3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of parathion. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

Many of the systemic effects observed following exposure to parathion discussed below under inhalation, oral, and dermal exposure (Sections 3.2.1, 3.2.2, and 3.2.3) are due to the inhibition by paraoxon (the active metabolite of parathion) of AChE at nerve terminals from the central, peripheral somatic, and autonomic divisions of the nervous system. Inhibition of AChE at these various levels triggers signs and symptoms that involve mainly, but not exclusively, the respiratory, cardiovascular, and gastrointestinal systems, and also induce ocular effects (see Section 3.5.2). Therefore, although listed under specific systems, the reader should keep in mind that these effects are secondary to a neurological effect, inhibition of the enzyme AChE. AChE inhibition is a biochemical feature common to all organophosphate pesticides.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death,

or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of parathion are indicated in Table 3-2 and Figure 3-2.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

Individuals who work with parathion are potentially exposed through inhalation of aerosols or dusts and through dermal contact. Minor oral exposure may also occur since inhaled materials can be swallowed through hand-to-mouth activities or deposited in the oral mucosa and either directly absorbed or swallowed. However, the specific contribution of each route of exposure is difficult to determine, especially in cases in which it is not known whether or not the workers were using protective clothing and/or respirators. Because technical parathion has relatively low vapor pressure, it is unlikely that workers would be subjected to saturated air for prolonged periods of time. On the other hand, agricultural

workers, particularly thinners and harvesters, had extensive contact between the fruit and their hands and less extensive contact between their arms and other parts of their body and the foliage (Milby et al. 1964; Quinby and Lemmon 1958). Therefore, studies of agricultural workers and other studies of humans in which no specific mention is made regarding which exposure route prevailed are summarized in Section 3.2.3, Dermal Exposure. This decision is somewhat arbitrary and is, in part, dictated by the document format, but the reader should keep in mind that both inhalation and dermal routes combined contributed to the effects described.

3.2.1.1 Death

Among 30 deaths that occurred in children due to parathion exposure in Florida from 1956 through 1964, one was due to inhalation of parathion powder (Eitzman and Wolfson 1967); in this lethal case, the time from contact of a hospital to death was 7 hours. Inhalation may have contributed to three additional deaths (Eitzman and Wolfson 1967).

A 1-hour LC_{50} of 137 mg/m^3 was calculated for technical parathion in female Sprague-Dawley rats (EPA 1978). All rats exhibited typical signs of cholinesterase inhibition, including salivation, lacrimation, exophthalmos, defecation, urination, and muscle fasciculations. An additional study reported a 4-hour LC_{50} of 84 mg/m^3 in male Sprague-Dawley rats exposed to technical-grade parathion (NIOSH 1974). Tremors, convulsions, and death occurred at concentrations ≥50 mg/m^3, but not at ≤35 mg/m^3. No lethality was observed in groups of four male beagle dogs exposed to up to 37.1 mg/m^3 aerosolized technical parathion for 4 hours followed by an observation period of 14 days (NIOSH 1974).

The LC_{50} values in female rats in the EPA (1978) study and in male rats in the NIOSH (1974) study are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.2 Systemic Effects

The highest NOAEL values and all reliable LOAEL values from each study for systemic effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1. No studies were located regarding hepatic, renal, endocrine, dermal, or ocular effects in humans or animals after inhalation exposure to parathion.

Table 3-1 Levels of Significant Exposure to Parathion - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL: Less Serious (mg/m³)	LOAEL: Serious (mg/m³)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Sprague-Dawley)	1 hr				137 F (1-hour LC50)	EPA 1978	
2	Rat (Sprague-Dawley)	4 hr				84 M (LC50)	NIOSH 1974	
Systemic								
3	Rat (Sprague-Dawley)	4 hr	Resp	35 M	50 M (respiratory difficulties)		NIOSH 1974	
			Gastro			26.1 M (diarrhea in 15/34 rats)		
4	Rat (Wistar)	1 hr	Resp		63 M (increased lung resistance after provocation test)		Pauluhn et al. 1987	No increase in lung resistance in the absence of provocation test
Neurological								
5	Rat (Sprague-Dawley)	4 hr			5.4 M (50% inhibition of RBC cholinesterase)	50 M (tremors in 8/34 rats)	NIOSH 1974	
6	Dog (Beagle)	4 hr			0.015 M (56% Inhibition RBC cholinesterase)	3.4 M (62% inhibition RBC cholinesterase)	NIOSH 1974	No apparent clinical signs.

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Table 3-1 Levels of Significant Exposure to Parathion - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
INTERMEDIATE EXPOSURE								
Systemic								
7	Rat (Sprague-Dawley)	6 wk 5 d/wk 7 hr/d	Hemato	0.74 M			NIOSH 1974	Hematology NOAEL is for hematocrit on week 6.
			Bd Wt	0.74 M				
8	Dog (Beagle)	6 wk 5 d/wk 7 hr/d	Bd Wt	0.2 M			NIOSH 1974	
Neurological								
9	Rat (Sprague-Dawley)	6 wk 5 d/wk 7 hr/d		0.01[b] M	0.1 M (35-40% decrease RBC cholinesterase during exposure)	0.74 M (85% decrease RBC cholinesterase on week 5)	NIOSH 1974	
10	Dog (Beagle)	6 wk 5 d/wk 7 hr/d		0.01 M	0.2 M (59% reduced RBC cholinesterase on week 6)		NIOSH 1974	

a The number corresponds to entries in Figure 3-1.

b Used to derive an intermediate-duration inhalation minimal risk level (MRL) of 0.00002 mg/m3 for parathion; the MRL was derived by dividing the duration-adjusted NOAEL by an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability).

Bd Wt = body weight; d = day; F = female; Gastro = gastrointestinal; Hemato = hematological;hr = hour; LC50 = lethal concentration, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; RBC = red blood cell; Resp = respiratory; wk = week

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Figure 3-1 Levels of Significant Exposure to Parathion - Inhalation

Acute (≤14 days)

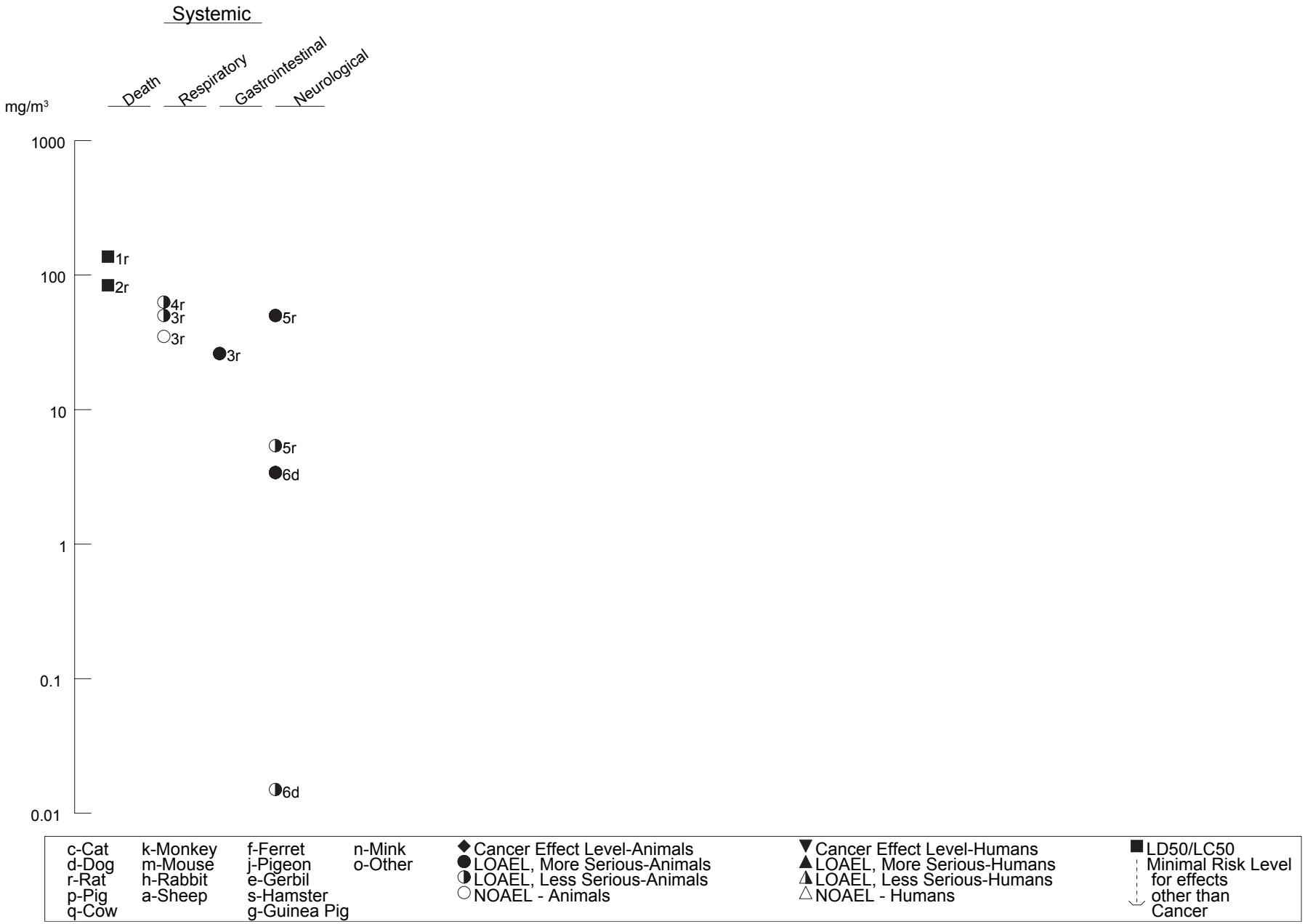

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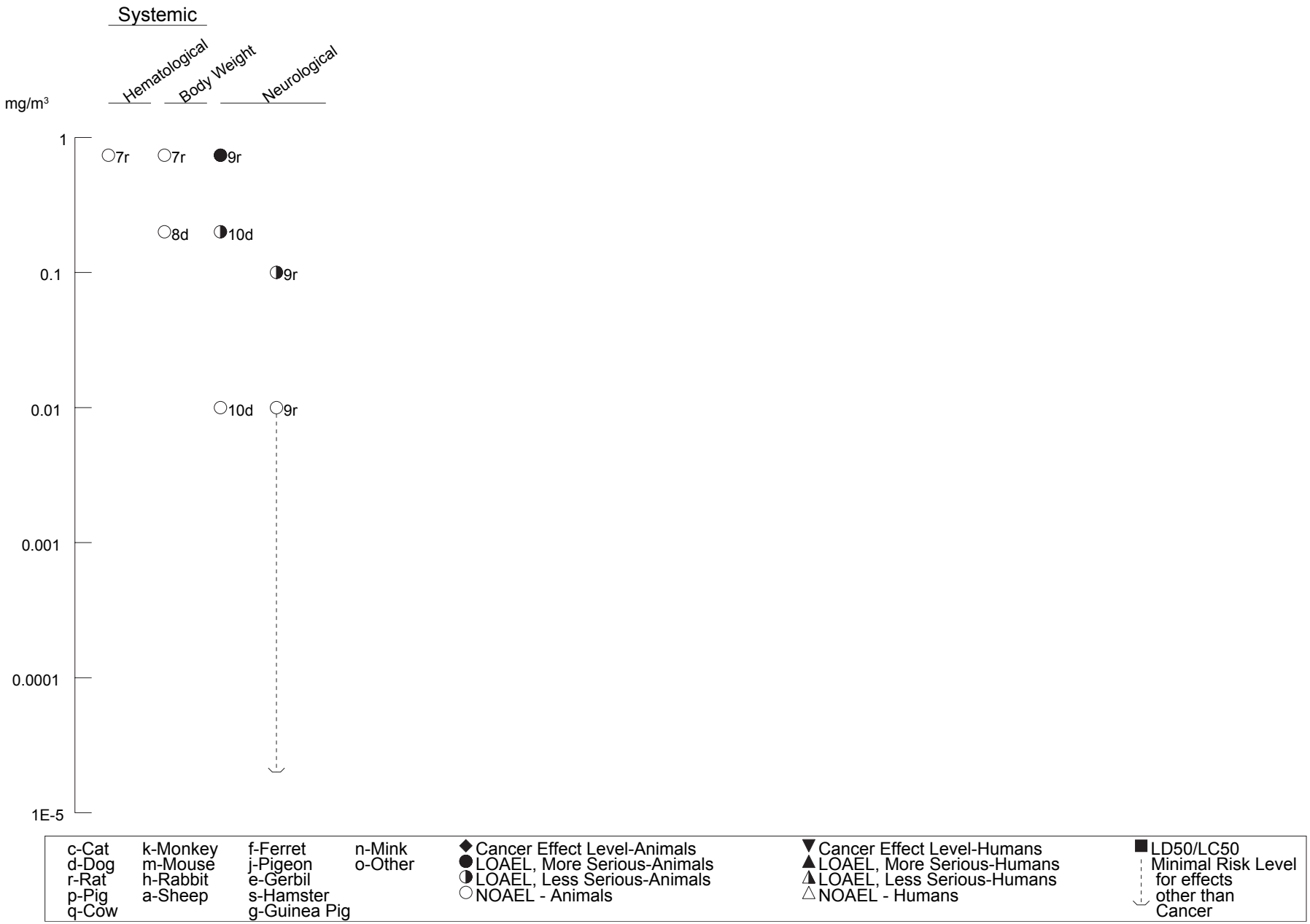
Figure 3-1 Levels of Significant Exposure to Parathion - Inhalation *(Continued)*

Intermediate (15-364 days)



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Respiratory Effects. Male Sprague-Dawley rats exposed to 50 mg/m^3 parathion aerosol for 4 hours showed respiratory difficulties; no such effect was observed at 35 mg/m^3 (NIOSH 1974). A study that examined the effects of technical-grade parathion on lung function in male Wistar rats reported that nose-only exposure to 63 mg/m^3 (the only concentration tested) aerosolized parathion did not affect basal lung resistance (Pauluhn et al. 1987). However, airway resistance was increased after a provocation test with acetylcholine.

Cardiovascular Effects. No explicit information regarding cardiovascular effects in animals was located in the limited number of inhalation studies available, most likely because cardiovascular end points were not monitored. Toxic doses of parathion high enough to induce a cholinergic crisis typically also affect heart rate and blood pressure, usually inducing hypertension.

Gastrointestinal Effects. Diarrhea was reported in 15 out of 34 male Sprague-Dawley rats exposed to 26.1 mg/m^3, the lowest concentration tested, technical parathion aerosol for 4 hours (NIOSH 1974). Gastrointestinal symptoms, such as nausea, abdominal pain and cramps, are commonly reported by humans after high exposure to parathion.

Hematological Effects. The only relevant information in the inhalation studies in animals available is that hematocrit was not altered in male Sprague-Dawley rats after exposure to 0.74 mg/m^3 (the highest concentration tested) technical parathion aerosol 7 hours/day, 5 days/week for 6 weeks (NIOSH 1974).

Musculoskeletal Effects. The available inhalation studies in animals do not provide any information regarding musculoskeletal effects following exposure to parathion. Muscle fasciculation tremors that occur following high exposure to parathion are of neurological origin and are discussed below in Section 3.2.1.4.

Body Weight Effects. Body weight was not affected in male Sprague-Dawley rats or male beagle dogs exposed to concentrations of 0.74 and 0.2 mg/m^3, respectively, of technical parathion aerosol (the highest concentrations tested) 7 hours/day, 5 days/week for 6 weeks (NIOSH 1974).

3.2.1.3 Immunological and Lymphoreticular Effects

No information was located regarding immunological and lymphoreticular effects in humans or animals following inhalation exposure to parathion.

3.2.1.4 Neurological Effects

Limited information regarding neurological effects in humans comes from two studies that assessed changes in red blood cell cholinesterase in subjects during exposure to parathion. The first study assessed the activity of plasma and red blood cell cholinesterase among workers at an industrial plant that manufactured concentrated parathion as well as dusts containing various concentrations of parathion (Brown and Bush 1950). Parathion was measured in air at different operations. The maximum concentration determined was 0.8 mg/m^3 and the estimated average was about 0.2 or 0.3 mg/m^3. The cohort consisted of 13 workers; only 1 worker was an unexposed person. No further data were provided regarding the study group. Due to the rotation of personnel, the 12 exposed subjects had only intermittent contact with parathion-contaminated air until July 1949, when production ceased. Therefore, the investigators noted that it was impossible to determine exactly what the total exposure had been. Analyses of blood from five subjects who provided successive blood samples over a 6-month period showed a decrease in plasma cholinesterase activity. However, the changes in red blood cell cholinesterase activity were less conclusive. The investigators noted that probably the most significant finding was the fact that measurements of cholinesterase activities conducted 5 months after the plant had stopped manufacturing parathion showed a marked increase in activities in almost all cases.

In the second study, two volunteers were exposed to various formulations of heated parathion dust or liquid technical parathion for periods of 30 minutes (Hartwell et al. 1964). The concentrations of parathion to which the subjects were exposed were not determined. Exposure to dusts heated to 82°F (~28°C) did not immediately reduced red blood cell cholinesterase activity, but it did so (15–20%) 7 hours after exposure began; levels returned to pre-exposure levels after 20 hours. Exposure to dust heated to 120°F (~49°C) reduced red blood cell cholinesterase activity (19%) in one subject immediately after exposure. A second exposure 24 hours later reduced the enzyme activity to 78% of pre-exposure levels. Exposure of one subject to vaporized technical parathion at 105°F (~41°C) had no significant effect on red blood cell cholinesterase; however, when the subject was exposed to the chemical at 120°F (~49°C) for 3 consecutive days, only the second exposure reduced the enzyme activity (29%) following exposure, and pre-exposure levels were achieved 20 hours later. No *p*-nitrophenol was detected in the urine after exposures at 82°F (~28°C), and only small amounts were detected at the higher temperatures.

Typical cholinergic signs were observed in acute toxicity studies with parathion in rats. Exposure of male Sprague-Dawley rats for 4 hours induced tremors and convulsions and eventually death (NIOSH 1974). The ED_{50} for red blood cell cholinesterase (exposure concentration that caused 50% inhibition) was 5.4 mg/m^3. The ED_{50} for tremors and convulsions were 73.7 and 110.6 mg/m^3, respectively. Exposure of groups of four male beagle dogs for 4 hours to up to 37.1 mg/m^3 parathion aerosol did not induce acute cholinergic signs; however, the lowest concentration tested, 0.015 mg/m^3, reduced red blood cell cholinesterase activity by 56% 48 hours after exposure, while 3.4 mg/m^3 inhibited the enzyme by 62% (NIOSH 1974).

In an intermediate-duration study, male Sprague-Dawley rats were exposed to 0.01, 0.1, or 0.74 mg/m^3 parathion aerosol 7 hours/day, 5 days/week for 6 weeks (NIOSH 1974). No clinical signs were seen in rats in the low- or mid-concentration groups. Some rats in the high-concentration group showed signs of parathion toxicity, but quantitative information was not available. The maximum decrease in red blood cell cholinesterase in the low-concentration group was approximately 30% and occurred on weeks 4 and 5. In the mid-concentration group, the maximum decrease in red blood cell cholinesterase was 43% and occurred on week 1. During the rest of the exposure period, red blood cell cholinesterase activity was 60–70% of pretest levels. In the high-concentration group, red blood cell cholinesterase activity was decreased 85% on week 5. In general, activities recovered during a 6-week post-dosing period. Groups of male beagle dogs were exposed in a similar manner to 0.001, 0.01, or 0.2 mg/m^3 parathion aerosol (NIOSH 1974). No information was provided regarding clinical signs in the dogs. No significant effects on levels of red blood cell cholinesterase were observed at the low exposure level. Exposure to 0.01 mg/m^3 parathion reduced red blood cell cholinesterase by 21% by the end of the second week of exposure. Red blood cell levels recovered and were <20% reduced the rest of the study. Exposure to 0.2 mg/m^3 parathion reduced red blood cell cholinesterase by 59% by the end of week 6 of exposure. Data regarding red blood cell AChE inhibition in rats were used to derive an intermediate-duration inhalation MRL for parathion.

The highest NOAEL values and all LOAEL values from each reliable study for neurological effects in each species and duration category are recorded in Table 3-1 and Figure 3-1.

No information was located regarding the following effects after inhalation exposure to parathion:

3.2.1.5 Reproductive Effects
3.2.1.6 Developmental Effects
3.2.1.7 Cancer

3.2.2 Oral Exposure

3.2.2.1 Death

Severe parathion poisoning can lead to death if not treated. Severe poisoning is generally characterized by unconsciousness, marked miosis and loss of pupillary reflex to light, muscle fasciculations, flaccid paralysis, secretions from the mouth and nose, moist rales in the lungs, respiratory difficulty and cyanosis, and serum cholinesterase levels <10% of normal value (Namba et al. 1971). If untreated patients poisoned with parathion are alive 24 hours after the onset, they usually recover (Namba et al. 1971). Death generally occurs due to respiratory failure attributed to excessive tracheobronchial and salivary secretions, nicotinic paralysis of the diaphragm and respiratory muscles and depression of central nervous system respiratory centers (Abou-Donia 1995).

There are numerous reports of deaths involving adults and children following ingestion of either a commercial parathion formulation or food prepared with contaminated components such as contaminated flour. Selected studies that reported multiple deaths are briefly summarized below; additional references can be found in those reports and in review articles (i.e., Gallo and Lawryk 1991).

Wishahi et al. (1958) reported that 8 out of 22 children who consumed parathion contaminated food in Egypt died. All fatal cases fell rapidly into a coma and died from respiratory failure 4–9 hours after the onset of symptoms. In a similar case of consumption of contaminated food, 17 out of 79 people died in Jamaica (Diggory et al. 1977). Deaths occurred within 6 hours of poisoning as a result of respiratory arrest. Postmortem examinations conducted in two cases showed pulmonary edema with intra-alveolar hemorrhage; no other significant gross findings were noted. In yet another report of ingestion of contaminated food, 14 out of 49 people exposed died in Sierra Leone (Etzel et al. 1987). Eitzman and Wolfson (1967) reported that 30 children died in the state of Florida from 1945 through 1964 due to exposure to parathion; 16 of the deaths resulted from ingestion of the pesticide. Six children ingested the parathion from improper containers such as a soft drink bottle. Another group of six children obtained the parathion from the floor or windowsill where it had been placed to kill roaches. The majority of the children were dead on arrival to the emergency room and the remainder died within 3 hours. Estimates of

lethal doses range from about 2 to 13 mg/kg in adults (assuming 70 kg body weight) and from 0.1 to 1.3 mg/kg in children (Gallo and Lawryk 1991). However, Gallo and Lawryk (1991) also indicate that there have been reports of patients who survived after ingesting 20,000–40,000 mg parathion and that prompt treatment with oximes can save some patients who have ingested as much as 50,000 mg parathion. In addition to potentially unreliable estimates, whether or not proper treatment was implemented after poisoning and how fast this was done probably impacted the outcome and contributed to the wide range of lethal doses reported for parathion in humans.

Several studies provide information regarding death in animals following oral exposure to parathion. Oral LD_{50} values of 14 and 7.9 mg/kg were reported in male and female Sprague-Dawley rats, respectively (EPA 1978). In another study with technical parathion in Sprague-Dawley rats, the 24-hour LD_{50} in males was 6.8 mg/kg, no deaths occurred at 4 mg/kg, and all rats in the group (n=10) dosed with 10 mg/kg died within 2 hours (NIOSH 1974). Gaines (1960) reported oral LD_{50} values of 13 and 3.6 mg/kg in male and female Sherman rats, respectively. To determine whether LD_{50} values underwent seasonal variations, Gaines and Linder (1986) conducted bimonthly determinations in male and female Sherman rats over a period of 1 year. The LD_{50} values ranged from 6.9 to 11.0 mg/kg in males and from 3.0 to 3.4 mg/kg in females, suggesting that LD_{50} values were little affected by the time of the year that the tests were conducted. Pasquet et al. (1976) reported 10-day LD_{50} values of 16 and 6 mg/kg for technical parathion in male and female CD rats, respectively. The results of these studies also suggested that female rats are more sensitive to the acute effects of parathion than male rats. Signs of poisoning reported in some of these studies included muscle fasciculation, excessive salivation, lacrimation, tremors, diarrhea, and involuntary urination. In a developmental study, administration of 1 mg parathion/kg/day to a group of 25 pregnant Wistar rats on gestation days 6 through 15 resulted in 13 deaths; no deaths occurred with 0.3 mg parathion/kg/day (Renhof 1984). The exact time of death was not indicated, but it was before the study termination on gestation day 20 (Renhof 1984). Deaths were attributed to respiratory and circulatory failure.

In a 6-week dietary study with technical parathion in Osborne-Mendel rats, two out of five males dosed approximately 14 mg parathion/kg/day died within 2 weeks and two out of five females dosed with approximately 7.6 mg parathion/kg/day died during the first week of the study (NCI 1979). No males dosed with 7 mg/kg/day or females dosed with 3.8 mg/kg/day died during the study.

In an intermediate-duration study in albino rats, feeding the animals a diet that provided approximately 5.3 mg parathion/kg/day (76.8% active ingredient) 6 days/week resulted in 24 out of 72 rats dying within

3 weeks of starting the experiment; the exact times of death were not specified (Barnes and Denz 1951). The 5.3 mg/kg/day dose was the lowest dose tested. In a group dosed with approximately 7.9 mg/kg/day, 59 out of 72 rats died within 27 days in the study. In a group dosed with approximately 10.5 mg/kg/day group, 65 out of 72 rats died in the first 19 days of the study.

A 36% mortality rate was reported among male C57BL/6N mice 2 days after receiving a single gavage dose of 16 mg technical parathion/kg (Casale et al. 1983). Mild to severe signs of toxicity, including tremors, muscle fasciculation, and excessive salivation, began 1–2 hours after dosing and lasted for 4–7 hours. In a 6-week dietary study with $B6C3F_1$ mice, all five males dosed with approximately 58 mg technical parathion/kg/day and four of the five females dosed with approximately 62 mg parathion/kg/day died during the second week of the study (NCI 1979). Doses of approximately 29 and 31 mg/kg/day were not lethal to males or females, respectively.

In male beagle dogs (4/group), the 24-hour LD_{50} for technical parathion administered in a capsule was 8.27 mg/kg (NIOSH 1974). No deaths occurred at 2.5 mg/kg; all dogs administered doses of 20 mg/kg died.

Reliable oral LD_{50} values and lethal doses are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

The highest NOAEL values and all reliable LOAEL values from each study for systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

Respiratory Effects. Commonly reported respiratory signs and symptoms occurring after oral exposure to parathion include excessive bronchial secretions, rhinorrhea, wheezing, edema, tightness of the chest, bronchospasms, bronchoconstriction, cough, and dyspnea. In cases of fatal intoxication in children described by Wishahi et al. (1958), hyperpnea was the earliest manifestation of respiratory failure, which in turn was the direct cause of death. Shallow respiration and pulmonary edema were reported in similar cases described by Eitzman and Wolfson (1967). Among 246 cases of acute parathion poisoning reported by Tsachalinas et al. (1971), 92 had increased bronchial secretions and 33 developed lung edema. Among 79 cases of intoxication due to ingestion of contaminated food in Jamaica, dyspnea was reported in the more severe cases (Diggory et al. 1977); deaths occurring in this study were due to respiratory arrest. Postmortem examination of 17 fatalities showed pulmonary edema and intra-alveolar

Table 3-2 Levels of Significant Exposure to Parathion - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Sprague-Dawley)	once (G)				14 M (LD50) 7.9 F (LD50)	EPA 1978	
2	Rat (Sherman)	once (GO)				13 M (LD50) 3.6 F (LD50)	Gaines 1960	
3	Rat (Sherman)	once (GO)				6.9 M (LD50) 3 F (LD50)	Gaines and Linder 1986	
4	Rat (Osborne-Mendel)	1 wk ad libitum (F)				14 M (2/5 deaths on week 2) 7.6 F (2/5 deaths on week 1)	NCI 1979	
5	Rat (Sprague-Dawley)	once (GO)				6.83 M (24-hour LD50)	NIOSH 1974	
6	Rat (CD)	once (G)				16 M (10-day LD50) 6 F (10-day LD50)	Pasquet et al. 1976	
7	Rat (Wistar)	10 d Gd 6-15 1 x/d (GW)				1 F (13/25 deaths)	Renhof 1984	
8	Mouse (C57BL/6N)	once (GO)				16 M (36% mortality rate)	Casale et al. 1983	

Table 3-2 Levels of Significant Exposure to Parathion - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
9	Mouse (C57BL/6N)	once				16 M (20% lethality)	Casale et al. 1984	
10	Mouse (B6C3F1)	2 wk ad libitum (F)				58 M (5/5 deaths on week 2) 62 F (4/5 deaths on week 2)	NCI 1979	
11	Dog (Beagle)	once (C)				8.27 M (24-hour LD50)	NIOSH 1974	
Systemic								
12	Rat (Long- Evans)	once (GO)	Bd Wt	4 M		7 M (9.7% body weight loss)	Moser 1995	
13	Rat (Wistar)	10 d Gd 6-15 1 x/d (GW)	Bd Wt	0.3 F		1 F (56% reduced weight gain during treatment)	Renhof 1984	1 mg/kg/day also caused lethality.
14	Mouse (BALB/c)	once (GO)	Hepatic	16 F			Kim et al. 2005	Hepatic NOAEL is for serum AST and ALT activities.
			Bd Wt	16 F				
15	Mouse (Swiss-Webster)	5 d 1 x/d (GO)	Bd Wt	5.3 M			Thomas and Schein 1974	

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Table 3-2 Levels of Significant Exposure to Parathion - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
16	Mouse (BALB/c)	8 d 1x/d (GO)	Bd Wt			2.2 F (20% reduction in body weight)	Wiltrout et al. 1978	
Immuno/ Lymphoret								
17	Mouse (C57BL/6N)	once (GO)			16 M (suppressed IgM PFC response)		Casale et al. 1983	Dose also caused lethality.
18	Mouse (C57BL/6N)	once		4 M	16 M (suppressed IgM response to SRBC)		Casale et al. 1984	The high dose also caused lethality.
19	Mouse CBA/J	5 d 1 x/d (GO)			0.4 F (increased response to allergens)		Fukuyama et al. 2010	
20	Mouse (BALB/c)	5 d 1 x/d (GO)			0.15 F (increased sensitivity to allergens)		Fukuyama et al. 2011	
21	Mouse C3H/HeN	5 d 1 x/d (GO)		0.15 F	1.5 F (decreased SRBC-specific IgM response in blood)		Fukuyama et al. 2012	
22	Mouse (BALB/c)	once (GO)		4 F	16 F (suppressed antibody response to SRBC)		Kim et al. 2005	

Table 3-2 Levels of Significant Exposure to Parathion - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
23	Mouse (BALB/c)	8 d 1x/d (GO)			2.2 F (suppressed humoral immune response)		Wiltrout et al. 1978	A 20% loss in body weight occurred at 2.2 mg/kg/day
Neurological								
24	Human NA	5 d 1 x/d (F)		0.028			Morgan et al. 1977	NOAEL is for clinical signs and RBC cholinesterase activity.
25	Monkey (Cynomolgus)	once (GO)			2 (50% depression of RBC cholinesterase)		Elkner et al. 1991	
26	Monkey (Rhesus)	once		0.5 M	1 M (abolished performance of a learned task)		Reiter et al. 1975	
27	Rat (Long- Evans)	once (GO)		4 M	7 M (altered neurological functions)		Moser 1995	
28	Rat (Sprague-Dawley)	once (GO)		4 M		5 M (tremors in 10/10)	NIOSH 1974	
29	Rat (Sprague-Dawley)	once (GO)		0.35 M	0.7 M (27.4% inhibition of RBC cholinesterase)	5.6 M (69.7% inhibition of RBC cholinesterase)	NIOSH 1974	

Table 3-2 Levels of Significant Exposure to Parathion - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
30	Rat (Sprague-Dawley)	once (GO)			2.8 M (56% inhibiton RBC cholinesterase 4 hours after dosing)		NIOSH 1974	
31	Rat (CD)	once (G)			1.1 F (50% inhibition of RBC cholinesterase)		Pasquet et al. 1976	Percent inhibition 24 hours after dosing.
32	Rat (Wistar)	10 d Gd 6-15 1 x/d (GW)		0.3 F		1 F (tremors in 8/25 rats)	Renhof 1984	1 mg/kg/day also caused lethality.
33	Mouse (C57BL/6N)	once (GO)				16 M (tremors, fasciculations)	Casale et al. 1983	Dose also caused lethality.
34	Mouse (Swiss-Webster)	once (G)			6 M (impaired learning of a passive avoidance task)		Reiter et al. 1973	
35	Dog (Beagle)	once (C)		2.5 M		6.3 M (tremors, ataxia, convulsions)	NIOSH 1974	
36	Dog (Beagle)	once (C)			0.5 M (29% inhibition of RBC cholinesterase)	2.5 M (64% inhibition of RBC cholinesterase)	NIOSH 1974	

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Table 3-2 Levels of Significant Exposure to Parathion - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
37	Dog (Beagle)	once (C)				2.5 M (64% inhibition of RBC cholinesterase 24 hours after dosing)	NIOSH 1974	
Reproductive								
38	Mouse (Swiss-Webster)	5 d 1 x/d (GO)		5.3 M			Thomas and Schein 1974	NOAEL is for testis and prostate weight and metabolism of testosterone.
Developmental								
39	Rat (Wistar)	10 d Gd 6-15 1 x/d (GW)		1 F			Renhof 1984	NOAEL is for standard developmental parameters.
40	Rabbit (Himalayan)	13 d Gd 6-18 1 x/d (GW)		0.3 F			Renhof 1985	NOAEL is for standard developmental parameters.
INTERMEDIATE EXPOSURE								
Death								
41	Rat (albino)	3 wk 6 d/wk (F)				5.3 (24/72 deaths within 3 weeks)	Barnes and Denz 1951	
Systemic								
42	Rat (NS)	90 d 1 x/d (GO)	Hepatic		2.6 M (mild liver histopathology)		Dikshith et al. 1978	
			Renal	2.6 M				

Table 3-2 Levels of Significant Exposure to Parathion - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
43	Rat (Wistar)	4-15 wk ad libitum (F)	Bd Wt	0.55 F			Ivens et al. 1998	
44	Rat (Osborne-Mendel)	6 wk ad libitum (F)	Bd Wt	3.8 F		7.6 F (body weight reduced 24%)	NCI 1979	
45	Rat (Sprague-Dawley)	6 wk 5 d/wk (GO)	Bd Wt	0.25 M			NIOSH 1974	
46	Mouse (B6C3F1)	6 wk ad libitum (F)	Bd Wt		29 M (body weight reduced 14%)		NCI 1979	
47	Dog (Beagle)	60 d 1 x/d (C)	Bd Wt	0.794			Atkinson et al. 1994	
48	Dog (Beagle)	6 wk 5 d/wk (C)	Bd Wt	0.5 M			NIOSH 1974	
Neurological								
49	Human (NA)	25-70 d 5 d/wk (NS)		0.1 F			Edson 1964	NOAEL is for RBC cholinesterase activity.

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Table 3-2 Levels of Significant Exposure to Parathion - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
50	Human (NA)	30 d 1 x/d (C)		0.09[b] M	0.11 M (22 and 37% reduced RBC cholinesterase in 2/5 subjects)		Rider et al. 1969	
51	Monkey Squirrel	148 d 1 x/d (C)			0.1 M (increased variability in hearing thresholds)		Reishchl et al. 1975	
52	Rat (albino)	3 wk 6 d/wk (F)				5.3 (fasciculations, tremors)	Barnes and Denz 1951	Dose also caused lethality.
53	Rat (NS)	90 d 1 x/d (GO)			2.6 M (50% reduced brain cholinesterase)		Dikshith et al. 1978	
54	Rat (Wistar)	4-15 wk ad libitum (F)		0.024 M	0.1 M (36-39% reduced RBC cholinesterase)	0.4 M (>80% reduced RBC cholinesterase)	Ivens et al. 1998	
55	Rat (Osborne-Mendel)	364 d ad libitum (F)				3.5 F (generalized body tremors)	NCI 1979	
56	Rat (Sprague-Dawley)	6 wk 5 d/wk (GO)		0.05 M	0.1 M (22% inhibition of RBC cholinesterase on week 4)		NIOSH 1974	

Table 3-2 Levels of Significant Exposure to Parathion - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
57	Dog (Beagle)	60 d 1 x/d (C)		0.794			Atkinson et al. 1994	NOAEL is for ocular function.
58	Dog Mixed breed	24 weeks ad libitum (F)		0.021	0.047 (25% reduced RBC cholinesterase)		Frawley and Fuyat 1957	
59	Dog (Beagle)	6 wk 5 d/wk (C)		0.1 M	0.5 M (25-58% inhibition of RBC cholinesterase)		NIOSH 1974	
Reproductive								
60	Rat (NS)	90 d 1 x/d (GO)			2.6 M (tubular atrophy in testes)		Dikshith et al. 1978	
Developmental								
61	Rat (CD)	34 d Gd 2-21 Ld 1-15 (GO)			0.01 (altered EKG in pups)		Deskin et al. 1979	

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Table 3-2 Levels of Significant Exposure to Parathion - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
CHRONIC EXPOSURE								
Systemic								
62	Rat (albino)	365 d 6 d/wk (F)	Resp	1.7			Barnes and Denz 1951	NOAELs are for organs histopathology.
			Cardio	1.7				
			Gastro	1.7				
			Hepatic	1.7				
			Renal	1.7				
			Endocr	1.7				
			Bd Wt	1.7				
			Other	1.7				
63	Rat (Osborne-Mendel)	80 wk ad libitum (F)	Resp	4.4 M			NCI 1979	NOAELs are for organ histopathology. Hematological NOAEL is for bone marrow.
			Cardio	4.4 M				
			Gastro	4.4 M				
			Hemato	4.4 M				
			Musc/skel	4.4 M				
			Hepatic	4.4 M				
			Renal	4.4 M				
			Endocr	4.4 M				
			Dermal	4.4 M				

Table 3-2 Levels of Significant Exposure to Parathion - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
64	Mouse (B6C3F1)	62-80 wk ad libitum (F)	Resp	27.6			NCI 1979	NOAELs are for organ histopathology. Hematology NOAEL is for bone marrow.
			Cardio	27.6				
			Hemato	27.6				
			Musc/skel	27.6				
			Hepatic	27.6				
			Renal	27.6				
			Endocr	27.6				
Immuno/ Lymphoret								
65	Rat (albino)	365 d 6 d/wk (F)		1.7			Barnes and Denz 1951	NOAEL is for histopathology of lymphoreticular organs.
66	Rat (Osborne-Mendel)	80 wk ad libitum (F)		4.4 M			NCI 1979	NOAEL is for histopathology of the spleen and lymph nodes.
67	Mouse (B6C3F1)	62-80 wk ad libitum (F)		27.6			NCI 1979	NOAEL is for histopathology of the spleen and lymph nodes.

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Table 3-2 Levels of Significant Exposure to Parathion - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Neurological								
68	Rat (albino)	365 d 6 d/wk (F)		1.7			Barnes and Denz 1951	NOAEL is for clinical signs and brain histopathology.
Reproductive								
69	Rat (albino)	365 d 6 d/wk (F)		1.7			Barnes and Denz 1951	NOAEL is for histopathology of ovaries and testis.
70	Rat (Osborne-Mendel)	80 wk ad libitum (F)		4.4 M 3.5 F			NCI 1979	NOAEL is for histopathology of the sex organs.
71	Mouse (B6C3F1)	62-80 wk ad libitum (F)		27.6			NCI 1979	NOAEL is for histopathology of the sex organs.
Cancer								
72	Rat (Osborne-Mendel)	80 wk ad libitum (F)				2.2 M (CEL:adrenal cortical adenoma or carcinoma)	NCI 1979	Comparison is between low-dose group and pooled controls.

a The number corresponds to entries in Figure 3-2.

b Used to derive an intermediate-duration oral minimal risk level (MRL) of 0.009 mg/kg/day for parathion; the MRL was derived by dividing the NOAEL by an uncertainty factor of 10 (for human variability).

ALT = alanine aminotransferase; AST = aspartate aminotransferase; Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; (F) = feed; F = Female; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; (GO) = gavage in oil; Hemato = hematological; IgM = immunoglobulin M; Immuno/Lymphoret = immunological/lymphoreticular; Ld = lactation day; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; Musc/skel = musculoskeletal; NA = not applicable; NOAEL = no-observed-adverse-effect level; NS = not specified; PFC = plaque-forming cells; RBC = red blood cell; Resp = respiratory; SRBC = sheep red blood cell; x = time(s); wk = week(s)

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Figure 3-2 Levels of Significant Exposure to Parathion - Oral

Acute (≤14 days)

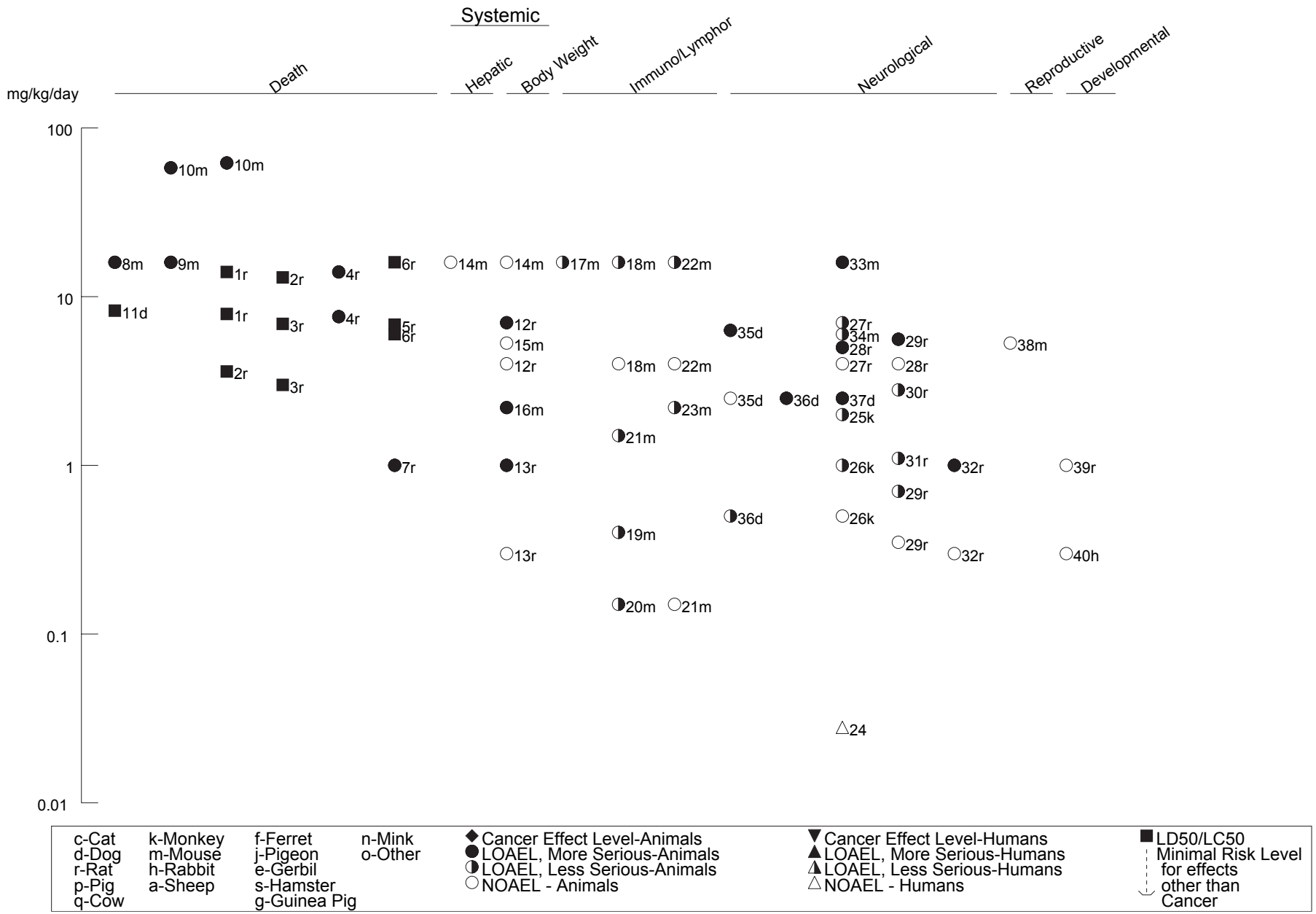

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Figure 3-2 Levels of Significant Exposure to Parathion - Oral *(Continued)*

Intermediate (15-364 days)

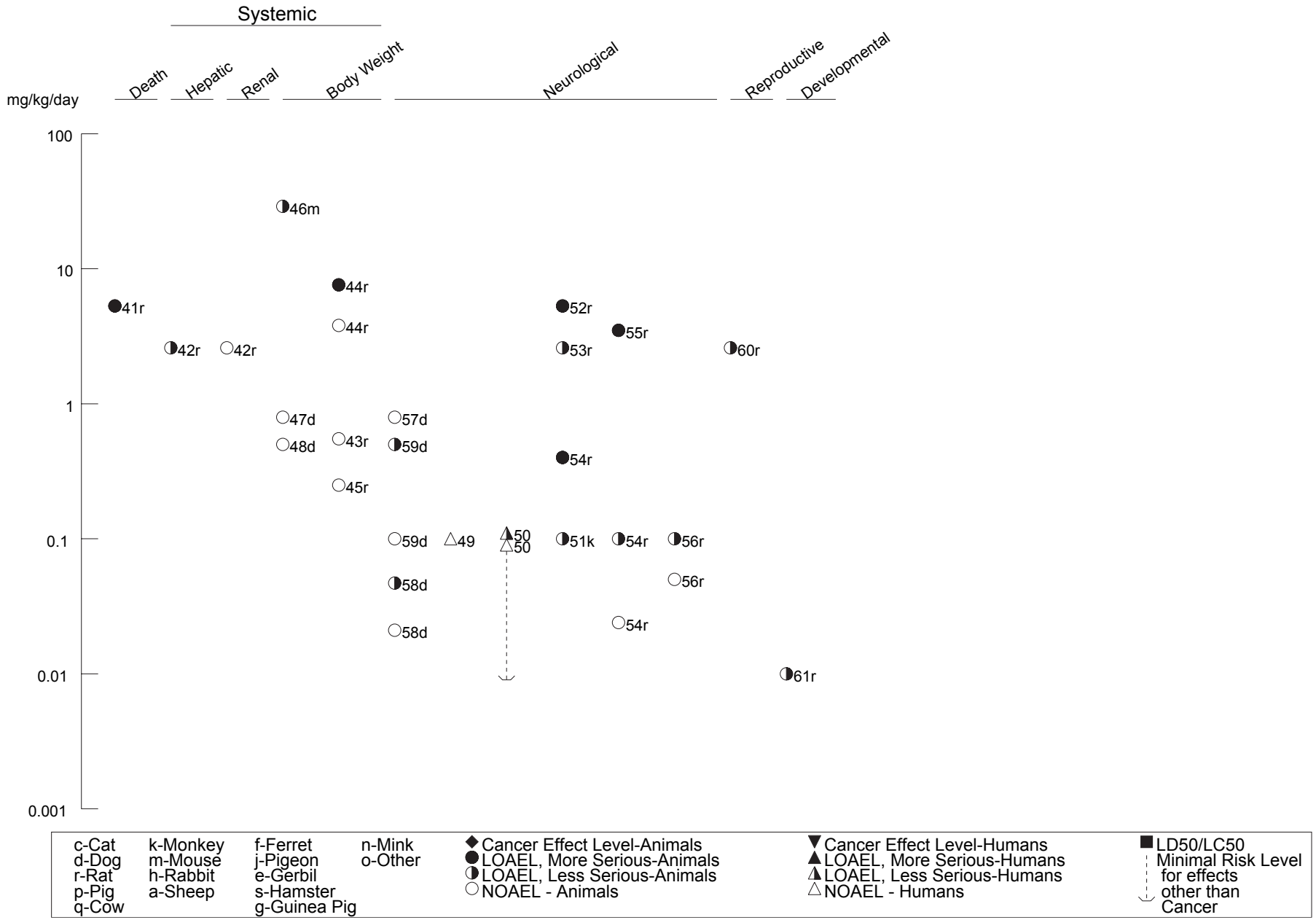

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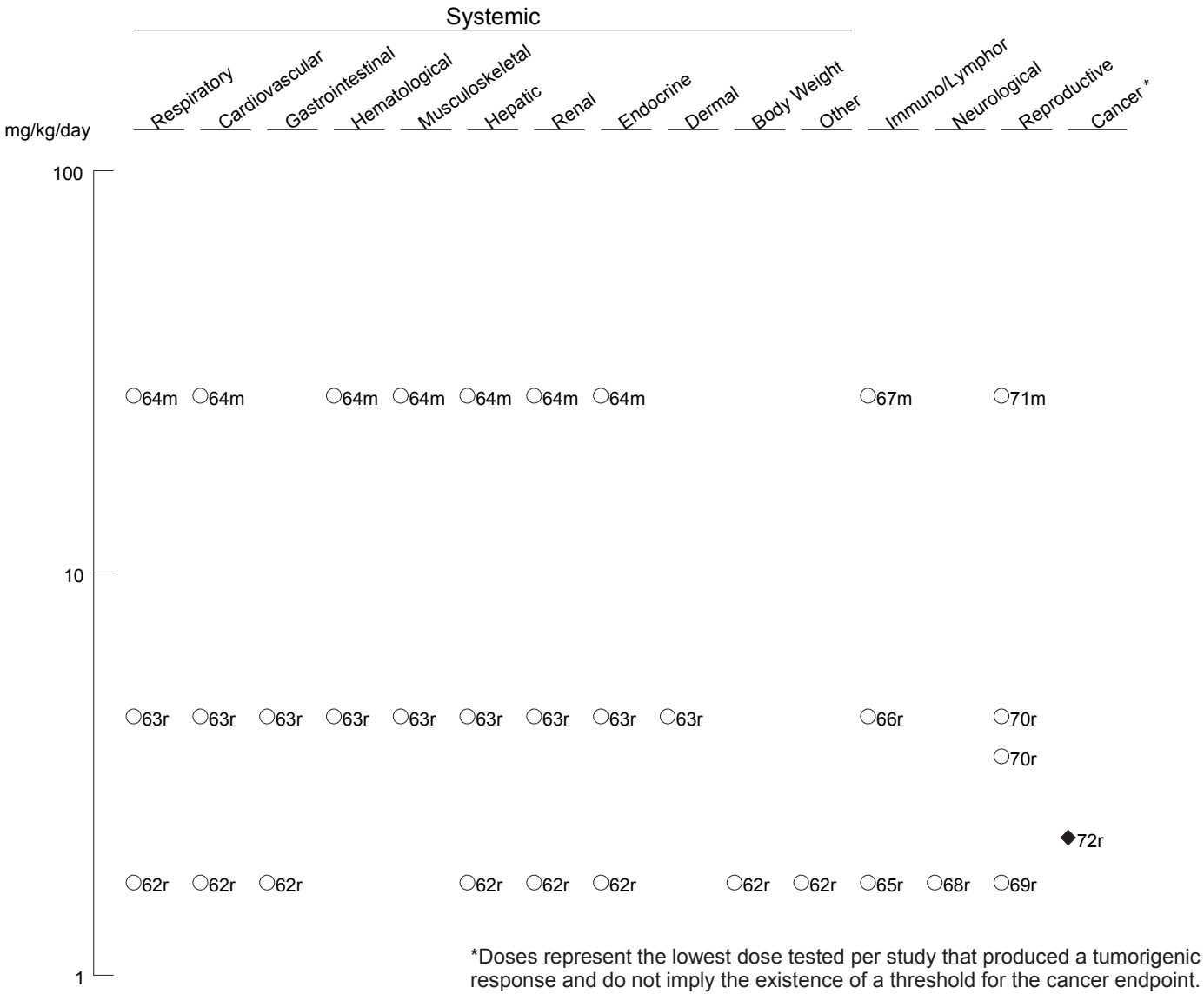
Figure 3-2 Levels of Significant Exposure to Parathion - Oral *(Continued)*

Chronic (≥365 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat d-Dog r-Rat p-Pig q-Cow	k-Monkey m-Mouse h-Rabbit a-Sheep	f-Ferret j-Pigeon e-Gerbil s-Hamster g-Guinea Pig	n-Mink o-Other	◆ Cancer Effect Level-Animals ● LOAEL, More Serious-Animals ◑ LOAEL, Less Serious-Animals ○ NOAEL - Animals	▼ Cancer Effect Level-Humans ▲ LOAEL, More Serious-Humans ◭ LOAEL, Less Serious-Humans △ NOAEL - Humans	■ LD50/LC50 Minimal Risk Level for effects other than Cancer

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hemorrhage in two of them. Edema of the lungs and bronchospasms were reported in a study of 68 cases of acute poisoning in China (He et al. 1998). Similar findings were reported in multiple cases of intoxication due to consumption of contaminated food in Sierra Leone (Etzel et al. 1987).

Chronic exposure of rats to up to 4.4 mg parathion/kg/day or of mice to up to 27.6 mg parathion/kg/day did not result in gross or microscopic alterations in the respiratory tract (Barnes and Denz 1951; NCI 1979).

Cardiovascular Effects. Inhibition of AChE can result in overstimulation of muscarinic and nicotinic receptors, both of which play a role in the control of blood pressure and heart rate. Therefore, individuals acutely poisoned with parathion can present with tachycardia or bradycardia and hypertension or hypotension. Six of eight fatal cases of children described by Wishahi et al. (1958) developed shock and two of them developed hypertension; hypertension was also reported in cases that survived. One of the cases studied by Lankisch et al. (1990) developed high blood pressure and tachycardia 1 week after ingesting parathion; the other case was in shock (no blood pressure measurable) on admission to the emergency room. Hypotension and bradycardia were seen among the children who died as described by Eitzman and Wolfson (1967). Bradycardia and hypertension were prevalent in a study of 246 acute poisonings in Greece (Tsachalinas et al. 1971). Among 79 cases of acute poisoning via contaminated food studied by Diggory et al. (1977), bradycardia was reported in the most severe cases. Circulatory insufficiency and bradycardia were seen in four out of six cases of acute poisoning studied by Eyer et al. (2003). Bradycardia was also reported in other single cases of poisoning (i.e., De Jager et al. 1981; Nisse et al. 1998).

Chronic exposure of rats to up to 4.4 mg parathion/kg/day or of mice to up to 27.6 mg parathion/kg/day did not result in gross or microscopic alterations in the heart (Barnes and Denz 1951; NCI 1979).

Gastrointestinal Effects. Nausea, vomiting, abdominal tightness, swelling and cramps, diarrhea, tenesmus, and fecal incontinence are typical signs and symptoms of acute intoxication with organophosphorus pesticides. Nausea, vomiting, and cramps have been reported in numerous studies of multiple and single cases of poisoning that included children and adults (i.e., Diggory et al. 1977; Eitzman and Wolfson 1967; Etzel et al. 1987; He et al. 1998; Hoffman and Papendorf 2006; Tsachalinas et al. 1971; Wishahi et al. 1958). Severe poisoning can rapidly induce loss of consciousness; therefore, information regarding gastrointestinal effects in these cases may not be available. In addition, some

reports only state that the victim suffered a cholinergic crisis without detailing specific signs and symptoms.

Diarrhea has been reported in animals in studies of acute toxicity of parathion. Diarrhea is neurological in origin and results from stimulation of parasympathetic autonomic post-ganglionic fiber innervating smooth gastrointestinal musculature. Chronic exposure of rats to up to 4.4 mg parathion/kg/day or of mice to up to 27.6 mg parathion/kg/day did not result in gross or microscopic alterations in the gastrointestinal tract (Barnes and Denz 1951; NCI 1979).

Hematological Effects. Very few reports of humans exposed orally to parathion provide information regarding hematological effects following oral exposure to parathion. In most cases, it is unknown whether hematological tests were conducted following poisoning with parathion or, if conducted, the results were unremarkable and were not discussed in the report. Leukocytosis was reported in one of the six cases described by Eyer et al. (2003), in a case described by Nisse et al. (1998), and in two cases described by Lankisch et al. (1990). Leukocytosis may occur secondary to increased catecholamine release from the adrenal medulla triggered by acetylcholine released by preganglionic fibers (Osmundson 1998). Effects on red blood cell cholinesterase are discussed in Section 3.2.4, Neurological Effects.

No information was located in the studies available regarding hematological effects in animals following oral exposure to parathion, except for lack of alterations in bone marrow from rats exposed to up to 4.4 mg parathion/kg/day and mice exposed to up to 27.6 mg parathion/kg/day in chronic-duration studies (Barnes and Denz 1951; NCI 1979).

Musculoskeletal Effects. Acute intoxication with parathion results in tremors, muscle fasciculations, and convulsions. These are signs of hyperstimulation of both nicotinic and muscarinic receptors in the central nervous system and of nicotinic receptors at the neuromuscular junction. Denervation potentials were recorded in the anterior tibial and gastrocnemius muscles and in the small hand muscles from a subject who developed polyneuropathy (see Section 3.2.2.4) after ingesting a large amount of parathion (De Jager et al. 1981). The subject had been in a coma for 7 weeks and the recordings were made 54 days after the poisoning. Denervation potentials were still seen in the anterior tibial muscles 1 year after poisoning. In another case of polyneuropathy, electromyography (EMG) of both anterior tibial muscles showed profuse fibrillations without voluntary motor unit potentials present several weeks after poisoning (Besser et al. 1993). Thenar EMG showed only few fasciculations with reduced recruitment. Microscopic examination of the quadriceps and deltoid muscles from a subject who

developed intermediate syndrome (see Section 3.2.2.4) after ingesting parathion showed small groups of atrophic fiber and mild fiber type grouping in the former (De Bleecker et al. 1992). Endplate staining for AChE and nonspecific esterase was absent in both muscles. Microscopic examination of the intercostal muscle 35 days after the poisoning showed a fair number of atrophic angulated fibers. Muscle potentials of reduced amplitude were reported in another study of cases that developed intermediate syndrome after ingestion of parathion (He et al. 1998).

Chronic-duration studies did not report gross or microscopic alterations in skeletal bone from rats exposed to up to 4.4 mg parathion/kg/day through the diet or in bone from mice similarly exposed to up to 27.6 mg parathion/kg/day (Barnes and Denz 1951; NCI 1979).

Hepatic Effects. No information was located regarding hepatic effects in humans following oral exposure to parathion. It is reasonable to assume that laboratory tests conducted in poisoned individuals may have included tests for liver function. Therefore, the lack of explicit information probably reflects the fact that liver function is usually not affected, except in the most severe cases in which death is due to multiorgan failure, as in, for example, cases described by Eyer et al. (2003).

Administration of a single dose of 16 mg parathion/kg, the highest dose tested, to Balb/c mice did not significantly alter alanine aminotransferase (ALT) or aspartate aminotransferase (AST) or the liver content of reduced glutathione 4 days after dosing (Kim et al. 2005). However, these parameters were altered by parathion in mice pretreated with phenobarbital, indicating that metabolic activation plays a role in parathion-induced hepatotoxicity. In an intermediate-duration study, daily treatment of male rats by gavage with 2.6 mg parathion/kg/day (only dose level tested) for 90 days resulted in mild changes in the liver consisting of hepatocyte swelling, congestion of blood vessels of the portal triads, and mild proliferation of fibroblasts around the bile ducts (Dikshith et al. 1978). Chronic exposure of rats to up to 4.4 mg parathion/kg/day or of mice to up to 27.6 mg parathion/kg/day did not result in gross or microscopic alterations in the liver (Barnes and Denz 1951; NCI 1979).

Renal Effects. As with hepatic effects, renal failure seems to develop in severe cases of poisoning with parathion that ultimately result in death (i.e., Eyer et al. 2003).

Treatment of male rats by gavage with 2.6 mg parathion/kg/day, the only dose level tested, for 90 days did not induce microscopic alterations in the kidneys (Dikshith et al. 1978). Chronic exposure of rats to

up to 4.4 mg parathion/kg/day or of mice to up to 27.6 mg parathion/kg/day did not result in gross or microscopic alterations in the kidneys (Barnes and Denz 1951; NCI 1979).

Endocrine Effects. No information was located regarding endocrine effects in humans following oral exposure to parathion. However, effects such as tachycardia, hypertension, and hyperglycemia, which have been reported in some cases of acute poisoning with parathion, may have been due in part to stimulation of the adrenal medulla by pre-ganglionic autonomic fibers.

No information was located in the available literature regarding endocrine effects in animals following oral exposure to parathion.

Dermal Effects. No information was located regarding dermal effects in humans following oral exposure to parathion.

Chronic dietary exposure of Osborne-Mendel rats to up to 4.4 mg parathion/kg/day or B6C3F$_1$ mice to up to 27.6 mg parathion/kg/day did not induce microscopic alterations in the skin (NCI 1979). No further information was located in the available literature.

Ocular Effects. Miosis and loss of pupillary reflexes resulting from the excess acetylcholine on parasympathetic autonomic post-ganglionic nerve fibers are typically seen in individuals acutely poisoned with parathion (see Section 3.2.2.4, Neurological Effects).

No information was located regarding ocular effects in animals following oral exposure to parathion.

Body Weight Effects. Effects on body weight would not be expected in humans following acute intoxication with parathion.

Acute-, intermediate-, and chronic-duration studies provide information regarding body weight in animals following oral exposure to parathion. In general, information regarding food consumption was not provided. In male Long-Evans rats, a single gavage dose of 7 mg parathion/kg induced a 9.7% reduction in body weight in 24 hours; this dose also caused tremors and gait changes (Moser 1995). In Balb/c mice, a single gavage dose of 16 mg parathion/kg did not affect body weight over a 4-day observation period (Kim et al. 2005), but 2.2 mg parathion/kg administered by gavage to female Balb/c mice for 8–13 days induced a 20% reduction in body weight (Wiltrout et al. 1978).

In intermediate-duration studies, the lowest LOAEL was 7.6 mg parathion/kg/day, a dose that induced a 24% decrease in body weight in female Osborne-Mendel rats following 6 weeks of dietary exposure (NCI 1979); the NOAEL was 3.8 mg parathion/kg/day. Mice appeared to be less sensitive, as doses of 31 mg parathion/kg/day in the diet for 6 weeks did not significantly affect the mean body weight of female $B6C3F_1$ mice, but 29 mg parathion/kg/day reduced body weight in males by 14% (NCI 1979). In a 6-week study in beagle dogs, 0.5 mg parathion/kg/day administered in a capsule 5 days/week did not significantly affect body weight (NIOSH 1974).

NCI (1979) reported that Osborne-Mendel rats fed a diet that provided approximately 4.4 mg parathion/kg/day to males and 3.5 mg/kg/day to females for 80 weeks had generally lower body weight than controls, but quantitative data were not provided. A similar observation was noted regarding male $B3C6F_1$ mice dosed with 27.6 mg parathion/kg/day for 62 weeks, but not in females receiving the same dose for 80 weeks (NCI 1979).

Metabolic Effects. No specific information was located regarding metabolic effects in humans following oral exposure to parathion. However, effects secondary to adrenal medulla stimulation, such as hyperglycemia, would not be unexpected. In addition, alterations in acid/base balance such as metabolic acidosis may be expected in severely poisoned subjects, particularly those exhibiting renal failure.

No information was located in the available literature regarding metabolic effects in animals following oral exposure to parathion.

Other Systemic Effects. Painless acute hemorrhagic pancreatitis was reported in two out of nine cases of acute parathion intoxication described by Lankisch et al. (1990). The investigators discussed the possibility that the condition was due to parathion-induced increase of pancreatic intraductal pressure and stimulation of pancreatic secretion.

3.2.2.3 Immunological and Lymphoreticular Effects

No information was located regarding immunological and lymphoreticular effects in humans following oral exposure to parathion.

A limited number of studies in animals have shown that oral exposure to parathion can affect immune function. Administration of eight doses of 2.2 mg parathion/kg/day to female BALB/c mice immunized on day 9 induced a statistically significant suppression of the humoral immune response in terms of plaque-forming cells per spleen 4 days after immunization (Wiltrout et al. 1978). In similar studies, Casale et al. (1983, 1984) showed that a single gavage dose of 16 mg parathion/kg, which induced severe cholinergic signs and caused some lethality in C57BL/6N mice, significantly suppressed the primary IgM response. However, when the mice received multiple lower doses of parathion, which produced no cholinergic signs, the primary IgM response was not suppressed. The results of additional experiments with arecoline, a cholinomimetic agent, suggested that cholinergic stimulation played a major role in parathion-induced suppression of the plaque-forming cells response. Kim et al. (2005) also reported that a single dose of 4 or 16 mg parathion/kg suppressed the antibody response to immunization with SRBCs in female Balb/c mice; no significant effects were reported at 1 mg/kg. More recently, Fukuyama et al. (2012) also showed that exposure to 1.5 mg parathion/kg/day for 5 days significantly decreased the SRBC-specific IgM response in blood from female C3H/HeN mice; no significant effect was seen at 0.15 mg parathion/kg. The IgM plaque-forming cell response to SRBC in splenocytes showed a decreasing trend, but the differences from the control were not statistically significant. In addition, parathion did not decrease the total cell counts in the spleen. Finally, the 1.5 mg/kg dose significantly decreased the ratio, but not the number, of IgM-positive lymphocytes and germinal center-positive B-lymphocytes in splenocytes.

Studies in mice have also shown that pretreatment with 0.4 mg parathion/kg/day (the lowest dose tested) for 5 days increased the response to allergens such as 2,4-D-butyl and eugenol (Fukuyama et al. 2010). Both agents were classified as moderate sensitizers after pretreatment with vehicle, corn oil, and as strong sensitizers after pretreatment with parathion. In a subsequent study, the same group of investigators showed that pretreatment with parathion (0.15 mg/kg/day, the lowest dose tested) aggravated T_H1- and T_H2-type allergy (Fukuyama et al. 2011). According to the investigators, the mechanism for these effects may involve alterations in the number of helper and cytotoxic T-cells, in levels of T_H1 and T_H2 cytokines, and in gene expression in lymph nodes. Since T_H1- and T_H2-helper cells direct different immune pathways, alteration of their normal ratio may result in an unbalanced immune response to a challenge. Excessive proinflammatory responses due to overactivation of T_H1-type cytokines may lead to uncontrolled tissue damage, whereas excess T_H-2 responses will counteract the T_H-1-mediated microbicidal action.

Long-term dietary studies in Osborne Mendel rats and B6C3F$_1$ mice dosed with up to approximately 4.4 and 27.6 mg parathion/kg/day, respectively, did not find gross or microscopic alterations in the spleen or lymph nodes (NCI 1979).

The highest NOAEL values and all LOAEL values from each reliable study for immunological and lymphoreticular effects in each species and duration category are recorded in Table 3-2 and Figure 3-2.

3.2.2.4 Neurological Effects

Information regarding neurological effects in humans is available from reports of intentional or accidental ingestion of formulations of the pesticide, ingestion of food accidentally contaminated with parathion, and studies in volunteers.

In a short communication, Edson (1964) reported that oral administration of up to approximately 0.1 mg parathion/kg/day 5 days/week for 25–70 days did not result in adverse clinical signs in a small group of volunteers. However, it did reduce red blood cell cholinesterase activity by 16% when administered to four females for 6 weeks. At this time, whole blood cholinesterase was inhibited 33% and plasma cholinesterase was inhibited 37%. No further information was provided. In another study of controlled oral exposure in five volunteers, administration of approximately 0.11 mg parathion/kg/day for about 20 days resulted in red blood cell cholinesterase levels reduced to 63, 78, and 86% of pretest levels and dosing was discontinued (Rider et al. 1969). In two subjects who completed the test period of 30 days, there was no significant effect on red blood cell cholinesterase. By the end of a 30-day post-test period, red blood cell cholinesterase activity had returned to pretest levels. No significant depression of red blood cell cholinesterase occurred in any subject who received doses ≤0.09 mg parathion/kg/day. No explicit information was provided regarding clinical signs. In a study that examined the urinary excretion of parathion metabolites, administration of 1 or 2 mg parathion (0.014 or 0.028 mg/kg/day assuming 70 kg body weight) to four volunteers for 5 consecutive days did not cause any symptoms or signs of parathion intoxication, nor did it alter red blood cell or plasma cholinesterase activities (Morgan et al. 1977). Data from the Rider et al. (1969) study regarding red blood cell AChE inhibition were used to derive an intermediate-duration oral MRL for parathion.

Subjects who ingested food contaminated with parathion usually show the typical signs and symptoms caused by inhibition of acetyl cholinesterase. For example, Diggory et al. (1977) reported that 79 persons were acutely poisoned by parathion in Jamaica in 1976 following consumption of contaminated wheat

flour. Signs and symptoms began 10 minutes to 4 hours after a meal; 17 people died. Severe cases had double vision, pinpoint pupils, muscle fasciculations, and convulsions. Mean red blood cell cholinesterase activity measured in nine patients 5 days from the onset of illness was 78% depressed. Measurements done in 50% of the patients on day 31 showed that red blood cell cholinesterase activity was still depressed by 47%. Similar findings were reported by Etzel et al. (1987) in 49 persons from Sierra Leone acutely poisoned following ingestion of bread baked with parathion contaminated flour, 14 of whom died. Most common signs and symptoms included loss of consciousness, excess sweating and salivation, muscle twitching, and convulsions. Tsachalinas et al. (1971) examined 246 cases following acute poisoning with parathion in Greece. Although not explicitly indicated, it appeared that most cases were due to consumption of contaminated food. Neurological signs and symptoms recorded included miosis and muscle cramps and spontaneous contractions. No data regarding cholinesterase activities were provided in the Etzel et al. (1978) or Tsachalinas et al. (1971) reports.

More recently, Eyer et al. (2003) studied the toxicokinetics of parathion in six acute oral poisoning cases and provided information regarding neurological effects and levels of red blood cell cholinesterase activity. Two of the subjects presented to the emergency room with cholinergic signs; red blood cell cholinesterase activity measured in one of them was reduced to <10% of normal. The other four subjects were unconscious when they arrived at the emergency room. Red blood cell cholinesterase activity in one of them was 3% of normal, whereas no activity could be detected in two of them. In all of the subjects, administration of obidoxime was able to reactivate the enzyme to some degree. Worth noting is the fact that estimates of the amount of parathion absorbed in four subjects (0.31, 0.13, 0.36, and 1.15 g) based on measurements of *p*-nitrophenol in the urine were more than 2 orders of magnitude lower than estimated from anecdotal reports of the amount ingested.

Data regarding brain AChE activity following intoxication with parathion is available in a study by Finkelstein et al. (1998). The investigators employed a computerized method of quantitative histochemical analysis to measure levels of the enzyme in the brain of a man and a woman who died following intentional ingestion of parathion. Brains from two subjects who died of unrelated causes were used as matched controls. In all cases, the postmortem delay did not exceed 32 hours. The results of the enzyme analysis showed that inhibition of brain AChE by parathion was regionally selective. Relative to controls, the biggest decreases (65–80%) occurred in the cerebellum, some thalamic nuclei, and the cortex. Moderate decreases of 10–30% were seen in the *substantia nigra* and basal ganglia; no significant changes were seen in the white matter. Macroscopic observation of the parathion-exposed brains showed

slight diffuse edematous changes; no other gross abnormalities were detected. Brain congestion and edema were also observed in fatal cases of children described by Wishahi et al. (1958).

A condition that has been reported infrequently in humans as a consequence of acute exposure to high amounts of parathion is the intermediate syndrome. The intermediate syndrome is termed as such because it occurs in the time interval (24–96 hours) between the end of the acute cholinergic crisis and the usual onset of delayed neuropathy, is thought to be due to persistent cholinesterase inhibition leading to combined pre- and post-synaptic impairment of neuromuscular transmission, and was first described by Senanayake and Karalliedde (1987) in a study of organophosphorus pesticides other than parathion. De Bleecker et al. (1992) described the first case due to acute parathion exposure. The syndrome was characterized by respiratory paresis, weakness in the territory of several motor cranial nerves, and weakness of proximal limb and neck flexor muscles, and persisted for 3 weeks. During this time, cholinesterase activity remained markedly depressed. Serial EMGs with repetitive nerve stimulation suggested a combined pre- and post-synaptic disorder of neuromuscular transmission. Among 68 cases of acute exposure to parathion studied by He et al. (1998), 7 developed intermediate syndrome (10.3%). Nisse et al. (1998) also described a case of intermediate syndrome following acute exposure to parathion. Besser et al. (1993) described a case of intermediate syndrome in a subject who later developed delayed neuropathy (see below).

Very few cases of parathion-induced delayed neuropathy have been described. Organophosphorus pesticide-induced delayed neuropathy (OPIDN) is a neurodegenerative disorder characterized by a delayed onset of prolonged ataxia and upper motor neuron spasticity (Abou-Donia 1995; Abou-Donia and Lapadula 1990; Johnson 1975). The lesion is a central-peripheral distal axonopathy caused by a Wallerian-type degeneration of the axon, followed by myelin degeneration of the central and peripheral nervous systems. De Jager et al. (1981) described the case of a man who ingested an estimated 150 g of parathion and became comatose. He was treated for the acute cholinergic crisis, but remained in a coma for 7 weeks. Upon recovering from the coma, he had flaccid paralysis of both legs and weakness of the muscles of both hands. The patient gradually recovered but after 3 months, there were still marked muscle wasting and weakness of dorsiflexors and plantar flexors of the feet. Besser et al. (1993) described an additional case of severe intoxication with coma, cholinergic crisis, and intermediate syndrome. After gradually recovering over a 28-day period, the patient complained of numbness and weakness in his feet and hands. Clinical examination showed signs of severe, symmetrical, distal sensorimotor polyneuropathy. The patient was unable to stand and walk. Gradual recovery was observed

during the next 5 weeks, more completely in the hands than in the feet. Eventually, the patient was able to walk without assistance, but distal weakness persisted in the legs.

Results from studies in animals support the findings in humans. The available studies provide information regarding enzyme activities, clinical signs, and neurobehavioral end points.

The lowest LOAEL for a >20% inhibition of red blood cell cholinesterase activity in an acute-duration study was reported in male beagle dogs administered a single dose of 0.5 mg parathion/kg in a capsule (NIOSH 1974). That dose inhibited the enzyme by 29% 24 hours after dosing. The ED_{50} (dose reducing for the enzyme activity to 50% of pretest levels) was 0.385 mg/kg and the ED_{50} for plasma cholinesterase was 1.67 mg/kg. A dose of 2.5 mg parathion/kg reduced red blood cell cholinesterase activity by 64%, but did not induce clinical signs. However, single doses ≥6.3 mg parathion/kg induced tremors, ataxia, convulsions, and prostration. A time-course experiment showed that 36 days after dosing with 2.5 mg/kg, red blood cell cholinesterase activity was reduced by 11% and plasma cholinesterase had recovered to pre-dosing levels (NIOSH 1974). Similar studies in male Sprague-Dawley rats showed that a doses of 0.7 and 5.6 mg parathion/kg reduced red blood cell cholinesterase activity by 27.4 and 69.7%, respectively, at an unspecified time after dosing (NIOSH 1974). The ED_{50} values for red blood cell and plasma cholinesterase were 2.60 and 2.55 mg/kg, respectively. Tremors occurred in 10/10 rats given a single dose of 5 mg parathion/kg, but not in rats dosed with 4 mg parathion/kg. In a time-course experiment in rats administered a dose of 2.8 mg parathion/kg, red blood cell and plasma cholinesterase activities were 44 and 35% of pretest values, respectively, 4 hours after dosing and 67 and 89% of pretest values, respectively, 14 days after dosing. A study in female CD rats reported ED_{50} values of 1.7, 1.1, and 1.1 mg parathion/kg for red blood cell cholinesterase activity 2, 5, and 24 hours after dosing, respectively (Pasquet et al. 1976). The corresponding ED_{50} values for brain cholinesterase were >3.6, 3.6, and >3.6 mg parathion/kg. The ratio of red blood cell cholinesterase ED_{50}/LD_{50} was about 1/5.

In a neurobehavioral study in male Rhesus monkeys, a single dose of 1 mg parathion/kg abolished performance of a learned task 5 hours after dosing, an effect that lasted 3–7 days (Reiter et al. 1975). That dose inhibited blood cholinesterase activity by 40–45%. A dose of 0.5 mg parathion/kg, which reduced blood cholinesterase activity by about 20%, did not affect performance of the learned task. The highest dose tested in the study, 2 mg parathion/kg, produced mild signs of toxicity consisting of decreased postural tone and slight vomiting. Neurobehavioral screening of male Long-Evans rats with tests that assessed autonomic function, neuromuscular function, sensorimotor domain, activity levels, and excitability showed that a single gavage dose of 7 mg parathion/kg, which induced tremors and gait

alterations, affected all of the neurobehavioral parameters measured; the largest magnitude of effects was obtained on the day of dosing and the NOAEL was 4 mg/kg (Moser 1995). Cholinesterase activity was not measured in this study. In male Swiss-Webster mice, administration of a single gavage dose of 6 mg parathion/kg (only dose tested) blocked learning of a one-trial passive avoidance task, but did not significantly affect memory (Reiter et al. 1973). The maximum effect of parathion occurred when it was given within the first hour before the learning trial and correlated with maximum changes in brain and blood true cholinesterase and pseudocholinesterase activities (50–60% depression).

A study that examined the effects of parathion on the dark-adapted pupil dilation in cynomolgus monkeys reported that a single gavage dose of 2 mg parathion/kg (only dose tested) reduced red blood cell AChE activity by approximately 50% 3–12 hours after dosing and that the maximum depression of plasma cholinesterase (65–80% of pre-dosing) occurred approximately 3 hours after dosing (Elkner et al. 1991). The only clinical sign observed was loss of appetite in three of the four monkeys, which corresponded with the time of maximum enzyme inhibition. The study did not find a consistent pattern of change in pupil/iris diameter ratios following exposure to parathion due to high dispersion of the data, which led the investigators to conclude that measurements of pupil dilation after dark adaptation is not a sensitive indicator for systemic exposure to organophosphorus pesticides.

In intermediate-duration studies, exposure of male Sprague-Dawley rats to 0.1 mg parathion/kg/day by gavage 5 days/week for 6 weeks resulted in a 22% decrease in red blood cell cholinesterase activity; no significant inhibition occurred with doses of 0.05 mg/kg (NIOSH 1974). The highest dose of parathion tested, 0.25 mg/kg, reduced red blood cell cholinesterase activity by 26% on week 1 and to 43–57% of control on weeks 4–6 of exposure and on week 1 post-exposure. Plasma cholinesterase was inhibited about 48% on weeks 5–6 of exposure. No toxic signs were observed in the rats in this study. In another intermediate-duration study, dietary exposure of male Wistar rats to 0.4 mg parathion/kg/day significantly inhibited red blood cell AChE (>80%), 0.1 mg/kg inhibited the enzyme by 36–67%, and <20% inhibition occurred in males dosed with 0.024 mg/kg (Ivens et al. 1998). In females, doses of 0.036, 0.152, and 0.550 mg parathion/kg/day reduced red blood cell cholinesterase activity by 27, 67, and 92%, respectively; brain cholinesterase was reduced 10% in high-dose females. On week 15, plasma cholinesterase was significantly reduced in males dosed with 0.4 mg/kg (44%) and in females dosed with 0.55 mg/kg (52%); the red blood cell enzyme was reduced in mid-dose males and females (36–44%) and high-dose males and females (85%); brain cholinesterase was not affected. In a group of rats exposed for 13 weeks and tested on weeks 45–49, all cholinesterase levels had recovered. Ivens et al. (1998) also subjected the rats to four learning and memory tests during the study and reported that exposure to

parathion did not affect the results of the tests. In yet another study in male rats (strain not reported), 90 daily gavage doses of 2.6 mg parathion/kg (only dose tested) reduced brain cholinesterase activity by 50% and blood cholinesterase by 74%; no clinical signs were observed in the rats (Dikshith et al. 1978). In a chronic-duration study in Osborne-Mendel rats, the investigators reported that there were no significant clinical signs during the first 6 months of the study. However, during the second 6 months, 25/50 female rats dosed with approximately 3.5 mg parathion/kg/day, the highest dose tested, had generalized body tremors (NCI 1979). No information was provided regarding rats dosed with lower doses.

A 6-week study in male beagle dogs reported that doses of 0.05 mg parathion/kg/day in a capsule (the lowest doses tested) induced a maximal inhibition of red blood cell cholinesterase of 30% on week 1 post-treatment (NIOSH 1974); at that time, the highest dose tested, 0.5 mg/kg/day, reduced the enzyme's activity by 50%. No toxic signs were seen in the dogs. Similar results had been reported by Frawley and Fuyat (1957) in mixed breed dogs exposed to parathion in the diet for 24 weeks. A study that examined the ocular toxicity of parathion in male and female beagle dogs reported that the highest dose tested, 0.794 mg parathion/kg/day, induced a maximum decrease in red blood cell AChE of about 20% on week 6 of the 6-month study (Atkinson et al. 1994). At study termination, retinal cholinesterase was depressed by about 50% in males and females, ocular muscle cholinesterase was not significantly affected, and pons and cerebellum cholinesterase was reduced by 23–25%. Routine ophthalmoscopic and slit lamp examinations, refraction and intraocular pressure determinations, and electroretinograms performed at various intervals during the study were not significantly altered by exposure to parathion and microscopic examination of the retina, optic nerve, ocular muscles, and ciliary body did not show changes indicative of ocular toxicity.

A study examined the effects of parathion on auditory detection behavior in male squirrel monkeys during a 148-day period in which the monkeys were given a daily capsule with 0.1 mg parathion/kg (Reischl et al. 1975). The dose of parathion did not induce signs of toxicity. Hearing thresholds were determined at 500, 1000, 2000, 4000, 8000, and 16,000 Hz. Exposure to parathion did not significantly change the mean hearing thresholds at any auditory frequency. However, the group exposed to parathion showed a significantly increased standard deviation in hearing thresholds after 40 days of parathion exposure. The investigators suggested that parathion disrupted the monkey's tone reporting behavior during hearing threshold testing. The disruption became significant at tones presented near the animal's hearing threshold, but not for tones presented 20–25 dB above the threshold.

Chronic exposure of rats to up to 4.4 mg parathion/kg/day or of mice to up to 27.6 mg parathion/kg/day did not result in gross or microscopic alterations in the brain (Barnes and Denz 1951; NCI 1979). In the NCI (1979) study, the investigators noted that during the first half of the second year, clinical signs among dosed rats were noted at a low or moderate incidence, and during the second half of the year, they increased. However, no quantitative data were presented. In addition, the investigators mentioned that by week 60 of the study, all high-dose male mice (approximately 27.6 mg parathion/kg/day) were showing signs of hyperexcitability, but no data were provided.

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans following oral exposure to parathion.

Limited information was located in the available studies regarding reproductive effects of parathion in animals. Thomas and Schein (1974) reported that daily gavage administration of up to 5.3 mg parathion/kg for 5 consecutive days to male Swiss-Webster mice did not significantly affect the weight of the testes or prostate. In an intermediate-duration study, daily gavage administration of 2.6 mg parathion/kg/day (only dose tested) to male rats (strain not reported) for 90 days caused tubular atrophy, necrosed spermatogenic cells, and enlargement of the interstitial space of the testes (Dikshith et al. 1978). There was also proliferation of new blood vessels in the interstitial space. Chronic exposure of rats to up to 4.4 mg parathion/kg/day or of mice to up to 27.6 mg parathion/kg/day did not result in gross or microscopic alterations in the reproductive organs (Barnes and Denz 1951; NCI 1979).

3.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans following oral exposure to parathion.

Limited information is available regarding developmental effects of parathion in animals. Conventional developmental end points were examined in Wistar rats (Renhof 1984) and Himalayan rabbits (Renhof 1985). The rats were exposed to 0.1, 0.3, or 1 mg technical parathion/kg on gestation days 6 through 15 and cesarean sections were performed on gestation day 20. Treatment with parathion did not significantly affect the number of live fetuses/litter, number of resorptions/litter, mean fetal weight/litter, number of fetuses with slight bone alterations/litter, number of runts/litter, or number of fetuses with malformations/litter. Maternal toxicity, including significantly reduced weight gain during treatment and tremors, occurred in the group dosed with 1 mg/parathion/kg/day. Pregnant rabbits were administered 0.03, 0.1, or 0.3 mg parathion/kg/day on gestation days 6–18 and cesarean sections were performed on

gestation day 29. Evaluation of the same end points examined in rats showed no significant embryotoxic or teratogenic effects in rabbits under the conditions of the study. No significant maternal toxicity occurred in the rabbits.

In an earlier study, pregnant CD rats were administered 0, 0.01, 0.1, or 1.0 mg parathion /kg/day from day 2 of gestation through day 15 of lactation (Deskin et al. 1979). On postnatal day 25, five pups per sex were evaluated for pseudocholinesterase and red blood cell cholinesterase activities and plasma renin. An EKG was also performed in anesthetized pups. EKG values for males and females were combined. No data were presented regarding maternal effects. Perinatal exposure to parathion did not significantly affect red blood cell AChE activity, but reduced pseudocholinesterase activity in female pups by 17–27%. Heart rate was not significantly affected by parathion exposure, but there were alterations in the EKG including a 43% reduction in atrial depolarization (P-R interval) in low-dose pups. Plasma renin was also reduced in a dose-related manner (60% with the lowest dose).

3.2.2.7 Cancer

No studies were located regarding cancer effects in humans following oral exposure to parathion.

A bioassay for parathion was conducted in Osborne-Mendel rats and $B6C3F_1$ mice (NCI 1979). Rats were fed diets that provided approximately 0, 2.2, or 4.4 mg parathion/kg/day to males and 0, 1.8, or 3.5 mg parathion/kg/day to females for 80 weeks; the rats were then observed for 32–33 weeks. In males, analyses of neoplastic lesions showed significant increased incidence of cortical adenomas of the adrenal gland in high-dose group using pool controls (2/80 vs. 9/46, $p<0.002$). Analysis of combined cortical adenomas and carcinomas showed significantly increased incidences in both low- and high-dose groups (3/80 pooled control vs. 7/49 and 11/46, respectively). Also significantly elevated in high-dose males were the incidences of follicular cell adenoma of the thyroid (5/76 pooled control vs. 8/43, $p=0.046$) and carcinoma of the pancreatic islet-cell (0/79 pooled control vs. 3/46, $p=0.048$). In females, the incidences of adrenal cortical adenomas or adrenal cortical adenomas and carcinomas were significantly elevated when compared with pooled controls (adenomas 4/78 pooled control vs. 11/42 high-dose, $p=0.001$; adenomas plus carcinoma 4/78 pooled control vs. 13/42 high-dose, $p=0.001$). The investigators concluded that, under the conditions of the study, parathion was carcinogenic to Osborne-Mendel rats. The dose of 2.2 mg parathion/kg/day in male rats is listed as a CEL in Table 3-2 and is plotted in Figure 3-2.

Mice were fed a diet that provided approximately 0, 13.7, or 27.6 mg parathion/kg/day (NCI 1979). Low-dose males were treated for 71 weeks, high-dose males for 62 weeks, and low- and high-dose females for 80 weeks. All mice were killed at 89 or 90 weeks. Gross and microscopic examination of organs and tissues showed that neoplastic lesions were distributed equally between dosed and control groups. Therefore, under the conditions of the assay, parathion was not carcinogenic to $B6C3F_1$ mice.

Based on the results of the NCI (1979) bioassay, the EPA placed parathion on Group C, possible human carcinogen (IRIS 2003). Under updated guidelines, the data regarding carcinogenicity of parathion are "suggestive evidence of carcinogenic potential" (EPA 2005). A quantitative estimated of carcinogenic risk from oral exposure is not available.

3.2.3 Dermal Exposure

3.2.3.1 Death

Deaths have been reported in humans following occupational or accidental dermal exposure to parathion. In a study of 40 occupationally exposed subjects, Grob et al. (1950) reported that six of them died due to contact with the pesticide. Exposure occurred during the synthesis or handling of various parathion formulations; inhalation exposure was also likely to have occurred. All the men who died had been exposed on ≥1 days during the month preceding the day on which symptoms occurred. The average exposure length for the whole cohort was 8 hours/day for 12 days. Four of the patients who died walked into the hospital and two died before they could be brought to a hospital. Ataxia, tremor, drowsiness, difficulty in concentrating, mental confusion, occasionally disorientation, and changes in speech developed, followed by profound coma with absence of all reflexes. The coma began, on average, 4 hours after the onset of symptoms and lasted an average of 3.5 hours before death occurred. The immediate cause of death was not known but, according to Grob et al. (1950), contributing factors may have been depression of the respiratory and circulatory centers in the medulla, weakness of the muscles of respiration, and pulmonary edema.

Among 20 deaths reported in children due to exposure to parathion in the state of Florida from 1959 through 1964, four were due to dermal contact with the pesticide (Eitzman and Wolfson 1967). Limited information provided regarding two of these cases indicates that both developed cholinergic crisis and died within 5 hours of being seen by a physician. Autopsy was performed on one of them and revealed increased bronchial secretions.

Lores et al. (1978) described the case of a young man whose death was attributed to dermal contact with parathion residue <24 hours after application of the pesticide to a tobacco field where he had worked. He was found unconscious at home and was taken to the hospital where he was pronounced dead on arrival.

The dermal LD_{50} values for technical parathion in male and female Sherman rats were 21 and 6.8 mg/kg, respectively, suggesting sex-related differences in susceptibility (Gaines 1960). In males, the minimal survival time was 24 minutes and the maximum survival time was 3 days. In females, the minimal survival time was 1 hour and the maximum survival time was 4 days. Signs of poisoning included muscle fasciculation, excessive salivation and lacrimation, tremors, diarrhea, and involuntary urination. The doses tested were not specified. A dermal LD_{50} of 49.4 mg/kg was reported for technical parathion in male Sprague-Dawley rats; the LD_{50} in females was 19.5 mg/kg (EPA 1978). No information was provided regarding whether the application site was occluded or washed at some point. All rats exhibited typical signs of cholinesterase inhibition including salivation, lacrimation, exophthalmos, defecation, urination, and muscle fasciculations; the doses tested were not specified. This study also supports the view that female rats are more susceptible than male rats to the acute effects of parathion. A similar study reported a dermal LD_{50} of approximately 8 mg/kg for female CD rats (Pasquet et al. 1976). The rats were fitted with a collar for 24 hours and the application site was washed with soap and lukewarm water; the observation period was 10 days. No further information was provided.

The LD_{50} values mentioned above are recorded in Table 3-3.

3.2.3.2 Systemic Effects

No studies were located that provide information regarding endocrine, dermal, or body weight effects in humans following dermal exposure to parathion. The only effects in animals recorded in Table 3-3 are dermal effects in rats following dermal exposure to parathion.

Respiratory Effects. Using the AHS, Hoppin et al. (2006) examined the association of 40 individual pesticides (parathion among them) with wheeze. The AHS is a prospective cohort study of nearly 90,000 private pesticide applicators (mostly farmers), their spouses, and commercial pesticide applicators in Iowa and North Carolina. Exposure and medical history of farmers and pesticide applicators was assessed by means of self-administered questionnaires. Pesticides were evaluated using logistic regression models adjusted for age, sex, state, smoking status, and body mass index. The final analysis included 17,920 farmers and 2,255 commercial applicators. Nineteen percent of farmers and 22% of

Table 3-3 Levels of Significant Exposure to Parathion - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
ACUTE EXPOSURE							
Death							
Rat (Sprague-Dawley)	once				49.4 M mg/kg (LD50)	EPA 1978	
					19.5 F mg/kg (LD50)		
Rat (Sherman)	once				21 M mg/kg (LD50)	Gaines 1960	
					6.8 F mg/kg (LD50)		
Rat (CD)	once				8 F mg/kg (10-day LD50)	Pasquet et al. 1976	
Systemic							
Gn Pig NS	5 d 1 x/d	Dermal		0.004 F mg/kg/day (hyperkeratinization of epidermis; thickening of stratum corneum)		Dikshith and Datta 1972	
Neurological							
Human (NA)	5 d 2 hr/d		100 mg			Hayes et al. 1964	NOAEL is for RBC cholinesterase activity.

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Table 3-3 Levels of Significant Exposure to Parathion - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
INTERMEDIATE EXPOSURE							
Systemic							
Gn Pig NS	15 d 1 x/d	Dermal		0.004 F mg/kg/day	(Hyperkeratinizationn of the dermis; proliferation of connective tissue)	Dikshith and Datta 1972	

d = day(s); F = Female; Gn pig = guinea pig; hr = hour(s); LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NA = not applicable; NOAEL = no-observed-adverse-effect level; NS = not specified; RBC = red blood cell x = time(s)

commercial applicators reported wheezing at least once in the year before enrollment. For parathion, 7% of farmers reported past use and 1% reported current use (ever used in the year prior enrollment); the corresponding percentages for commercial applicators were 3 and 1%. The odds ratio (OR) was elevated for farmers, but did not achieve statistical significance (OR=1.37, 95% confidence interval [CI] 0.93–2.03). An OR was not calculated for parathion because there were fewer than five exposed cases. In a more recent report, the same group of investigators examined the association between pesticide exposure and allergic and non-allergic asthma among 19,704 male farmers in the AHS (Hoppin et al. 2009). Parathion was found to be significantly associated with allergic asthma (OR=2.05, 95% CI 1.21–3.46), although there was no exposure-response trend. Exposure to parathion was not associated with non-allergic asthma (OR=1.11, 95% CI 0.75–1.66). Exposure-response was evaluated using three measures of cumulative pesticide exposure: total years of use, lifetime days of use, and intensity-adjusted lifetime days of use.

Respiratory difficulty suggestive of bronchospasm, excessive bronchial secretions, and pulmonary edema with cyanosis were reported in workers acutely exposed to parathion during the synthesis or handling of various formulations of the pesticide (Grob et al. 1950). Pulmonary edema was reported in a child who later died following dermal exposure to parathion; shallow respiration was reported in a child who survived dermal exposure to the pesticide (Eitzman and Wolfson 1967).

Cardiovascular Effects. Elevated blood pressure was reported in most workers with moderate or severe symptoms described by Grob et al. (1950). The investigators noted that decreased blood pressure was not noticed, except shortly before death. EKGs performed in four patients who recovered did not show alterations. Eitzman and Wolfson (1967) reported hypotension in two children who eventually died following dermal contact with parathion; a third child who survived also had hypotension and bradycardia.

Gastrointestinal Effects. Anorexia, nausea, vomiting, abdominal cramps, and diarrhea were commonly reported in workers exposed during synthesis and handling various parathion formulations (Grob et al. 1950) and in agricultural workers exposed to parathion (Milby et al. 1964; Quinby and Lemmon 1958). Nausea and vomiting were also reported in two poisoning cases in children; one of them also complained of abdominal pain (Eitzman and Wolfson 1967).

Diarrhea and defecation were reported in rats given high dermal doses of parathion in LD_{50} studies (EPA 1978; Gaines 1960).

Hematological Effects. Hematological tests conducted in four workers who recovered from severe poisoning symptoms following exposure to parathion revealed no appreciable abnormality regarding red blood count, hematocrit, hemoglobin, and sedimentation rate (Grob et al. 1950). In 12 patients in whom the leucocyte count was determined, there was slight to moderate leukocytosis. The differential count showed a slight increase in the percent of mature polymorphonuclear leucocytes (Grob et al. 1950).

Musculoskeletal Effects. No direct effects on muscle or bone were reported in humans following dermal exposure to parathion. Clinical signs such as muscle fasciculation, twitching, and tremors are of neurological origin, as mentioned in Section 3.2.3.4.

Hepatic Effects. The only information located in the available literature regarding hepatic effects in humans following exposure to parathion is in the study of workers by Grob et al. (1950). Laboratory test performed in four subjects who recovered after severe symptoms showed cephalin flocculation, serum alkaline phosphatase activity, total serum protein, and albumin/globulin values within normal limits.

Renal Effects. Normal blood urea nitrogen (BUN) was reported in the four workers who recovered from severe parathion intoxication described by Grob et al. (1950). No further relevant information was located.

Dermal Effects. In a study in 30 female guinea pigs, the animals were applied 1 mL of a 1-ppm solution of parathion in 50% ethanol to a clipped latero-abdominal area daily for 5 or 10 days (approximately 4 μg/kg/day based on a body weight of 0. 250 kg) (Dikshith and Datta 1972). Ten guinea pigs were killed 24 hours after the 5th, 10th, and 15th application, and the skin was prepared for gross and microscopic examination. Treatment with parathion did not induce adverse clinical signs. Gross examination of the skin did not show dermatitis or any other noticeable changes. Microscopic examination showed hyperkeratinization of the epidermal layer and thickening of the stratum corneum after 5 days of treatment. Five applications also induced mild damage to the endothelial cells of the blood vessels. Ten days of treatment resulted in scattered infiltration of mononuclear cells in the dermis. The dermis also showed mild proliferation of connective tissue around hair follicles and sebaceous glands. Additional applications induced changes such as thickening of the wall of the blood vessels and swelling of the endothelial cells. A mild perivascular inflammatory infiltrate was also present.

Ocular Effects. Clinical signs such as miosis, unresponsive pupils, and blurred vision are caused by alterations in the neural control of the eye, but can be exacerbated by directly touching the eyes with contaminated objects or the hands.

3.2.3.3 Immunological and Lymphoreticular Effects

No information was located regarding immunological and lymphoreticular effects in humans following dermal exposure to parathion, except for the report of an association (OR=2.05, 95% CI 1.21–3.46) between exposure to parathion and allergic asthma in participants in the AHS (Hoppin et al. 2009). Exposure to parathion was not associated with non-allergic asthma (OR=1.11, 95% CI 0.75–1.66).

3.2.3.4 Neurological Effects

Neurological effects have been studied in volunteers exposed to controlled amounts of parathion, in adults and children acutely exposed to high amounts of parathion, and in workers exposed chronically to lower levels of parathion. In addition, data are available regarding neurobehavioral effects in agricultural workers and on the possible role of parathion exposure and Parkinson's disease.

In an early study, Grob et al. (1950) described the effects of parathion in 38 men and 8 women involved in the synthesis or handling of various parathion formulations. About half of the subjects began to have symptoms while they were still exposed to parathion. The remaining subjects developed symptoms 0.5–8 hours after their last exposure to parathion. The severity of the acute cholinergic crisis seemed to depend on the severity of the intoxication. In cases of marked intoxication, ataxia, tremor, drowsiness, difficulty in concentrating, mental confusion, occasionally disorientation, and changes in speech developed. In the most severe cases, coma gradually developed. The average durations of the acute effects were 25 hours in four patients who had severe symptoms and 12 hours in 30 patients who had less severe effects. Patients who recovered from severe illness showed giddiness, uneasiness, headache, anxiety, insomnia, and weakness for 48–72 hours after their last exposure to parathion. In patients suspected of spraying or rubbing parathion into the eyes, it took several weeks for pinpoint pupils to return to normal size. Four patients who survived severe symptoms had red blood cell AChE activity reduced to11–22% of normal activity. In six subjects with less severe symptoms, red blood cell AChE activity ranged from 12 to 28% of normal. When 18 subjects whose enzyme had been depressed were removed from further exposure, red blood cell AChE activity increased at an average rate of approximately 10% of normal activity during the first 3 days and diminished to between 1 and 2% per day

by the fourth day. The rate of recovery subsequently remained fairly constant until a normal level of activity was reached.

Milder effects were described by Quinby and Lemmon (1958) among >70 subjects who had contact with parathion residues. The workers were engaged in picking, thinning, cultivating, and irrigating various crops. Dermal exposure appeared to have been favored by the removal of protective clothing and by the persistent wearing of contaminated clothing. Weakness, twitching of arm and leg muscles and of the eyelids, and some cases of miosis were reported. Effects such as headache, weakness, miosis, blurred vision, and dizziness were reported in a study of 186 peach orchard workers (Milby et al. 1964). Measurements of parathion residues in the fruit, on the subjects' skin, and in the air from two orchards that had produced the highest rates of clinical illness led to an estimate of total exposure by a picker of <4 mg; dermal exposure contributed the highest amount, approximately 3.2 mg. Ingestion and inhalation contributed only 0.5 and 0.3 mg, respectively.

Weakness, headache, muscle fasciculations, tremors, and severely depressed red blood cell AChE activity were reported in three children dermally exposed when a burlap sack heavily contaminated with parathion and filled with old clothing was used as a swing (Eitzman and Wolfson 1967).

Hayes et al. (1964) conducted a series of experiments with volunteers exposed to controlled amounts of parathion. Application of 5 g of 2% parathion dust at a constant temperature of 105°F onto the right hand and forearm for 2 hours on 5 consecutive days resulted in a maximum decrease of 14% in red blood cell cholinesterase activity 24 hours after exposure; no adverse clinical signs were noted. The daily amount applied was 100 mg parathion (5 g dust x 2 mg parathion/100 mg dust). This amount is listed as a NOAEL in Table 3-3. In additional experiments in which volunteers were exposed to other parathion formulations (4 L of 2% emulsion for 70 minutes at 81°F; unspecified amount of 47.5% emulsifiable concentrate for 120 minutes at 69°F or 90 minutes at 103°F), no significant depression of red blood cell cholinesterase was seen. Whole-body exposure of volunteers to 7 pounds of 2% parathion dust (~7 hours) or to parathion vapor (3 hours) did not significantly depress red blood cell AChE (<20%) activity, and no clinical signs were observed. Exposure of volunteers for 3 hours to filter paper pads containing 40–50 g of parathion did not result in significant depression of red blood cell AChE. In general, plasma cholinesterase was more affected than the red blood cell enzyme in all experiments.

The association between exposure to pesticides, parathion included, and self-reported hearing loss was examined among private pesticide applicators in the AHS (MacCrawford et al. 2008). Pesticide exposure

and medical history were assessed by questionnaires. The final sample available for analysis consisted of 14,229 applicators. Of these, 4,926 met the case inclusion criterion of self-reporting hearing troubles and 9,303 met the criterion to serve as controls. Logistic regression was performed with adjustment for state, age, and noise, solvents, and metals. Exposure to organophosphate pesticides was modestly associated with hearing loss, with a 17% increase in odds in the highest quartile of exposure (OR=1.17, 95% CI 1.03–1.31). Analysis of individual organophosphate pesticide using traditional logistic regression showed elevated odds for various pesticides, marginally significant for parathion (OR=1.21, 95% CI 1.04–1.40). Strengths of the study included a large population, an internal control group, detailed information on pesticide exposure, and information on additional potential causes of hearing loss.

The association between pesticide exposure and behavioral function has also been examined in the AHS (Starks et al. 2012). The cohort consisted of 701 males with a mean age of 61 years who were administered nine neurobehavioral tests to assess memory, motor speed and coordination, sustained attention, verbal learning, and visual scanning and processing. Associations between pesticide (parathion among them) use and neurobehavioral tests performance were estimated with liner regression controlling for age, height, education, state, smoking status, alcohol consumption, head injury, current antidepressant use, caffeine consumption, and exposure to other potentially neurotoxic substances. The results showed that parathion exposure was associated with better verbal learning and memory and better performance on a test of sustained attention. The investigators noted that given the large number of statistical tests performed, the possibility existed that the results were due to chance; however, a non-monotonic dose-effect function of parathion could not be ruled out.

Two studies were located that examined the relationship between exposure to parathion and Parkinson's disease. Neither one found a significant risk associated with exposure to parathion. In an ongoing population-based, case-control study of Parkinson's disease in western Washington State, Firestone et al. (2005) assessed occupational and home-base exposure using a structured interview. The final cohort consisted of 250 incident Parkinson's disease case patients and 388 healthy controls with a participation rate of 73% for cases and 66% for controls. ORs and 95% CIs were determined using logistic regression models controlling for age, sex, and smoking. Analysis of only the occupationally exposed subjects (156 cases and 241 controls) showed that parathion had the highest OR among organophosphate pesticides (OR=8.08, 95% CI 0.92–70.85). Analysis of the entire cohort showed an OR for the organophosphate class of 0.83 (95% CI 0.60–.16). Manthripragada et al. (2010) examined the association between Parkinson's disease and parathion (among other organophosphate pesticides) and the influence of a functional polymorphism at position 55 in the coding region of the PON1gene (PON1-55). Studies

have suggested that individuals with low PON1 activity might be at higher risk for organophosphate toxicity (reviewed in Costa et al. 2013). The cohort consisted of 351 incident cases and 363 controls from three rural California counties in a population-based, case-control study. Residential exposure was estimated for each study participant using their residential history and a geographic information system. The results showed no increased risk of Parkinson's disease for people exposed to parathion, and risk did not increase in carriers of the variant MM PON1-55 genotype. In a more recent study, the same group of investigators examined whether single nucleotide polymorphisms $PON1_{Q192R}$ and $PON1_{C-108T}$ impact the association between Parkinson's disease and residential exposure to parathion (Lee et al. 2013). $PON1_{Q192R}$ affects catalytic efficiency of PON1, whereas $PON1_{C-108T}$ has been associated with lower expression levels. The results of the analyses showed no significant increased risk associated with the variant genotypes studied (effect estimates included the null).

Acute toxicity studies aimed at determining dermal LD_{50} values in rats have reported cholinergic signs such as muscle fasciculations and tremors, but the dose levels at which these signs were observed were not provided (EPA 1978; Gaines 1960).

3.2.3.5 Reproductive Effects

A study of Chinese workers exposed to parathion and methamidophos reported that the workers (n=20) had a modestly lower sperm count, lower sperm concentration, and lower percentage of motile sperm than an unexposed control group (n=23) (Padungtod et al. 2000). However, the results should be interpreted with caution due to the small sample size. In addition, when the exposed subjects were assigned to a high- or low-exposure group based on job titles, measurements of exposure could not differentiate between them. Exposure was assessed by attaching a piece of gauze onto nine body areas, attaching a pump to the lapel of the subject's shirt to asses potential inhalation, and measuring urinary *p*-nitrophenol in five samples collected at the end of the shift. While the results were suggestive, the role of parathion, if any, remained unclear. It should also be noted that the mean number of pregnancies fathered by the exposed subjects was four, compared to two for the unexposed group, suggesting that fertility was not impaired.

3.2.3.6 Developmental Effects

Limited information is available regarding developmental effects in humans exposed to parathion. Eskenazi et al. (2004) studied the effects of organophosphate pesticide exposure during pregnancy on fetal growth and gestational length in a cohort of 488 low-income Latina women living in an agricultural

community in the Salinas Valley, California. Although exposure by multiple routes is likely to have occurred, touching produce sprayed with pesticides was likely the most significant exposure route. Exposure to parathion was assessed by measuring *p*-nitrophenol in the urine during pregnancy and by measuring cholinesterase in whole blood and butyl cholinesterase in plasma from the mothers during pregnancy and delivery and from umbilical cord. The investigators acknowledged that *p*-nitrophenol can also be derived from exposure to methyl parathion and other non-pesticide chemicals. Infant birth weight, crown-heel length, head circumference, and gestational length were obtained from medical records and hospital delivery logs. Linear regression models were used to test for associations between exposure measurements and length of gestation, birth weight, length, head circumference, and ponderal index. Logistic regression was used to test for associations between exposure measurements and low birth weight, preterm delivery, and small for gestational age births. The results of the analyses did not show an adverse association between fetal growth and any measure of in utero exposure to parathion, if there was true exposure to parathion. It is also possible that there was no exposure to parathion since, as mentioned above, *p*-nitrophenol may be derived from exposure to other chemicals.

3.2.3.7 Cancer

Dennis et al. (2010) examined the potential association between exposure to 50 agricultural pesticides, parathion among them, and the incidence of cutaneous melanoma in the AHS cohort of pesticide applicators along with ever-use of older pesticides that contain arsenic. Pesticide applicators completed an enrollment questionnaire that sought information on ever use of 50 pesticides and on a number of potential confounders. Pesticide applicators also completed an additional "take home" questionnaire that sought more extensive information on occupational activities. Logistic regression was used to examine ORs and 95% CIs associated with pesticide exposure adjusted for age, sex, and other potential confounders. A total of 150 cases of melanoma were identified among 24,704 subjects who completed the "take home" questionnaire. The investigators found no association between melanoma incidence and organophosphate insecticides as a class. However, there was a significant association between melanoma and parathion (≥56 days of exposure; OR, 2.4; 95% CI 1.3–4.4; p =0.003) based on 11 cases. The study also found a higher OR of 7.3 (95% CI 1.5–34.6) among those who had used arsenical pesticides. The investigators noted that strengths of the AHS include a prospective design, comprehensive pesticide exposure assessment, completeness of followup, and high participation rates. A limitation of the Dennis et al. (2010) study, noted by the investigators, was the small number of subjects who used parathion for at least 56 days and had melanoma (n=11). Overall, the study could not rule out the possibility that cutaneous melanoma was caused by exposure to arsenical pesticides. The investigators suggested that

more research is needed regarding chemicals and other environmental factors that may increase the risk of cutaneous melanoma.

3.2.4 Other Routes of Exposure

A series of publications from Slotkin and coworkers have provided evidence of neurological effects of parathion, as well as other organophosphate pesticides, dissociated from parathion-induced inhibition of AChE. The experiments were conducted in male and female Sprague-Dawley rats. To avoid the high first-pass removal by the liver, neonatal rats were injected with parathion subcutaneously on postnatal days 1–4 and neurochemical parameters were evaluated in various brain regions within days of dosing or during adulthood. Parathion was tested at doses of 0.02–0.1 mg/kg/day, which were below the threshold for overt toxicity as defined by mortality data. While parathion did not compromise the development of neuritic projections or emergence of the cholinergic phenotype in the forebrain and brainstem in 5-day-old pups (Slotkin et al. 2006a), it did decrease serotonin $5HT_{1A}$ receptors in the brainstem and forebrain but had no significant effect on $5HT_2$ receptors or serotonin transporter in 5-day-old pups (Slotkin et al. 2006b). In another study, examination of rats at older ages showed that parathion upregulated $5HT_{1A}$ receptors with a peak in the frontal/parietal cortex at about 60 days of age, followed by a decrease of the effect in most brain regions and eventually inducing deficits at 100 days of age (Slotkin et al. 2009a). The investigators also showed that parathion produced lasting alterations in acetylcholine markers in the frontal/parietal cortex, temporal/occipital cortex/ midbrain, hippocampus, and striatum in adolescence and adulthood; in general, effects were more pronounced in males than in females (Slotkin et al. 2008). In additional studies, parathion induced long-lasting selective behavioral alterations in adolescence and adulthood, some of which were sex-dependent (Levin et al. 2010; Timofeeva et al. 2008).

Subcutaneous administration of parathion to neonatal rats also caused a metabolic dysregulation in adult rats characterized in males by a net anabolic response at a low dose (0.1 mg parathion/kg) and a catabolic response at a higher dose (0.2 mg parathion/kg) and by a greater sensitivity to catabolic effects in females; the metabolic alterations were consistent with a pre-diabetic state (Lassiter et al. 2008). A likely mechanism for the pre-diabetic state involved a persistent parathion-induced disruption of adenylyl cyclase (the enzyme that synthesizes cyclic adenosine monophosphate [cAMP] from adenosine triphosphate [ATP]) signaling in peripheral tissues, particularly in the liver (Adigun et al. 2010). Studies also showed that a high-fat diet in adulthood reversed the parathion-induced alterations in acetylcholine systems and lessened some of the effects of parathion on serotonin synaptic function (Slotkin et al. 2009b, 2009c), presumably due to global changes in the composition of synaptic membrane lipids. Furthermore,

early exposure to parathion was found to disrupt major control points of lipid metabolism and induced an inflammatory response in adipose tissue. The changes in lipid metabolism were found to interact with deficits in synaptic function which, according to the investigators, may contribute to impaired behavioral performance (Lassiter et al. 2010).

Behavioral effects and effects on the brain morphology have been described following subcutaneous injections of parathion into neonatal rats. For example, treatment of 5-day-old rats with 1.3 or 1.9 mg/kg/day parathion until postnatal day 20 did not affect developmental landmarks such as eye opening or incisor eruption (Stamper et al. 1988). That treatment affected only one out of four tests of reflex development before weaning. However, evaluations conducted post-weaning showed small deficits in tests of spatial memory in both the T-maze and radial arm maze. These effects were associated with significant decreases in brain AChE activity (35–68%) and muscarinic receptor density, but not affinity, in the cerebral cortex. The investigators concluded that early exposure to parathion affected the performance of spatial memory tasks by interfering with the development of the cholinergic system. In a similar study, neonatal rats were administered 0.882 mg/kg/day parathion on gestation days 5–20 and morphological, histochemical, and biochemical tests were conducted in the hippocampus collected on gestation day 21 (Veronesis and Pope 1990). The results showed hippocampal damage consisting of cellular disruption and necrosis in the dentate gyrus, CA4, and CaA3c regions. On postnatal day 12, hippocampal AChE was depressed 73% while muscarinic receptor binding was depressed by 36%. Since the dosing period coincided with a time critical to hippocampal neurogenesis and synapse formation, the resulting changes may translate into permanent neurobehavioral alterations.

3.3 GENOTOXICITY

No information was located regarding genotoxic effects in humans exposed specifically to parathion. A study of 25 male workers in India occupationally exposed to organophosphate pesticides (parathion among them), organochlorine pesticides, and fertilizers reported that the frequency of chromosomal aberrations and sister chromatid exchanges in peripheral lymphocytes were significantly elevated compared to unexposed control subjects; however, the role of parathion, if any, cannot be determined (Rupa et al. 1988). *In vivo* studies in animals have yielded negative results in tests for induction of micronuclei, chromosomal aberrations, and dominant lethality (Table 3-4). Intraperitoneal administration of a single dose of 10 mg parathion/kg to male mice did not significantly increase the frequency of chromosomal aberrations in bone marrow cells or spermatogonia assessed 12, 24, and 36 hours after dosing (Degraeve and Moutschen 1984). Gavage administration of a single dose of 2.2 mg parathion/kg

Table 3-4. Genotoxicity of Parathion *In Vivo*

Species (test system)	End point	Results	Reference
Mammalian cells:			
Mouse spermatogonial cells	Chromosomal aberrations	–	Degraeve and Moutschen 1984
Mouse bone marrow cells	Chromosomal aberrations	–	Degraeve and Moutschen 1984
Mouse bone marrow cells	Micronuclei	–	Kevekordes et al. 1996
Eukaryotic organisms:			
Male mice	Dominant lethal	–	EPA 1977
Male mice	Dominant lethal	–	Degraeve and Moutschen 1984

– = negative results

to male mice or 1.5 mg/kg to female mice did not significantly increase the frequency of micronuclei in bone marrow cells assessed 48 hours after dosing (Kevekordes et al. 1996). Dietary administration of parathion to male mice for 7 weeks at levels of 62.5, 125, or 250 ppm followed by mating for 8 weeks did not provide evidence of mutagenicity by the dominant lethal procedure (EPA 1977). Females were sacrificed at midterm of pregnancy and were scored for early and late fetal deaths and for living fetuses. The lowest dose was intended to induce up to 20% weight loss, mild but transient clinical signs, no inhibition of breeding performance, and no mortality. In another study, male mice were given a single intraperitoneal injection of 10 mg parathion/kg and were then mated with untreated females for 7 consecutive weeks (Degraeve and Moutschen 1984). Pregnant females were sacrificed on day 14 of pregnancy and the numbers of corpora lutea, implants, live embryos, and dead fetuses were recorded. The results showed no significant effects of parathion on the end points monitored. However, administration of 0.3 mg paraoxon/kg resulted in a low number of live embryos per litter with females mated during the first and seventh week after dosing; the former case appeared to be associated with a higher number of pre-implantation loss and the latter case with a lower number of corpora lutea. The differences with control values were not statistically significant. Without specifying, Degraeve and Moutschen (1984) noted that the doses of parathion and paraoxon used, 10 and 0.3 mg/kg, respectively, induced obvious signs of intoxication, but little or no mortality. The data available in animal studies indicate that exposure to environmentally relevant levels of parathion is unlikely to represent a genotoxic hazard. No useful data in humans were found.

Studies of the genotoxic potential of parathion *in vitro* have yielded negative results, with one exception (Table 3-5). Parathion did not induce mutations in *Salmonella typhimurium*, *Escherichia coli*, or *Serratia marcescens* (Fahrig 1974; Mohn 1973; Simmon et al. 1976). Parathion also did not induce mitotic recombination, mitotic gene conversions, or forward mutations in yeast (Fahrig 1974; Gilot-Delhalle et al. 1983; Simmon et al. 1976). Only Gilot-Delhalle et al. (1983) conducted their test of forward mutation in *Schizosaccharomyces pombe* both with and without metabolic activation. In studies with mammalian cells *in vitro*, Nishio and Uyeki (1981) reported the only positive data for an increase frequency of sister chromatid exchanges in cultures of Chinese hamster ovary cells in the presence of parathion and in the absence of an activating system; no tests were conducted with metabolic activation. Tests conducted with equimolar concentrations of paraoxon also yielded positive results, and there was no significant difference in sister chromatid exchange frequencies induced by parathion and paraoxon (Nishio and Uyeki 1981). However, incubation of human lymphocytes with parathion for up to 48 hours did not result in an increase in the frequency of sister chromatid exchanges relative to controls (Kevekordes et al. 1996). The difference between the results of Nishio and Uyeki (1981) and Kevekordes et al. (1996) may be due, in

Table 3-5. Genotoxicity of Parathion *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	–	No data	Simmon et al. 1976
Escherichia coli K-12	Forward mutation	No data	–	Fahrig 1974
E. coli K-12	Forward mutation	No data	–	Mohn 1973
Serratia marcescens	Reverse mutation	No data	–	Fahrig 1974
Eukaryotic organisms:				
Saccharomyces cerevisiae	Mitotic recombination	–	No data	Simmon et al. 1976
S. cerevisiae	Mitotic gene conversion	No data	–	Fahrig 1974
Schizosacchromyces pombe	Forward mutation	–	–	Gilot-Delhalle et al. 1983
Mammalian cells:				
Cultured human lymphocytes	Sister chromatid exchanges	–	–	Kevekordes et al. 1996
Chinese hamster ovary cells	Sister chromatid exchanges	No data	+	Nishio and Uyeki 1981

+ = positive results; – = negative results

part, to the different test systems used (Chinese hamster ovary cells vs. human lymphocytes) and the difference in the concentrations of parathion tested (mM range vs. μM range, respectively). The studies available suggest that parathion is not a mutagenic compound.

3.4 TOXICOKINETICS

Data on the absorption of parathion after inhalation and oral exposure are very limited, and do not allow for the estimation of absorption rates or fractional uptake by these exposure routes. However, volunteer studies and poisoning incidents confirm that parathion is absorbed through both the respiratory and gastrointestinal tracts. The dermal uptake of parathion has been well-studied in a variety of systems and has been shown to be highly variable, ranging from 9 up to 100%, depending on anatomical site, in one study of volunteers. Dermal uptake is also affected by parathion formulation, ambient temperature, relative humidity, and airflow across the exposed skin.

Few data on the distribution of parathion in body tissues are available; these data are limited to oral and intravenous or intraperitoneal exposure routes. Available *in vivo* data show high affinity of parathion for adipose tissue and the liver, with lower levels seen in the kidney, muscles, lung, and brain of animals exposed *in vivo*. Transplacental transfer of the parathion or its metabolite(s) has been demonstrated in sheep. In blood, as much as 94–99% of parathion is bound to proteins, particularly albumin.

The metabolism of parathion is important in assessing its toxicity, as bioactivation to the paraoxon metabolite is a key step in the toxicity associated with AChE inhibition. Metabolism of parathion reflects complex interactions among a number of cytochrome P450 isozymes capable of both bioactivation and detoxification, as well as detoxification by carboxylesterases and A-esterases, and elimination facilitated by glucuronidase, glutathione transferase, and other conjugating enzymes. The involvement of numerous bioactivating and detoxifying enzymes suggests that polymorphisms in the genes encoding these enzymes might lead to substantial interindividual variability; in addition, sex, age, and pregnancy status have been shown to change the metabolism of parathion in animals.

Parathion is eliminated primarily through metabolism and subsequent excretion of metabolites in urine; a small proportion of metabolites is eliminated through the feces. Excretion of metabolites in urine has been shown to continue for days after exposure has concluded.

3.4.1 Absorption

There are few data on the absorption of parathion after inhalation and oral exposure. Available information on inhalation exposure is limited to early volunteer studies in humans, in which the exposure levels were not measured; these studies indicate only that parathion can be absorbed via the respiratory tract. Likewise, an occupational study, volunteer studies, and case reports of poisonings in which AChE inhibition or parathion metabolites were detected confirm the oral absorption of parathion, but do not indicate the rate or degree of absorption. The oral bioavailability of parathion in dogs has been estimated to range from 1 to 29%. The dermal uptake of parathion has been extensively studied in humans, animals, and *in vitro*; the data indicate large variability in dermal absorption rates, which depend on ambient temperature, relative humidity, anatomical area of skin exposed, and skin condition (Gosselin et al. 2005). Depending on the anatomical region, between 9 and 100% of the applied dose was absorbed across the skin of volunteers in a study by Maibach et al. (1971).

3.4.1.1 Inhalation Exposure

The parathion metabolite, *p*-nitrophenol, was detected in a volunteer exposed to parathion via inhalation of vapors for 5 consecutive days (Hartwell et al. 1964). The exposure concentrations were not measured or estimated, but were generated by spreading fresh technical parathion over 36 square inches of area and heating to 105–150°F. The authors also measured decreases in cholinesterase activity in erythrocytes and plasma in volunteers exposed to parathion as vapor or dust, or from a chamber sprayed with an insecticide sprayer (exposure concentrations were neither measured nor estimated). These data indicate that parathion vapor and dust are absorbed across the respiratory tract, but do not provide enough information to estimate the fractions absorbed. An occupational study determined a maximum concentration of parathion in air of 0.8 mg/m^3 and an estimated average of about 0.2 or 0.3 mg/m^3 (Brown and Bush 1950). This level of exposure appeared to be associated with reduced levels of both plasma and erythrocyte cholinesterase activities.

3.4.1.2 Oral Exposure

Case reports of humans accidentally or intentionally poisoned by ingestion of parathion (i.e., Eyer et al. 2003 and others in Section 3.2.2) provide evidence of gastrointestinal absorption, but do not include measurements of parathion intake. For example, the concentration of parathion in plasma was 318 ng/mL in a 72-year old man hospitalized after ingesting an unknown amount of parathion in a suicide attempt (Hoffman and Papendorf 2006). Furthermore, absorption estimates made from poisoning incidents would

not be predictive of absorption in the absence of medical interventions that are intended to interfere with absorption (e.g., induced vomiting, gastric lavage, or activated charcoal). Morgan et al. (1977) measured metabolites of parathion in the urine of volunteers who ingested 1 or 2 mg/day of parathion in corn oil for 5 consecutive days, also providing evidence for oral absorption. Blood samples were not collected. Urine samples were not collected during the 5-day exposure period (collected only during the 2-day postdosing period); in addition, the average cumulative excretion of metabolites was not reported. Thus, these data do not provide a reliable estimate of oral absorption.

Braeckman et al. (1983) estimated the oral bioavailability of parathion to be between 1 and 29% in seven mongrel dogs given 10 mg/kg ^{14}C parathion via gavage. Bioavailability was estimated based on the ratios of the areas under the serum concentration-time curves after oral and intravenous administration. The peak serum concentrations in the seven treated dogs varied from 0.01 to 0.41 μg/mL, and time to peak ranged from 30 minutes to 5 hours, indicating substantial interindividual variability. Urinary excretion of radioactivity was similar after oral and intravenous administration of parathion to two dogs; the oral absorption estimates were 57 and 98% based on the ratios of the percentages of dose excreted after the two exposures.

Puga and Rodrigues (1996) estimated LD_{50} values in order to evaluate the effect of different solvents on the oral absorption of parathion in rats. Administration of 2% parathion (w/v) in arol vehicle yielded a slightly higher oral LD_{50} (130 mg/kg; 95% CI 98–152) than administration in xylene (LD_{50} of 102 mg/kg; 95% CI 78–121), suggesting greater oral absorption from xylene than from arol; however, the difference was small and the CIs overlapped.

3.4.1.3 Dermal Exposure

Evidence of dermal absorption of parathion is available from poisoning incidents in the general population (Eitzman and Wolfson 1967; Lores et al. 1978) and among agricultural workers (Grob et al. 1950; Milby et al. 1964; Quinby and Lemmon 1958). Lores et al. (1978) reported that the concentration of parathion in the whole blood was 0.034 ppm in a 13-year-old boy who died from organophosphate poisoning via dermal contact with treated tobacco; the only metabolite detected was diethyl phosphorothioate (0.26 ppm). In agricultural workers, Milby et al. (1964) estimated that a picker exhibiting cholinergic signs was exposed to a total of <4 mg/day and that dermal exposure contributed the highest amount, approximately 3.2 mg.

Available data indicate wide variability in the rates and fractional absorption of parathion across human skin. Dermal absorption rates vary depending on the form of parathion in contact with skin (e.g., vapor or dust), the temperature and relative humidity of the ambient air, the anatomical location of the exposed skin, and the condition of the exposed skin (Hayes et al. 1964; Maibach et al. 1971; Gosselin et al. 2005).

Measurement of urinary metabolites has suggested that dermal exposure may yield higher absorbed doses than respiratory exposure during parathion spraying activities. Durham et al. (1972) estimated the dermal and respiratory exposure of workers engaged in spraying parathion in orchards, and measured urinary excretion to estimate absorption. The average dermal exposure, extrapolated from measurements of parathion on pads worn by the workers on clothing near bare skin, was 137.9 mg. Based on total urinary excretion of *p*-nitrophenol (other metabolites were not measured), average dermal absorption was estimated to be 1.23% after subtracting the estimated respiratory exposure of 0.24 mg. The study authors also conducted controlled experiments to assess respiratory and dermal exposure separately with workers wearing either protective clothing or a respirator during exposure to an airblast spray machine. In these experiments, dermal exposure proved to be much greater (0.497–0.666 mg absorbed dose based on excretion of *p*-nitrophenol) than respiratory exposure (0.006–0.088 mg).

Higher ambient temperature is associated with higher dermal penetration of parathion in humans. Funckes et al. (1963) and Hayes et al. (1964) conducted a series of experiments investigating dermal absorption of parathion in various forms and at different ambient temperatures. Funckes et al. (1963) observed temperature-related increases in the urinary excretion of *p*-nitrophenol after dermal exposure to 2% parathion dust on one hand and forearm of each of four volunteers. Peak rates of *p*-nitrophenol excretion, occurring 5–6 hours after exposure, averaged 9.7, 25, 21.9, and 88.6 μg/hour at 58, 70, 82, and 105°F (respectively), indicating temperature-dependent increases in dermal penetration. Likewise, experiments by Hayes et al. (1964) showed markedly increased urinary excretion of *p*-nitrophenol at higher temperatures after 3 hours of exposure to filter paper pads containing 46–51 g of parathion. The total excretion of parathion over the 3 days after exposure was ~3- and 5-fold higher at 80 and 104°F, respectively, compared with similar exposure at 52°F.

Maibach et al. (1971) observed substantial variation in the absorption of parathion across skin from different anatomical regions of volunteers. In each experiment, six volunteers received a topical application of 4 μg/cm^2 ^{14}C-parathion, and urine was collected for 5 days after dosing. Based on total urinary excretion of radioactivity (and corrected for incomplete urinary recovery) the highest absorption estimates, ~100 and ~64% of the applied dose, were observed when the dose was applied to scrotal and

axillary skin, respectively; the lowest absorption estimates, ~9 and 12% of applied dose, were across the skin of the forearm and palm, respectively. Areas of the face and scalp exhibited relatively high absorption (about 30–40%); the study authors suggested that the presence of follicles in these areas enhanced penetration.

Experiments with animal skin tested *in vitro* also demonstrate the dependence of dermal uptake on ambient temperature and relative humidity. Chang and Riviere (1991) showed that increased humidity enhanced the penetration of parathion across porcine skin *in vitro*; the mean absorption efficiency increased more than 2-fold when relative humidity was increased from 60 to 90% in 8-hour experiments using doses from 4 to 400 μg/cm^2. Higher penetration was also observed when the air or perfusate temperature was increased from 37 to 42°C (Chang and Riviere 1991).

Knaak et al. (1984) evaluated dermal absorption of ^{14}C-parathion applied to the clipped backs of male and female rats. Male rats received 44 μg/cm^2 parathion over 13.7 cm^2 of skin, while females received 48 μg/cm^2 parathion over 12.5 cm^2 of skin. Recovery of radioactivity from excreta, plasma, liver, kidney, heart, and remaining carcass indicated that 59.2 and 57% of the applied dose was absorbed by males and females, respectively. The authors estimated permeability rates of 0.33 and 0.49 μg/hour/cm^2 for males and females, respectively, based on elimination from plasma; permeability constants (K_p) were estimated as 7.5x10^{-3} cm/hour for males and 1x10^{-2} cm/hour for females.

Based on quantification of radioactivity in plasma and excreta, Qiao and Riviere (1995) estimated the mean systemic bioavailability of parathion (40 μg/cm^2) applied to the back and abdomen of weanling pigs to be 14.7–19.7 and 8.9–9.2%, respectively.

Puga and Rodrigues (1996) estimated dermal LD_{50} values in order to evaluate the effects of different solvents on the percutaneous absorption of parathion in rats. Parathion (2% w/v) in arol or xylene vehicle was applied to the shaved back of rats over a 16 cm^2 area. As was seen with the oral study by these authors, administration in arol yielded a slightly higher LD_{50} (310 mg/kg; 95% CI 298–359) than administration in xylene (LD_{50} of 242 mg/kg; 95% CI 220–276), suggesting greater dermal absorption from xylene than from arol. However, plasma cholinesterase activity did not differ significantly in the rats treated with the different vehicles; thus, it is not clear that absorption differences were entirely responsible for the different lethality estimates.

Nabb et al. (1966) measured AChE inhibition to estimate dermal absorption of parathion by male albino rabbits. Technical parathion was applied to the animals' clipped sides while the rabbits were lightly anaesthetized; the parathion was left in place until symptoms of poisoning appeared between 5 and 8 hours later. Comparison of AChE inhibition rates after dermal exposure with rates observed with intravenous exposure yielded absorption rate estimates of 0.021–0.39 μg/minute/cm^2 (Nabb et al. 1966).

The flux of ^{14}C-parathion across freshly harvested skin flaps from weanling Yorkshire pigs peaked at about 4 hours after the end of dosing, reaching a peak rate of almost 0.01% of the dose/minute (Williams et al. 1990). The flux rate declined to about half the peak rate by 10 hours postexposure (Williams et al, 1990). A 5 cm^2 area of the skin flap was treated with 40 μg/cm^2 parathion for 8 hours. The authors estimated the fraction of dose that would be absorbed through 6 days postdosing to be 0.066, which compared favorably with the fraction of dose absorbed by whole animals exposed by topical application of 40 μg/cm^2 parathion on the abdomen (0.064, calculated from total excretion of radioactivity over 6 days).

In vitro estimates of parathion permeability across skin are shown in Table 3-6. Available *in vitro* data (Chang and Riviere 1993; Miller and Kasting 2010; Moody et al. 2007; Wester and Maibach 1985) suggest that the mass of parathion absorbed across the skin increases in proportion to applied dose up to doses as high as 3,200 μg/cm^2. Van der Merwe et al. (2006) measured the flux of parathion across porcine skin over the course of a 480-minute exposure at six different applied doses (6.3, 11.1, 22.5, 43.3, 106.9, and 209.1 μg/cm^2). The peak flux rate, as measured in percent of dose per hour, occurred at 180 minutes after the beginning of exposure; the rate remained the same for the remainder of the exposure time.

Qiao et al. (1996) studied how the interactions between two different solvents, dimethyl sulfoxide (DMSO) and acetone, the surfactant sodium lauryl sulfate (SLS), the rubefacient methyl nicotinate (MNA), and the reducing agent stannous chloride ($SnCl_2$) affected the absorption of radiolabeled parathion through isolated perfused porcine skin. The investigators used a full 2x4 factorial design to study treatment effects and potential interactions. The results showed that more radiolabeled was absorbed with DMSO than with acetone. SLS increased absorption of parathion in both DMSO and acetone vehicles, while MNA reduced absorption rates in DMSO and acetone without significantly changing total absorption. Stannous chloride blocked absorption of parathion and increased residue level on the skin surface and in the stratum corneum. Overall, the study showed interactive effects at multiple levels that need to be considered when studying dermal absorption of a chemical in a mixture.

Table 3-6. *In Vitro* Estimates of Parathion Dermal Permeability

Reference	Applied dose	Duration	Basis for absorption/ permeability estimate	Percent absorption of applied dose	Permeability coefficient (K_p; cm/hour)	Conditions
Wester et al. 2000	4 μg/cm^2	96 hours	Receptor liquid only	1.78±0.41	1.89x10^{-4}	Permeability of ^{14}C-parathion through human skin samples (1 cm^2 and 500 μm thick) measured in flow-through skin diffusion cells
				0.29±0.17	2.04x10^{-5}	As above with dry uniform material covering
				0.65±0.16	6.16x10^{-5}	As above with wet uniform material covering
Miller and Kasting 2010	0.4 μg/cm^2 4 μg/cm^2 41 μg/cm^2 117 μg/cm^2	76 hours	Receptor liquid + dermis	29.9±4.2 31.8±4.1 32.1±4.4 24.3±4.9	Not reported	Permeability of ^{14}C-parathion through human skin samples (400 μm thick) measured in modified Franz skin diffusion cells; unoccluded
	0.4 μg/cm^2 4 μg/cm^2 41 μg/cm^2 117 μg/cm^2			57.2±8.5 60.0±5.3 53.0±4.2 35.2±3.6	Not reported	As above, but occluded
Boudry et al. 2008	15 μg/cm^2	24 hours	Receptor liquid only receptor liquid + skin + stratum corneum	7.5±7.3 23.4±10.6	Not reported	Permeability of ^{14}C-parathion in ethanol through human abdominal skin samples (0.84 cm^2 and 420–550 μm thick) measured in dynamic glass diffusion cells; unoccluded
Shehata-Karam et al. 1988	38 μg/cm^2	48 hours		78.64	Not reported	Permeability of ^{14}C-parathion through fresh human newborn full thickness (882–1,093 μm) foreskin samples measured in modified static diffusion cell system

Table 3-6. *In Vitro* Estimates of Parathion Dermal Permeability

Reference	Applied dose	Duration	Basis for absorption/ permeability estimate	Percent absorption of applied dose	Permeability coefficient (K_p; cm/hour)	Conditions
Chang and Riviere 1991	4 μg/cm^2 40 μg/cm^2 400 μg/cm^2	8 hours	Receptor liquid only	7.69 1.91 0.46	Not reported	Permeability of ^{14}C-parathion through weanling pig skin (0.32 cm^2 and 500 μm thick) samples measured in Bronaugh flow-through Teflon diffusion cells at 60% relative humidity
	4 μg/cm^2 40 μg/cm^2 400 μg/cm^2			16.91 5.25 1.18	Not reported	As above, but 90% relative humidity

3.4.2 Distribution

Few data on the distribution of parathion in body tissues are available; only oral, intravenous, or intraperitoneal exposure routes were used in the available studies. Available *in vivo* data show high affinity of parathion for adipose tissue and the liver (Garcia-Repetto et al. 1995; Poore and Neal 1972) that is also seen in *in vitro* studies (Jepson et al. 1994; Sultatos 1990). Lower levels of parathion and/or paraoxon have been detected in the kidney, muscles, lung, and brain of animals exposed *in vivo* (Nielsen et al. 1991; Poore and Neal 1972). Radioactivity has been detected in ovine fetal blood and amniotic fluid after maternal intravenous exposure to ^{14}C-parathion, indicating transplacental transfer of the compound or its metabolite(s) (Villaneuve et al. 1971). In blood, parathion is largely bound to proteins, particularly albumin; a number of studies have suggested that 94–99% of parathion is protein-bound at equilibrium (Braeckman et al. 1983; Foxenberg et al. 2011; Nielsen et al. 1991).

3.4.2.1 Inhalation Exposure

No data on the distribution of parathion after inhalation exposure were located in the available literature.

3.4.2.2 Oral Exposure

Garcia-Repetto et al. (1995) measured the tissue concentrations of parathion at intervals between 4 hours and 20 days after dosing male Wistar rats via gavage with parathion in olive oil at 1/3 the oral LD_{50}. Parathion and paraoxon in various tissues were analyzed by gas chromatography. Parathion was detectable in adipose tissue and muscle 4 hours after dosing, and in these tissues as well as the liver and brain 4 days postdosing. Tissue:blood partitioning coefficients estimated for seven different time intervals showed increasing distribution to all four tissues; the highest partition coefficients (4.11–20.76) were in the liver at ≥12 days postdosing. Paraoxon appeared in the blood and adipose tissue within 4 hours of dosing and in the liver 8 days after dosing. When paraoxon was administered orally, only the liver showed tissue:blood partition coefficients >1 (ranging up to ~23 at 12–14 days postdosing).

Poore and Neal (1972) measured bound ^{35}S in the tissues of rats given an oral dose of 29 mg/kg ^{35}S parathion and sacrificed 35 minutes later; the results are shown in Table 3-7. The highest concentrations of ^{35}S were (in descending order) in the liver, intestine, kidney, muscles, lung, and brain.

Table 3-7. Concentration of Bound ^{35}S in Tissues of Male Sprague-Dawley Rats 35 Minutes After a Single Dose of 29 mg/kg ^{35}S Parathion Orally

Tissue	Tissue concentration (pmol ^{35}S bound/mg precipitate)
Liver	31.97±4.25
Intestine	13.75±6.41
Kidney	7.51±1.36
Intercostal muscle	5.44±0.26
Lung	3.99±1.02
Leg muscle	1.73±0.54
Brain	1.04±0.36
Heart	0.93±0.40
Diaphragm	0.70±0.09

Source: Poore and Neal 1972

3.4.2.3 Dermal Exposure

No data on the distribution of parathion after dermal exposure were located in the available literature.

3.4.2.4 Other Routes of Exposure

When piglets of different ages were administered 0.5 mg/kg ^{14}C-radiolabelled (ring-2,6) parathion intravenously, radioactivity was detected in the plasma as well as the kidney, liver, lung, brain, heart, and muscle tissues (Nielsen et al. 1991). Age-related differences in tissue distribution were observed; newborn piglets (1–2 days) exhibited much higher concentrations in all tissues than 1- or 8-week-old piglets. Mean tissue concentrations are shown in Table 3-8 for all three age groups, as are tissue:plasma concentration ratios; concentrations of parathion and its metabolites in plasma, liver, and kidney are shown in Table 3-9. Braeckman et al. (1983) estimated the hepatic extraction ratio as the percentage difference in parathion concentration (parent compound, measured by gas chromatography) in the femoral artery (as a surrogate for the portal vein concentration) compared with the hepatic vein. In anaesthetized dogs given intravenous administration of parathion in a foreleg vein, the hepatic extraction ratio was estimated to be 82–97%.

In vitro estimates of parathion partitioning to various tissues confirm the high partitioning to adipose tissue and liver (Jepson et al. 1994; Sultatos 1990); the available values are listed in Table 3-10.

Studies of pregnant animals demonstrate changes in toxicokinetics associated with physiological changes during gestation, and also show that parathion can cross the placenta. The distribution of parathion from blood to liver after intraperitoneal injection of 5 mg/kg was lower in pregnant mice (liver:blood ratio of 1.42) than in virgin mice (liver:blood ratio of 15.35) (Weitman et al. 1986). The authors postulated that the higher blood concentrations of parathion in pregnant mice would be available for extrahepatic activation and result in the higher toxicity of this compound in pregnant animals. Villeneuve et al. (1971) showed that ^{14}C-parathion administered intravenously at a dose of 1 mg/kg to pregnant sheep resulted in transfer of radioactivity to the fetal blood and amniotic fluid.

In the blood at equilibrium, 94–99% of parathion is bound to proteins, while only 60% of paraoxon is bound. Available information suggests that the degree of binding does not vary at parathion or paraoxon concentrations up to 50 μM. Nielsen et al. (1991) reported that 97% of parathion administered

Table 3-8. Tissue Distribution of ^{14}C in Piglets 3 Hours After a Single Dose of 0.5 mg/kg ^{14}C-Parathion Intravenously

	Tissue concentration (ng/g or mL)			Tissue:plasma ratio		
Tissue	Newborn	1 Week old	8 Weeks old	Newborn	1 Week old	8 Weeks old
Plasma	262±145	120±24	69±11	–	–	–
Kidney	1,360±546	746±129	509±83	5.2±0.6	6.5±2.3	7.4±0.8
Liver	1,254±638	156±10	46±8	5.4±2.1	1.3±0.3	0.67±0.04
Lung	421±92	78±9	47±5	1.6±0.3	0.66±0.10	0.71±0.18
Brain	215±76	38±10	16±4	0.8±0.3	0.33±0.15	0.25±0.10
Heart	302±85	53±3	22±2	1.1±0.3	0.50±0.19	0.32±0.07
Muscle	484±92	110±38	13±2	1.8±0.3	0.95±0.43	0.19±0.04

Source: Nielsen et al. 1991

Table 3-9. Concentrations of Parathion and its Metabolites in Piglets 3 Hours After a Single Dose of 0.5 mg/kg ^{14}C-Parathion Intravenously

	Tissue concentration (ng/g or mL)		
Tissue	Newborn	1 Week old	8 Weeks old
Total ^{14}C			
Plasma	262±145	120±24	69±11
Kidney	1,360±546	746±129	509±83
Liver	1,254±638	156±10	46±8
Parathion			
Plasma	83±47	11±5	3±1
Kidney	272±122	37±15	5±3
Liver	840±426	17±28	1±1
Paraoxon			
Plasma	2±0.8	0.06±0.11	0.14±0.06
Kidney	Not detected	Not detected	Not detected
Liver	6±5	0.2±0.5	Not detected
p-Nitrophenol			
Plasma	14±4	4±0.5	3±0.7
Kidney	762±136	433±127	81±15
Liver	100±50	12±9	1±1

Source: Nielsen et al. 1991

Table 3-10. Partition Coefficients for Parathion in Mice and Rats

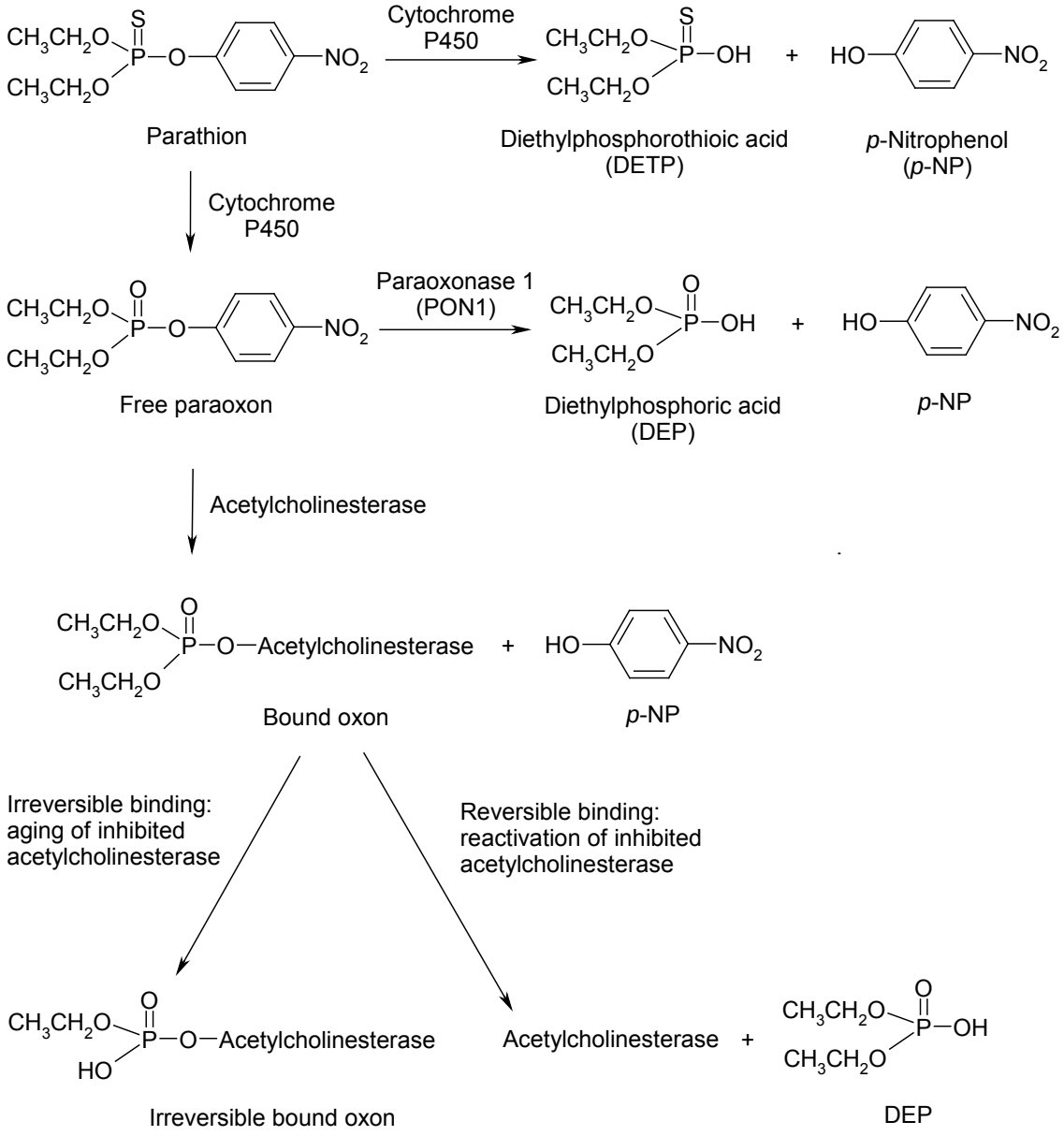
Tissue	Mouse	Rat	Method	Reference
Blood:saline		54.2	High pressure ultrafiltration	Jepson et al. 1994
Fat: saline		5,365	High pressure ultrafiltration	
Muscle:saline		136	High pressure ultrafiltration	
Liver:saline		270	High pressure ultrafiltration	
Skin: saline		160–180	High pressure ultrafiltration	
Liver: blood	6.56		Equilibrium dialysis	Sultatos 1990
Lung: blood	2.55		Equilibrium dialysis	
Brain: blood	3.51		Equilibrium dialysis	
Diaphragm: blood	1.37		Equilibrium dialysis	
Fat: blood	11.84		Equilibrium dialysis	
Rapidly perfused tissue:blood	6.56		Equilibrium dialysis	
Slowly perfused tissue:blood	4.51		Equilibrium dialysis	

intravenously to piglets was bound to plasma proteins. The fraction protein-bound did not differ by age (newborn and 1- and 8-week-old piglets were tested) or plasma concentration of parathion (ranging from 10 to 250 ng/mL). Foxenberg et al. (2011) evaluated parathion (25 or 50 μM) and paraoxon (10, 25, or 50 μM) binding to human serum albumin using the equilibrium dialysis method. Equilibrium was reached at about 60 minutes for both compounds; at this time, about 94% of parathion was bound to albumin and about 6% remained free at both concentrations, while about 60% of paraoxon was bound and 40% was free (at all concentrations). Sultatos et al. (1984) also used the equilibrium dialysis method to assess binding of parathion to fatty acid-free bovine serum albumin; the authors reported an apparent Kd value of 11.1 μM. Braeckman et al. (1983) obtained similar results (protein binding of 99%) in both human and dog serum treated with parathion *in vitro*; the fraction bound did not vary with parathion concentration in the range tested (0.2–30 μg/mL). When human albumin solution containing a typical albumin concentration was used instead of human serum, the measured protein binding fraction was also high (98%), indicating that parathion is largely bound to albumin in serum (Braeckman et al. 1983).

3.4.3 Metabolism

The metabolism of parathion is important in assessing its toxicity, as bioactivation to the paraoxon metabolite is a key step in the toxicity associated with AChE inhibition. Figure 3-3 outlines the metabolic pathways for parathion. Metabolism of parathion reflects complex interactions among a number of cytochrome P450 isozymes capable of both bioactivation and detoxification, as well as detoxification by carboxylesterases and A-esterases, and elimination facilitated by glucuronidase, glutathione transferase, and other conjugating enzymes. The complexity of these metabolic pathways is increased by the potential for both induction of P450 enzymes and inhibition of P450 enzymes by a sulfur radical produced when parathion is metabolized. The involvement of numerous bioactivating and detoxifying enzymes suggests that polymorphisms in the genes encoding these enzymes might lead to substantial interindividual variability; this variability has been seen in studies of paraoxon formation after incubation of human liver microsomes from a number of donors with parathion (Mutch and Williams 2006; Mutch et al. 2003). Sex, age, and pregnancy status have been shown to change the metabolism of parathion in animals. Pregnancy has been shown to alter the metabolism of parathion, possibly by increasing the systemic availability of parathion for extrahepatic metabolism (Weitman et al. 1983). In addition, an age-related decline in parathion toxicity was postulated to occur via enhancement of detoxification by A-esterases (Benke and Murphy 1974). Enhanced toxicity in female rats was associated with decreased detoxification of parathion (Benke and Murphy 1974).

Figure 3-3. Metabolism of Parathion



Sources: Buratti et al. 2003; Gosselin et al. 2004; El-Masri et al. 2004; Mutch et al. 2003

The bioactivation of parathion is generally well-understood, and is similar to other phosphorothionates. The initial step in bioactivation of parathion is desulfuration by cytochrome P450, yielding a theoretical unstable intermediate compound (phosphoxythiiran) that decomposes to paraoxon and a sulfur (S:) atom. Paraoxon may react with AChE to form the bound oxon and free *p*-nitrophenol, or it may be detoxified by A-esterase (also known as paraoxonase) to diethylphosphoric acid and *p*-nitrophenol. The bound oxon has two potential fates: irreversible binding to AChE, leading to "aging" of inhibited AChE (irreversible inhibition; see Section 3.5 for further details) or reversible binding with the release of free AChE and diethylphosphoric acid (Gosselin et al. 2005).

Cytochrome P450 isozymes are also responsible for detoxification of parathion via dearylation. This process, which may represent an alternative fate for the putative phosphoxythiiran intermediate (Tang et al. 2006), yields diethylphosphorothioic acid and *p*-nitrophenol. Diethylphosphorothioic acid may undergo desulfuration to diethylphosphoric acid. *p*-Nitrophenol may be eliminated as is or conjugated with glycine, glutathione, glucuronic acid, or sulfuric acid for excretion in urine. Paraoxon, diethylphosphorothioic acid, and diethylphosphoric acid were also shown to be formed non-enzymatically by a homogeneous preparation of rabbit liver cytochrome P450 (Kamataki et al. 1976). The three metabolites appeared to have been formed by breakdown by different pathways of a common enzymatically formed intermediate thought to be a sulfine derivative of parathion.

Neal (1967) first suggested that different cytochromes are involved in the formation of paraoxon and diethylphosphorothionate by showing that some enzyme inhibitors inhibited the formation of one parathion metabolite but not the other. For example, *p*-chloromercuribenzoate, Cu^{2+}, and 8-hydroxy-quinoline inhibited the formation of diethylphosphorothionate more than the formation of paraoxon. A number of investigators have attempted to identify the primary cytochrome isoforms involved in bioactivation and detoxification of parathion using recombinant human cytochromes (Buratti et al. 2003; Foxenberg et al. 2007; Mutch et al. 2002, 2003) and human liver microsomes (Mutch and Williams 2006; Mutch et al. 2003). Estimates of K_m, V_{max}, and in some cases, intrinsic clearance rate (V_{max}/K_m) for individual cytochrome enzymes have been calculated by Foxenberg et al. (2007), Mutch et al. (2006), and Buratti et al. (2002). Example estimates from Foxenberg et al. (2007) are shown in Table 3-11. Other studies have examined correlations between paraoxon formation and cytochrome-specific enzyme reactions, enzyme activities, or other cytochrome-specific markers (Buratti et al. 2002; Mutch et al. 1999) or by measuring the change in paraoxon formation that occurs when specific cytochromes are inhibited (Buratti et al. 2003; Butler and Murray 1997; Huhr et al. 2000). Taken together, the available data suggest that CYP1A2, CYP2B6, CYP2C19, and CYP2C8 may be important producers of paraoxon at low

Table 3-11. CYP-Specific Metabolism of Parathion by Recombinant Human P450s

Parameters	V_{max} (pmol/minute/nmol P450)	K_m (μM)	V_{max}/K_m (Clint)
Paraoxon formation			
CYP1A2	6,131±90.1	1.63±0.13	3.755
CYP2B6	4,827±134	0.61±0.08	7.875
CYP2C9	1,140±47.7	9.78±1.63	0.117
CYP2C19	4,879±73.7	0.56±0.04	8.705
CYP3A4	14,009±767	65.5±6.83	0.214
CYP3A5	2,020±540	43.2±27.1	0.047
CYP3A7	–	–	–
p-Nitrophenol formation			
CYP1A2	5,656±83.9	2.15±0.16	2.637
CYP2B6	1,804±46.4	0.74±0.10	2.447
CYP2C9	742±63.3	12.1±4.01	0.061
CYP2C19	2,338±65.4	0.60±0.09	3.872
CYP3A4	15,738±488	31.2±2.40	0.504
CYP3A5	1,175±1,039	68.2±121	0.017
CYP3A7	1,739±201	37.3±10.6	0.047

Source: Foxenberg et al. 2007

parathion exposures, and CYP2C9, CYP2D6, and CYP3A4/5 become more important at higher parathion exposures.

The initial step in bioactivation of parathion is desulfuration by cytochrome P450, yielding a theoretical unstable intermediate compound (phosphoxythiiran) that decomposes to paraoxon and a sulfur (S:) atom. While paraoxon is the active inhibitor of AChE, the free sulfur atom, S, is reactive, and can damage nearby proteins including the cytochromes (Tang et al. 2006).

The primary urinary metabolites identified in humans and animals exposed to parathion are *p*-nitrophenol, diethylphosphoric acid, and diethylphosphorothioic acid. Morgan et al. (1977) quantified levels of *p*-nitrophenol, diethylphosphoric acid, and diethylthiophosphate in the urine of four volunteers who ingested 1 or 2 mg/day parathion in corn oil for 5 consecutive days. Urinary excretion of diethylphosphoric acid represented between 3 and 13% of the daily dose of parathion during the first 24 hours postdosing and between 3 and 9% during the second 24 hours; *p*-nitrophenol and diethylthiophosphoric acid excretion represented 2–8 and 1–3% of the administered dose, respectively, during the first 24 hours and were not detected during day 2 (Morgan et al. 1977).

Urinary metabolites of parathion in male Sprague-Dawley rats given three consecutive daily gavage doses of 3.73 or 37.3 mg parathion/rat/day in peanut oil included diethylphosphoric acid, diethylphosphorothioic acid, and *p*-nitrophenol. The study authors estimated that diethylphosphoric acid and diethylphosphorothioic acid excretion represented 39.6 and 41.2% of the low and high administered doses, respectively, while *p*-nitrophenol excretion represented 37.3 and 11.8%, respectively, of the administered doses (Bradway et al. 1977).

Conjugates of *p*-nitrophenol have also been detected in the urine of humans exposed to parathion. Oneto et al. (1995) detected the sulfate and glucuronide conjugates of *p*-nitrophenol in the urine of a 20-year-old woman who died of parathion ingestion. *p*-Nitrophenol glururonide, *p*-nitrophenol sulfate, and free *p*-nitrophenol constituted approximately 7, 80, and 13%, respectively, of the total urinary *p*-nitrophenol.

Nielsen et al. (1991) measured urinary metabolites in newborn, 1-week-old, and 8-week-old piglets given a single dose of 0.5 mg/kg ^{14}C-parathion intravenously. The primary metabolites identified in urine were *p*-nitrophenyl-glucuronide (85% of the excreted radioactivity), *p*-nitrophenyl-sulfate (6%), and free *p*-nitrophenol (1%). The structural location of the radioactive label was not reported, but is presumed to be on the ring based on the detected urinary metabolites, which did not include diethylphosphoric acid or

diethylthiophosphoric acid. Hollingworth et al. (1973) identified the glutathione conjugate of *p*-nitrophenol when rat or mouse liver soluble fraction was incubated with paraoxon.

Parathion is bioactivated primarily in the liver, but also in extrahepatic tissues including the lung and brain. Norman and Neal (1976) observed metabolism of parathion to paraoxon and diethylphosphoric acid in rat lung microsomes incubated with ^{14}C-parathion ($5x10^{-5}$ M) in the presence of an NADPH-generating system *in vitro*, and this metabolism was inhibited by cytochrome inhibitors SKF-525A and piperonyl butoxide. The authors also detected paraoxon and diethylphosphoric acid when the 250,000 g centrifugal precipitate from rat brain was incubated with $15x10^{-5}$ M parathion. Poore and Neal (1972) detected radioactivity in the liver, lung, and brain of rats given an intraperitoneal dose of ^{35}S, ^{32}P-parathion (18 mg/kg). In addition, the authors measured ^{35}S in a number of tissues after oral administration of 29 mg/kg ^{35}S-parathion to adult male rats; radioactivity was detected in the liver, intestine, kidney, intercostal muscle, lung, leg muscle, brain, heart, and diaphragm. While it is possible that metabolites were formed in the liver and subsequently transported to other tissues, the authors postulated that this was unlikely given the reactivity of the sulfur formed by metabolism of parathion. Neal (1967) incubated microsomes with the 9000 g supernatant from four tissues with 0.35 μM parathion and measured the metabolites formed by each using thin-layer chromatography. The highest rate of metabolism was in the liver (98, 417, and 353 μmol/hour/g tissue formation of paraoxon, diethylphosphorothionate, and diethylphosphate, respectively) followed by kidney (32, 13, and 3 μmol/hour/g tissue), lung (13, 9, and 3 μmol/hour/g tissue), and brain (4, 9, and 2 μmol/hour/g tissue).

There is some evidence for dose-dependence of parathion metabolism to *p*-nitrophenol. Bradway et al. (1977) reported that the fractional urinary excretion of *p*-nitrophenol was lower (11.8% of administered dose) in male rats given 37.3 mg parathion/day for 3 days than in those given 3.73 mg/day for 3 days (37.3% of administered dose). The fractional excretion of diethylphosphoric acid and diethylphosphothioic acid (combined) was similar (~40%) at both doses (Bradway et al. 1977).

Physiological changes during pregnancy may alter the metabolism of parathion. Weitman et al. (1986) observed no differences between pregnant (gestation day 19) and nonpregnant mice in the levels of parathion, paraoxon, or *p*-nitrophenol measured in liver perfusate after 45 minutes of perfusion, suggesting that pregnancy does not alter the total hepatic metabolism of parathion. However, Weitman et a l. (1983) observed higher concentrations of both parathion and paraoxon in the blood and brain of pregnant (gestation day 19) mice given a single intraperitoneal dose of 5 mg parathion/kg compared with virgin mice given the same treatment; the higher levels of paraoxon correlated with significantly greater

inhibition of plasma and brain cholinesterase and with greater cholinergic toxicity in the pregnant mice. The authors suggested the possibility that extrahepatic metabolism of parathion might be a partial explanation for the enhanced toxicity in pregnant animals (Weitman et al. 1983).

Benke and Murphy (1975) observed an age-related decline in parathion toxicity (intraperitoneal LD_{50}) in rats tested at 1, 12–13, 23–24, and 35–40 days of age; no additional decline occurred in those 56–63 days of age. In addition, there was a sex difference in LD_{50} values; females were more sensitive. The authors attempted to correlate the changes in toxicity with changes in enzyme activities for bioactivation and detoxification. The age changes in toxicity were not explained by variation in cholinesterase inhibition or in oxidative bioactivation in the liver; however, the increasing LD_{50} values did correlate with increasing A-esterase activity in the rat liver and plasma, suggesting greater detoxification potential in older rats (Benke and Murphy 1975). The soluble liver fractions from adult male and female rats exhibited significant differences in GSH-dependent dearylation of parathion, with significantly ($p<0.005$) less dearylation in females; this correlated with increased toxicity in female rats.

3.4.4 Elimination and Excretion

Parathion is eliminated primarily through metabolism and subsequent excretion of metabolites in urine; a small proportion of metabolites is eliminated through the feces. Excretion of metabolites in urine has been shown to continue for days after exposure has concluded. Parathion elimination from the blood of a poisoned patient was estimated to exhibit a biphasic pattern, with a half-life of 3.1 hours for the early phase and 17.2 hours for the later phase. For the later phase; however, these elimination kinetics were likely substantially altered by the patient's treatment with gastric lavage and activated charcoal.

3.4.4.1 Inhalation Exposure

Urinary excretion of *p*-nitrophenol was measured in a volunteer exposed for 30 minutes each day for 4 days, followed by a 10-minute exposure on the fifth day (Hartwell et al. 1964). The exposure was reported as 1 mL of fresh technical parathion spread over 36 square inches of area and heated to 105–115°F (the first 4 days) or 5 mL spread over 80 square inches and heated to 150°F (day 5; exposure was terminated at 10 minutes due to illness of the volunteer). Daily urinary excretion of *p*-nitrophenol reached a peak of 3,517 μg/day on the fifth day of exposure and then declined to 300.5 μg/day 2 days later. When excretion of *p*-nitrophenol was highest, plasma and erythrocyte cholinesterase activities were 2 and 17% of pre-exposure values, respectively.

3.4.4.2 Oral Exposure

The concentration of parathion in plasma dropped rapidly (from 318 to <50 ng/mL) in the first 10 hours after a 72-year-old man was hospitalized after ingesting an unknown amount of parathion in a suicide attempt (Hoffman and Papendorf 2006). Blood levels declined more gradually thereafter. The study authors estimated a half-life of 3.1 hours for the early phase and 17.2 hours for the later phase. The elimination kinetics were likely altered by the patient's treatment with gastric lavage and activated charcoal, and do not represent elimination kinetics in the absence of treatment.

Morgan et al. (1977) measured metabolites of parathion in the urine of four volunteers (age and sex not specified) who ingested 1 or 2 mg/day of parathion in corn oil for 5 consecutive days. The same volunteers were exposed to both doses, separated by 4 undosed days; these volunteers had also been exposed to methyl parathion over two 5-day treatment periods prior to the experiments with parathion. Daily 24-hour urine samples were collected for analysis; mean levels of the three metabolites quantified in urine are provided in Table 3-12. Urinary excretion of diethyl phosphate peaked between 4 and 8 hours postdosing, and remained at detectable levels through the 2-day postdosing observation period. The study authors estimated that urinary excretion of diethyl phosphate represented between 3 and 13% of the daily dose of parathion during the first 24 hours postdosing and between 3 and 9% during the second 24 hours. *p*-Nitrophenol excretion was highest during the first 4 hours after dosing and declined rapidly thereafter to negligible levels by 24 hours postdosing. Diethylthiophosphate followed a similar pattern. The authors estimated that *p*-nitrophenol and diethylthiophosphate excretion represented 2–8 and 1–3% of the administered dose, respectively, during the first 24 hours; these metabolites were not detected during day 2.

3.4.4.3 Dermal Exposure

Hayes et al. (1964) assessed the time course of urinary excretion of *p*-nitrophenol over five consecutive daily 2-hour exposures to of 2% parathion dust (5 g) applied to the right hand and forearm of volunteers. The hourly rate of excretion of *p*-nitrophenol peaked (at ~60–80 μg/hour) 4–10 hours after each exposure and dropped rapidly thereafter.

The rate of urinary *p*-nitrophenol excretion in an individual with whole-body exposure (apart from the head) to parathion dust (2%) for 7 hours was 247.1 μg/hour in the first 24 hours after the start of exposure and dropped to 58.7 and 21.3 μg/hour in the subsequent 2 days (Hayes et al. 1986). No estimate of the total dermal dose was made.

Table 3-12. Urinary Excretion of Parathion Metabolites in Volunteers Exposed Via Ingestion

	Urine concentration (mg/L)		24-Hour excretion (mg)		Creatinine-adjusted excretion (mg/g)	
Metabolite	1 mg/day	2 mg/day	1 mg/day	2 mg/day	1 mg/day	2 mg/day
p-Nitrophenol	0.06	0.17	0.13	0.30	0.07	0.18
Diethyl phosphate	0.07	0.17	0.16	0.30	0.09	0.18
Diethylthiophosphate	0.03	0.07	0.06	0.12	0.03	0.07

Source: Morgan et al. 1977

3.4.4.4 Other Routes of Exposure

The total clearance of parathion from the body was age-dependent in piglets given 0.5 mg/kg ^{14}C-parathion intravenously; clearance rates of 7, 35, and 121 mL/minute/kg were estimated for newborn, 1-week-old, and 8-week-old piglets, respectively (Nielsen et al. 1991). The study authors estimated elimination rate constants (k_e) of 0.0038, 0.0265, and 0.0771 minute^{-1}, respectively, for elimination from the central compartment. Urinary excretion of radioactivity within the first 3 hours after dosing accounted for 18, 48, and 82% of the administered dose in the newborn, 1-week-old, and 8-week old piglets; all three groups excreted a small amount of radioactivity (0.1–0.2%) in the bile (Nielsen et al. 1991).

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of

toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

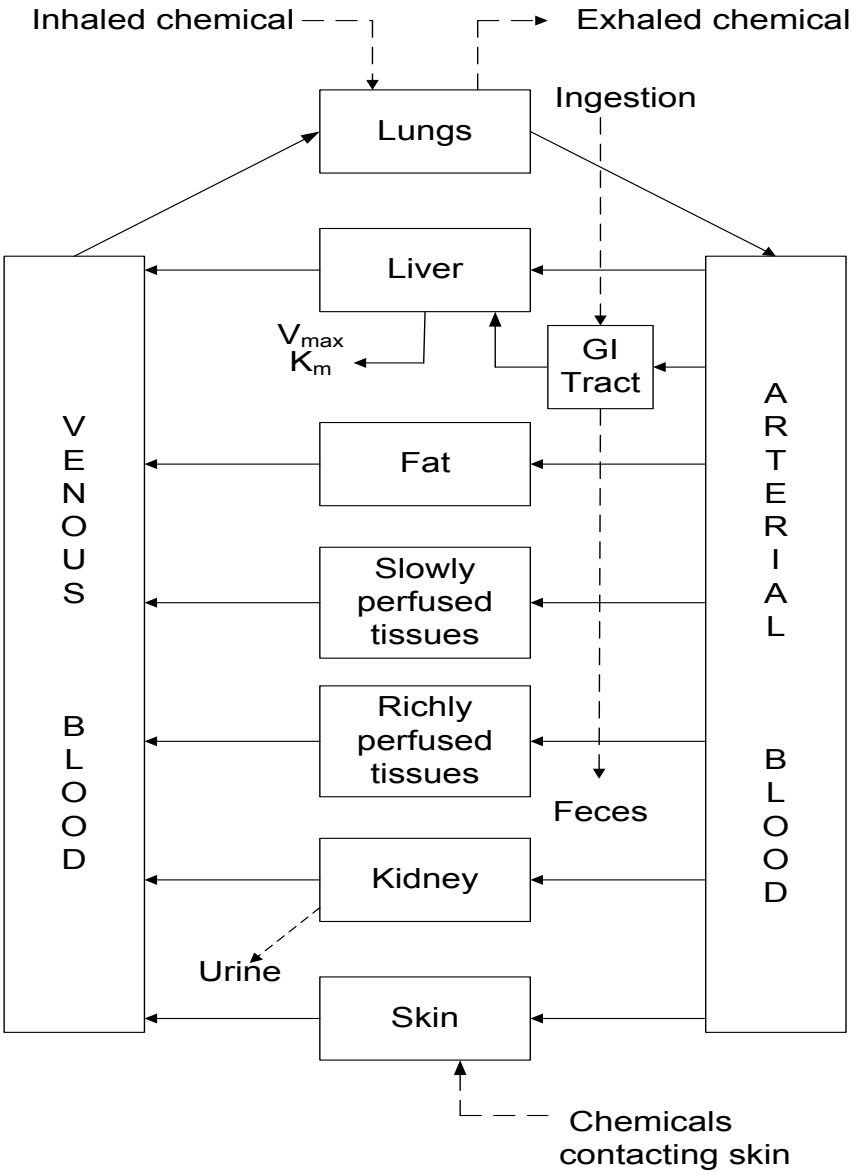
PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-4 shows a conceptualized representation of a PBPK model.

If PBPK models for parathion exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

PBPK models for parathion have been developed for a variety of purposes (El-Masri et al. 2004; Foxenberg et al. 2011; Gearhart et al. 1994; Gentry et al. 2002; Gosselin et al. 2005; Qiao et al. 1994; Sultatos 1990; van der Merwe et al. 2006). However, these models are inadequate for purposes of quantitative risk assessment.

Sultatos (1990) developed a PBPK model for parathion in the mouse based on parameters determined *in vitro*. The model included eight compartments (gastrointestinal tract, lungs, brain, diaphragm, fat, rapidly perfused tissue, slowly perfused tissues, and liver). Previous results from *in vitro* studies using mouse hepatic microsomes were used to estimate tissue/blood distribution coefficients (K_p) by equilibrium

Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Source: adapted from Krishnan and Andersen 1994

dialysis and calculate Michaelis-Menten kinetic constants V_{max} and K_m used in the model (Sultatos 1986). Sources for physiological parameters used for the mouse were not specified in the study report. Application of the PBPK model to the calculation of a hepatic extraction ratio of parathion in the mouse yielded a value in agreement with that obtained from parathion-perfused mouse livers *in situ*. The development of the Sultatos (1990) PBPK model for parathion demonstrates the usefulness of *in vitro* data.

Gearhart et al. (1994) developed a PBPK model for the inhibition of AChE by organophosphate esters. The model was developed for diisopropylfluorophosphate pharmacokinetics and AChE inhibition in rats, mice, and humans, but included an adaptation for modeling parathion and its toxic metabolite, paraoxon. The model included eight compartments (lungs, brain, liver, kidney, rapidly perfused tissue, fat, slowly perfused tissue, and diaphragm). Major determinants of parathion and paraoxon disposition in the model were metabolism of parathion to paraoxon, hydrolysis of paraoxon by esterases, binding of paraoxon to esterases, and tissue solubility of parathion and paraoxon; parameters for these determinants were based on *in vivo* and *in vitro* results from rodent studies (Chemnitius et al. 1983; Maxwell et al. 1987; Pla and Johnson 1989; Wallace and Dargan 1987). Metabolism of parathion to paraoxon was described in the model to occur in the liver and kidney via Michaelis-Menten kinetics. Initial K_m and V_{max} were based on intrinsic metabolic clearance of parathion and paraoxon from livers of rodents (Wallace and Dargan 1987). Partitioning estimates in the rat brain were based on measured concentrations of parathion and paraoxon following intravenous injection of parathion (Eigenberg et al. 1983); partitioning to other tissues was estimated *in vitro* by equilibrium dialysis (Jepson et al. 1992). Simulations of parathion and paraoxon kinetics in brain, liver, and blood after intravenous injection generally reflected the measured concentrations of Eigenberg et al. (1983). The simulation of fat tissue required the addition of diffusion limitation to achieve agreement with experimental data. Sources for physiological parameters used for rats, mice, and humans were not specified.

Gentry et al. (2002) used the PBPK model for parathion and paraoxon developed by Gearhart et al. (1994) in combination with Monte Carlo analysis to incorporate information on polymorphisms in the PON1 gene into the analysis of variability in tissue dose of paraoxon. The results of the analyses suggested that polymorphisms in the PON1 gene make only a minor contribution to the overall variability in paraoxon tissue dose. Sensitivity analysis showed that the estimation of the area under the curve (AUC) was most sensitive to changes related to the polymorphism of paraoxonase. Other parameters with the greatest impact on the arterial AUC were the V_{max} and K_m for paraoxonase in the blood compartments. However, many other parameters also had a significant impact on the AUC, reducing the impact of the

polymorphism on the total variability. Overall, the results were consistent with *in vivo* studies in animals, which suggest that PON1 polymorphism has little impact on the differences in paraoxon toxicity (see Costa et al. 2013 for review).

El-Masri et al. (2004) developed PBPK rat models for parathion and paraoxon to estimate blood concentrations of paraoxon. These models were used in conjunction with PBPK models for chlorpyrifos and its desulfuration metabolite (chlorpyrifos-oxon) and linked to an AChE kinetics model to produce an overall PBPK model intended to describe interactions between parathion and chlorpyrifos in the rat. The parathion and paraoxon PBPK models each include eight compartments (lung, rapidly perfused tissue, slowly perfused tissue, fat-diffusion limited, diaphragm, brain, kidney, and liver). Physiological parameters and tissue/blood coefficients for parathion and paraoxon were obtained from the report of Gearhart et al. (1994). Metabolic and biochemical reaction parameters for parathion were based on parameters reported in earlier reports (Gearhart et al. 1994; Ma and Chambers 1994). Hydrolysis of parathion by esterases was assumed to occur in all compartments except for fat, slowly perfused tissue, and diaphragm. The overall model was designed to evaluate interactions between chlorpyrifos and parathion in AChE inhibition in rats.

Gosselin et al. (2005) developed a multi-compartment model to describe the kinetics of parathion and its urinary metabolites, *p*-nitrophenol and alkyl phosphates, in order to assess worker exposure and health risks. The model was designed to estimate the dose of parathion absorbed under dermal, oral, or inhalation exposure routes under various temporal exposure scenarios. Model compartments represent body burdens and excreta of parathion and its metabolites. Model parameter values were determined from statistical fits to published *in vivo* human kinetic data. The model was developed for the purpose of biological monitoring.

Foxenberg et al. (2011) converted an existing PBPK/PD model for chlorpyrifos that used metabolism parameters from rat liver into human cytochrome-based/age-specific models for chlorpyrifos using chemical-specific recombinant human cytochrome kinetic parameters (V_{max}, K_m), hepatic cytochrome content, and plasma binding measurements to estimate AChE and butyrylcholinesterase inhibition. These models were used to simulate single oral exposures of adults and infants to chlorpyrifos (0.1, 1.0, and 10 mg/kg) or parathion (0.005, 0.025, and 0.1 mg/kg).

Van der Merwe et al. (2006) developed a PBPK model to simulate the absorption of organophosphate pesticides, including parathion, through porcine skin with flow-through cells. Parameters related to the

structure of the stratum corneum and solvent evaporation rates were independently estimated. Solvent evaporation rate, diffusivity, and mass transfer factor parameters were optimized based on experimental dermal absorption data. Diffusion cell studies were conducted to validate the model under a range of parathion doses (6.3–106.9 μg/cm^2), a variety of solvents (ethanol, 2-propanol, acetone), different solvent volumes, occlusion versus nonoccluded administration, and corneocyte removal.

Qiao et al. (1994) developed a pharmacokinetic model to quantify disposition of parathion and its major metabolites following dermal or intravenous administration to weanling pigs. The model quantitates evaporative loss, dosing device binding, percutaneous absorption, first-pass metabolism, and distribution and excretion of parent compound and its metabolites.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

As discussed in detail in Section 3.4 (Toxicokinetics), parathion is absorbed following inhalation, oral, or dermal exposure. No studies were located in which mechanisms of parathion absorption were evaluated. It is expected that absorption occurs via passive diffusion. In blood, parathion binds reversibly to plasma proteins and can subsequently be distributed throughout the body (Foxenberg et al. 2011). Parathion is lipophilic, and thus has a higher affinity for adipose tissue compared with blood or saline solution (Jepson et al. 1994; Sultatos 1990). In addition, depending on exposure route, a large fraction of parathion may distribute to the liver (Braeckman et al. 1983), where it is bioactivated to paraoxon. The bioactivation of parathion to its active metabolite paraoxon may vary widely among individuals due to variations in P450 isozymes and their activities. When human liver microsomes from 27 individuals were incubated with 200 μM parathion, there was an 18-fold range (0.038–0.683 nmol/minute/mg protein) in formation of paraoxon and a 90-fold range in formation of *p*-nitrophenol (0.023–2.10 nmol/minute/mg protein; Mutch and Williams 2006; Mutch et al. 1999, 2003). No information was located regarding mechanisms of elimination and excretion of parent compound or metabolites of parathion.

3.5.2 Mechanisms of Toxicity

Effects Mediated by AChE Inhibition. The most salient systemic effects of exposure to parathion are related to its direct effect on the nervous system and the secondary effects that result from it. Parathion is known to exert direct systemic effects through inhibition of cholinesterase, specifically AChE in the central and peripheral nervous systems. Inhibition of AChE is common to all organophosphorus

pesticides (OPs), but there are mechanisms of toxicity of OPs that should be kept in mind when addressing toxic effects. AChE is also present in erythrocytes. Thus, inhibition of erythrocyte AChE is commonly used as a surrogate indicator of the extent of inhibition of neural AChE. In addition, cholinesterases can be found in plasma. In humans, plasma cholinesterase is almost exclusively composed of butyrylcholinesterase. Although butyrylcholinesterase is capable of hydrolyzing acetylcholine and butyrylcholine *in vitro*, the *in vivo* substrate of plasma cholinesterase is unknown. Parathion is bioactivated *in vivo* and *in vitro* to its oxygen analog form, paraoxon (e.g., Buratti et al. 2003; Forsyth et al. 1989; Lessire et al. 1996; Mutch et al. 1999; Sultatos and Minor 1986; Zhang et al. 1991). Paraoxon phosphorylates a hydroxyl group on serine at the active site of AChE. Under normal circumstances, AChE rapidly and efficiently degrades the neurotransmitter, acetylcholine, following its release at the nerve synapse or at a neuromuscular junction; however, the phosphorylated AChE enzyme cannot degrade acetylcholine and the neurotransmitter accumulates at the ending of cholinergic nerves, resulting in repetitive firing of postsynaptic fibers (Abou-Donia 1995; Bajgar 2004; Costa 2008).

Cholinergic terminals play an important role in the normal function of the neuromuscular, central nervous, endocrine, immunological, and respiratory systems (Carrier and Brunet 1999). Thus, the inhibition of the enzyme AChE by paraoxon may have profound and wide-ranging systemic effects. Acetylcholine can be found in the autonomic, somatic motor, and central nervous systems. In the autonomic nervous system, accumulation of acetylcholine leads to the overstimulation of the muscarinic receptors of the parasympathetic nervous system, which can lead to effects on the exocrine glands (increased salivation, perspiration, lacrimation), eyes (miosis, blurred vision), gastrointestinal tract (nausea, vomiting, diarrhea), respiratory system (excessive bronchial secretions, wheezing, tightness of chest), and cardiovascular system (bradychardia, decrease in blood pressure) (Abou-Donia 1995; Bajgar 2004; Costa 2008).

Stimulation of the nicotinic receptors in the parasympathetic or sympathetic nervous system may also cause adverse effects on the cardiovascular system such as tachycardia, pallor, and increased blood pressure. In the somatic nervous system, nerve fibers innervate the skeletal muscles motor end-plates. Accumulation of acetylcholine in the somatic nervous system may be manifested as muscle fasciculations, cramps, paralysis, and flaccid or rigid tone, among other signs and symptoms. Overstimulation of the nerves in the central nervous system, specifically the acetylcholine receptors of the brain, by the accumulation of acetylcholine may result in lethargy, drowsiness, and mental confusion among other effects. More severe effects on the central nervous system include a state of coma without reflexes, depression of the respiratory centers, and cyanosis (Abou-Donia 1995; Bajgar 2004; Costa

2008). It has been recognized that, after repeated exposures to organophosphate insecticides, humans and other animal species may develop a tolerance to the appearance of cholinergic signs (Costa et al. 1982). It has been proposed that this tolerance to the effect of excess acetylcholine develops by the down-regulation of postsynaptic cholinergic receptors. This reduces the apparent cholinergic symptoms even in the presence of marked reductions in erythrocyte AChE activity (Sultatos 1994).

Which effects may dominate depends on the sensitivity of the target enzyme at various synapses and the level of the ultimate toxic molecule, paraoxon, which may be produced at or near the nerve from parathion or transported from the site of parathion activation such as the liver, lung, or kidney. While distribution of paraoxon is poorly understood, it undoubtedly depends on the route of exposure to parathion.

Phosphorylated AChE may be reactivated or irreversibly inhibited through a process known as 'aging". The bond between AChE and the phosphorous atom is very stable, but may be hydrolyzed by water over the course of hours or days (Abou-Donia 1995; Bajgar 2004; Costa 2008). Some hydroxylamine derivatives known as oximes facilitate AChE dephosphorylation and are used to treat poisoning with organophosphates such as parathion. Phosphorylated AChE may not be reactivated if "aging" of the inhibited enzyme occurs; "aging" refers to the hydrolysis of one of the two alkyl groups of the organophosphate (Costa 2008), leading to irreversible inhibition of AChE.

Recent studies have demonstrated that plasma AChE levels in animals treated with organophosphate compounds may not only return to pretreatment levels, but may also increase to as much as 250% over pretreatment levels (Duysen and Lockridge 2011). Plasma AChE levels were 150% of pretreatment levels in male mice 3 days after a single subcutaneous dose of 12.5 mg parathion/kg (Duysen and Lockridge 2011). The study authors postulated that organophosphate treatment induces apoptosis, which then triggers induction of AChE leading to plasma levels that exceed pretreatment levels.

Effects Mediated by Mechanisms Other Than AChE Inhibition. As described in Section 3.2.4, a series of studies by Slotkin and coworkers have shown that exposure to parathion and other organophosphate pesticides can affect the nervous system via mechanisms not directly related to inhibition of AChE (Slotkin 2011; Slotkin et al. 2006a, 2006b, 2008, 2009a). In these studies, neonatal rats received subcutaneous injections of parathion and were evaluated at later times up to adulthood. Administration of parathion affected the development of neurotransmitter systems involved in critical functions such as learning and memory (cholinergic) and in the expression of emotion, appetite, and sleep patterns

(serotoninergic), and induced behavioral alterations (Levin et al. 2010; Timofeeva et al. 2008). These effects occurred with doses of parathion that did not induce significant inhibition of AChE. In addition, the fact that the effects of parathion differed from those of other organophosphate pesticides regarding brain areas targeted and sex selectivity supported the view that the mechanism(s) involved, although not elucidated, was not directly related to AChE inhibition. Additional studies showed that early-life exposure to parathion caused a metabolic dysregulation in adult rats that was consistent with a pre-diabetic state (Lassiter et al. 2008). The mechanism for the metabolic effects appeared to involve disruption of adenylyl cyclase signaling in peripheral tissues, particularly in the liver (Adigun et al. 2010). Adenylyl cyclase is the enzyme that synthesizes cAMP from ATP. Interestingly, some of the effects of early parathion exposure were ameliorated or reversed by a high-fat diet, including effects on lipid peroxidation associated with synaptic activity (Slotkin et al. 2009b, 2009c). This was presumably due to global changes in the composition of synaptic membrane lipids. The fact that effects on lipids interacted with deficits in synaptic function suggested that the former may contribute to impaired behavioral performance (Lassiter et al. 2010; Slotkin 2011).

3.5.3 Animal-to-Human Extrapolations

While there are clear parallels between the toxicokinetics (and toxicity) of parathion in animals and humans, little is known about how well the toxicokinetics of parathion in animals predicts its behavior in humans due to the lack of studies examining humans (or human tissues) and animals under the same conditions. Recent work suggests that the desulfuration of parathion to paraoxon in human liver is mediated by a large number of cytochromes (CYP1A2, CYP2B6, CYP2C19, CYP2C8, CYP2C9, CYP2D6, and CYP3A4/5), which show different affinities for the substrate (see Section 3.4.3). Significant variations in the activities of these cytochromes among humans and laboratory animal species would be expected to result in differences in parathion metabolism.

In addition, there are four esterase enzymes in rodent blood (carboxylesterase, butyrylcholinesterase, AChE, and paraoxonase-1) that can detoxify organophosphate compounds, while human blood contains only three, lacking carboxylesterase (Duysen et al. 2012). Carboxylesterase has been shown to exert a protective effect against the toxicity of parathion in rats and mice. Karanth and Pope (2000) showed that the enhanced sensitivity of neonatal, juvenile, and aged rats (relative to adult) to the acute lethality of parathion was correlated with plasma carboxylesterase activity in these different age groups. Duysen et al. (2012) observed significantly enhanced inhibition of plasma AChE in carboxylesterase knockout mice

(73.1% inhibited), compared with their wild type counterparts (56% inhibited), after subcutaneous administration of 12.5 mg/kg parathion, but not after subcutaneous administration of 0.2 mg/kg paraoxon.

The efficiency of the parathion detoxification by serum paraoxonase (PON1) varies in laboratory animals. For example, PON1 knockout mice are no more sensitive to parathion toxicity (as measured by cholinesterase inhibition) than their wild type counterparts, while injection of rabbit PON1 into rats protects against paraoxon toxicity (Furlong et al. 2000). Furlong et al. (2000) suggested that these disparate findings suggest that rabbit PON1 has a higher catalytic efficiency for paraoxon hydrolysis than mouse PON1. Furlong et al. (2000) also performed *in vitro* measurements of paraoxon hydrolysis products after incubation of paraoxon with human serum from individuals with polymorphic PON1 genotypes ($PON1_{192QQ}$ and $PON1_{192RR}$). These experiments revealed variations in human PON1 K_m (0.36 and 0.42 mM for QQ and RR genotypes, respectively) and V_{max} (284 and 1,400 units/L, respectively), and showed that the QQ PON1 form is kinetically similar to the mouse PON1 (K_m of 0.34 mM and K_m of 300 units/L), while the RR form is more similar to the rabbit PON1 (K_m of 0.23 mM and K_m of 2,372 units/L).

There are indications that the response of the human nervous system to paraoxon exposure may differ from that in animals. Van den Beukel et al. (1998) observed greater sensitivity of the human nicotonic ACh receptor of SH-SY5Y neuroblastoma cells to blockage by paraoxon (as measured via membrane currents) than the nACh receptor of either mouse neuroblastoma cells or locust thoracic ganglion cells. Ecobichon and Comeau (1973) measured the *in vitro* inhibition of plasma cholinesterases by paraoxon in 11 different mammalian species; IC_{50} values (concentration necessary to achieve 50% enzyme inhibition) ranged from $0.2x10^{-7}$ M in hamster to $7.9x10^{-7}$ M in swine. IC_{50} values in humans, male rats, female rats, and mice were relatively similar, at $1.1x10^{-7}$, $1.4x10^{-7}$, $1.8x10^{-7}$, and $1.3x10^{-7}$ M, respectively (Ecobichon and Comeau, 1973).

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a

naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

The effects of parathion on the uptake and metabolism of [^{3}H]-testosterone in the prostate from mice were studied (Thomas and Schein 1974). Gavage administration of up to 5.3 mg parathion/kg/day for 5 days did not significantly affect the uptake of labeled testosterone or the ability of the prostate to transform the androgen into its main metabolites. In addition, the *in vitro* metabolism of testosterone by hepatic microsomes from mice treated with parathion was not significantly affected, except for an increase in the polar metabolite, androstanediol. Furthermore, hepatic testosterone hydroxylase activity was not affected by treatment with parathion, as judged by unchanged amounts of 6-, 7-, and 16-hydroxytestosterone derivatives.

Several studies have examined potential interactions of parathion with the estrogen receptor (ER) and androgen receptor (AR) *in vitro*. The AR antagonistic activity of parathion was examined *in vi*tro in HepG2 human hepatoma cells transfected with human AR plus an androgen-responsive luciferase reported gene, MMTV-luc (Tamura et al. 2003). Dose-shift experiments were conducted by adding set

concentrations of parathion across a complete dose-response range of the natural ligand dihydrotestosterone (DHT). The results showed that parathion lacked sufficient AR antagonistic activity to determine potency in the dose-shift experiments. Parathion was reported to have weak anti-androgenic effects in a reporter gene assay in Chinese hamster ovary cells (CHO-K1) relative to DHT alone (Kojima et al. 2004). The concentration of parathion showing 20% inhibition on the androgenic activity induced by 10^{-10} M DHT was 2.2×10^{-6} M. In the same study, parathion did not exhibit androgenic transcriptional activity or agonism or antagonism to the two human estrogen receptor subtypes, hERα and hERβ. In yet another *in vitro* study, parathion exhibited anti-androgenic activity when tested using a human AR reporter gene assay in an African monkey kidney cell line CV-1 transiently transfected with the constructed reporter gene plasmid pMMTV-chloramphenicol acetyltransferase (CAT) and the human AR expression plasmid AR/pcDNA3.1 (Xu et al. 2008). The anti-androgenic activity was assessed by measuring the ability of parathion to inhibit the induction of the CAT product by DHT. At concentrations $\geq 10^{-7}$ M, parathion significantly inhibited DHT-induced CAT activity in a concentration-related manner. The concentration of parathion that induced 50% inhibition was approximately 2×10^{-7} M. In the same study, parathion did not exhibit androgenic activity, which is consistent with the results of Kojima et al. (2004).

Parathion was a potent activator of the constitutive androgen receptor (CAR) in HepG2 cells *in vitro* with an EC_{50} of 1.43 μM (Mota et al. 2010). CAR is a transcription factor that regulates several detoxification enzymes. Cells were cotransfected with a mCAR expression plasmid and a luciferase reporter plasmid containing the CYP2B6 PBREM and then were cotreated with the inverse agonist, dihydroandrosterone (DHA) plus parathion. In studies *in vivo* with wild type (WT) and CAR-null mice, administration of a dose of 5 mg parathion/kg did not activate CAR in hepatic microsomes of either strain (Mota et al. 2010). However, parathion was significantly more toxic to CAR-null mice than to WT mice, suggesting that CAR has a protective role against parathion toxicity. According to the investigators, the lack of CAR activation in hepatic microsomes by parathion may have been due to its short half-life possibly preventing reaching the required hepatic concentration to activate CAR (Mota et al. 2010).

The potential role of parathion in human breast cancer has been studied by Calaf and coworkers using human breast epithelial cells *in vitro*. The investigators reported that parathion alone and in combination with 17β-estradiol (E2) induced malignant transformation of an immortalized human breast cell line, MCF-10F (Calaf and Roy 2007a); E2 alone did not induce malignant transformation. Malignancy was confirmed by increased anchorage independent growth and invasive capabilities. It was also shown that parathion increased the expression of a few proteins associated with signaling pathways and mutant p53

proteins. Of particular interest was the increase in epidermal growth factor receptor (EGFR) since growth factors and their receptors are functionally related to cell proliferation. Studies of gene expression using an array-based approach to monitor genes involved in a wide range of functions including apoptosis, cell cycle, cell growth and differentiation, signal transduction pathway, and other cancer-related genes showed that a significant number of genes were altered by either parathion, E2, or the combination of both (Calaf and Roy 2007b, 2008a, 2008b; Calaf et al. 2009). These results suggested that E2 and parathion can induce changes in gene expression in breast epithelial cells influencing the process of carcinogenesis. In a related publication, Calaf and Roy (2007c) showed that the parathion-induced effects could be significantly diminished by the muscarinic acetylcholine antagonist atropine. However, how parathion could alter gene expression by a cholinergic mechanism was not discussed.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example,

infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The fetus/infant has an immature (developing) blood-brain barrier that past literature has often described as being leaky and poorly intact (Costa et al. 2004). However, current evidence suggests that the blood-brain barrier is anatomically and physically intact at this stage of development, and the restrictive intracellular junctions that exist at the blood-central nervous system interface are fully formed, intact, and functionally effective (Saunders et al. 2008, 2012).

However, during development of the blood-brain barrier, there are differences between fetuses/infants and adults which are toxicologically important. These differences mainly involve variations in physiological transport systems that form during development (Ek et al. 2012). These transport mechanisms (influx and efflux) play an important role in the movement of amino acids and other vital substances across the blood-brain barrier in the developing brain; these transport mechanisms are far more active in the developing brain than in the adult. Because many drugs or potential toxins may be transported into the brain using these same transport mechanisms—the developing brain may be rendered more vulnerable than the adult. Thus, concern regarding possible involvement of the blood-brain barrier with enhanced susceptibility of the developing brain to toxins is valid. It is important to note however, that this potential selective vulnerability of the developing brain is associated with essential normal physiological mechanisms; and not because of an absence or deficiency of anatomical/physical barrier mechanisms.

The presence of these unique transport systems in the developing brain of the fetus/infant is intriguing; as it raises a very important toxicological question as to whether these mechanisms provide protection for the developing brain or do they render it more vulnerable to toxic injury. Each case of chemical exposure should be assessed on a case-by-case basis. Research continues into the function and structure of the blood-brain barrier in early life (Kearns et al. 2003; Saunders et al. 2012; Scheuplein et al. 2002).

Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948).

Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

Acute parathion exposure affects children in the same manner as it affects adults. Regardless of the route of exposure, children exposed to high amounts of parathion exhibit the typical cholinergic signs and symptoms described in previous sections. Some examples are provided below.

Eitzman and Wolfson (1967) reported 30 deaths that occurred in children in the state of Florida from 1959 through 1964. The average age was 2.9 years and deaths occurred within hours of poisoning. Exposure occurred through ingestion of parathion from improper containers, through ingestion of parathion from the floor or windowsills where it was place to kill roaches, or due to inhalation or skin contact. There also have been numerous cases of children poisoned though ingestion of contaminated food. For example, Wishahi et al. (1958) reported that 200 people were accidentally poisoned in Cairo, Egypt, following ingestion of contaminated flour; 22 of them were children. There were eight fatalities and all were children. Signs and symptoms included abdominal pain, vomiting, and convulsions. All fatal cases fell into a deep coma accompanied by shock in six cases and hypertension in two cases. Death, which occurred 4–9 hours after the onset of symptoms, was due to respiratory failure. Postmortem examination of five cases selected at random showed cyanosis of the lips, conjunctiva, and face, and miosis of the pupils. The heart was enlarged and the lungs showed variable degrees of acute edema; some congestion and edema was reported in the brain. Diggory et al. (1977) reported that 79 cases of poisoning occurred in Jamaica due to ingestion of contaminated flour and involved an unspecified number of children. Illness began 10 minutes to 4 hours after a meal and the first symptoms were nausea, cramps and vomiting. Severe cases showed sialorrhea, diplopia, pinpoint pupils, “giddiness,” muscle fasciculation, dyspnea, bradycardia, coma, and convulsions. Deaths generally occurred within 6 hours as a result of respiratory arrest. Although the number of children involved was not specified, the investigators noted that case-fatality ratios (40%) were highest in children ≤4 years of age. Etzel et al. (1987) reported similar episodes in Sierra Leone that involved children eating bread baked with

parathion-contaminated flour. Signs and symptoms included loss of consciousness, shortness of breath, excess sweating, frothing of the mouth, wheezing, excess tearing, excess salivation, muscle twitching, convulsions, diarrhea, vomiting, increased urination, chest pains, and abdominal cramps. Children between the ages of 1 and 10 years had the highest rates of illness; however, this may have been due to higher consumption of bread than adults rather than increased susceptibility.

A study of women living in an agricultural community in California did not find a significant association between exposure to parathion and adverse developmental effects in the offspring including length of gestation, birth weight, length, head circumference, and ponderal index (Eskenazi et al. 2004). However, it should be noted that exposure to parathion was assessed by measuring urinary *p*-nitrophenol, which can also be produced as a result of exposure to substances other than parathion.

Gestational exposure of rats to up to 1 mg parathion/kg or of rabbits to up to 0.3 mg parathion/kg did not cause embryotoxicity or teratogenicity (Renhof 1984, 1985). However, another study in which rats were exposed to parathion during gestation and lactation reported that pups showed EKG alterations, even at the lowest dose of parathion tested, 0.01 mg/kg/day (Deskin et al. 1979).

Numerous studies in rats have demonstrated that younger animals are more sensitive to the effects of parathion than mature animals. This difference is likely due to age-related differences in toxicokinetics. It should be noted, however, that in all of these studies, the rats were administered parathion by either subcutaneous or intraperitoneal injection, both non-relevant routes of exposure. For example, Gagné and Brodeur (1972) showed that weanling rats given parathion intravenously are more susceptible to parathion than adult rats mainly because of deficient mechanisms of degradation of parathion and paraoxon. In addition, the brain from adult male rats appeared to be less sensitive to paraoxon than the brain from weanling rats. Another study in male rats of various ages (1–80 days old) given parathion intraperitoneally reported that the specific activities of AChE in cerebral cortex and of liver aliesterases increased with age, thus providing significantly more protection against parathion toxicity (Atterberry et al. 1997). The increase in specific activity of brain AChE occurred without developmental changes in sensitivity of the enzyme to paraoxon, but liver aliesterase sensitivity to inhibition by paraoxon decreased with age. Benke and Murphy (1975) examined how the rates of several alternate metabolic pathways affected the toxicity of parathion in rats of five ages. The pathways studied included oxidative activation and cleavage, hydrolysis by A-esterases, glutathione-S-alkyl-, and -S-aryl-transfer, and binding of paraoxon to tissue constituents. It was found that, in general, increasing LD_{50} values with age obtained after intraperitoneal injection of parathion correlated better with changes in rates of reactions that

represented detoxification pathways for paraoxon than for reactions that represented direct metabolism of parathion. The LD_{50} values were about 6 and 3 times higher in adult males and females than in the neonatal male and female rats, respectively. This suggests that the use of an uncertainty factor of 10 to protect susceptible populations, such as the young, is probably adequate, at least in rats. Benke and Murphy (1975) also found that age differences in susceptibility were not related to differences in sensitivity of cholinesterase to inhibition by paraoxon *in vitro*. Similar findings were reported by Pope et al. (1991) who found that neonatal rats were more sensitive than adults to the acute toxicity of parathion. However, the maximal brain AChE inhibition was similar in both age groups (>78%). In a subsequent paper, Pope and Chakraborti (1992) reported that ED_{50} estimates of brain AChE and plasma ChE (dose that inhibits 50% of enzyme activity) were highly correlated with maximal tolerated doses in neonates and adults. In their study of male rats of varying ages, Atterberry et al. (1997) also reported a progressive increase in activities of P 450-mediated activation (desulfuration, 6–14-fold) and detoxification (dearylation, 2–4-fold) as well as concentrations of P450 (7-fold) and protein (2-fold) between neonate and adult hepatic microsomes. It was also reported that microsomal pentoxyresorufin (PROD) activity increased 16-fold between neonates and adults, whereas ethoxyresorufin (EROD) activity increased 16-fold until 21 days of age and then decreased in adulthood to a 10-fold higher than neonates. Atterberry et al. (1997) noted that their results suggested that the lower levels of hepatic aliesterase-mediated protection and P450-mediated dearylation contribute significantly to the greater sensitivity of young rats to parathion toxicity. Karanth and Pope (2000) conducted a similar study but included an aged group of rats (24 months old). The maximum tolerated dose (i.e., the dose that caused 0% mortality 7 days after a subcutaneous injection of parathion) was 2.1, 4.8, 18, and 6 mg/kg in neonatal, juvenile, adult, and aged rats, respectively. The levels of carboxylesterases and A-esterases in liver, plasma, and lung from neonatal and juvenile rats were significantly lower than in adults. Aged rats had A-esterases levels in tissues and plasma similar to adults and carboxylesterase levels in liver and lung similar to adults, but had significantly lower carboxylesterase levels than adults in plasma. The authors concluded that carboxylesterase activity may play a more critical role in the differential sensitivity to parathion.

Harbison (1985) determined an intraperitoneal LD_{50} of 8.8 mg/kg for parathion in adult male rats vs. 1.8 mg/kg in newborn rats. Pretreatment of the newborns with the microsomal inducer phenobarbital increased the LD_{50} to 4.8 mg/kg. Since microsomal enzymes cannot only activate parathion to paraoxon, but can also detoxify parathion to *p*-nitrophenol and diethylphosphorothioic acid (DEPTA), the results suggested that inducing the metabolism of parathion in the newborn enhanced detoxification rather than bioactivation. A study in newborn pigs showed age-related distribution of parathion and metabolites in tissues (Nielsen et al. 1991). Three hours after an intravenous injection of 0.5 mg/kg ^{14}C-parathion to

newborn, 1-week-old, and 8-week-old piglets, tissues and plasma from newborns had significantly more ^{14}C than 1-week-old piglets, which in turn had more ^{14}C than 8-week-old animals. This could be explained by differences in body clearance (7, 35, and 121 mL/minute/kg with increasing age) and urinary excretion (18, 42, and 82% of the dose with increasing age) rather than by age-related differential affinity between parathion and the tissues.

As previously mentioned, a series of studies in which neonatal rats were administered subcutaneous doses of parathion that did not induce significant inhibition of AChE reported alterations in the development of neurotransmitter systems and metabolic dysregulation that were evident at later times up to adulthood (see Section 3.2.4, Other Routes of Exposure for references). Since the various organophosphorus pesticides tested seemed to induce effects of opposing direction, the investigators suggested that organophosphorus pesticides can affect the developing nervous system via mechanisms not directly related to AChE inhibition.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of the U.S. population to environmental chemicals using biomonitoring. This report is available at http://www.cdc.gov/exposurereport/. The biomonitoring data for parathion from this report are discussed in Section 6.5. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly

found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to parathion are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by parathion are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10, Populations That Are Unusually Susceptible.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Parathion

The most specific biomarkers for exposure to parathion are the parent compound itself and its metabolites in tissues and body fluids. Diethylphosphoric acid, diethylthiophosphoric acid, and *p*-nitrophenol are metabolic products of the *in vivo* degradation of parathion and have been detected in urine of humans under field and experimental conditions after oral, dermal, or otherwise unspecified exposure. For instance, Morgan et al. (1977) detected these metabolites in the urine of volunteers as early as 4 hours after they ingested 1 or 2 mg parathion/kg. Diethylphosphoric acid and diethylthiophosphoric acid can be detected after exposure to other organophosphate insecticides. Due to its rapid appearance in urine, *p*-nitrophenol was suggested early on as a biomarker for parathion (Arterberry et al. 1961; Denga et al. 1995; Wolfe et al. 1970) and remains in use for this purpose today (Arcury et al. 2007; Kissel et al. 2005). However, it should be noted that *p*-nitrophenol can also be derived from exposure to methyl parathion, O-ethyl O-4-nitrophenyl phenylphosphonothioate (EPN), and other non-pesticide chemicals. Davies et al. (1967) reported urinary *p*-nitrophenol concentrations in 14 fatal and 9 nonfatal cases of parathion poisonings in Dade County, Florida during the years 1962–1965; a number of these cases were children. The mean concentration of *p*-nitrophenol in the fatal cases was 40.3 ppm (range 2.4–122 ppm), while the

concentration in nonfatal cases averaged 10.79 ppm (range 0.7–22 ppm). In a recent study, Arcury et al. (2007) analyzed urine samples from 60 Latino children of farm workers for pesticide metabolites, including *p*-nitrophenol. *P*-nitrophenol was present in 90% of the urine samples at a mean creatinine-adjusted concentration of 1.25 μg/g. Kissel et al. (2005) measured organophosphate metabolites in urine samples from 13 children in Washington State who had been identified as having potentially elevated organophosphate exposure. Urine samples were collected before bed, during first morning void, after lunch, and before dinner in two seasons. A total of 96% of the samples contained *p*-nitrophenol, suggesting possible parathion exposure. The authors reported that the first morning void samples were the best predictors of the volume-weighted daily average (Kissel et al. 2005).

Noort et al. (2009) described a liquid chromatography-tandem mass spectrometry method for analyzing organophosphorothioate pesticides bound to albumin in blood. The method was able to detect covalent binding of parathion and other compounds to albumin at concentrations that did not inhibit butyryl-cholinesterase. The study authors suggested that measurement of protein adducts in blood might provide a better indication of chronic, low-level exposure than urinary metabolites because the adducts accumulate over time. Further evaluation and application of this method are needed to firmly establish its utility and reliability.

In humans, inhibition of cholinesterases in erythrocytes and plasma may be a useful marker of higher levels of exposure. However, Arterberry et al. (1961) detected significant quantities of urinary *p*-nitrophenol in individuals with occupational parathion exposure, in whom plasma and erythrocyte ChE levels were normal, indicating that urinary *p*-nitrophenol is a more sensitive indicator of exposure than plasma or erythrocyte AChE activity.

3.8.2 Biomarkers Used to Characterize Effects Caused by Parathion

Diagnosis of organophosphate poisoning, including parathion, can be made by the presence of characteristic clinical signs and measurements of serum (plasma) cholinesterase and red blood cell AChE activities. Enzyme inhibition, however, is not specific for organophosphates since exposure to carbamate insecticides also results in cholinesterase inhibition. Nonspecific cholinesterase (pseudocholinesterase, butyrylcholinesterase) is present in myelin, liver, and plasma, whereas AChE is present in the central and peripheral nervous systems and in red blood cells. Plasma cholinesterase activity can be inhibited by 20–25% without significant physiological consequences (Abou-Donia 1995). Parathion is a stronger inhibitor of plasma cholinesterase than of red blood cell AChE (Maroni et al. 2000). Plasma cholinesterase regenerates at a more rapid rate than red blood cell AChE, about 25% regeneration occurs

in the first 7–10 days, and is regenerated by the liver in about 2 weeks (Abou-Donia 1995). After severe poisoning, plasma cholinesterase activity remains depressed for up to 30 days, which corresponds to the time that it takes the liver to synthesize new enzymes. Although a more sensitive indicator of exposure to organophosphates than red blood cell AChE, plasma cholinesterase is less specific since the levels may also be suppressed due to genetic factors and a variety of conditions and diseases (Abou-Donia 1995; Tafuri and Roberts 1987). The rate of decrease of red blood cell AChE correlates better with appearance of symptoms than the absolute value reached after exposure (Maroni et al. 2000). Reduction of red blood cell AChE after severe exposure lasts up to 100 days, reflecting the time of production of new cells. Red blood cell AChE levels are representative of AChE levels in the nervous system and, therefore, may be a more accurate biomarker of the neurological effects of chronic, low-level exposure of humans to parathion (Midtling et al. 1985). Tafuri and Roberts (1987) proposed a classification of organophosphate poisoning as follows. Clinical signs and symptoms of intoxication may occur when plasma cholinesterase levels drop to below 50% of the normal value. Mild poisoning, with the patient still ambulatory, may occur when plasma cholinesterase levels are 20–50% of normal; moderate poisoning with inability to walk may occur at levels 10–20% of normal; and severe poisoning with respiratory distress and unconsciousness may occur with levels <10% of normal.

Several methods for measuring red blood cell AChE and plasma cholinesterase are available (see Chapter 7). Baseline data are often collected for workers, preferably three values, but these data would not be available for environmentally exposed people. Inferences made by comparing values of exposed subjects with a reference population may be erroneous since values at the upper limit of the normal range may be 200% higher than those at the lowest one (Maroni et al. 2000). Therefore, it is useful to conduct a long-term, sequential determination of cholinesterase activity to confirm enzyme inhibition (Coye et al. 1987). Plasma cholinesterase is preferred over red blood cell AChE since it recovers more quickly and an increase in activity is more likely to occur over shorter observation periods.

3.9 INTERACTIONS WITH OTHER CHEMICALS

Numerous studies in animals have examined how co-exposure to parathion and other substances, particularly other pesticides, affect the toxicity of parathion. Of particular interest has been the study of substances that affect the metabolism of parathion, as this plays a crucial role in the toxicity of parathion. Some examples are summarized below. Overall, the data suggest that results from *in vivo* studies do not always parallel the results from *in vitro* studies, so that caution should be exercised when extrapolating

from *in vitro* to *in vivo* situations. Worth noting also is that the sequence of exposure can influence the toxic outcome of the interaction.

In mice, administration of three daily doses of DDT followed 24 hours later by a single dose of parathion reduced parathion's 24-hour LD_{50} from 10 to 7.2 mg/kg (Chapman and Leibman 1971). Four pre-doses of 3-methylcholanthrene (3-MC) increased the LD_{50} to 15 mg/kg, whereas pre-dosing with chlordane increased the LD_{50} to 35 mg/kg. Examination of the metabolism of parathion *in vitro* in mice pre-treated with DDT, 3-MC, or chlordane showed that DDT treatment preferentially enhanced the formation of DEPTA (parathion's detoxification pathway), 3-MC decreased DEPTA production, and chlordane enhanced both the activation and detoxification pathways. Since the *in vivo* results did not parallel the changes in the microsomal metabolism of parathion, the investigators suggested that factors other than metabolism, possibly tissues and plasma binding of parathion, contribute to the toxicity of parathion (Chapman and Leibman 1971). In a similar study, pretreatment of mice with DDT protected against parathion-induced lethality and inhibition of plasma, whole-blood, and brain cholinesterase; however, no protection was afforded against paraoxon toxicity (Bass et al. 1971). *In vitro* experiments showed that, contrary to what was expected from the *in vivo* results, pre-treatment with DDT increased the activation of parathion, as shown by a greater inhibition of cholinesterase, but did not significantly affect the detoxification of paraoxon (Bass et al. 1971). These seemingly inconsistent results could be explained by an increased rate of conversion of parathion to DEPTA by DDT pre-treatment, although this was not tested in the study.

Srivastava et al. (1976) studied the effect of di(2-ethylhexyl) phthalate (DEHP) on the toxicity of parathion. A single oral dose of parathion was given to rats 18 hours after receiving a single intraperitoneal dose of DEHP. Brain and whole-blood cholinesterase activities were assayed 30 minutes after parathion administration. The results showed that DEHP significantly decreased the parathion-induced inhibition of the two enzymes. Since the administration of DEHP followed by paraoxon did not affect the paraoxon-induced enzyme inhibition, the results suggested that DEHP increased the rate of conversion of parathion to paraoxon. Yasoshima and Masuda (1986) reported that carbon disulfide (CS_2) administered to mice 1 hour before a single parathion dose significantly potentiated the inhibitory effect of parathion on plasma cholinesterase activity, suggesting that CS_2 increased activation of parathion to paraoxon. This was consistent with the results of an experiment in which liver microsomes from mice treated with CS_2 incubated with parathion showed decreased *p*-nitrophenol production. However, in that experiment, cholinesterase inhibition was reduced, suggesting that although paraoxon formation may have been increased by pretreatment with CS_2, a greater stimulation of the detoxification pathway may

have also occurred *in vitro*. Studies by Kuntz et al. (1990) and Chaturvedi et al. (1991) showed that mixtures of toxaphene and parathion and 2,4-dichlorophenoxyacetic acid (2,4-D) reduced the parathion-induced decrease in serum cholinesterase activity in mice possibly by increasing the release of enzyme from the liver. In addition, the results suggested that toxaphene had the potential to reduce the toxicity of parathion and paraoxon by increasing the NADP-dependent metabolism of these substances to poor binders of acetylcholine esterase. Triolo and Coon (1966) showed that administration of aldrin to mice resulted in increased toxicity of parathion 1 hour after aldrin dosing, but it protected against parathion toxicity from 16 hours to 12 days after aldrin dosing. The investigators suggested that the initial effect of aldrin may have been due to inhibition of parathion detoxification, although this was not investigated. The second phase could be explained in part by a 38% increase in A-esterase activity in the liver and 24% increase in B-esterase in plasma, but it appeared that other factors, possibly involving the central nervous system, played a role in aldrin's protective effect against parathion poisoning.

In an intermediate-duration study, treatment of male rats with parathion plus lindane for 90 days resulted in more severe testicular toxicity than in a group treated with parathion alone, but no quantitative data were provided (Dikshith et al. 1978). Measurements of enzyme activities showed that parathion alone induced a 50% decrease in brain cholinesterase activity, whereas the parathion/lindane combination induced a 79.5% decrease; lindane did not significantly affect the parathion-induced inhibition of blood cholinesterase (about 80% in both cases) (Dikshith et al. 1978).

In a more recent study, Karanth et al. (2001) showed that the interactive toxicity of parathion and chlorpyrifos can be influenced by the sequence of exposure. Gavage administration of chlorpyrifos to rats followed by parathion 4 hours later resulted in significant more cholinergic toxicity than if the sequence was reversed. This suggested that in the former case, more inhibitor (the respective oxons) was allowed to reach the target tissues. Studies *in vitro* suggested a differential role of carboxylesterases and A-esterases in the detoxification of chlorpyrifos oxon and paraoxon. Carboxylesterases were found to detoxify both chlorpyrifos oxon and paraoxon, while A-esterases only detoxified chlorpyrifos oxon. In liver from rats pretreated with parathion, A-esterases still detoxified chlorpyrifos oxon, while the liver from rats pretreated with chlorpyrifos had little apparent effect on paraoxon. Similar findings were reported in a later study in neonatal rats (Kacham et al. 2006).

El-Masri et al. (2004) developed a PBPK model to estimate an interaction threshold for the joint toxicity between parathion and chlorpyrifos in the rat. The investigators first developed PBPK models for each chemical to estimate the blood concentration of their respective metabolites, paraoxon and chlorpyrifos

oxon. The estimated levels of metabolites were then linked to a model for AChE kinetics describing enzyme synthesis, degradation, binding to the metabolites of both chemicals, and aging after binding. The resulting overall PBPK model described interactions between parathion and chlorpyrifos at the levels of the P450 enzymatic activation site and at AChE binding sites. Calibration of the model was performed using interaction data from published studies. The results of the modeling showed that less-than-additive and additive interactions occurred at different dose ranges. The overall model simulations indicated that additivity is obtained at oral doses <0.8 mg/kg of each chemical, the interaction threshold.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to parathion than will most persons exposed to the same level of parathion in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of parathion, or compromised function of organs affected by parathion. Populations who are at greater risk due to their unusually high exposure to parathion are discussed in Section 6.7, Populations with Potentially High Exposures.

Although no studies were found in humans regarding the role that diet plays in the toxicity of parathion, several studies in animals have examined this issue. The issue is relevant as it may impact people living in developing countries where both malnutrition and the use of certain pesticides, including parathion, are widespread. For example, in an early study, Boyd (1969) showed that reducing the amount of dietary casein from 26 to 3.5% during 28 days to male Wistar rats increased the acute oral toxicity of parathion by about 7 times. In a similar study, Casterline and Williams (1971) reported that exposure of protein-deprived Osborne-Mendel rats to parathion for 28 days resulted in much greater inhibition of serum and brain cholinesterase and serum and liver triacetinesterase than in rats exposed to parathion alone. The investigators speculated that reduced dietary protein resulted in reduced detoxifying enzymes. In another study in rats, food restriction simultaneous with daily parathion intake for 28 days increased the inhibition of plasma cholinesterase and plasma and liver carboxylesterase by parathion compared to rats fed a normal diet (Villenueve et al. 1978). Bulusu and Chakravarty (1986, 1987) studied the effects of a low-protein diet on the activities of liver β-glucuronidase and acid and alkaline phosphatases. Male rats were kept on normal or low-protein diets for 3 weeks and were given daily doses of parathion at the same time. Parathion increased β-glucuronidase and acid phosphatase activities and decreased alkaline phosphatase activity in rats on the normal protein diet. Maintaining the rats on a low-protein diet aggravated the effect of parathion on the enzyme's activities. The investigators speculated that the effects of parathion on

β-glucuronidase and acid phosphatase may be due to parathion-induced damage to lysosome membranes leading to enzyme leakage into the cytoplasm. The decrease in alkaline phosphatase was attributed to a possible action at the cell membrane level affecting transport mechanisms involving phosphate.

A more recent study provided evidence suggesting that people who consume excessive amounts of sugar may be at a higher risk from exposure to parathion based on the results of studies in rats (Olivier et al. 2001). The investigators provided rats with tap water or 15% glucose in tap water for up to 21 days. On day 7, the rats received a single subcutaneous injection of parathion or paraoxon, and signs of toxicity were recorded for the next 13 days. Exposure to high glucose resulted in a significant decrease in food intake (50%) and increase in total caloric consumption (20%). Rats exposed to glucose showed more severe and long-lasting signs of toxicity due to parathion than rats drinking tap water only. However, the excess glucose had no apparent effect on the toxicity induced by paraoxon. Glucose feeding also increased the magnitude and duration of the inhibition of brain and plasma cholinesterase by parathion, but not by paraoxon. Also, glucose feeding did not affect the biotransformation of parathion or paraoxon. Finally, while parathion exposure down-regulated total muscarinic receptor binding in the cortex of control and glucose-fed rats, a much greater reduction (43%) was noted in glucose-fed rats. The investigators suggested that the glucose-induced reduction in food intake, particularly of amino acids, may limit the *de novo* synthesis of AChE and consequent recovery of synaptic transmission. Liu et al. (2005) conducted a similar study in adult and juvenile rats provided with either tap water or 15% high fructose corn syrup (HFCS) in drinking water. The results showed that the cholinergic toxicity of parathion was significantly enhanced by feeding HFCS in both adults and juvenile rats. However, consumption of HFCS had no significant effect on parathion-induced AChE inhibition in the frontal cortex or the diaphragm. The latter suggested that differences in enzyme inhibition may not account for the greater parathion toxicity observed in sugar-fed rats than in water-only rats. Since feeding HFCS significantly reduced food intake in rats, the effects of parathion were examined in a pair-fed group of rats. The results showed that food restriction alone did not exacerbate parathion toxicity. In a later study, Liu et al. (2007) reported that the exacerbation of parathion toxicity by glucose feeding was associated with significant increases in nitric oxide and reductions in high-energy phosphates/metabolites in the brain. According to the investigators, these biochemical responses may be involved in the modulation of parathion toxicity by glucose feeding, but the precise contribution remains unclear. The investigators noted that their results may be particularly important in children because children often consume relatively higher proportions of sugar in their diets.

Paraoxonase (PON1), the A-esterase that hydrolyzes paraoxon, the active metabolite of parathion, is polymorphically distributed in humans, suggesting that there might be a genetically based differential susceptibility to the toxicity of parathion and similar organophosphorus pesticides. The information below has been extracted from a recent review of the topic by Costa et al. (2013). The reader is referred to references cited therein for more detailed information. Human PON1 displays two polymorphisms in the coding region, Q192R and L55M. A significant additional polymorphism found in the non-coding regions of the PON1 gene is that at position –108, with the 108C allele providing levels of PON1 about twice as high as those seen with the 108T allele. While the Q192R polymorphism significantly affects the catalytic efficiency of PON1 and is substrate-dependent, the L/M polymorphism at position 55 has been associated with plasma PON1 protein levels, with $PON1_{M55}$ being associated with low plasma PON1. The latter appears to be related to linkage with the low efficiency –108T allele of the –108 promoter region polymorphism. Both the Q192R and the –108 (C/T) polymorphisms contribute to determine an individual's PON1 "status". For adequately predicting risk of organophosphate toxicity, it is important to know the two variables of PON1 (192 genotype and level), as high catalytic efficiency and high concentrations of PON1 are the two determinants of PON1 protection. Plasma PON1 activity can vary up to 40-fold in a given population, and differences in PON1 protein levels up to 13-fold are also present within a single $PON1_{192}$ genotype in adults. Human studies have shown that PON1 activity is very low at birth and increases over time reaching a plateau between 6 months and a few years of age. There are also data indicating that PON1 activity may be even lower before birth as determined in premature babies compared to term babies. While this suggests that fetuses and young children may be at higher risk of organophosphate toxicity, it may not be the case regarding parathion based on data from studies with rat liver microsomes and human liver microsomes that have suggested that PON1 is not functionally important at the toxicologically relevant concentrations of paraoxon (Chambers et al. 1994; Mutch et al. 1999).

Studies in animals have shown that administration of rabbit PON1 (high PON1 activity) afforded significant protection against the cholinergic effects of oxons, including paraoxon. However, knockout ($PON1^{-/-}$) mice, which have no paraoxonase activity in plasma and liver, unexpectedly showed no increased sensitivity to paraoxon. Moreover, intravenous injection of purified human $PON1_{Q192}$ or $PON1_{R192}$ to $PON1^{-/-}$ mice did not afford protection against paraoxon toxicity. These results were explained by the fact that with paraoxon, the $PON1_{R192}$ alloform is much more efficient than the $PON1_{Q192}$ alloform, but its overall catalytic efficiency is too low to protect against paraoxon toxicity. This strongly suggested that PON1 may not degrade paraoxon efficiently *in vivo* and as such, it does not play an important role in modulating sensitivity to paraoxon toxicity. This is consistent with results of studies

that examined the association between Parkinson's disease and parathion and the influence of functional polymorphisms at position 55 in the coding region of the PON1 gene (PON1-55) (Manthripragada et al. 2010) and also the single nucleotide polymorphisms $PON1_{Q192R}$ and $PON1_{C-108T}$ impact (Lee et al. 2013) (see Section 3.2.3.4). The results showed no increased risk of Parkinson's disease for people exposed to parathion, and risk did not increase in carriers of the variant MM PON1-55 genotype or the variant genotypes $PON1_{Q192R}$ or $PON1_{C-108T}$.

The results of a series of studies in which neonatal rats were administered subcutaneous doses of parathion that did not induce significant inhibition of AChE suggested that young organisms may be especially sensitive to the effects of organophosphorus pesticides, parathion included (see Section 3.2.4 for references). These studies reported alterations in the development of neurotransmitter systems and metabolic dysregulation that were evident at later times up to adulthood. Since the various organophosphorus pesticides tested seemed to induce effects of opposing direction, the investigators suggested that organophosphorus pesticides can affect the developing nervous system via mechanisms not directly related to AChE inhibition.

A few studies suggest that female rats are more susceptible to the acute effects of parathion than males. For example, Gaines (1960) reported oral LD_{50} values of 13 and 3.6 mg/kg in male and female Sherman rats, respectively. To determine whether LD_{50} values underwent seasonal variations, Gaines and Linder (1986) conducted bimonthly determinations in male and female Sherman rats over a period of 1 year. The LD_{50} values ranged from 6.9 to 11.0 mg/kg in males and from 3.0 to 3.4 mg/kg in females. Pasquet et al. (1976) reported 10-day LD_{50} values of 16 and 6 mg/kg for technical parathion in male and female CD rats, respectively. Whether this reflects sex-related differences in toxicokinetics is unknown. Making inferences to possible sex-related differences in humans based on these limited data would be inappropriate.

Studies have shown that many cytochromes are involved in the hepatic metabolism of parathion and that glutathione S-transferases also participate in the elimination of parathion. Since the metabolism of parathion plays a key role in its toxicity, genetic polymorphisms may influence health outcomes and place certain individuals at a higher risk of parathion exposure. Studies have suggested that cytochrome CYP3A4 is the main human cytochrome involved in the metabolism of parathion (Butler and Murray 1997; Mutch et al. 1999). Although there is growing evidence for functional polymorphisms in CYP3A4, evidence is too preliminary to predict with certainty the extent to which polymorphism might impact parathion metabolism (Haber et al. 2002).

See also Section 3.7, Children's Susceptibility for related information regarding susceptibility of younger organisms to parathion toxicity.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to parathion. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to parathion. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to organophosphate pesticides:

Goldfrank LR, Flomenbaum NE, Lewin NA, et al., eds. 2002. Goldfrank's toxicologic emergencies. 7th ed. Stamford, CT: Appleton and Lange.

Shannon MW, Borron SW, Burns MJ, eds. 2007. Haddad and Winchester's clinical management of poisoning and drug overdose. 4th ed. Philadelphia, PA: WB Saunders Company.

Viccellio P, Bania T, Brent J, et al., eds. 1998. Emergency toxicology. 2nd ed. Philadelphia, PA: Lippincott-Raven Publishers.

3.11.1 Reducing Peak Absorption Following Exposure

The following information was extracted from the books listed above; specific chapters were written by Aaron (2007), Clark (2002), and Osmundsen (1998).

The first priority following parathion intoxication should be airway management with frequent suctioning of secretions and respiratory support. Intubation may be required to facilitate control of secretions and for ventilator support if respiratory failure occurs. Patients with liquid contamination of skin and clothing may pose a skin contact risk, so health care personnel should wear neoprene or nitrile gloves. To prevent further dermal absorption, the patient should be disrobed as soon as possible and the skin should be washed thoroughly with alkali soap and water. The eyes should be irrigated copiously with water or saline. The removal of clothing should eliminate 85–90% of a contamination hazard. Although hypochlorite solutions deactivate organophosphate pesticides *in vitro*, their use on human tissues is not recommended because it may cause corneal burns and other toxicity. Agents such as soil, flour, or talcum powder may be applied to the skin followed by mechanical removal. Cutaneous absorption can also

occur as a result of contact with vomitus or diarrhea. Following oral poisoning, evacuation of the stomach contact is recommended if vomiting has not occurred. However, induced emesis should be avoided. If the patient is alert and asymptomatic, a single dose of activated charcoal is recommended (usually 1 g/kg) because ileus may develop during atropine therapy. Osmundsen (1998) points out that Ipecac should not be used for organophosphate poisoning. Cathartics may be unnecessary, as intestinal motility is greatly increased.

3.11.2 Reducing Body Burden

No information was located regarding reducing the body burden of parathion, or organophosphates, following exposure. As mentioned in Section 3.4, parathion is eliminated relatively rapidly, such that short-term exposures will not result in accumulation of the pesticide.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Seizure activity should be rapidly controlled with intravenous diazepam, midazolam, or lorazepam. Initial recommendations include the use of at least 10 mg intravenous diazepam or 5–10 mg intramuscular midozalam in adults (pediatric dose is 0.1–0.2 mg/kg intravenous diazepam or 0.1–0.3 mg/kg intramuscular midozalam). After stabilization of the patient and decontamination, the next priority should be to control excessive muscarinic activity with atropine. Atropine is a competitive antagonist at muscarinic receptor sites and since it crosses the blood-brain barrier, it also treats the central nervous system effects. Glycopyrrolate, a quaternary ammonium compound, has been suggested as an alternative to atropine. Unlike atropine, glycopyrrolate does not cross the blood-brain barrier and, therefore, has fewer central nervous system effects. Intravenous doses of atropine should begin at 1–5 mg in adolescents and adults and at 0.05 mg/kg in children up to adult doses, and should be repeated every 2–3 minutes until atropinization occurs. The latter is achieved when the patient exhibits dry skin and mucous membranes, decreased or absent bowel sounds, tachycardia, reduced secretions, no bronchospasm, and usually mydriasis. Patients with severe toxicity may require 75–100 mg atropine. Direct ocular exposure to parathion may respond only to topical ophthalmic atropine. Atropine does not antagonize nicotinic effects; therefore, pralidoxime (2-PAM) is needed for treatment of muscle weakness and respiratory depression. 2-PAM is a quaternary amine oxime that can reverse the phosphorylation of AChE and thereby restore activity. It may also prevent continued toxicity by detoxifying the organophosphate molecule and has an anticholinergic effect. 2-PAM and other oximes function by nucleophilic attack on the phosphorylated enzyme; the oxime-phosphonate is then split off, leaving the regenerated enzyme. 2-PAM should be administered as soon as the diagnosis is made. The initial dose is

1–2 g for adults and 25–50 mg/kg for children administered intravenously over 30–60 minutes. The dose can be repeated in 1 hour and then every 8–12 hours until clinical signs have diminished and the patient does not require atropine. Some patients may require multiple doses, as enzyme regeneration depends on plasma levels of the organophosphate. A 2-PAM serum level of 4 mg/L is suggested as the minimum therapeutic threshold. 2-PAM is considered a very safe drug with few side effects. In addition to 2-PAM, obidoxime has been used successfully to treat parathion poisoning (Eyer et al. 2003). Treatment consisted of an initial 250 mg intravenous bolus followed by continuous infusion at 750 mg/24 hours. This resulted in plasma obidoxime concentrations between 10 and 20 μmol/L.

Although the emergency medicine textbooks cited above do not specifically mention the use of substances to antagonize the nicotinic effects induced by organophosphotus pesticides, studies in animals provide some information. For example, Mehrani et al. (2008) reported that in rats treated intraperitoneally with paraoxon, simultaneous administration of atropine plus the nicotinic receptor antagonist, mecamylamine, resulted in less signs of toxicity (involuntary movements) than in rats treated with paraoxon and only atropine. Studies in animals also provide information regarding other types of treatments to interfere with parathion toxicity. Petrikovics et al. (1999) showed that intravenous injection of recombinant phosphotriesterase encapsulated in sterically stabilized liposomes into mice 1 hour prior to a subcutaneous injection of paraoxon significantly increased the 24-hour LD_{50} from 0.9 to 125 mg/kg. Combining the phosphodiesterase treatment with either atropine or 2-PAM further increased the LD_{50} to 540–550 mg/kg. An even higher LD_{50} of 920 mg/kg was obtained when the mice were pretreated with phosphotriesterase plus atropine and 2-PAM. Evron et al. (2007) reported that mice injected with the human AChE variant, AChE-R, exhibited reduced toxicity to a lethal dose of paraoxon than control mice. AChE-R was produced from plant-optimized cDNA in *Nicotiana benthamiana* plants and showed the same affinity for paraoxon as the mammalian cell culture-derived AChE. Yet another potentially useful mitigating agent was studied by Bird et al. (2008). These investigators reported that a single intravenous injection of a recombinant bacterial organophosphate hydrolase (OpdA), cloned from *Agrobacterium radiobacter*, to rats immediately or 10 minutes after an oral dose of parathion did not prevent lethality. However, repeated doses at 45 and 90 minutes after poisoning significantly improved survival to 62.5%. Administration of a single dose of OpdA in combination with 2-PAM therapy improved survival to 75%. Time-course experiments showed that OpdA maintained clinically relevant enzymatic activity *in vivo* for several hours.

3.12 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of parathion is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of parathion.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

3.12.1 Existing Information on Health Effects of Parathion

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to parathion are summarized in Figure 3-5. The purpose of this figure is to illustrate the existing information concerning the health effects of parathion. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Most of the literature reviewed concerning the health effects of parathion in humans described case reports and case series of occupational exposure and accidental or intentional ingestion of parathion. There are also a few studies of the general population and of controlled exposure in volunteers. The cases of occupational exposure to parathion concerned exposures of acute, intermediate, and chronic durations. The predominant route of exposure in the occupational case reports/series was believed to be dermal, but the possibility of some degree of inhalation exposure could not be ruled out. The information on current

Figure 3-5. Existing Information on Health Effects of Parathion

	Death	Acute	Intermediate	Chronic	Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
	Systemic									
Inhalation	●	●	●			●				
Oral	●	●	●			●				
Dermal	●	●	●	●		●	●	●		●

Human

	Death	Acute	Intermediate	Chronic	Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
	Systemic									
Inhalation	●	●	●			●				
Oral	●	●	●	●	●	●	●	●	●	●
Dermal	●	●	●	●	●	●				

Animal

● Existing Studies

human exposure in the United States is limited because production and all uses of parathion in the United States were cancelled in 2003. The precise duration and level of exposure to parathion in the human studies available generally cannot be quantified from the information presented in the reports.

There is more information in the open literature regarding the health effects of parathion following acute and intermediate oral exposure in experimental animals than regarding chronic exposure. Also, as can be seen in Figure 3-5, considerably less information is available on the effects of inhalation and dermal exposure to parathion in animals. There is no evidence suggesting that the toxicity of parathion is route-specific. However, ingested parathion should reach the liver sooner.

People living near hazardous waste sites may be exposed to parathion primarily via dermal contact with, or through ingestion of, contaminated soils since parathion is found bound to soil particles. Another possible mechanism for oral exposure to parathion is the ingestion of pesticide-laden dust from a waste site. Ingestion of contaminated water is not expected to be a significant route of exposure since parathion is poorly soluble in water and is generally not found in groundwater. Likewise, inhalation exposure to parathion is not a major route of exposure due to its low volatility. Before the use of parathion was banned, the primary route of exposure to parathion for the general population was probably via ingestion of low-level residues on contaminated foods.

Recommendations made below regarding conducting additional studies where data gaps exist need to be balanced by the fact that the current risks of exposure have diminished significantly since all uses and production of parathion were cancelled several years ago.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. Information is available regarding the effects of acute-duration exposure in humans following inhalation (Hartwell et al. 1964), oral (De Bleecker et al. 1992; De Jager et al. 1981; Diggory et al. 1977; Eitzman and Wolfson 1967; Etzel et al. 1987; Eyer et al. 2003; Hayes et al. 1964; He et al. 1998; Hoffman and Papendorf 2006; Morgan et al. 1977; Tsachalinas et al. 1971; Wishahi et al. 1958), and dermal exposure (Grob et al. 1950; Hayes et al. 1964; Milby et al. 1964; Quinby and Lemmon 1958). Parathion may be lethal to humans and animals by all routes of exposure studied, depending on the dose (Diggory et al. 1977; Eitzman and Wolfson 1967; EPA 1978; Etzel et al. 1977; Gaines 1960; Gaines and Linder 1986; Lores et al. 1978; NIOSH 1974; Pasquet et al. 1976; Wishahi et al. 1958). The main target of toxicity in humans and animals following acute, high-level exposure by any route is the

nervous system (Diggory et al. 1977; Eitzman and Wolfson 1967; EPA 1978; Etzel et al. 1977; Gaines 1960; Gaines and Linder 1986; Lores et al. 1978; NIOSH 1974; Pasquet et al. 1976; Wishahi et al. 1958). Adverse systemic effects (respiratory, cardiovascular, and gastrointestinal) reported in most cases of acute exposure to high amounts of parathion in humans and in animals are likely to be secondary to the serious neurological effects (i.e., tremors, seizures). Acute oral exposure to parathion also induced neurobehavioral alterations in animals at doses higher than those that inhibited cholinesterase activity (Moser 1995; Reiter et al. 1973, 1975). Acute-duration oral studies in mice also showed that parathion can affect immune function by inhibiting the production of antibodies and increase the sensitivity to allergens (Casale et al. 1983, 1984; Fukuyama et al. 2010, 2011, 2012; Kim et al. 2005; Wiltrout et al. 1978). In some studies, this was observed in mice treated with relatively low doses, comparable to doses that inhibited AChE activity (Fukuyama et al. 2011, 2012). It would be helpful to try to replicate these findings to add confidence to the results. An acute-duration study also reported that parathion altered the microscopic appearance of the skin of guinea pigs when applied directly onto the skin for 5 days (Dikshith and Datta 1972). As discussed in Section 2.3, studies of cholinesterase inhibition have shown that it takes approximately 21–28 days for inhibition of cholinesterase activity to reach a steady state and that values obtained in single-dose or short-duration studies carry great uncertainty. For this reason, and also based on data collected on enzyme inhibition for a great number of organophosphate pesticides (EPA 2006), acute-duration inhalation MRLs were not derived for parathion. However, as explained in Section 2.3, the intermediate-duration MRLs are protective of acute effects.

Intermediate-Duration Exposure. No intermediate-inhalation studies in humans were located. Only one intermediate-duration inhalation study in animals was located, and it provided information on neurological effects in rats and dogs during whole-body, intermittent exposure to parathion aerosol for 6 weeks (NIOSH 1974). An intermediate-duration inhalation MRL was derived for parathion from data regarding changes in red blood cell AChE in rats in the NIOSH (1974) study. Information is available regarding the effects of intermediate-duration exposure in humans in two studies that evaluated changes in blood cholinesterase levels in humans during controlled oral exposure to parathion (Edson 1964; Rider et al. 1969). Studies in animals provided information regarding death (Barnes and Denz 1951), systemic effects (hepatic, renal, and body weight) (Atkinson et al. 1994; Dikshith et al. 1978; NCI 1979; NIOSH 1974), neurological effects (Barnes and Denz 1951; Dikshith et al. 1978; Frawley and Fuyat 1957; Ivens et al. 1998; NCI 1979; NIOSH 1974; Reischchl et al. 1975), reproductive effects (Dikshith et al. 1978), and developmental effects (Deskin et al. 1979). The data from Rider et al. (1969) regarding changes in red blood cell AChE activity in volunteers exposed to parathion in a capsule for 30 days were used to derive an intermediate-duration oral MRL for parathion. It should be mentioned that in the

developmental study in rats by Deskin et al. (1979), the lowest dose tested, 0.01 mg parathion/kg/day, administered to pregnant rats during gestation and lactation induced alterations in EKGs in 25-day-old pups. Since this is not a developmental end point routinely tested in guideline developmental studies, it would be helpful to try to replicate these results. An acute-duration study also reported that parathion altered the microscopic appearance of the skin of guinea pigs when applied directly onto the skin for 15 days (Dikshith and Datta 1972). Additional intermediate-duration studies do not seem necessary at this time.

Chronic-Duration Exposure and Cancer. One study was located that provided information regarding changes in plasma cholinesterase and red blood cell AChE activity in workers at an industrial plant that manufactured the concentrated material and dusts containing various concentrations of parathion (Brown and Bush 1950). Uncertainties regarding exposure data and the extent of the changes in red blood cell AChE activity precluded the use of this study for derivation of a chronic-duration inhalation MRL for parathion. No chronic-duration inhalation studies in animals were found. Also, no chronic-duration oral data in humans were located. Two chronic-duration oral studies were located in the open literature (Barnes and Denz 1951; NCI 1979). Barnes and Denz (1951) reported that dietary exposure of rats to up to approximately 1.7 mg parathion/kg/day did not induce adverse clinical signs or gross or microscopic changes in organs or tissues. The NCI (1979) study reported that exposure of rats to up to 4.4 mg parathion/kg/day or mice to up to 27.6 mg parathion/kg/day did not cause gross or microscopic alterations in organs and tissues. However, the investigators noted that during the first half of the second year, clinical signs among dosed rats were noted at a low or moderate incidence, and during the second half of the year, they increased, but no quantitative data were presented. In addition, by week 60 of the study, all high-dose male mice were showing signs of hyperexcitability, but no data were provided. However, because these studies did not monitor red blood cell AChE activity and there is uncertainty in the NCI (1979) study regarding the incidence of clinical signs in rats and mice during the second year of exposure, they are inadequate for derivation of a chronic-duration oral MRL for parathion. A chronic-duration oral study with interim determinations of plasma, red blood cell, and brain cholinesterase activity would be valuable to confirm that enzyme activities reach a steady state and do not continue to decrease during long-term exposure to low-to-moderate doses of parathion. Data regarding chronic dermal exposure to parathion were provided in the AHS. The AHS provided information regarding respiratory effects (Hoppin et al. 2006, 2009), hearing loss (MacCrawford et al. 2008), behavioral function (Starks et al. 2010), and cancer (Dennis et al. 2010). A study of Chinese workers exposed to parathion provided information regarding reproductive effects (Padungtod et al. 2000).

Studies of the general population provided data regarding Parkinson's disease (Firestone et al. 2005; Manthripragada et al. 2010) and developmental effects (Eskenazi et al. 2004).

Very limited information is available regarding exposure to parathion and cancer. Dennis et al. (2010) examined the potential association between exposure to 50 agricultural pesticides, parathion among them, and the incidence of cutaneous melanoma in the AHS cohort of pesticide applicators along with ever-use of older pesticides that contain arsenic. The study found no association between melanoma incidence and organophosphate insecticides as a class. However, there was a significant association between melanoma and parathion (≥56 days of exposure; OR=2.4; 95% CI 1.3–4.4; p=0.003) based on 11 cases. The study also found a higher OR of 7.3 (95% CI 1.5–34.6) among those who had used arsenical pesticides. A limitation of the study was the small number of subjects who used parathion for at least 56 days and had melanoma (n=11). Since the AHS is a prospective study, continuous monitoring of the cohort will provide useful information. The carcinogenicity of parathion has been studied in a chronic oral bioassay using rats and mice (NCI 1979). That study concluded that parathion was carcinogenic to rats based on an increased incidence of combined adrenal cortical adenomas and carcinomas in males and females. Parathion was not carcinogenic in mice. No further information was located in the open literature. Additional studies do not seem necessary at this time.

Genotoxicity. No reliable data on humans exist to indicate whether parathion may act by a genotoxic mechanism. The results from available *in vivo* animal studies and *in vitro* studies showed that parathion is not a mutagenic or clastogenic agent (Degraeve and Moutschen 1984; EPA 1977; Fahrig 1974; Gilot-Delhalle et al. 1983; Kevekordes et al. 1996; Simmon et al. 1976). Additional studies do not seem necessary at this time.

Reproductive Toxicity. No studies were located regarding reproductive effects in humans after oral or inhalation exposure to parathion. A small study of Chinese workers exposed to parathion and methamidophos reported that the workers (n=20) had a modestly lower sperm count, lower sperm concentration, and lower percentage of motile sperm than an unexposed control group (n=23) (Padungtod et al. 2000). While the results were suggestive, the role of parathion, if any, remained unclear. Evaluation of participants in the AHS could provide valuable information regarding a possible association between exposure to parathion and reproductive effects. Since the AHS includes evaluation of pesticide applicators and their spouses, information could be collected regarding possible effects in males and females exposed to parathion in the past. No information was located regarding reproductive effects in animals following inhalation or dermal exposure to parathion and very limited data were available

regarding oral exposure. Parathion induced histological alterations in the testes of rats in an intermediate-duration oral study (Dikshith et al. 1978). However, chronic-duration oral studies in rats and mice did not find gross or microscopic alterations in the reproductive organs from male or female animals treated with higher doses of parathion than in the intermediate-duration study. It would be useful to try to replicate the findings of the intermediate-duration study of Dikshith et al. (1978). In addition, a 2-generation study in rats would provide valuable information.

Developmental Toxicity. No information was located regarding developmental effects in humans following inhalation or oral exposure to parathion. A study of Latina women living in an agricultural community in California did not find significant associations between several measures of *in utero* exposure to parathion and fetal growth (Eskenazi et al. 2004). However, as mentioned before, exposure to parathion was assessed by measuring urinary *p*-nitrophenol, which can also be produced as a result of exposure to substances other than parathion. It should be mentioned that studies of other pesticides with mechanisms of action similar to parathion (i.e., chlorpyrifos, diazinon) have reported neurodevelopmental alterations in children following maternal environmental exposure (for references, see Bouchard et al. 2011; Eskenazi et al. 2007; Rauh et al. 2011). Therefore evaluation of women participants in the AHS or other similarly exposed cohorts could provide important information regarding possible effects of exposure to parathion on various developmental end points. No studies were located regarding developmental effects in animal following inhalation or dermal exposure to parathion. In developmental studies in rats exposed orally to up to 1 mg parathion/kg on gestation days 6–5 and rabbits exposed to up to 0.3 mg parathion/kg on gestation days 6–18, there was no evidence of embryotoxicity or teratogenicity (Renhof 10984, 1985). In another study, 25-day-old pups from rats exposed to relatively low doses of 0.01 mg parathion/kg/day during gestation and lactation showed alterations in the EKGs (Deskin et al. 1979). Since this is not an end point routinely evaluated in standard developmental studies, it would be useful to conduct developmental studies to evaluate traditional end points in addition to examining the pups for possible cardiotoxicity.

Immunotoxicity. No information was located regarding immunological and lymphoreticular effects in humans following dermal exposure to parathion, except for the report of a significant association between exposure to parathion and allergic asthma in participants in the AHS (Hoppin et al. 2009). No information was located on immunotoxic effects in animals exposed to parathion by inhalation or dermally. Several studies in mice reported that acute oral exposure to parathion suppressed the antibody response to immunization with SRBC (Casale et al. 1984; Fukuyama et al. 2012; Kim et al. 2005; Wiltrout et al. 1978). The recent study by Fukuyama et al. (2012) reported that significant suppression

occurred with doses of 1.5 mg parathion/kg/day, but not 0.15 mg parathion/kg/day, for 5 days. In the Casale et al. (1984) study, significant suppression was observed with a single dose of 16 mg parathion/kg, but not 4 mg/kg, suggesting that repeated dosing may be necessary to induce immune suppression. Casale et al. (1984) also showed that cholinergic stimulation played a major role in the parathion-induced effect; further studies that examine the mechanism(s) involved would be valuable. It would also be useful to determine whether the parathion-induced immune suppression leads to increased susceptibility to infection by microorganisms. In addition, the possibility that immune suppression occurs also in longer-term studies may need to be examined.

Neurotoxicity. Information in both humans and animals indicates that the nervous system is the main target of parathion-induced toxicity following acute exposure by any route. This is particularly evident after exposure to high doses of parathion, as has occurred, for example, in cases of accidental or intentional ingestion of parathion formulations (Diggory et al. 1977; Eitzman and Wolfson 1967; Etzel et al. 1987; Eyer et al. 2003; He et al. 1998; Hoffman and Papendorf 2006; Tsachalinas et al. 1971; Wishahi et al. 1958) or in cases of high occupational exposure in workers involved in the manufacture or use of parathion (Diggory et al. 1977; Eitzman and Wolfson 1967; Etzel et al. 1987; Eyer et al. 2003; Grob et al. 1950; He et al. 1998; Hoffman and Papendorf 2006; Milby et al. 1964; Quinby and Lemmon 1958; Tsachalinas et al. 1971; Wishahi et al. 1958). As an organophosphate pesticide, parathion inhibits the activity of the enzyme, AChE, as well as that of plasma cholinesterase. The inhibition of AChE at various levels within the nervous system produces a characteristic set of signs and symptoms, including respiratory distress, bradycardia, increased bronchial secretions, excessive salivation, lacrimation, pupillary constriction, fasciculations, abdominal cramps, and diarrhea (Abou-Donia 1995; Ecobichon 1994). Most of these signs and symptoms have been observed in the cases listed above. A few cases of intermediate syndrome and induced delayed neuropathy have also been reported following exposure to parathion (Besser et al. 1993; De Bleecker et al. 1992; De Jager et al. 1981; He et al. 1998; Nisse et al. 1998). Information is also available regarding behavioral function in humans exposed to parathion. Starks et al. (2012) evaluated 701 male participants in the AHS with a series of neurobehavioral tests and found that parathion exposure was associated with better verbal learning and memory and better performance on a test of sustained attention. A possible explanation was that given the large number of statistical tests performed, the results may have been due to chance. Followup evaluations of this cohort may provide valuable information. A study of controlled administration of parathion in capsules to volunteers identified NOAEL and LOAEL values for inhibition of red blood cell AChE of 0.06 and 0.11 mg/kg/day, respectively (Rider et al. 1969). This study confirmed the findings of an earlier study in volunteers (Edson 1964) and was used to derive an intermediate-duration oral MRL for parathion.

Information is lacking on long-term effects of acute high exposure to parathion. This information can only be obtained from evaluation of cohorts exposed only to parathion, but data from subjects exposed to a few organophosphates would also be helpful.

Studies in animals support the findings in humans. In addition to measurements of cholinesterase activity and monitoring clinical signs, a few oral studies have examined the effects of parathion on neurobehavioral parameters and showed that effects occurred at dose levels that induced significant depression of blood cholinesterase activity and/or induced clinical signs (Moser 1995; Reiter et al. 1973, 1975). Should additional chronic studies be conducted, it would be valuable to monitor long-term changes in red blood cell and brain AChE activities. Also, a subgroup of animals could be tested for possible subtle neurobehavioral alterations of long-term, low-level exposure. Finally, pilot studies should be designed to evaluate possible neurodevelopmental effects of gestational and lactational exposure to parathion. It should be noted that studies have been conducted that examined neurodevelopmental endpoints in rats treated as neonates with parathion by subcutaneous injection, see Section 3.2.4, Other Routes of Exposure for a summary of the findings.

Epidemiological and Human Dosimetry Studies. Most of the literature reviewed concerning the health effects of parathion in humans described case reports of occupational exposure, accidental or intentional ingestion of parathion, or accidental dermal exposure to the pesticide (Diggory et al. 1977; Eitzman and Wolfson 1967; Etzel et al. 1987; Eyer et al. 2003; He et al. 1998; Hoffman and Papendorf 2006; Tsachalinas et al. 1971; Wishahi et al. 1958), studies of workers involved in the manufacture of parathion (Brown and Bush 1950; Grob et al. 1950; Padungtod et al. 2000), studies of agricultural workers (MacCrawford et al. 2008; Dennis et al. 2010; Hoppin et al. 2006, 2009; Milby et al. 1964; Quinby and Lemmon 1958; Starks et al. 2012), members of the general population (Eskenazi et al. 2004; Firestone et al. 2005; Manthripragada et al. 2010), and a few controlled exposure studies with volunteers (Edson 1964; Hartwell et al. 1964; Hayes et al. 1964; Morgan et al. 1977; Rider et al. 1969). The predominant route of exposure in the occupational studies is believed to be dermal exposure (workers involved in pesticide manufacture, formulation, and application). Some studies of agricultural workers examined possible associations between exposure to parathion (and additional pesticides) and health outcomes such as respiratory effects (Hoppin et al. 2006, 2009), hearing loss (MacCrawford et al. 2008), behavioral function (Starks et al. 2012), and cutaneous melanoma (Dennis et al. 2010). The information from occupational studies is limited because of the possibility of concurrent exposure to other pesticides or other toxic substances, and the duration and level of exposure to parathion generally were not quantified. Likewise, exposure levels in cases of acute intentional or accidental exposure to high amounts

of parathion were generally not available. Because all production and uses of parathion were cancelled in the United States (EPA 2000a, 2007), it is difficult to identify a subpopulation currently at risk of significant exposure to parathion.

Biomarkers of Exposure and Effect.

Exposure. Available data indicate that urinary levels of *p*-nitrophenol may serve as biomarkers of ongoing exposure to parathion. Further research on biomarkers of low-level exposure to parathion is needed. Noort et al. (2009) described a method for measuring organophosphorothioate pesticides bound to albumin, and proposed that this method might be suited to evaluation of chronic, low-level exposure; however, further testing and application of this method is needed to establish its suitability to this purpose.

Effect. There are no biomarkers of effect specific for parathion. As an organophosphate pesticide, parathion, in sufficient amounts, produces typical signs and symptoms of cholinergic stimulation. Plasma and red blood cell cholinesterase levels are widely used as biomarkers of exposure to organophosphates, but alone, their levels do not predict whether adverse health effects will occur, except in cases of significant inhibition (Maroni 2000). Because baseline data for plasma and red blood cell cholinesterase are not usually available for non-occupationally exposed individuals, additional studies of normal values by age and sex are needed for assessing potential adverse effects, if useful for other pesticides.

Absorption, Distribution, Metabolism, and Excretion. Little is known about absorption after oral or inhalation exposure and the distribution of parathion and its metabolites throughout the body; these areas represent significant data gaps in the toxicokinetics of parathion. Data on the dermal absorption, metabolism, and excretion of parathion are generally adequate to describe these elements of the toxicokinetics of this compound in humans. Additional information on the role of specific cytochromes on bioactivation and detoxification of parathion in *in vivo* systems exposed to a range of parathion doses and exposure routes would be useful to better predict the interindividual variability in parathion toxicity and/or identify new strategies for therapeutic intervention. Available studies focusing on the role of specific cytochrome isozymes have given varying results (e.g., Buratti et al. 2003; Foxenberg et al. 2007; Mutch and Williams 2006; Mutch et al. 2002, 2003).

Comparative Toxicokinetics. No studies were located that directly evaluated the comparative toxicokinetics of parathion in animals and humans. Because human blood lacks the carboxylesterase

enzyme found in rodent blood, and this enzyme is capable of detoxifying parathion, further information on the importance of this enzyme in predicting parathion toxicity would serve to inform the relevance of rodent models to human toxicokinetics.

Recent work suggests that the desulfuration of parathion to paraoxon in human liver is mediated by a large number of cytochromes (CYP1A2, CYP2B6, CYP2C19, CYP2C8, CYP2C9, CY 2D6, and CYP3A4/5), which show different affinities for the substrate (see Section 3.4.3). Significant variations in the activities of these cytochromes among humans and laboratory animal species would be expected to result in differences in parathion metabolism; additional information is needed to inform this question.

Methods for Reducing Toxic Effects. There is good information on the procedures used to limit absorption and to interfere with the mechanism of action of organophosphates, including parathion, after acute exposures (Aaron 2007; Clark 2002; Osmundsen 1998). Case reports of acute poisoning with parathion involved the use of various drugs in different combinations and sequences as the specific situations required. The effectiveness of these drugs varied from case to case and probably depended on the time elapsed between poisoning and initiation of treatment and on the amount of parathion taken. Publishing treatments that have proven to be effective in randomized controlled trials in medical journals could help decrease the number of fatalities resulting from parathion poisoning, particularly in countries where it is still widely used. Research leading to the development of more efficient oximes should be encouraged. Studies in animals showed that treatment with phosphotriesterase encapsulated in liposomes, a bacterial organophosphorus hydrolase, or plant-derived AChE-R reduced the acute toxicity of parathion or paraoxon (Bird et al. 2008; Evron et al. 2007; Petrikovics et al. 1999). Further research on these and similar strategies would provide valuable information. No information is available on dealing with long-term, low-level exposures to parathion. This may be due, in part, to the limited information on toxic effects associated with such exposures. If additional information becomes available indicating adverse health effects of long-term exposures, then studies examining methods for mitigating the effects of such exposures would become a data need.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

Information on the effects of parathion in children is derived mainly from case reports of accidental ingestion or dermal contact with commercial formulations (i.e., Diggory et al. 1977; Eitzman and

Wolfson 1967; Etzel et al. 1987; Wishahi et al. 1958). In all of these cases, exposure to parathion resulted in the characteristic signs and symptoms of organophosphate poisoning: increased salivation and lacrimation, miosis, nausea, vomiting, abdominal cramps and diarrhea, excessive bronchial secretions and dyspnea, bradycardia and low blood pressure, and muscle fasciculations. These case reports do not provide enough information to determine whether or not children are more susceptible to parathion exposure than adults. However, studies in animals have shown that young animals are more susceptible to the toxicity of high doses of parathion and that this is related to the metabolism and disposition of parathion and paraoxon rather than to differences in sensitivity to AChE inhibition (Atterberry et al. 1997; Benke and Murphy 1975; Gagné and Brodeur 1972; Harbison 1985; Karanth and Pope 2000; Nielsen et al. 1991).

Limited information is available regarding developmental effects of parathion in humans. A study of Latina women living in an agricultural community in California did not find an association between exposure to parathion and fetal growth and gestational length (Eskenazi et al. 2004). Evaluation of pregnancy outcomes in women participants in the AHS could provide valuable information regarding exposure to organophosphate pesticides and possible developmental effects. Conventional developmental studies in which female rats and rabbits were exposed during gestation showed that parathion was not embryotoxic or teratogenic (Renhof 1984, 1985). In a study in rats, 25-day-old pups from rats exposed to relatively low doses of 0.01 mg parathion/kg/day during gestation and lactation showed alterations in the EKGs (Deskin et al. 1979). Since this is not an end point routinely evaluated in standard developmental studies, it would be useful to try to replicate the results. Cross-foster studies in animals could provide information regarding differential transfer of parathion and/or metabolites through the placenta and the mother's milk.

There are no adequate data to evaluate whether pharmacokinetics of parathion in children are different from adults. However, to the extent that various cytochromes P450 that are involved in the metabolism of parathion in humans (Buratti et al. 2003; Foxenberg et al. 2007; Mutch and Williams 2006) are developmentally regulated (Tateishi et al. 1997), the metabolism of parathion in neonates and infants will likely differ from adults. Whether or not this would result in increased susceptibility of the young is not totally clear because cytochromes participate in both activation (desulfatation) and detoxification (dearylation) of parathion. No information was located regarding levels of parathion (or metabolites) in human milk. There is indirect evidence in animals that parathion (or its metabolites) can be transferred across the placenta and/or via breast milk to the offspring (Deskin et al. 1979; Villeneuve et al. 1972).

Further information on the dynamics of parathion and metabolites during pregnancy and lactation would be useful.

Biomarkers of exposure need to be further studied in order to better estimate human exposure at all age levels following acute or chronic exposure to parathion. There are no data on the interaction of parathion with other chemicals in children. Studies in animals have suggested that malnutrition, as may occur among some sectors of the general population, may exacerbate the toxicity of parathion (Boyd 1969; Bulusu and Chakravarty 1986, 1987; Casterline and Williams 1971; Villenueve et al. 1978). Further studies on children from malnourished populations should be conducted to explore this issue. The information available indicates that methods to reduce peak absorption of parathion and to interfere with the mechanism of action used for intoxication in adults are applicable to children.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

Information regarding ongoing research regarding parathion is presented in Table 3-13.

Table 3-13. Ongoing Studies on Parathion

Principal Investigator	Study topic	Institution	Sponsor
Baldwin, W	Role of CYP2B in the metabolism, fate, and toxicity of parathion using a CYP2B-knockdown mouse model; determine whether individuals with low CYP2B are sensitive to parathion	Clemson University, Clemson, South Carolina	National Institute of Environmental Health Sciences
Bird, SB	Determine the physiologic and electromyographic efficacy of the nicotinic receptor antagonist, rocurorium, in preserving function and architecture of the neuromuscular junction in parathion poisoning	University of Massachusetts, Worcester, Massachusetts	National Institute of Neurological Disorders and Stroke
Bird, SB	Test the safety and efficacy of a novel organophosphorus pesticide degrading enzyme, OpdA, in a nonhuman primate model	Harvard University, Boston, Massachusetts	National Center for Research Resources
Cerasoli, DM	Define, characterize, and develop a drug formulation that will afford post-exposure protection to victims of organophosphorus poisoning; the drug formulation will include a catalytic scavenger enzyme	U.S. Army Medical Research Institute for Chemical Defense, Aberdeen Proving Ground, Maryland	National Institute of Neurological Disorders and Stroke
Delorenzo, RJ	Develop a rat model to evaluate parathion toxicity and use this model to investigate mechanisms of toxicity that can be targeted to develop agent s to reverse these mechanisms and prevent morbidity and mortality	Virginia Commonwealth University, Richmond, Virginia	National Institute of Neurological Disorders and Stroke
Ford, BD	Evaluate the therapeutic benefit of the administration of neuroregulin-1, a neuroprotective anti-inflammatory compound alone or as a complement to standard therapy against parathion poisoning	Morehouse School of Medicine, Atlanta, Georgia	National Institute of Neurological Disorders and Stroke

Table 3-13. Ongoing Studies on Parathion

Principal Investigator	Study topic	Institution	Sponsor
Fryer, AD	Test the hypothesis that organophosphorus-induced airway hyper-reactivity in sensitized animals is mediated by the compounds affecting chemotactic factors and adhesion molecules that enhance eosinophil recruitment or nerves, and also compound-induced eosinophil activation	Oregon Health and Science University, Portland, Oregon	National Institute of Environmental Health Sciences
Garcia, GE	Evaluate novel sugar-linked reactivators of acetylcholinesterase with broader specificity, improved pharmacokinetics, and potential to cross the blood brain barrier; initial *in vitro* testing will be followed by testing in rodent species	U.S. Army Medical Research Institute for Chemical Defense, Aberdeen Proving Ground, Maryland	National Institute of Neurological Disorders and Stroke
Laskin, JD	Identify the precise site of action of a novel low-toxicity drug in the cytochrome P-450 system and test its efficacy in mitigating parathion toxicity in a rodent model	University of Medicine and Dentistry of New Jersey, Piscataway, New Jersey	National Institute of Neurological Disorders and Stroke
Lein, PJ	Elucidate the mechanism(s) by which organophosphorus compounds induce airway hyper-reactivity using physiological measurements *in vivo* and primary nerve cell cultures	University of California, Davis, California	National Institute of Environmental Health Sciences
Lein, PJ	Test the hypothesis that AMPA receptor antagonists and/or inhibitors of soluble epoxide hydrolases will significantly improve outcome following acute parathion poisoning	University of California, Davis, California	National Institute of Neurological Disorders and Stroke
Lein, PJ	Develop *in vivo* rodent models for assessing persistent neurological damage after seizures induced by parathion; identify effective therapeutic strategies for mitigating neurological damage	University of California, Davis, California	National Institute of Neurological Disorders and Stroke

Table 3-13. Ongoing Studies on Parathion

Principal Investigator	Study topic	Institution	Sponsor
Linney, EA	Examine how exposure of the developing nervous system to parathion can affect learning and/or behavior later in life; develop and use model vertebrate systems to examine possible mechanisms	Duke University, Durham, North Carolina	National Institute of Environmental Health Sciences
Pope, CH	Evaluate the role of endocannabinoid signaling in the expression of anticholinesterase toxicity of organophosphorus compounds and determine whether its differential modulation participates in selective toxicity	Oklahoma State University, Stillwater, Oklahoma	National Institute of Environmental Health Sciences
Wulff, H	Synthesize, characterize, test, and optimize the pharmacokinetic properties and central nervous system penetration of two distinct classes of therapeutic agents, sEH inhibitors and K-Ca channel activators	University of California, Davis, California	National Institute of Neurological Disorders and Stroke

Source: RePORTER 2013