III. BIOLOGIC EFFECTS OF EXPOSURE

Anticholinesterase compounds, including methyl parathion, exert their generally recognized acute toxic effects by inhibiting the enzyme responsible for hydrolyzing acetylcholine, with the subsequent accumulation of endogenous acetylcholine. [1] Acetylcholine is the substance which mediates the transmission of nerve impulses in preganglionic autonomic fibers, postganglionic parasympathetic fibers, and in some postganglionic sympathetic fibers. [2] These fibers innervate the heart, irises, salivary glands, stomach, small intestine, urinary bladder, bronchial glands, eccrine sweat glands, and other organs and tissues. [2] Acetylcholine also has a transmitter function at neuromuscular junctions (motor endplates) and at certain synapses between neurons within the central nervous system (CNS). [2]

In humans, there are two principal types of enzymes which hydrolyze choline esters: acetylcholinesterase, or true cholinesterase, and butyrylcholinesterase, frequently called plasma cholinesterase, serum cholinesterase, or pseudocholinesterase. [2] Acetylcholinesterase is found in neurons, at the neuromuscular junction, in erythrocytes, and in certain other tissues. Butyrylcholinesterase is found in various types of glial cells in the central and peripheral nervous systems, as well as in the plasma, liver, and other organs. [2]

As with other phosphorothioates, methyl parathion has only a slight inhibitory action on acetylcholinesterase and butyrylcholinesterase, but its active metabolite, methyl paraoxon, is a potent inhibitor of both these enzymes. [3,4] Because the resultant phosphorylated enzyme is stable,

hydrolysis leading to reactivation of the enzyme occurs slowly. [5] Recovery of cholinesterase activity is thought to occur as a result of hydrolysis of inhibited enzyme and by synthesis of fresh enzyme. [5,6] Hydrolysis is limited by another spontaneous reaction, aging, which leads to a stable phosphorylated cholinesterase refractory to spontaneous or induced hydrolysis. [2] Aging of the inhibited enzyme has been explained as a partial dealkylation of the phosphoryl moiety which results in a more stable phosphorylated enzyme. [2,7]

Extent of Exposure

Methyl parathion (O,O-dimethyl O-p-nitrophenyl phosphorothioate) is a nonproprietary organophosphorus insecticide. [8] Depending on storage conditions and on the use of stabilizing ingredients, methyl parathion isomers and methyl paraoxon may also be present. Some physical properties of methyl parathion are presented in Table XVI-1. Common trade names and synonyms for methyl parathion appear in Table XVI-2.

Methyl parathion is produced by the esterification of phosphorus pentasulfide with methanol, chlorination of the ester with molecular chlorine, and condensation of the chlorinated ester with the sodium salt of paranitrophenol to form methyl parathion. [9] Technical methyl parathion was produced by four US manufacturers in 1974. [9] Emulsifiable liquid, wettable powder, and dust products were being prepared from technical methyl parathion by 84 formulators registered with the US Environmental Protection Agency as of October 1974. Of these, 32 were registered for 5 or more different formulations. [10] Many of these formulations were mixtures of methyl parathion with other organophosphorus and organochlorine

insecticides. Methyl parathion is presently registered as a "restricted use" pesticide by the US Environmental Protection Agency. According to the 1972 amendments to the Federal Insecticide, Fungicide, and Rodenticide Act of 1947, "restricted use" pesticides may only be used by or under the supervision of a certified applicator. State certification programs must meet federal standards issued by the US Environmental Protection Agency. These standards appeared in the Federal Register 39:36446-52, 1974.

In 1972, methyl parathion was produced in greater quantity than any other organophosphorus insecticide in the United States. [9] Domestic production was estimated at 51 million pounds in 1972. Of the 40 million pounds of methyl parathion used domestically in 1972, 33.5 million pounds were used on cotton, 3.1 million pounds on soybeans, and the balance on other field, vegetable, and fruit crops. [9] In 1972, an estimated 75% of the methyl parathion applied in the United States was used in the south central states. [9] Regional distribution of methyl parathion use in 1972 is shown in Figure XVI-1. [11]

A list of occupations with potential exposure to methyl parathion is provided in Table XVI-3. [12] NIOSH estimates that approximately 150,000 US workers are potentially exposed to methyl parathion in occupational settings.

Historical Reports

The first indications that some organophosphorus compounds might be highly toxic appeared during the early 1930's when symptoms of acetylcholine poisoning were experienced by persons synthesizing dimethyl and diethyl phosphorofluoridates. In 1936, Schrader studied the phosphorus

compounds during an investigation of synthetic insecticides, and in 1937 he patented the general formula for organophosphorus (OP) contact insecticides. [13]

Although organophosphorus insecticides induce compound-specific toxic effects, those studied have at least one characteristic in common. All are cholinesterase inhibitors in mammalian systems. Because of this common toxic property, the signs and symptoms of acute systemic poisoning by organophosphorus compounds have been well characterized experimentally and from case studies. From these data, Holmstedt [14] compiled a table of signs and symptoms of anticholinesterase effects in humans. These effects are shown in Table XVI-4. Reports cited in this document strongly suggest that this list is applicable to acute systemic poisoning by methyl parathion.

Methyl parathion was introduced in 1949 by Farbenfabriken Bayer, Germany, as indicated by Spencer. [15] The first reported cases of methyl parathion intoxication in an occupational setting apparently occurred in 1956. Grigorowa [16] wrote that the incidence of reported systemic cholinergic symptoms among examined workers occasionally was as high as 38-83% during 1959 in a Wofatox (methyl parathion) dust-formulating plant in Germany. Symptoms typical of those observed for systemic poisoning by anticholinesterase compounds were accompanied by depression of plasma cholinesterase activity. Erythrocyte cholinesterase activities were not reported. Systemic poisonings were attributed to inadequate work practices and engineering controls. Workers wore neither gloves nor protective clothing and did not use respirators. Exposed skin and clothing showed yellowish-green discoloration. Although the author [16] reported that

pesticide dust measurements performed in the plant showed levels far in excess of "permissible amounts," the actual concentrations were not reported. Intoxications were especially frequent during hot summer periods. The author [16] stated that the symptoms of intoxication rapidly subsided in a majority of cases after seasonally high temperatures ended. Details provided on some of the cases indicated that poisoning had been responsible for actual injuries or had increased the risk of physical injury from other sources. For example, one employee who became dizzy in a mixing room was injured in a fall down some steps.

Effects on Humans

Fazekas [17] and Fazekas and Rengei [18,19] performed autopsies on 30 persons who died of methyl parathion poisoning between 1964 and 1969 in Hungary. Twenty-six cases involved ingestion (primarily suicidal) of an estimated 50-300 g of material, while four cases involved poisoning by dermal and respiratory exposures during spraying. The 20 male and 10 female victims ranged in age from 18 to 82 years. The victims died 2 hours-9 days after exposure; exact times of death were not reported. Autopsies were performed 4-12 hours after death. All organs were examined for gross and microscopic changes. Results of the autopsies indicated generalized edema, together with pooling and stagnation of blood and petechial hemorrhages in the liver, heart, and spleen. The gastrointestinal tract was severely irritated and exhibited petechial hemorrhages and inflammation. The pharynx, larynx, bronchi, and trachea were inflamed, and pulmonary edema and focal pneumonitis were evident. Petechial hemorrhages, congestion, and edema were observed in the kidneys

and adrenals. Blood stagnation and edema in the brain were also observed. These papers do not give any clear indications of the incidences of these various changes. Microscopic examination confirmed the gross autopsy findings, providing evidence of severe cellular degeneration. According to the authors, the most severe changes were found in the liver, kidneys, and brain, and in the vascular systems of these organs. Fazekas [17] acknowledged that the gross and microscopic changes observed could not be regarded as specific to methyl parathion poisoning. The author also indicated that the role, if any, of cholinesterase inhibition or of therapeutic agents in the observed changes was unclear. The possible role of ingested solvents (where present) in producing or altering toxic effects was not discussed. Since the report did not specify precisely how soon after death the autopsies were performed, it is impossible to separate the damage caused by methyl parathion poisoning from post-mortem changes.

Case histories [18,19] were provided for 7 of the 30 victims. None of the case histories described any of the four occupational fatalities, nor were cholinesterase activities reported. A 50-year-old woman ingested 200 g of methyl parathion while drunk and died 24 hours later, despite treatment of an unspecified nature. [19] A 28-year-old woman developed signs of anticholinesterase effects (eg, severe perspiration, vomiting, diarrhea) 30 minutes after drinking 100 g of methyl parathion in water. [19] Despite therapy with atropine (a competitive inhibitor of response to acetylcholine by effectors innervated by the parasympathetic nervous system), she died 27 hours later from respiratory complications. A 44-year-old man experienced nausea, vertigo, vomiting, and salivation immediately after drinking 300 ml of a methyl parathion solution of unknown

concentration. [19] Although atropine and pralidoxime chloride (a cholinesterase reactivator) were administered, the patient's condition suddenly deteriorated 70 hours after ingestion, and he died. A 31-year-old man ingested 100-200 g of methyl parathion and received atropine plus pralidoxime chloride therapy when hospitalized later in the day. [19] Thirty-three hours after ingesting the pesticide, he had muscle spasms over his entire body along with an "attack of asphyxia" and died within a few minutes, despite cardiac and respiratory support. A 57-year-old man ingested methyl parathion with wine while drunk. [19] Upon admission to a hospital 5 hours later, he exhibited skeletal muscle spasms, nausea, miosis, and, later, mydriasis. Although he received atropine and pralidoxime chloride therapy, he died from cardiac failure and pulmonary edema 20 hours after the ingestion. A sixth case involved a patient, age unknown, who displayed miosis along with severe perspiration and diarrhea upon hospital admission. [19] Although atropine, pralidoxime chloride, and strophanthin (a cardiac glycoside used to increase the force of myocardial contraction) were given, the victim died from respiratory arrest 65 hours after ingestion. A 50-year-old man drank an estimated 1.8 g of methyl parathion suspended in an unspecified liquid and was discovered dead. [18]

Organs examined in these cases showed detectable quantities of paranitrophenol (PNP), a degradation product of methyl parathion. [18,19] The examinations included the brain (pons, medulla oblongata, white matter, gray matter, stem ganglia, and cerebellum), stomach, spleen, liver, heart, lungs, small intestine, blood, kidneys, and urine. The sudden and unexpected death of many of the patients was not explained, but Fazekas [17] suggested that pericapillary hemorrhages observed in the myocardium

and medulla oblongata were probably involved. From these reports, it appears that the lethal dose for adults ingesting methyl parathion is less than 1.8 g.

During February and July 1959, Grigorowa [16] investigated methyl parathion poisonings in a German plant producing Wofatox. Eighteen of the 47 workers examined in February reported mild symptoms regardless of length of employment, which ranged from a few months to 6 years. Symptoms included lack of appetite, gastric distress, visual disturbances, lack of sleep, fatigue, nervousness, and slight headaches. Plasma cholinesterase activity was apparently measured but not reported. Of 35 persons examined in July, 29 reported severe symptoms. Twenty-seven of these had plasma cholinesterase activities that were between 31.6 and 89.0% of their winter measurements; 21 workers had inhibitions of more than 30%. Preexposure baseline activities were not available. Cholinesterase activity was measured by a colorimetric method. The author [16] stated that signs indicative of CNS involvement were most frequent. Symptoms included headache, dizziness, nausea, insomnia, fatigue, visual disturbances, increased perspiration, shooting pains in the heart, loss of appetite, vomiting, and stomach pains. Fibrillar muscular twitches in the eyelids and numbness of the legs, arms, or fingers were also reported frequently. No times of onset of these specific symptoms were provided. While the routes of exposure were not specified, a yellowish-green discoloration was observed on fingernail edges, fingertips, interdigital skin areas, lower arms, and neck, as well as on work shirts and foot wrappings of many workers. None of the workers in the plant wore respirators, gloves, or other protective clothing. The increase in the number and severity of

poisoning episodes in the summer over those in the winter apparently had been observed in the previous 3 years also.

Grigorowa [16] speculated that the greater incidence and severity of poisonings in hot weather were caused by increased respiratory rate, greater volatilization of methyl parathion, and increased conversion of methyl parathion to its active oxon and isomeric forms at elevated temperatures. Not mentioned was the possibility that skin absorption also may have been increased if clothing worn in summer months was either lighter in weight, left more skin exposed, or if dilation of superficial blood vessels had taken place. Since all workers had been employed in the factory for at least a few months and some for as long as 6 years, plasma cholinesterase activities measured during the winter probably were below those that would have been determined prior to exposure to anticholinesterase compounds. As a result, the actual extent of inhibition of cholinesterase activity in the summer examinations may have been greater than those reported. Correlation of the severity of poisoning with inhibition of plasma cholinesterase cannot be made because preexposure levels were not reported.

The author [16] described in slightly greater detail three of the poisoning incidents. In the first, a worker became dizzy, fell down the steps of the mixing room, and suffered considerable injuries. No additional details on this case were given. In the second, a 49-year-old worker with 5 years' work experience developed a headache and became weak and dizzy while filling bags with methyl parathion dust. The worker lost consciousness for a few minutes at home after work. Identical symptoms recurred when work was resumed. He reported that his left hand had been

numb for a few days, that his right hand had little feeling, and that he had experienced frequent twitching of the eyelids. At the first examination, in February, he had complained of insomnia. Plasma cholinesterase activity determined during the July examination showed an inhibition of 64.4% (reduction to 35.6%) of the winter value. In the third case, a 27-year-old worker with a year's experience had no complaints during the February examination, but he complained of severe headaches, loss of appetite, nausea, watering of the eyes, and insomnia at the July examination. For 8 weeks, he had experienced arm and leg numbness, with arm impairment sometimes involving only two or three fingers. While he was chopping wood at home, the ax had fallen from his hands. In the July examination, his plasma cholinesterase activity was inhibited by 60.2% (reduction to 39.8%) of the winter value.

During a period of approximately 10 years, Rider and Moeller, [20] Rider et al, [21-25] and Moeller and Rider [26-28] performed dose-response studies with several organophosphorus insecticides on prisoners to determine the minimum effective dose for significant cholinesterase depression. One of the substances tested was methyl parathion, which was administered orally in capsules containing corn oil. For each test dose, a different group of seven men, five as test subjects and two as controls, were utilized. Erythrocyte and plasma cholinesterase activities were determined over a 41-day pretest period. Methyl parathion was then ingested on a daily basis for approximately 30 days. Cholinesterase activity determinations were made twice weekly during the test period. All cholinesterase tests were done by the electrometric method of Michel. [29] The dose was increased in successive studies from 1 mg/day, [26] by

increments of 1/2, 1, [23] or 2 mg, to 30 mg/day. [25] The authors considered cholinesterase activities significant if 20-25% lower than preexposure baselines. Doses of 1-22 mg/day did not inhibit either erythrocyte or plasma cholinesterase activities significantly. After 24 mg/day, [24] two of five men had significant cholinesterase inhibitions. Maximum decreases in cholinesterase activities during the test for these individuals were 24 and 23% of plasma preexposure baselines and 27 and 55% of their erythrocyte preexposure baselines. After 26 mg/day, [24] two of five men showed significant cholinesterase inhibition, with maximum decreases of 25 and 37% of the preexposure baseline activities of the erythrocytes; their plasma cholinesterase activities were decreased less than 20%, however. After 28 mg/day, [25] three of five men had significant inhibitions of erythrocyte cholinesterase activity at the end of the test period. Plasma cholinesterase activities were not reported after the 28 and 30 mg/day doses. With 30 mg/day, [25] the mean maximum inhibition of erythrocyte cholinesterase activity for all five men during the 30-day test period was reported to be 37%. The authors stated that, by contrast, the mean maximum erythrocyte cholinesterase inhibition was 18% with the 26 mg/day dose [24] and 19% with the 28 mg/day dose. The study with the 30 mg/day dose [25] did not give individual cholinesterase activities for the subjects given methyl parathion. Since, for the 30 mg/day methyl parathion study, the only values reported were the mean maximum cholinesterase inhibitions (no cholinesterase activities during various times of the test period were given), it is impossible to determine whether cholinesterase inhibition was still changing at the end of the test period or had leveled off. The inconsistent reporting of individual values [25] also makes it

impossible to determine whether methyl parathion-induced inhibition of cholinesterase activities varied as greatly between test individuals as did those induced by parathion itself in a similar study performed by Rider et al. [23] In the study with parathion, [23] a 7.5 mg/day dose inhibited plasma cholinesterase activities in five volunteers to 97, 82, 69, 52, and 50% of their preexposure baselines by day 16 of the test interval. At this point, the two subjects with the lowest values withdrew from the study to avoid acute intoxication. A third volunteer was dropped on day 23 when his erythrocyte cholinesterase activity had declined to 54% of baseline. The two volunteers completing the 35-day test had plasma cholinesterase activities depressed to 86 and 78% of their preexposure levels. While Rider et al [25] did not report any clinical illnesses, observation for effects other than those associated with acute inhibition of cholinesterases was not reported.

Van Bao and Szabo [30] examined the abilities of organophosphorus insecticides to produce mutations in the lymphocytes of persons exhibiting systemic anticholinesterase effects. They reported that 14 of 15 exposed individuals (3 had been poisoned by methyl parathion) showed chromosomal anomalies in a mean of 2.53% (p less than 0.001) of metaphase cells examined. Only 4 of 10 controls exhibited chromosomal anomalies in 0.5% of control metaphase cells examined. None of the 15 poisonings was fatal. Anomalies in the methyl parathion cases were not quantified separately.

In a subsequent paper, Van Bao et al [31] examined lymphocyte cultures from 31 persons having systemic poisoning from organophosphorus insecticides. Nine of these persons were occupationally exposed. Of the 31 cases, 5 (4 men, 1 woman) had been exposed to Wofatox (methyl

parathion). Three blood samples were taken, the first 3-6 days after exposure, the second at 30 days, and the third at 180 days. The authors stated that the severity of intoxication was serious in two of the individuals, medium in one, and mild in the other two. All patients had received 16-360 mg of atropine (over unspecified time intervals), and one patient in each severity category had received an experimental cholinesterase reactivator, Toxogonin (bis (4-hydroxyiminomethylpyridinium-1-methyl) ether dichloride). Lymphocyte cultures from 15 controls (13 men, 2 women) were also examined. One hundred cells in metaphase were scored from each blood sample after incubation and air-drying. In the 3- to 6-day samples, 9.80% of 51 metaphase preparations karyotyped ($p = 0.05$) had translocations or deletions. In the 30-day samples, 26.00% of 50 cells in metaphase karyotyped ($p = 0.001$) had translocations or deletions. In the 6-month samples, 10.20% of 49 metaphase cells karyotyped had translocations or deletions. This last figure was not significantly different ($p = 0.05$) from that for the controls. The control group showed a deletion frequency of 3.33% and no translocations.

When data from all 31 patients were pooled without regard to the organophosphorus compound to which the patients had been exposed, the cells collected 3-6 days after the exposure had 17.83% deletions and translocations when the cells were arrested in metaphase. [31] In this sample, 34.30% of the cells with a deletion also included an acentric fragment. The cells collected at 30 days and 6 months after the exposures had 22.00% and 5.60%, respectively, metaphase cells with translocations or deletions. The increases in the percentage of cells with translocations and deletions were statistically significant in every severity group for

methyl parathion, phosdrin, malathion, and trichlorfon in both the 3- to 6-day and the 30-day samples. The frequencies of chromosomal aberrations did not differ significantly between the occupationally exposed and the suicidal cases. In their examination of case histories of the exposed and control individuals, the authors did not adequately document exposure to known mutagenic agents or high doses of ionizing radiation that could have accounted for, or contributed to, the observed abnormalities. Moreover, possible effects of pesticide solvents and vehicles, atropine, or Toxogonin were not properly considered. However, Toxogonin had been administered to only 12 of the 31 patients, and the authors [31] stated that atropine had not shown mutagenic activity in an unpublished study performed in their laboratory. The possibility of mutagenic effects occurring in response to preintoxication factors was rejected because of the correlation of systemic intoxication with subsequent increases in chromosomal aberrations in the 3- to 6-day and 30-day samples and the return to near control levels in the 6-month samples. While the number of cases in each severity group was too small to make a firm judgment, the authors noted that a dose-effect relationship was not apparent. The investigation did not determine whether chronic exposure to low levels of the compounds also would have produced chromosomal aberrations. This possibility merits investigation, because aberrations were observed in this study, even in cases of mild poisoning.

Yoder et al [32] studied chromosomal aberrations in lymphocytes of 16 crop dusters, formulators, spray rig operators, and farmers exposed to a variety of insecticides. Sixteen control individuals were selected from nonexposed, nonagricultural occupations. Experimental subjects had been exposed to a mixture of insecticides, in which the constituents, other than

parathion, were unknown. Methyl parathion, however, was included in a group of insecticides to which experimental subjects had been exposed less often. The number of chromosomal breaks in cultured lymphocytes of peak-season (high exposure) experimental subjects was five times higher than that for off-season (low exposure) experimental subjects, for off-season controls, and for peak-season controls. Because of the variety of possible substances (including solvents), the indeterminate exposure of test subjects, and the inadequacy of controls, no conclusions about the mutagenicity of methyl parathion can be drawn from this study.

A summary of the effects on humans from methyl parathion exposure is shown in Table XVI-5.

Epidemiologic Studies

No epidemiologic studies of persons occupationally exposed to methyl parathion were found. Exposure studies in which cotton checkers entered methyl parathion-treated fields are described in Chapter IV.

Animal Toxicity

Results of studies on the anticholinesterase effects of methyl parathion are presented immediately below. Other manifestations of methyl parathion toxicity, not necessarily related to cholinesterase inhibition, are discussed later in this section.

Gaines [33] reported that a single oral administration to rats of technical methyl parathion (80% methyl parathion in xylene) in peanut oil in graded doses produced LD50 values of 14 and 24 mg/kg for 68 male and 60

female animals, respectively. The sex-related difference observed is consistent with the observation by Johnsen and Dahm [34] that liver microsome fractions from male rats were more efficient at converting methyl parathion to methyl paraoxon than were those of female rats. This sex difference in LD50's was statistically significant in the study by Gaines [33]; confidence limits at the 0.95 level were 12-17 mg/kg for males and 22-28 mg/kg for females. Application of methyl parathion dissolved in xylene to the intact skin of 69 male and 50 female rats produced LD50 values of 67 mg/kg for both sexes, with confidence limits of 63-72 mg/kg at the 0.95 level.

Kimmerle and Lorke [35] reported that the median lethal concentrations (LC50) of technical methyl parathion for 20 male rats after 1- and 4-hour inhalation exposures were 0.2 mg/liter (200 mg/cu m) and 0.12 mg/liter (120 mg/cu m), respectively. Concentrations of methyl parathion in the air within the exposure chamber were determined by an unspecified analytical technique. Exposure was limited to breathing via the inhalation apparatus of Niessen et al. [36] The postexposure observation period lasted 14 days.

Newell [37] examined the acute systemic toxicity in rats of technical methyl parathion (74-76%) administered in propylene glycol. Fasting rats weighing approximately 200 g were used for all tests. The oral LD50's were 12.0 mg/kg for males and 18.0 mg/kg for females. The iv LD50's were 9.0 mg/kg for males and 14.5 mg/kg for females. The dermal LD50's were 110 mg/kg for males and 120 mg/kg for females. The 1-hour LC50's were 257 mg/cu m for males and 287 mg/cu m for females. The author stated that these LC50 values corresponded to 1.23 mg/kg and 1.38 mg/kg, respectively.

Miyamoto et al [38] examined the acute toxicities and anticholinesterase effects of methyl parathion, Sumithion, and their oxon derivatives in rats, guinea pigs, and mice. Test materials were synthesized in the laboratory and administered in aqueous emulsions. LD50 values were calculated after a 5-day observation period. The oral and iv LD50 values determined for methyl parathion and methyl paraoxon appear in Table III-1.

TABLE III-1

ACUTE TOXICITIES OF METHYL PARATHION AND METHYL PARAOXON
(LD50, MG/KG)

Animal (Sex)	Route	Methyl Parathion	Methyl Paraoxon	Potency Ratio
White rat (M)	Oral	24.5	4.5	5.4
(170-190 g)	iv	4.1	0.5	8.2
Guinea pig (M)	Oral	417	83	5.0
(290-320 g)	iv	50	2.2	22.7
Mouse (M,F)	Oral	17	10.8	1.6
(13-18 g)	iv	13	-	-

*Adapted from Miyamoto et al [38]

Miyamoto et al [38] also examined the iv dose required to reduce the cholinesterase activities in brain and plasma in rats and guinea pigs to half their initial values within 1 hour after dosing. Results of these tests appear in Table III-2.

TABLE III-2

SINGLE INTRAVENOUS DOSES REQUIRED TO REDUCE CHOLINESTERASE ACTIVITIES TO 50% OF INITIAL VALUES

Animal	Methyl Parathion (mg/kg)	Methyl Paraoxon (mg/kg)	Potency Ratio
White rat			
plasma	1.8	0.4	4.5
brain	2.1	0.3	7.0
Guinea pig			
plasma	24	2.0	12.0
brain	28	1.5	18.7

Adapted from Miyamoto et al [38]

From the data in Tables III-1 and III-2, it appears that, in rats and in guinea pigs, the single iv dose of methyl parathion required to reduce plasma cholinesterase activity to 50% of the initial value was 43.9% and 48.0% of the iv LD50 dose, respectively. For methyl paraoxon, the single iv dose sufficient to produce the same inhibition was 80% of the iv LD50 dose in rats and 91% in guinea pigs. These comparisons show a relatively small margin between the doses of methyl paraoxon necessary to inhibit the cholinesterase activity by 50% in the plasma and to kill 50% of the animals. Methyl parathion had a 1.8 times bigger margin between these two doses than its oxon analog in the rat and 1.9 times in the guinea pig. Furthermore, it is also clear that in each species tested methyl paraoxon was significantly more toxic than methyl parathion when given iv or orally in acute doses--the potency ratio ranged from 1.6 (oral LD50, mice) to 22.7 (iv LD50, guinea pigs). This [38] and other LD50 studies [33,35,37,39-42]

for methyl parathion are summarized in Table XVI-6.

Williams et al [43] fed technical methyl parathion to mixed breed dogs for 90 days at levels of 5, 20, or 50 ppm. Five plasma and five erythrocyte cholinesterase activity determinations were performed on four control and two experimental animals during a 4-week pretreatment period. Similar tests were performed every 2 weeks during the 90-day test period and at unspecified intervals during the 8-week recovery period. The investigators found the cholinesterase activities of the erythrocytes to be about 65% and 60% of baseline values in animals ingesting the 20- and 50-ppm diets, respectively. These decrements were statistically significant. Erythrocyte cholinesterase activities for animals on the 20- and 50-ppm diets were still declining at the end of the test period. This led the authors [43] to suggest that the cumulative inhibition (from an excess of inhibition over recovery) caused by methyl parathion probably was more pronounced than had been observed for Chlorthion, Dipterex, or Diazinon. The return of erythrocyte cholinesterase activity during the recovery period occurred at a rate of approximately 1%/day in the dogs fed the two highest dosages. This recovery rate is consistent with that reported by Grob et al [6] for related organophosphorus compounds (see Chapter IV).

Metcalf and March [41] compared the oral toxicities of methyl parathion and its heat-induced isomers in mice. Mortality over a twenty-four-hour period was used to determine LD50 values. Purified methyl parathion, heated for 4 hours at 150 F, was 84.7% "isomerized." While no separate quantifications of S-phenyl and S-methyl isomers were reported, the authors stated that analysis by paper chromatography showed that the S-methyl isomer was the principal constituent. The isomer mixture was 80

S-methyl isomer was the principal constituent. The isomer mixture was 80 times more effective than purified methyl parathion at inhibiting mouse brain cholinesterase in vitro. However, the same isomer mixture was reported to have been significantly less toxic than purified methyl parathion when given orally to mice in propylene glycol. The LD50 for purified methyl parathion was stated to be 100-200 mg/kg, while that for the isomer mixture reportedly was greater than 200 mg/kg. The investigators also demonstrated that UV radiation could isomerize purified methyl parathion. This finding was not confirmed in the study of UV-irradiated methyl parathion performed by Koivistoinen and Merilainen. [44] These authors separated the mixture by paper chromatography and reported only one product of UV-irradiated methyl parathion with anticholinesterase activity, which they suggested was methyl paraoxon. Metcalf and March [41] postulated that the difference between in vivo and in vitro inhibitions of cholinesterase observed for several isomerized alkyl phosphorus compounds was because of the far greater instability to hydrolytic detoxification of the isomer mixtures than of the purified normal forms. Examination showed that the hydrolysis of the mixed isomers of methyl parathion was incomplete. Therefore, the postulate of Metcalf and March [41] was not supported by their study. The results indicate the importance of using purified methyl parathion in investigations of mammalian toxicity to obtain the true toxicity of isomer-free methyl parathion. These data do not support the suggestion by Grigorowa [16] that heat-induced isomerization of methyl parathion contributed to the increased incidence of human intoxications during formulating runs in the summer.

Brodeur and DuBois [39] examined the effects of age on susceptibility to intoxication by organophosphorus compounds, including methyl parathion, in weanling (23-day-old) and adult male rats. Methyl parathion was administered ip to 58 weanlings and to 24 adults. The LD50's were 5.8 mg/kg for adults and 3.5 mg/kg for weanlings. Systemic toxicity at earlier ages was not examined. Since the rate of conversion of organophosphorous compounds to their active metabolites in the liver of immature animals has been shown to be lower than that in adults, [45] the increased toxicity observed in weanlings was assumed by the authors to be attributable to an incompletely developed ability to metabolize and detoxify these compounds, in this case, methyl parathion. [46]

Vandekar et al [47] examined the correlation of the ED50 (median effective dose for producing a selected first sign) for methyl paraoxon with its LD50 in dose-response tests with rats. Animals were injected iv or im with methyl paraoxon dissolved in propylene glycol or glycerol; ED50 values were measured by observing the slightest evoked tremors in rats dropped at 5-minute intervals from heights of 5-10 cm, and LD50 values were determined from 24-hour mortalities. Four rats were injected at each dose level. The LD50 for methyl paraoxon administered iv was about five times its corresponding ED50 (0.457 mg/kg and 0.084 mg/kg, respectively). The LD50 for methyl paraoxon administered im was about four times its corresponding ED50 (1.69 mg/kg and 0.402 mg/kg, respectively).

Tolerance of rabbits to repeated doses of methyl parathion was reported in 1967 by Orlando et al. [48] Twenty test animals were divided into two groups. One group received one dose of 15 mg/kg of methyl parathion by the im route 7 days before receiving 12.5 mg/kg methyl

parathion iv. The other group was treated with 15 mg/kg im every 15 days during a 5-month period prior to receiving a lethal iv dose, 12.5 mg/kg. After receiving the lethal dose, neither group of test animals showed the marked ECG changes found in animals given only the lethal dose in previous studies. Correspondingly, the other clinical signs of intoxication were comparatively mild. The first group of test animals showed some ECG abnormalities, including extrasystolic bigeminy as well as slight alterations in ventricular and atrial repolarization, which tended to disappear within 1 hour. The ECG tracings returned to normal after 2 hours and remained normal for 10 days. Clinical signs in this group included agitation, tremor, dyspnea, lacrimation, excessive salivation, and urinary and fecal discharges. The signs of acute intoxication disappeared after 1 hour. In the second group of test animals, no repolarization disorders were observed. One rabbit had a brief (less than 5 minutes) run of extrasystolic trigeminy. Signs of intoxication by the iv dose were limited to dyspnea and diffuse tremors lasting only a few minutes. This study shows that rabbits may develop tolerance to methyl parathion; however, because only one species was studied, extrapolation of these results to humans is impossible.

In 1957, Frawley et al [49] simultaneously administered EPN (O-ethyl-O-p-nitrophenyl benzenethiophosphonate) and malathion to rats and dogs. There was more inhibition of whole blood cholinesterase in rats and plasma and erythrocyte cholinesterase in dogs than would have been expected from the known inhibitory potencies of the components of the mixture. No studies were found demonstrating similar potentiation for methyl parathion in combination with either other pesticides or common pharmaceuticals.

Investigations into the toxic effects on humans of the interactions of methyl parathion with active co-ingredients in the most popular formulations (eg, with parathion or with toxaphene) have not been found in the published literature. In 1975, Plapp [50] described the increasingly popular combination of methyl parathion with chlordimeform, a synergist. While no human data have been found, he indicated that, when methyl parathion and chlordimeform were applied in equal amounts (1:1), the toxicity of methyl parathion to tobacco budworms was increased 2.8 times. These data cannot be extrapolated to humans.

Gaines [51] examined 30 organophosphorus and 9 carbamate compounds for evidence of neurotoxic effects in chickens. In this screening study, the latency of effects from these compounds was compared to the characteristic 8- to 14-day delay in onset of flaccid paralysis of legs observed in chickens, cats, and humans as discussed by Davies, [52] for the known neurotoxic compound, triorthocresyl phosphate. The highest no-effect dose of methyl parathion tested was 32 mg/kg. The lowest effective dose tested, 64 mg/kg, was approximately 1/3 of the lowest lethal dose tested, 200 mg/kg. The number of chickens tested was not specified. Although no histologic examinations were performed in this study, the neurotoxic signs in chickens treated with methyl parathion did not show the characteristic latency or persistence of classical neurotoxic agents. Onset of leg flaccidity at this dose occurred within 24 hours and lasted 3-28 days. These results suggest that methyl parathion is not a demyelinating agent and confirm those reported earlier by Barnes and Denz. [53]

Shcherbakov [54] reported in 1970 that 24 rats given single ip doses of 4 mg/kg of methyl parathion, said to be nonlethal, developed signs of

mild poisoning. These disappeared within 2 hours, but animals killed and examined at that time were said to have exhibited evidence of interference with the integrity of both central and peripheral neurons. These included swelling, chromatolysis, shrinkage and fragmentation of neurons, and swelling and fragmentation of myelin sheaths.

In a later paper, the same author [55] injected 40 rats ip with 20 mg/kg of methyl parathion. These rats died after 12-15 minutes. Despite the short times of survival, Shcherbakov [55] reported changes similar to those in the previous study [54] with the smaller dose of methyl parathion but a longer period of survival. In this paper, [55] the results of some experiments with another organophosphorus compound, tabun, rather than methyl parathion, are reported. One of the findings was that administration of atropine prevented death from poisoning by tabun, but did not decrease the degree of hydration of neurons in the rats. Shcherbakov [55] suggested, therefore, that the alterations in the neurons following administration of cholinesterase inhibitors are in part due to changes in the electrolyte composition of the nerve cell and are only indirectly related to the pseudocholinergic activity of the cholinesterase inhibitors. This suggestion is supported by reported alterations in the activities of acid phosphatase and adenosinetriphosphatase (ATPase) within neurons.

These two papers by Shcherbakov [54,55] are difficult to interpret. The rapidity with which the reported changes must have taken place has not been corroborated. In particular, the fragmentation of myelin within 2 hours after a nonlethal dose of methyl parathion reported by the author is difficult to believe because changes in myelin typically require days rather than hours. [53,56-59] Furthermore, the unfixed tissues used for

histochemical study of enzymes in brain and peripheral nerve [55] are of only questionable value in demonstrating localization of hydrolytic enzymes. Shcherbakov used these structural changes in the neurons and the results of the histochemical study as partial justification for suggesting that cholinesterase inhibition may produce structural changes in the neuronal terminals as a secondary effect.

The first paper [54] did not mention the use of control rats for comparison, but the second [55] stated that six control animals were used. Presumably, therefore, the reported changes in the rats given methyl parathion or tabun were different from those seen in the control rats; however, no actual information on the controls was provided. Therefore, NIOSH is skeptical about the validity of these observations but would welcome additional study of the early effects of nonfatal doses of methyl parathion on neuronal and glial integrities.

Akhmedov and Danilov [60] reported pathologic changes in the organs of adult rats in a 3-month inhalation study in which animals were exposed for 24 hours/day, 7 days/week, to aerosolized methyl parathion. Fifteen rats were exposed at each of the airborne levels tested: 0.072, 0.024, and 0.008 mg/cu m. An additional group of 15 rats served as controls. With the highest level tested (0.072 mg/cu m), the most marked changes were reported in the CNS. They were described as perivascular, pericellular edema, and vacuolar dystrophy of the cytoplasm of the ganglionic cells. In addition, the authors reported several other histopathologic effects at this dose level. Lymphoid infiltrations in the connective tissue of the heart were noted. The liver showed hemorrhagic, dystrophic, and necrotic changes. Hyperplasia of the red pulp of the spleen and moderate atrophy of

the follicles were observed. In the adrenal glands, the authors reported atrophy of the zona glomerulosa, granular dystrophic changes in the zona fasciculata, and hyperchromatosis in the cortical cells. At the intermediate level, 0.024 mg/cu m, similar but less marked changes were observed in these organs, but CNS effects were not reported. With the lowest level tested, 0.008 mg/cu m, the authors found no significant changes in the organs of experimental animals by comparison with those of controls. On the basis of these data, the authors recommended a maximum allowable concentration (MAC) of 0.008 mg/cu m for methyl parathion in urban atmospheric air. Interpretation of this study is impossible because of inadequate characterization of pathologic changes.

Street and Sharma [61] reported that immunosuppressive effects of recrystallized methyl parathion were observed in dose-response studies with rabbits. Groups of seven animals each received methyl parathion-treated feed to yield doses of 0.036, 0.162, 0.519, or 1.479 mg/kg/day for 28 days before challenge with injected sheep erythrocytes. A control group of eight animals received untreated feed. While the test rabbits were continued on treated feed, immunosuppression was assessed during the following 28 days by a fluorescent antibody technique. The investigators examined leukocyte count, hemolysin titer, hemagglutinin titer, the concentration of gamma globulin in serum and its ratio to transferrin, the response of skin cells to intradermal injection of tuberculin, atrophy of germinal centers of the spleen, thymus cortical atrophy, and lymph node fluorescence. In the majority of these tests, results were inconclusive, inconsistent, or not statistically significant. Since only a relatively small number of animals of only one species was used and positive controls

were absent, definite conclusions cannot be drawn from this study. However, these data suggest that there may be a slight tendency for methyl parathion to suppress cell-mediated immune responses in rabbits.

Lybeck et al [62] demonstrated that methyl parathion had an inhibitory effect on iodine uptake by adult female rat thyroid in a study of 25 experimental animals, 25 positive controls (propylthiouracil), and 15 negative controls. Five experimental, five positive-control, and three negative-control rats were used for each of five test periods. Each experimental animal received ip 0.15 mg of methyl parathion in 3 ml of distilled water. The negative-control animals received ip injections of 3 ml of distilled water. The positive controls received ip 1 mg of propylthiouracil in 3 ml of distilled water. A few drops of acetic acid were added to each liter to stabilize the test solution. The animals were killed 8, 12, 16, 20, or 24 hours after receiving their injections. Two hours before the rats were killed, a dose of 20 microcuries of 131I was administered ip. Methyl parathion did not inhibit as much of the uptake of inorganic iodine by the thyroid gland as propylthiouracil, but it still produced a demonstrable effect: maximum inhibition by propylthiouracil was 95.5%, and that by methyl parathion was 65%. Both maximum effects occurred 8 hours after administration of the experimental compounds. A curious phenomenon observed in this study was that both propylthiouracil and methyl parathion apparently produced greater inhibition of uptake of iodine by the thyroid at 20 hours after the experimental compounds were given than at either 16 or 24 hours. The action of methyl parathion on the uptake of iodine by the thyroid therefore appears to be largely, if not completely, an indirect one. Lybeck et al [62] suggested that methyl parathion may

have an influence on the hypothalamic-pituitary level of control of thyroid activity, acetylcholine being thought to be the chemical mediator that initiates liberation of thyrotropin-releasing factor from the hypothalamus.

Mohn [63] examined the mutagenic potential of methyl parathion in Escherichia coli. Induction of 5-methyltryptophan-resistant mutations was determined by examining plate cultures after treatment with 0.01 M methyl parathion for 0, 60, 120, 180, or 240 minutes. Survival times were determined simultaneously on other plates without 5-methyltryptophan. The frequency of occurrence of mutant colonies in the cultures exposed to methyl parathion was not significantly different from that in the control cultures with doses which did not inactivate cells. For treatment times of 0 and 60 minutes, the survival fractions were 100 and 93%, and the mean numbers of mutant colonies/plate were 9.7 $\pm$ 6.5 and 11.3 $\pm$ 7.6, respectively. However, at higher doses, the frequency of occurrence of mutant colonies was significantly increased with a parallel decrease in survival. After contact times of 120, 180, and 240 minutes, survival fractions were 81, 69, and 53%, and mean numbers of mutant colonies/plate were 18.0 $\pm$ 7.9, 19.3 $\pm$ 7.6, and 12.7 $\pm$ 6.2, respectively. Mohn concluded in an abstract [64] of this study that methyl parathion was "probably mutagenic" and indicated [63] the need for further testing in several other mutation systems.

Plate tests with methyl parathion and eight other organophosphorus insecticides were performed by Dean [65] using E coli WP2. The author reported that methyl parathion did not induce reverse mutations in this system. Nine positive control compounds (alkylating agents known to be mutagenic) produced reverse mutations in this strain of bacteria; however,

two compounds, caffeine and urethane, which induce reverse mutations in certain bacterial test systems, had no mutagenic influence on E coli WP2. These findings confirmed the necessity for testing methyl parathion in several mutation systems before a firm judgment on its mutagenic potential can be made.

Simmon et al [66] examined the mutagenic potential of methyl parathion in several systems. The authors stated that their data showed no significant mutagenic effects in B subtilis, E coli, mice (dominant lethal test), Salmonella typhimurium (TA 98, TA 100, TA 1535, TA 1537, TA 1538), and unscheduled DNA biosynthesis. Results were reportedly inconclusive in a yeast test system. In the bacterial test systems, methyl parathion and methyl paraoxon were tested separately, since these organisms cannot perform desulfuration of the thio compound to form the oxon. Results in these test systems were negative for methyl paraoxon as well as for methyl parathion.

Huang [67] reported that, when three different human hematopoietic cell lines were treated with methyl parathion in concentrations of 25, 50, 75, and 100 μg/ml, the percentage of chromosomal aberrations in metaphase preparations was not increased in treated cultures. Doses of 5, 10, and 20 mg/kg of methyl parathion administered ip to mice did not produce significant chromosomal changes in cultures of bone marrow stopped in metaphase and examined 24 hours after exposure to methyl parathion.

Lobdell and Johnston [68] used the three-generation study design described by Fitzhugh [69] in 1959 to examine the effects of methyl parathion on reproductive performance in rats. Methyl parathion (99% pure) was administered by incorporation into the diets of experimental animals

(10 male and 20 female rats/group on each of two concentrations). [68] Daily dietary intake levels were adjusted to provide 10 ppm (1 mg/kg) and 30 ppm (3 mg/kg). With 30 ppm, tremors were observed only in a few of the original parent rats (F0). Reductions in survival time were observed in the first generation-first litter (F1a) and first generation-second litter (F1b) weanlings in the group fed the 30-ppm methyl parathion diet, and in the third generation-first litter (F3a) weanlings of that fed the 10-ppm diet. Of the second generation-second litter (F2b) females in the 30-ppm group, only 41% had litters after the second mating. Littering by the F2b females of the group fed 10 ppm was comparable with that of F2b controls. Rats in the 30-ppm F1b group had an elevated total number of stillbirths, but at each mating the numbers of litters containing stillborn pups did not differ significantly between the control and the treated groups. However, the authors concluded that dietary concentrations of 30 ppm or less of methyl parathion did not produce a consistent or dose-related toxic effect on the rat. This conclusion was based in part upon unpublished data from their laboratory.

Tanimura et al [70] examined the effects on rat and mouse fetuses of single doses of methyl parathion, dissolved in carboxymethyl cellulose, administered to the mothers. The material was injected ip into pregnant rats on day 12 of gestation and into pregnant mice on day 10 of gestation. Signs of cholinergic intoxication were seen approximately 30 minutes after administration at each dose (5, 10, and 15 mg/kg for rats; 20 and 60 mg/kg for mice). The animals were killed near term, on day 21 in rats, and on day 18 in mice. The fetuses were examined for intrauterine death, external malformations, and skeletal abnormalities. In rats, the only effects

observed were suppression of fetal growth and ossification. However, cleft palates were observed in fetuses of mice given doses of 20 mg/kg and 60 mg/kg. The higher dose was reported by the authors to be close to the LD50 for mice. With the lower dose, two cleft palates were observed among 143 fetuses. With the higher dose, 13 of 112 fetuses had cleft palates. In addition, fetal deaths in mice were dose-related: 22.3%, 4.2%, and 2.9% for the 60-, 20-, and 0-mg/kg groups, respectively. The authors suggested that the transient depression of maternal food intake in combination with the chemical action of this compound or its metabolites may have been responsible for the adverse prenatal effects. Since the distribution of deformities among the litters was not shown, the data presented cannot be properly analyzed. In addition, the single dose on day 10 (mice) or day 12 (rats) does not represent exposure to methyl parathion during all phases of organogenesis.

Fish [71] studied the effects on fetuses of methyl parathion given ip to pregnant rats. Single doses of 4 and 6 mg/kg in a vehicle of ethanol (20%) and propylene glycol (80%) were administered on day 9 or 15 of gestation. Controls received injections of the vehicle only. The author reported that the LD50 for methyl parathion in female rats was 24 mg/kg. Signs of acute intoxication appeared 5-10 minutes later and most maternal deaths occurred within the first 30 minutes after injection. Eight test animals (two treated with 4 mg/kg on day 9 of gestation, three treated with 4 mg/kg on day 15 of gestation, and three treated with 6 mg/kg on day 9 of gestation) and two controls were killed on day 21 of gestation. The author found no resorption or gross abnormalities in any of these animals; however, a diminished cerebral cortical cholinesterase activity was

observed in all fetuses of the treated groups. The fetal brain cholinesterase activity was determined using a histochemical technique. The data are qualitative, as no quantitation of the depth of staining was possible with this technique. The other rats, two test animals (one each treated with 4 mg/kg on days 9 and 15 of gestation) and two controls, were allowed to deliver. The author found no significant differences between pups derived from control and treated animals when measured by stillbirths, neonatal deaths, and gross developmental abnormalities. The small number of litters obtained from any one regimen makes any assessment of embryotoxicity speculative. However, the observed reduction in the cholinesterase activity of the fetal cerebral cortex suggested that methyl parathion had passed the placental barrier.

Ackermann and Engst [72] examined the passage of methyl parathion and methyl paraoxon across the placental barrier 1-3 days before parturition in four pregnant rats weighing 270 g. Methyl parathion, 3 mg dissolved in 0.4 ml ethanol (11.1 mg/kg), was administered orally. The placentas as well as the embryos were removed by surgery 30 minutes after administration. Maternal and fetal twitching, an early sign of methyl parathion poisoning, was observed at this dose. Animals were killed 30 minutes after the dose; maternal liver and placenta and fetal brain, liver, and muscle were sampled for assay. Methyl parathion and methyl paraoxon were found in fetal brain, liver, and muscle. Maternal liver and placenta contained residues of methyl parathion, but the authors stated that their analytical technique was not sufficiently sensitive to detect methyl paraoxon in these tissues. The presence of methyl paraoxon in fetal liver but not in maternal liver tissue was attributed to the threefold greater esterase activity observed

in maternal liver than was found in fetal liver.

Miyamoto [73] used 32P-labeled methyl parathion in tissue distribution studies. In male rats killed 2.5 minutes after iv injection, methyl parathion and methyl paraoxon were found in all tissues assayed (brain, liver, lung, heart, kidney, spleen, muscle, and blood). Methyl paraoxon was found primarily in liver tissue. Similar results were found in the guinea pig 2.5 minutes after iv injection of methyl parathion. Conversion of methyl parathion to methyl paraoxon and degradation of methyl paraoxon by liver microsomes have been confirmed in vitro for eight mammalian species by Johnsen and Dahm. [34]

Hollingworth et al [74] identified several metabolites of methyl parathion in the urine of mice. Single doses of 3 or 17 mg/kg of 32P-labeled methyl parathion in 0.15 ml of olive oil were fed to male mice. Urine collected during the following 24 hours was analyzed by ion-exchange chromatography. Excretion products from the 17 mg/kg dose were reported by percentage of radioactivity in urine as follows: dimethyl phosphoric acid, 31.9%; O-methyl-O-p-nitrophenyl phosphate, 23.1%; O-methyl-O-p-nitrophenyl phosphorothioate, 18.8%; dimethyl phosphorothioic acid, 12.9%; phosphoric acid, 5.8%; unknown, 3.1%; methyl paraoxon, 2.4%; methyl phosphoric acid, 2.0%. After both doses tested, 65-75% of the 32P administered was excreted in the urine within 18 hours. The authors suggested that urinary excretion of methyl paraoxon may be an important detoxification mechanism. The appearance of methyl paraoxon, but not of methyl parathion, in the urine was attributed [74] to the much higher water solubility of methyl paraoxon. [75] On the basis of this study, the authors proposed the metabolic pathways shown in Figure XVI-2.

Table XVI-7 summarizes the effects on animals reported in the studies discussed above other than LD50 studies.

Correlation of Exposure and Effect

Methyl parathion is absorbed through the gastrointestinal tract, [17-19,24,25,33,37,38,41,43,61,68] the skin, [33,37,76,77] and the respiratory tract. [35,37,60] Parathion is absorbed through conjunctivae, cuts, and abrasions [6]; methyl parathion is probably also absorbed through these routes.

The only confirmed effects on humans of exposure to methyl parathion are the signs and symptoms characteristic of systemic poisoning by cholinesterase-inhibiting organophosphorus compounds observed in the case studies cited. [16-19] These studies of methyl parathion intoxication, however, do not provide sufficient data for correlating plasma or erythrocyte cholinesterase activity with the various signs and symptoms of intoxication by methyl parathion. Despite this deficiency, the oral ingestion studies performed by Rider et al [24,25] suggest that manifestations of acute methyl parathion toxicity are absent in humans whose erythrocyte cholinesterase activity has been reduced to as little as 45% of their preexposure baselines. A study with dogs by Williams et al [43] lends support to this range of erythrocyte cholinesterase inhibition within which humans showed no clinical manifestations of acute intoxication by methyl parathion. Inhibition of erythrocyte cholinesterase to 60-65% of the preexposure baseline activities in dogs fed methyl parathion was not reported to produce signs of systemic intoxication. This range of inhibition within which humans and dogs appear asymptomatic is consistent

with the data compiled by Namba et al [78] (reported in Chapter IV), which showed that persons having symptoms of poisoning by parathion had more than 50% inhibition of the cholinesterase of their erythrocytes. Since no preexposure baseline determinations were made in the study by Grigorowa, [16] the range of plasma cholinesterase activities reported for workers with symptoms of methyl parathion intoxication may not reflect the true extent of cholinesterase inhibition. For this reason, true inhibition values were probably greater than those reported in the paper. In addition, the author reported only plasma cholinesterase activities. Plasma cholinesterase activity appears to return to baseline, after exposure to methyl parathion ceases, more promptly than erythrocyte cholinesterase activity. [43]

In their 1970 and 1971 studies, Rider et al [24,25] found that ingestion by humans of doses of methyl parathion of 24, 26, and 28 mg/day (five subjects each level) for 30 days was sufficient to inhibit erythrocyte cholinesterase activity by at least 20-25% in two, two, and three test subjects, respectively. With a 30 mg/day dose, the mean maximum cholinesterase activity of the erythrocytes for the test group was inhibited to 63% of the baseline activity. Return of erythrocyte cholinesterase activity to baseline value occurred at a rate of approximately 1%/day. Since Rider et al [25] did not consider the failure or possible failure of cholinesterase inhibition to level off at the end of the test intervals, 30 mg/day may not be the threshold doses for significant cholinesterase inhibition. The true threshold dose appears to be lower than 30 mg/day and may even be lower than 22 mg/day--the dose at which some of the test subjects in the 1970 study by Rider and coworkers

[24] first had inhibition of more than 25% of their erythrocyte cholinesterase by the end of the test.

Since none of the human experimental studies presented in this chapter examined the toxicity of methyl paraoxon, the active metabolite of methyl parathion, it is not possible from these data to confirm for humans the oxon:thion toxicity ratios observed in the Miyamoto et al [38] study with animals.

The data of Vandekar et al [47] from the rat show a relatively narrow range between median effective and median lethal doses for methyl paroxon. Data from human poisonings by methyl parathion, however, are not sufficiently detailed to identify the range between the doses producing first symptoms and those producing severe or fatal intoxication. Moreover, the routes of administration used in the animal study, iv and im, were not ones of occupational significance (ie, as compared to dermal, oral, and respiratory). From the data on human fatalities, [17-19] the minimum lethal dose of methyl parathion for adults appears to be less than 1.84 g.

No reports of inhalation or percutaneous dose-response studies with humans were found for methyl parathion. However, the data on rats by Newell [37] suggest that the methyl parathion is several times more toxic by inhalation than by oral administration.

None of the noncholinergic effects of methyl parathion reported in animal studies have been adequately confirmed or refuted, nor has their applicability to humans been determined.

No reports of investigations of the carcinogenic potential of methyl parathion in humans or in laboratory animals were found. However, Bedford and Robinson [79] have found that methyl parathion has about 1/300 and that

methyl paraoxon has about 1/160 the ability of dimethyl sulfate to methylate 4-(p-nitro benzyl) pyridine, a model of generalized protein with an aromatic type of amino group in a reactive form at pH 7.5. Although meaningful interpretation of the practical importance of alkylating activity is uncertain at best, one can guess that neither methyl parathion nor its oxon metabolite would be remarkably potent as either a mutagen or a carcinogen. Preussmann et al, [80] who stated that a strong positive response was given by methyl parathion as an alkylating agent, used a method of study that can be characterized as semiquantitative at best. Bedford and Robinson [79] modified the method to make it more reproducible and quantifiable. Their judgment on the alkylating propensity of methyl parathion is probably more reliable than that of Preussmann et al. [80]

Data from the reproductive studies considered [68,70-72] were not sufficient to reach a judgment on the teratogenic potential of methyl parathion. The reduced survival time observed in the study by Lobdell and Johnston [68] might be accounted for by the increased susceptibility of younger rats to methyl parathion, [45] especially in light of the report by Ackermann and Engst [72] that methyl parathion crosses the placental barrier in rats. This factor does not seem adequate, for the reasons given below, to explain the prevalence of cleft palates observed by Tanimura et al [70] in mouse fetuses from dams given a near-LD50 dose of methyl parathion ip on day 10 of gestation. On the other hand, the teratogenic potential for humans of methyl parathion can be neither confirmed nor refuted by the latter study, since the distribution of deformities within the litters was not shown. The work of Fish [71] does not determine whether or not methyl parathion has a teratogenic potential in rats. In

addition, it is impossible to determine from the study of Tanimura et al [70] whether terata and early fetal deaths were a direct result of exposure to methyl parathion (or its metabolites) or were simply a result of impaired maternal health. Furthermore, methyl parathion was not administered in the studies of Fish [71] and Tanimura et al [70] during all phases of organogenesis.

The study by Mohn [63] with bacterial cultures did not adequately confirm the mutagenic potential suggested by the human data of Van Bao and Szabo, [30] Van Bao et al, [31] and Yoder et al, [32] in light of negative results that Simmon et al [66] found in several test systems. However, the study by Huang [67] with cultures of mouse bone marrow and human hematopoietic cells and the dominant lethal (mouse) test by Simmon et al [66] do not conclusively demonstrate an absence of mutagenic potential in humans, but they do suggest the need for further research in this area.