parathion given for 9 days, of 2 mg/kg given for 2 days, and of 5 mg/kg given for 4 days.

In 1973, Reiter et al[83] reported the effects of parathion on ChE activities and learning in mice. A single-trial, passive-avoidance task was used to evaluate learning. Blood and brain ChE activities were determined. Mice used in the acute phase of the study were starved for 18 hours prior to oral administration by intubation of parathion (in polyethylene glycol) at a dose of 6 mg/kg. At least 20 animals in each group were used for the behavioral tests and at least 6 animals per group for the ChE determinations. This dose produced death in about 10% of the animals, usually within 15 minutes of administration. The animals died with typical signs of parathion poisoning: tremors, convulsions, and respiratory distress. Parathion was administered at various times prior to the learning trial and at various times prior to retesting. The maximum effect on performance, at a dose of 6 mg/kg, occurred when parathion was administered within the first hour before the learning trial; about 31% of the mice treated within this period remained on the platform during retesting.

Parathion in oral doses of 4 mg/kg, 8mg/kg, and 12 mg/kg was also administered to groups of mice 45 minutes prior to the learning trial. As in the case of the 6 mg/kg dose, these doses of parathion resulted in impairment of performance. However, the 8 mg/kg dose killed 19% of the mice while 12 mg/kg killed 40% of the animals tested. Thirty minutes after administration of an oral dose of 6 mg/kg of parathion, AChE and ChE activities in both blood and brain declined to 30-55% of controls. To determine the effects of subacute parathion treatment, mice were given daily sc injections of parathion for a period of 6 days in doses ranging from 1 to 4 mg/kg. This treatment had no effect on passive-avoidance learning. However, marked effects were seen on AChE and ChE activities in blood and brain. It is interesting to note that when measured 18 hours after the sixth sc injection, the 2 mg/kg dose produced the same degree of inhibition which was present ½ hour after the 6 mg/kg acute dose, namely, a 55% reduction in brain AChE, with no effect on learning.

The results indicated that acute exposure to high doses of parathion impaired passive-avoidance learning in mice and that maximal changes in ChE activity correlated closely with peak behavioral effects. Contrary to these results were those in mice exposed subacutely to parathion. No effects on learning were observed in mice injected subcutaneously with parathion for 6 days despite the fact that significant depressions in blood and brain ChE's occurred. The authors concluded that these animals were able to compensate for increased amounts of ACh at central synapses.

The possibility that potentiation may occur with combinations of two or more anticholinesterase agents has been discussed by DuBois.[84] Of particular interest has been the combination of EPN and malathion which causes potentiation of the acute toxicities of the components.[85] DuBois reported that parathion showed simple, additive acute toxicity with EPN, Dipterex, Systox, Co-Ral, and Di-Syston and less than simple, additive acute toxicity with malathion and Guthion. Potentiation of toxicity was not observed for parathion.

Other factors which may influence sensitivity to parathion include diet and preexposure levels of plasma and RBC ChE. In a study of the effects of diet, Casterline and Williams[86] found that low protein diets increased the susceptibility of rats to parathion. An intraperitoneal dose of 4.5 mg/kg resulted in 100% mortality in rats fed a casein-free diet for 30 days vs one of 67% in rats fed a 15% casein diet for a similar period. Casterline and Williams found that single doses of parathion inhibited liver and brain AChE to a greater extent when animals were fed an essentially protein-free diet.

The importance of plasma ChE and RBC ChE in the protection of acetylcholinesterase at tissue sites was clearly demonstrated by Karczmar and Koppanyi.[71] In one series of experiments, they infused groups of 10-18 atropinized dogs with either isotonic saline, Tyrodes' solution, glucose, or a 0.2% solution of ACh in saline. Plasma and RBC ChE's were measured manometrically before and after the infusions. The maximal hemodilution produced by the infusions (concentration of hemoglobin decreased by $26 \pm 6\%$) was accompanied by a decrease in the activities of the ChE's of the blood of $28 \pm 7\%$. When these dogs were given iv injections of standard doses of ACh and BCh (benzoylcholine), the pressor responses elicited in that way were about twice those obtained in the same dogs before the infusions. Reduction of the activities of the ChE's of the blood by dilution with the saline solutions was concluded to have enhanced the abilities of choline esters degradable by these enzymes to stimulate sympathetic ganglia and the adrenals.

In a second series of 34 experiments, normal dogs were transfused serially for up to 15 times with blood from heparinized, atropinized dogs that had been given iv doses of DFP (2.5 to 4.0 mg/kg) to inactivate the ChE's in their bloods. In this way, the bloods of the recipient dogs were diluted with respect to both RBC and plasma ChE's without any general hemodilution. In this series of experiments, a 40% decrease in the activities of the ChE's in the blood enhanced the pressor response to injected ACh but not that to injected methacholine (MCh). When additional transfusions had reduced activities of the ChE's of the blood to 10 to 15% of the normal values, the responses to both injected ACh and MCh were increased fourfold. At this time, iv injections of DFP (0.25 to 0.50 mg/kg) into these dogs enhanced still further the responses to injected BCh but not those to injected ACh, presumably by inhibiting all, or at least most, of the remaining BuChE in the blood and tissues of these dogs.

A third group of 15 atropinized dogs was given sufficient DFP to inhibit completely the ChE's of all tissues. The responses to injected ACh and BCh were increased. These animals were then transfused with blood from normal dogs to restore the activities of the ChE's of their bloods to approximately normal levels. Although the ganglionic AChE and the BuChE of the intestine were still in-

hibited in these dogs, the responses to injected ACh and BCh were similar to those recorded before the administration of DFP.

Eight control experiments were carried out in which both the donor and the recipient dogs were normal. The responses in the recipient dogs to injected ACh, BCh, MCh, nicotine, and epinephrine varied from the mean pre-transfusion magnitudes by less than 15% after a series of transfusions.

These experiments demonstrated apparently the ability of the ChE's of the blood to react with circulating cholinergic and anticholinesterase compounds and to reduce their impacts upon active sites of effector organs. Accordingly, plasma and RBC ChE's have been considered to have important buffer-like roles in protecting the AChE at neuroeffector junctions and other active sites from the inhibitory actions of parathion and other organophosphorus anticholinesterase compounds.

Recovery of an animal from parathion poisoning and its reestablishment of normal susceptibility to intoxication by OP compounds depend upon the reestablishment of normal activities of ChE's in blood and tissues. Although parathion is classified as an "irreversible" inhibitor of ChE, the reaction is reversible, at least initially.[87] Grob[87] demonstrated in vitro that the combination between parathion and ChE's from human plasma, RBC's, and brain was partially reversible during approximately the first 3 hours after addition of the compound to the enzyme, and irreversible after that time. DuBois et al[80] demonstrated that in rats acutely poisoned with 5 mg/kg of parathion brain ChE activity regenerated from an observed low of 5.8% after ½ hour to 97% of normal activity in 4 hours.

In animals, parathion has not been shown to exert toxic effects other than those related to inhibition of AChE. The ability of some OP compounds to produce persistent paralysis accompanied by toxic changes in distal axons and demyelination of the sheath of the damaged axon has focused a great deal of interest on the part of researchers in this effect. Barnes and Denz[88] found that neither parathion nor paraoxon produced a paralytic effect in chickens whereas TOCP, DFP, and mipafox did. Severe cholinergic responses but no demyelinating lesions were observed in chickens after 3 successive doses of parathion (amount unspecified). The chickens were observed for 3 weeks after the third dose.

In 1972, Kibler[89] reported that parathion-induced histologic evidence of skeletal muscle necrosis in rats. Thirteen Sprague-Dawley rats received daily ip injections of parathion at a dose of 1 mg/kg and 13 received 1.3 mg/kg. All injected rats demonstrated several signs of acute parathion poisoning 20-30 minutes after each injection. These signs included tremors, respiratory difficulty with tachypnea and mild cyanosis, lethargy, and decreased responsiveness to sensory stimuli. These lessened in severity during the next 2-3 hours. Animals receiving both doses were sacrificed on days 1, 3, 5, 8, 10, 12, and 14 after the first injection. Six rats died as the result of parathion toxicity (the doses administered represented approximately 0.5-0.75 the LD50 for this species); 26 animals were killed by injection of pentobarbital sodium; then sections of their quadriceps, gastrocnemius, and soleus muscles were prepared and studied microscopically.

According to the author, the animals receiving 1 mg/kg of parathion/day developed a progressive myopathy which reached a peak on day 8, after which the number of new lesions diminished. Rats receiving 1.3 mg/kg/day developed large numbers of completely disrupted and phagocytized muscle fibers by the 3rd day. Although the appearance of new lesions progressed to day 5, their fibers did not continue to undergo degeneration and subsequent phagocytosis. Kibler reported that less than 5% of muscle fibers studied were affected and that this percentage "is far too small to produce clinical weakness." The effects observed were postulated by the author to be due to excessive ACh at the neuromuscular junction caused by inhibition of AChE. The doses of parathion administered were very large. No mention was made of demyelination of nerves, but the author may not have looked at nerves.

In 1975, Johnson[90] published a review article on neurotoxicity caused by organophosphorus esters. The study by Barnes and Denz[88] was referenced along with a later study by Johnson on the neurotoxicity of certain organophosphorus compounds, including paraoxon. Paraoxon did not produce ataxia. Johnson's paper provides an interesting discussion of the possible mechanism of delayed neurotoxicity.

The stress of exposure to parathion has been shown[91] to result in increased plasma levels of free corticosterone (PFC) in rats. Murphy[91] attributed this action of parathion to its stimulation of pituitary ACTH since depletion of adrenal ascorbic acid accompanied the increase in PFC. The 2 lowest doses of parathion tested (1.0 and 1.5 mg/kg) produced 20-25% inhibition of brain ChE and approximately 50% inhibition of RBC ChE. No gross signs of poisoning were observed in these animals. PFC levels increased by about 75% two hours after injection of parathion.

In 1974, Thomas[92] reported on the effects of parathion and other pesticides on the mouse reproductive system. Parathion in daily oral doses of 1.3, 2.6, or 5.3 mg/kg was administered by gastric intubation for 5 days to male mice and effects on the reproductive system determined by measuring the uptake and subsequent metabolism of 1, 2-3H-testosterone by the prostate gland. The uptake of tritiated testosterone was not affected by pretreatment with parathion. Regardless of the dose of parathion, no changes in the pattern of radiosteroids were detected. Thomas concluded that parathion did not produce any significant changes upon the uptake and metabolism of androgens by sex accessory organs of the mouse.

(c) New Unpublished Research Data

Based on the limitations of the aforementioned data, the decision was made by NIOSH in late 1973 that extrapolation from the then existing data to a recommended safe environmental limit (ie, safe airborne concentration) for man could not be achieved with an acceptable degree of scientific reliability. In order to obtain additional information regarding the inhalation toxicity of parathion in experimental animals, and particularly inhalation-to-oral toxicity ratios, research was undertaken for the Institute by the Toxicology Division, Biomedical Laboratory, Edgewood Arsenal. The research described below was conducted during the period July 1974-December 1975, during which time the parathion criteria document was held in abeyance.

The research consisted of 2 parts: (1) acute inhalation and oral toxicity studies on rats and dogs; and (2) subacute inhalation and oral toxicity studies using the same 2 species. Purebred adult male beagle dogs and adult male Sprague-Dawley/Wistar strain rats were used in the experiments. The effects of the insecticide on both RBC and plasma ChE were used to calculate inhalation-to-oral toxicity ratios for rats and dogs.

These ratios were then used by NIOSH to determine a predicted safe environmental limit for man based on a known safe human ingestion dose (see Chapter V, Development of Standard).

Technical grade parathion of 99.3% purity was used in the inhalation and oral experiments. Chamber air samples collected during the inhalation phases of the work were analyzed to determine whether any paraoxon was present in the air being breathed by the animals. No paraoxon or any other more toxic derivative of parathion was detected in any samples. Particle diameters of the aerosols generated, as determined by use of a Rochester cascade impactor, were 1.0-2.0 microns. RBC and plasma ChE activity levels were determined by an automated colorimetric procedure. Normal horse serum was analyzed periodically as a standard.

(1) Acute Inhalation Toxicity in Male Rats and Male Dogs

In acute inhalation toxicity tests, groups of 34 rats were exposed during 4 hours to concentrations of parathion ranging from 0.04 to 230.0 mg/m^3 in a 1,000-liter dynamic flow chamber. Groups of 4 dogs were exposed to parathion for 4 hours at 5 concentrations ranging from 0.015 to 37.1 mg/m^3. The LC50 for rats was estimated to be 84.0 mg/m^3 while that for dogs was determined to be greater than 37.1 mg/m^3 (the highest dose tested). The ChE50 for RBC ChE in the rat was 5.4 mg/m^3 and that for plasma ChE was 7.3 mg/m^3. In the experiment with dogs, the investigators were unable to estimate a ChE50 dose for this species because of the pronounced and inconsistent effects on blood ChE activity by the exposure concentrations used. No deaths occurred in dogs exposed to the aforementioned concentrations. However, blood ChE activities were significantly depressed by all exposure concentrations. These data seem rather remarkable because the lowest concentration produced nearly the same inhibitions of the activities of RBC and plasma ChE's as the largest concentration, and in close to the same times (1.5 days vs 1.0 day). The concentrations referred to above differed by a factor of more than 2,470 times, so that the Ct doses of parathion received by the groups of dogs exposed to the two extreme concentrations would be expected to differ by this same factor.

At the lowest exposure level for dogs, 0.015 mg/m^3, the RBC and plasma ChE activities after 4-hour exposure were 62% and 18% of normal, respectively. Twenty hours after the termination of exposure the values were 49% and 14% of normal, respectively. Fourteen days after exposure the RBC and plasma ChE activity levels had risen to 58% and 75% of normal, respectively. The data are presented in Tables XVI-5 through XVI-8. Similar results were observed at airborne parathion concentrations of 0.15 mg/m^3 and greater; the RBC and plasma ChE activities were significantly depressed.

Tremors, convulsions, respiratory difficulty, and excessive salivation were seen in rats exposed to parathion concentrations of 50 mg/m^3 or greater. The EC50's for tremors and convulsions were estimated to be 73.7 mg/m^3 and 110.6 mg/m^3, respectively. At the lowest level tested, 26.1 mg/m^3, occasional sneezing, diarrhea, urination (scrotal area

wet with urine), lethargy, and "wet dog shakes" were observed in test animals. The highest concentration that did not cause deaths was 35.0 mg/m^3. A no-effect concentration was not determined for the rat in this series of exposures. At the end of the 4-hour exposure to parathion at a concentration of 0.04 mg/m^3, the average RBC ChE activity of the exposed rats was 84% of normal.

(2) Subacute Inhalation Toxicity in Male Rats and Male Dogs

Groups of 80 male rats were exposed for 7 hours/day, 5 days/week for 6 weeks to aerosol concentrations of 0.01, 0.10, and 0.74 mg parathion/m^3. Toxic signs were not seen in the rats exposed to the 0.01 and 0.10 mg/m^3 concentrations of parathion except for 1 animal that died on the first day of exposure to 0.01 mg parathion/m^3. Microscopic examination revealed congestion of the lungs with the highest parathion concentration used, 0.74 mg/m^3. Two rats died, one on the 10th day of exposure and one on the 28th day of exposure. Both animals had congested lungs on gross examination. The lowest dose tested, 0.01 mg/m^3, was estimated from the acute toxicity phase of the study to be a no-effect dose.

The average blood hematocrit value, 47.2 mg%, obtained from 9 exposed rats after the last exposure to parathion at a concentration of 0.74 mg/m^3 was not significantly different from that obtained from 4 control animals. The exposed rats gained weight throughout the exposure and postexposure periods with all 3 parathion concentrations.

With the lowest exposure level, 0.01 mg/m^3, the greatest effect of the parathion on RBC ChE occurred during week 4, when the activity decreased to 69% of normal. In contrast, the RBC ChE activity decreased to 57% and 58% of normal after one week of exposure to 0.10 and 0.74 mg parathion/m^3, respectively. After 5 weeks' exposure to an airborne parathion concentration of 0.74 mg/m^3 the RBC ChE activity had been lowered to 16% of normal. In almost all cases, the RBC ChE was inhibited to a greater extent than the plasma ChE. Of the 3 concentrations, 0.01 mg parathion/m^3 produced the least inhibition of the ChE activity of either RBC's or plasma. The intermediate concentration, 0.10 mg parathion/m^3, exerted a moderate inhibitory effect whereas the highest, 0.74 mg/m^3, concentration produced marked inhibition. Table XVI-9 summarizes these data.

The results of the acute studies with dogs indicated that this species is more sensitive than rats to inhaled parathion, as shown by the effects on ChE activities in the blood of the dog. Accordingly, groups of 6 dogs were exposed to airborne parathion concentrations of 0.001, 0.01, and 0.20 mg/m^3 for 7 hours/day, 5 days/week, for 6 weeks. RBC and plasma ChE activity determinations were made after 1 day and 1, 2, 3, 5, and 6 weeks of exposure to aerosols of parathion and at various times following cessation of exposure. The RBC and plasma ChE activity values are shown in Table XVI-10. With the 0.001 mg/m^3 exposure concentration both the RBC and plasma ChE activities were not inhibited significantly during the 6-week exposure period and the 6-week postexposure period. Thus, the lowest exposure concentration constituted a safe level on the basis of inhibition of blood ChE's. After 2 weeks of exposure to 0.01 mg/m^3, of parathion the RBC and plasma ChE activities were 79% and 70% of normal, respectively (average of 6 dogs). After a 6-week exposure to 0.01 mg/m^3, the corresponding activities were 101% and 58%, respectively. With 0.20 mg parathion/m^3, the effects on RBC and plasma ChE were great. After 2 weeks of exposure to this concentration, the RBC and plasma activities were 54% and 26% of normal, respectively. The RBC and plasma ChE activities decreased to 41% and 36% of normal, respectively, after 6 weeks of exposure; the RBC ChE activity did not return to normal until 4 weeks after termination of the exposure. All 3 parathion concentrations exerted greater inhibitory effects on the plasma enzyme than on that of the RBC's. Other than effects on blood ChE's, no toxic signs were observed in the dogs with any of the 3 exposure concentrations.

(3) Acute and Subacute Oral Toxicity in Male Rats and Male Dogs

Preliminary to subacute feeding studies, 50 adult male rats and 20 adult male dogs were used to determine the acute 24-hour LD50's following single oral doses of parathion in corn oil. These values were 6.85 (6.18-7.60) mg/kg and 8.27 (4.79-14.29) mg/kg for male rats and male dogs, respectively. The results indicate that parathion is approximately equitoxic when administered orally to these 2 species. Tables XVI-11 and XVI-12 list the dose levels administered, the percent of animals of each species responding at each level, the average percent inhibition of RBC and plasma ChE's, and the Bliss statistical analysis of the data.

Acute ChE50 values were also determined for male rats and male dogs. The dose range for groups of 10 rats was 0.18 to 7.0 mg/kg of parathion; 7 different doses in corn oil being used. Dogs in groups of 4 were given the following oral doses of parathion in corn oil: 0.50, 1.26, 2.5, and 10.0 mg/kg. In both species, blood samples for

ChE activity determinations were taken 24-hours postexposure.

For male rats the RBC and plasma ChE50 values were determined to be 2.6 (2.1-3.1) and 2.5 (2.1-3.0) mg/kg, respectively. In male beagle dogs, the ChE50 values for RBC and plasma were found to be 1.5 (1.1-2.1) and 1.7 (0.9-3.0) mg/kg, respectively. Like the LD50 data, the ChE50 values estimated for the 2 species indicate that orally administered parathion is approximately equitoxic in male rats and male beagle dogs. These data are presented in Tables XVI-13 through XVI-16.

Two hundred and forty rats received daily doses of 1 ml/kg of corn oil, 5 days/week, for 6 weeks and served as controls. Another 240 animals were divided into 3 groups of 80 rats each. These groups of rats received 1 ml/kg of corn oil containing parathion in concentrations of 0.25 mg/ml, 0.10 mg/ml, or 0.05 mg/ml. All rats were weighed daily on 5 days/week and dosed, presumably by stomach tube, on a ml/kg basis. With the highest dose, 10 control and 10 exposed rats were sacrificed for blood sampling at 1, 3, 4, 5, and 6 weeks during oral dosing, and at 1, 2, 4, and 6 weeks postexposure. With the 2 lowest doses, RBC and plasma ChE activities were determined at 1, 2, 4, and 6 weeks during exposure and at various times following the cessation of oral dosing until complete restoration of enzyme activity had occurred. The results of this study are given in Table XVI-17. The highest daily dose, 0.25 mg/kg of parathion inhibited about 54% of RBC ChE activity and about 48% of plasma activity by the end of the 6-week exposure period. No significant blood ChE inhibition resulted from the 0.05 mg/kg dose. The lowest RBC ChE activity observed at the intermediate dose of 0.10 mg/kg was 78% of normal (following the 4th week of exposure). Inhibition of plasma ChE was less marked in general than that of the RBC enzyme. Thus, the 2 lowest doses exerted no profound effects on either the RBC or the plasma ChE and can be considered safe oral doses for rats. None of the test animals exhibited toxic signs of parathion poisoning either during or after exposure. Weight gained by parathion-treated rats was not significantly different from that gained by the control animals.

In a similar manner, the effects of orally administered parathion on the RBC and plasma ChE's of dogs were determined. Twenty-four adult male beagle dogs in groups of 6 each were dosed orally 5 days/week for 6 weeks with one of 4 concentrations of parathion in corn oil: 0.00, 0.05, 0.10, or, 0.50 mg/kg. All dogs were weighed weekly. Blood samples for ChE determinations were taken at 1, 2, 4, and 6 weeks during exposure and at 1, 2, 4, and 6 weeks postexposure, if required.

The effects on the blood ChE's of the dog by these daily doses of parathion are presented in Table XVI-18. The 0.50 mg/kg dose produced after 6 weeks about 58% inhibition of RBC ChE and about 85% inhibition of the plasma enzyme.

With respect to the RBC ChE activity, the 0.10 mg/kg and 0.05 mg/kg dose levels produced approximately equivalent results. With 0.10 mg/kg of parathion, the RBC activity was 80% of normal after 6 weeks of exposure and with the 0.05 mg/kg dose, 83%. However, the plasma ChE activity was inhibited to a significantly greater extent by the daily dose of 0.1 mg/kg of parathion than by the lower dose. No toxic signs were observed in any of the test or control dogs during or after exposure to these oral doses of parathion. There was no significant effect on body weight in any of the exposed animals. The only conclusion from these results is that a daily dose of 0.05 mg/kg may not produce any deleterious inhibition of the ChE's of the blood of the dog during 6 weeks and that the 2 highest doses probably are not safe for repeated daily oral ingestion.

Experiments were performed also to determine the rates of recovery of the RBC and plasma ChE activities in male rats and dogs. A total of 60 rats and 4 dogs were administered single oral doses of parathion in corn oil of 2.8 mg/kg and 2.5 mg/kg, respectively. Twenty control rats received corn oil alone. No mention was made by the investigators of control dogs. Rats were bled at 4 hours and 1, 2, 3, 7, and 14 days postexposure; dogs were bled at 1, 11, 15, 29, and 36 days postexposure. The rates of recovery for both RBC and plasma ChE activities agree with those reported previously in the criteria document. The RBC ChE activity recovered at the following rates: 1.7%/day and 1.6%/day for male rats and male dogs, respectively. For rats, the plasma ChE activity returned at a rate of 3.1%/day whereas in dogs the rate was 5.5%/day. The results are presented in Tables XVI-19 and XVI-20.

Carcinogenicity, Mutagenicity, Teratogenicity

Malformations in the embryonic skeleton of the Japanese quail by parathion have been reported by Lutz-Ostertag et al[93] and by Meiniel et al.[94] These workers either injected eggs with a 0.1% solution of parathion or immersed the eggs in a 2% solution of parathion-in-acetone for 30 seconds. The dose received by the embryos cannot be calculated

but must have been very high. All embryos from eggs treated with parathion by immersion showed abnormalities, principally localized in the cervical region. Malformations of the axial skeleton and reductions in the length of the spine were common. Similar results from studies on hen and quail eggs were published by Meiniel in 1973.[95]

Khera and Bedok[96] obtained similar results in chick and duck embryos. They injected 1 mg of parathion (presumably in propylene glycol) into the yolk sac of preincubated or 4-day-incubated chick eggs and 4-day-incubated duck eggs. Control eggs of both species were injected with sterile propylene glycol. Parathion treatment resulted in characteristically tortuous and shortened vertebral columns composed of abnormal vertebral bodies (fused neural arches).

Kimbrough and Gaines[97] reported the effects of parathion on the rat fetus. Intraperitoneal injections of parathion in dams in doses of 3.0 and 3.5 mg/kg caused a high incidence of resorptions and reduced the weights of the fetuses and placentas. With the higher dose, one edematous fetus was observed out of a total of 28. However, the investigators emphasized that only those doses of parathion that produced toxic symptoms in the dams affected the fetus.

Talens and Woolley[98] reported the effects of exposure to parathion during gestation on development in the rat. Female rats were subcutaneously injected with 2 mg/kg of parathion for 4 days and then killed at various intervals for determination of blood and brain AChE activities. Cholinergic signs of poisoning, including salivation, lacrimation, diarrhea, and tremors were observed in the test animals; 2 of 28 animals died after the second injection. Twenty-six hours after the last injection, the AChE activities in the neocortex, brain stem, remaining brain, and blood were 21%, 40%, 19%, and 49% of control activities, respectively. To determine effects on development, pregnant rats were injected subcutaneously with 1.5 or 2.0 mg/kg parathion daily for 4 days beginning on day 1, 7, or 13 of gestation. Signs of poisoning were seen in the dams (most severe when administered during the third trimester). Four of 52 rats injected with the highest dose died, 3 during the third trimester. In surprising contrast to the effects in the dams, the brain AChE activity of the pups at birth was normal in all treated groups. At birth, the average litter size of the parathion-treated dams did not differ from that of controls. Thus, fetal resorption did not occur, in contrast to the results previously reported by Kimbrough and Gaines.[97] Average birth weights of pups born to dams injected with parathion during the latter third of pregnancy were significantly lower than those of controls. By the second week, body weights were normal in pups from all treated groups. The authors' statement that the slower body growth and later development may be attributable to poor maternal behavior appears reasonable in light of the fact that brain AChE activity was normal in the pups at birth and also to their observations that the parathion-treated dams appeared to pay less attention to their pups than unpoisoned mothers.

In 1975, Harbison[99] reported the results of his study on parathion-induced toxicity during prenatal development in mice. Mice were injected ip with parathion at doses of 4, 8, 10, 11, or 12 mg/kg. Control mice were injected with corn oil. Laparotomy was performed on pregnant mice on gestational day 19. At this time, the number and positions of live, dead, and resorbed fetuses were noted and then fetuses were removed and weighed. The greatest effects were observed when parathion was injected during gestational days 12, 13, and 14. During this period, a dose of 12 mg/kg killed 90% of the fetuses, while the same dose was lethal to 27% of the fetuses in utero during gestational days 8, 9, and 10, the period of early organogenesis. A dose of 12 mg/kg was not lethal to the maternal animal. The body weights of surviving fetuses were significantly reduced by parathion treatment. As in the case of resorptions, a gestational period susceptibility was observed; the developing organism appeared to be more susceptible to parathion-induced reduction in body weight during late organogenesis or the fetal maturation period. Data were also presented showing that phenobarbital greatly protected the developing fetus from the effects of ip-injected parathion.

Weis and Weis[100] subjected fertilized killifish (*fundulus heterclitus*) eggs to parathion at concentrations of 1 and 10 ppm. The insecticide was dissolved in acetone and added to dishes containing the eggs at the 8-16 cell stage, in filtered sea water. When controls had reached the 19-20 cell growth stage, embryos in 1 ppm parathion were in stage 17-18 while those in 10 ppm were in stage 17. Sixty-four percent of eggs exposed to 10 ppm of parathion successfully formed axes vs 93% in controls. After 3 days in parathion, 10-12 embryos were removed from the 1- and 10-ppm dishes, washed, and placed in clean sea water. Fifty percent of the group exposed to 10 ppm parathion developed a thin feebly-beating tube with rudimentary chambers stretching between the yolk and the embryo, instead of a normal heart. The authors

stated that other tissues and spontaneous movement were normal. The concentrations of parathion did not produce death in the embryos. Because of the species used and the nature of the exposure, it is impossible to extrapolate these results to man.

In 1973, Mohn[101] reported on the mutagenic potential of parathion and other insecticides. The induction of 5-methyltryptophan (5-MT) resistant mutations in *Escherichia coli* by parathion was evaluated. A concentration of 20μg 5-MT/ml was used and the inoculum contained 300,000-500,000 cells/ml. Cultures with a low spontaneous mutation frequency were used. The test system was subjected to 0.043 M parathion with no increased mutagenic activity observed; the number of 5-MT resistant mutations did not differ significantly from the spontaneous value.

In a 1975 review article dealing with mutagenesis by pesticides, Fahrig[102] compared the results of 4 studies with parathion. One of the mutagenicity studies was his own, which was published in 1973 and is described above.[101] Two of the 4 studies were apparently not published and are referenced in Fahrig's review as personal communications. The fourth study was performed by the author but the data had not been published. Results in the 3 unpublished studies were obtained using the following procedures: (1) spot test for back-mutations to prototrophy in the 2 auxotrophic strains, a 21 and a 742, of *S. marcescens* and for forward mutations to galactose prototrophy in the phenotypic galactose-negative Gal RS strain of *E. coli*; (2) a liquid holding test for forward mutations to streptomycin resistance in *E. coli*; and (3) a liquid holding test for mitotic gene conversion at the ade2 and trp5 loci of *S. cerevisiae*. Fahrig reported that parathion was negative in all test systems.

No papers have been found reporting the production of carcinomas or other malignancies by parathion. However, parathion is presently undergoing carcinogenesis bioassay by the National Cancer Institute. (H Kraybill, written communication, March 1976) Male Osborne-Mendel rats have been fed parathion (ad libitum) for 18 months in their diets at concentrations of 30 and 60 ppm. Females of the same species and strain were fed parathion in their diets at 20 and 40 ppm for the same length of time. Male and female mice (B_6C_3F1 strain) were fed 80 and 160 ppm parathion in their diets for 16 months. The observation periods for rats and mice were 6 months and 5 months, respectively. Although a preliminary report was anticipated during the Fall of 1975, it did not materialize. Results of the carcinogenesis bioassay of parathion have not been made available to NIOSH as of July 1, 1976. When the bioassays are completed and the results made available to NIOSH, appropriate action will be taken.

The data presented in this section relating to the testing of parathion for carcinogenic, mutagenic, and teratogenic activity in various mammalian and nonmammalian species indicate that the compound is not active in these respects. It is concluded that parathion is unlikely to produce mutations, terata, or cancer in humans.

Correlation of Exposure and Effect

Evaluation of the literature pertaining to the effects of overexposure to parathion indicates that parathion is absorbable after inhalation,[37,38] ingestion,[34-36] and impingement on conjunctival, cutaneous, or mucous membranes,[11,16,39-41] and induces either subclinical (ie, depression of blood ChE activity) or toxic (ie, signs/symptoms of parathion poisoning) effects, or both, depending on the amount absorbed. It is probable that the high solubility of parathion in lipoid media influences its absorption through the skin and its distribution in the body. Parathion is converted in vivo and oxidized in the air to paraoxon[22-25] which then reacts with, and inhibits the activity of, ChE enzyme throughout the body.[1] When the ChE activity of tissues is inhibited to a certain degree, resulting in a disruption of normal enzyme function, a local condition of excess concentration of acetylcholine results. The signs and symptoms of parathion poisoning arise from the totality of these individual local effects.[1] The signs and symptoms most frequently seen in parathion poisoning are presented in Table III-1.

Effects of parathion due to actions other than inhibition of AChE are not proved. Karczmar and Koppanyi[71] demonstrated that both RBC and plasma ChE's may serve as "buffers," protecting neuroeffector ChE's from inhibition by parathion. Experiments[81,91,103] have shown that in animals poisoned with parathion, the blood ChE's are depressed along with tissue ChE's. Similar results in humans have been reported[17,18]; a decrease in the activities of the blood ChE's reflects concomitant, but not necessarily proportional, reductions in tissue ChE's. Grob et al[18] made a comparison of the ChE activities of various tissues of two subjects who died after exposure to parathion with the average ChE activities of 8 subjects who had received no exposure to parathion or any other anti-ChE agent and who had no disease of the CNS, liver, kidneys, or blood-forming organs. In

the 2 parathion-poisoned individuals, the percentage of control ChE activities for various tissues were: plasma—2.5%, RBC's—16.5%, liver—60%, kidney—88% (one patient), cerebral cortex—26.5%, thalamus—52%, cerebellum—52.5%, pons—41%, and medulla—39%. Both patients developed marked signs/symptoms of parathion poisoning prior to death. The blood ChE's and the urinary excretion of PNP are the only well-demonstrated and practical measures, from the viewpoint of a compliance standard, of subclinical parathion exposure presently available. Chronic occupational exposures to parathion are typified by repeated absorption of amounts of parathion which in single doses would not produce signs/symptoms of poisoning. However, the effect of repetition during a sufficiently long period of high enough doses is to progressively inhibit tissue acetylcholinesterase to the point where decreased enzyme function results in the development of signs/symptoms of poisoning.[80] Neither parathion nor its metabolites cumulate in the body.[50,51,104] However, the effects of parathion on ChE's may be cumulative depending on the rate of enzyme inhibition. Grob et al[18] demonstrated in humans that after inhibition by parathion, RBC ChE activity returned at an average daily rate of 1-2% while the plasma ChE activity returned at the average rate of 3-4%/day. If the daily inhibition of blood enzymes exceeds the daily recovery of ChE activity, there will ultimately be a progressive decrease in tissue enzyme activity to a level where signs/symptoms of poisoning will probably occur. Thus, monitoring of the RBC and plasma ChE activities serves as an indicator of what is happening to tissue ChE activity levels. A review of the data presented[18,34,35,39,87,103] reveals that both plasma and RBC ChE activity may be depressed simultaneously upon exposure to parathion, with the plasma enzyme inhibited to a greater extent (ie, preferentially inhibited). Thus, plasma ChE activity is usually depressed more promptly and extensively and recovers more rapidly than RBC ChE activity when parathion is administered in uniform oral daily doses. Accordingly, if RBC ChE activity rather than plasma ChE activity were being monitored on a fixed routine it might provide a better warning that excessive absorption of parathion was occurring than would be provided by monitoring of plasma ChE activity. In the situation where absorption of parathion occurs on each of several serial days and then ends a few days before a preset monitoring time, plasma ChE activity could return to normal before the blood sample was collected whereas the RBC ChE activity would be more likely to remain depressed.

For this reason, selection of RBC ChE activity for routine biologic monitoring of blood to detect exposure to parathion seems appropriate. Determining both plasma and RBC ChE activities would not significantly increase the likelihood of detecting progressive inhibition of tissue ChE above that provided by monitoring RBC enzyme activity alone.

In summary, progressive depression of tissue ChE activity to a dangerous extent is believed to be preventable by a biologic monitoring program involving measurement of the activity of RBC ChE. Thus, by preventing a chronic but sustained progressive depression of this blood enzyme, poisoning by parathion may be prevented. However, it is highly unlikely that infrequent blood ChE monitoring can prevent the occurrence of acute poisoning caused by a relatively massive exposure with a resultant precipitous decline in both the blood and the tissue ChE's. In cases of serious overexposures to parathion, such as by contamination of a large area of skin by spills or splashes of concentrate material, it is recommended that both the RBC and the plasma ChE's be measured, because the latter type of ChE is more sensitive to inhibition by single large doses of parathion than the RBC ChE, the latter retaining still its value as an indicator of cumulative absorption of parathion during a relatively long period of time.