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 diazinon. 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.

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

Systemic effects common to humans and laboratory animals exposed to diazinon by all natural exposure routes (inhalation, oral, dermal) are primarily attributable to the inhibition of acetylcholinesterase (AChE) by diazoxon, the active metabolite of diazinon. Inhibition of AChE at nerve terminals in central and peripheral nervous tissues triggers cholinergic signs and symptoms that are particularly apparent in respiratory, cardiovascular, and gastrointestinal systems. Although listed under specific systemic effects sections, many of the systemic effects listed are likely the direct result of AChE inhibition. Some cases of human exposure to diazinon may include mixed (inhalation, oral, and/or dermal) exposure routes; in such cases, a cumulative dose of diazinon would be expected to be the result of absorption by all relevant exposure routes.

The Toxicological Profile for Diazinon deals with diazinon-induced health effects in humans and animals. Most controlled animal studies were performed using technical-grade diazinon (purity ranging from 87 to essentially 100%). Other ingredients in technical-grade diazinon were not typically specified. Some animal studies employed particular diazinon pesticide formulations such as 60EC (a 60% emulsifiable concentration of diazinon) or 25WP (a 25% wettable powder). Summaries of animal data in the Toxicological Profile for Diazinon focus on health effects in animals exposed to technical-grade diazinon. MRLs were derived from results of studies that employed technical-grade diazinon. Available controlled human studies were performed using technical-grade diazinon of high purity. However, most of the available human data derive from case reports of intentional or accidental exposure to various diazinon formulations. Exposure of pesticide applicators often included exposure to other pesticides as well. Thus, some signs and symptoms resulting from a particular exposure scenario may be at least partly attributable to compounds other than diazinon. Furthermore, the extent and rate of absorption of diazinon may vary greatly depending on the source of diazinon exposure (i.e., technical grade or particular formulation of an end-use product).

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.

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.

The principal toxic effect of diazinon in humans and laboratory animals is inhibition of acetylcholinesterase (AChE), which results in the accumulation of acetylcholine at acetylcholine receptors leading to cholinergic responses in the peripheral (muscarinic and nicotinic) and central nervous system and neuromuscular junctions. In this Toxicological Profile for Diazinon, AChE inhibition of magnitude 20–59% is considered a less serious adverse effect in the absence of more serious signs of neurotoxicity. AChE inhibition ≥60% is considered a more serious effect independent of the presence or absence of other neurotoxicity indicators.

3.2.1 Inhalation Exposure

Diazinon has a low volatility; thus, inhalation exposure is likely to be to diazinon aerosols rather than vapor. In one of the studies described below, animals were exposed to diazinon in inhalation chambers (Holbert 1989). It is possible that some of the exposure under these conditions was by the dermal route and/or the oral route (grooming).

3.2.1.1 Death

There are no reports of deaths in humans or animals exposed by inhalation to diazinon alone. One case report described the death and autopsy results of a 51-year-old man who had been exposed to an insecticide mixture that contained diazinon and malathion, another anticholinesterase insecticide that is more acutely potent than diazinon (Wecker et al. 1985). The death was attributed to irreversible cardiac arrest, despite atropine therapy. Autopsy revealed mild pathologic changes in intercostal muscle tissue, including muscle fibers with subsarcolemmal grouped granular basophilic inclusions and scattered areas of necrosis. The victim's neuromuscular AChE activity was one-half that of muscle from unexposed persons.

No deaths were reported in Sprague-Dawley rats (5/sex) exposed to 2,330 mg/m^3 diazinon for 4 hours in inhalation chambers and observed for a further 14 days (Holbert 1989), or in hybrid rats (10/sex/group) exposed to air concentrations of 0.05, 0.46, 1.57, or 11.6 mg/m^3 diazinon (nose-only) for 6 hours/day, 5 days/week for 3 weeks (Hartman 1990).

3.2.1.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, endocrine, dermal, ocular, or body weight effects in humans after inhalation exposure to diazinon. A single study described mild degenerative changes in the muscles in a human acute-duration exposure to a mixture of diazinon and malathion (Wecker et al. 1985). No studies were located regarding gastrointestinal, musculoskeletal, or dermal effects in animals after inhalation exposure to diazinon. The systemic effects observed in humans and animals after inhalation exposure to diazinon are discussed below. The highest NOAEL and all LOAEL values from each reliable study for systemic end points in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. Nasal discharge was observed in Sprague-Dawley rats exposed to 2,330 mg/m^3 diazinon for 4 hours in an inhalation chamber (Holbert 1989). A statistically significant increase in lung-to-body weight ratio was observed in hybrid female rats exposed to 0.46 and 1.57 mg/m^3 diazinon (nose-only) for 6 hours/day, 5 days/week for 3 weeks (Hartman 1990). This effect was not seen in male rats or in female rats exposed at 11.6 mg/m^3, so its toxicological significance is unclear. No gross or histological evidence of treatment-related damage to nasal tissues or the lungs was observed at the termination of this study.

Cardiovascular Effects. No gross or histological evidence of treatment-related damage to the heart was observed in hybrid rats (10/sex) exposed to 11.6 mg/m^3 diazinon (nose-only) 6 hours/day, 5 days/week for 3 weeks (Hartman 1990).

Hematological Effects. No statistically significant effects on hematological parameters (erythrocyte count, hemoglobin, packed red cell volume) were seen in hybrid rats (10/sex/group) exposed to up to 11.6 mg/m^3 diazinon (nose-only) for 6 hours/day, 5 days/week for 3 weeks (Hartman 1990).

Musculoskeletal Effects. Mild pathologic changes in the intercostal muscle tissue, including muscle fibers with subsarcolemmal grouped granular basophilic inclusions and scattered areas of necrosis were reported in the autopsy of a 51-year-old man who died from high acute-duration exposure, via inhalation, to a commercial insecticide spray containing diazinon and malathion. Neuromuscular AChE activity was one-half that of muscle from unexposed persons (Wecker et al. 1985).

Table 3-1 Levels of Significant Exposure to Diazinon - 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								
Systemic								
1	Rat (Sprague-Dawley)	4 hr	Resp		2330 M (nasal discharge; 3/5)		Holbert 1989	
			Renal		2330 F (polyuria; 3/5)			
			Bd Wt	2330				
Neurological								
2	Rat (Sprague-Dawley)	4 hr			2330 (decreased activity, 2/5; salivation, 2/5)		Holbert 1989	
INTERMEDIATE EXPOSURE								
Systemic								
3	Rat (Hybrid)	3 wk 5 d/wk 6 hr/d	Resp	11.6			Hartman 1990	
			Cardio	11.6				
			Hemato	11.6				
			Hepatic	11.6				
			Renal	11.6				
			Endocr	11.6				
			Ocular	11.6				
			Bd Wt	11.6				
Immuno/ Lymphoret								
4	Rat (Hybrid)	3 wk 5 d/wk 6 hr/d		11.6			Hartman 1990	

Table 3-1 Levels of Significant Exposure to Diazinon - 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
Neurological								
5	Rat (Hybrid)			1.57[b]	11.6 (36-39% RBC AChE inhibition)		Hartman 1990	

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

b Used to derive an intermediate-duration inhalation minimal risk level (MRL) of 0.01 mg/m3 for diazinon. The concentration (1.57 mg/m3) was adjusted for intermittent exposure and converted to a human equivalent concentration as described in detail in Appendix A. The resulting duration-adjusted human equivalent concentration was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans using dosimetric adjustment and 10 for human variability).

AChE = acetylcholinesterase; Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = Female; Gastro = gastrointestinal; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; RBC = red blood cell; Resp = respiratory; wk = week(s)

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

Acute (≤14 days)

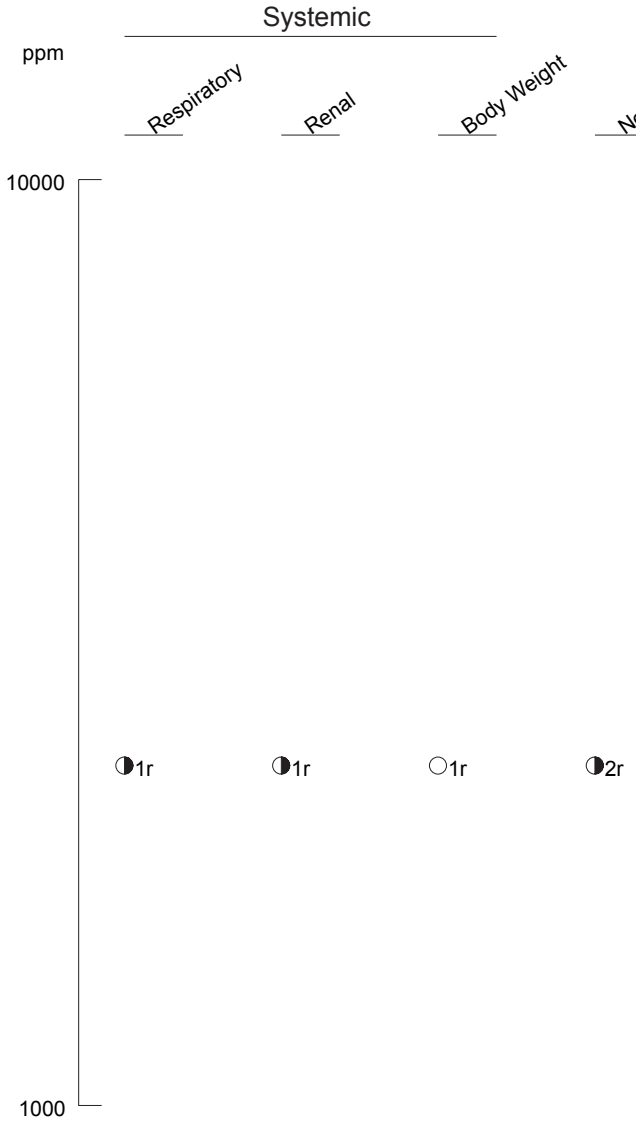
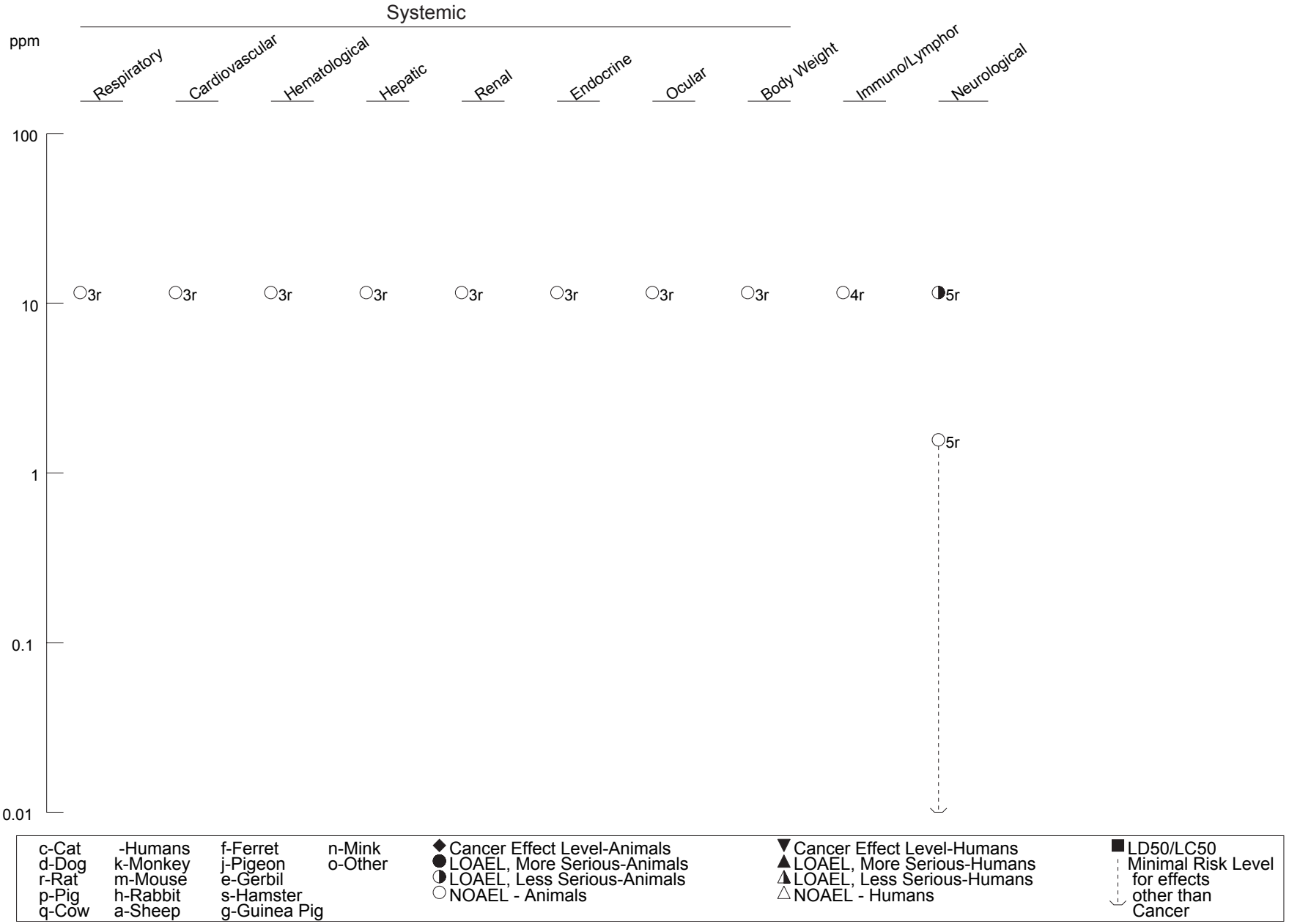


Figure 3-1 Levels of Significant Exposure to Diazinon - Inhalation *(Continued)*

Intermediate (15-364 days)



Hepatic Effects. No gross or histological evidence of treatment-related damage to the liver was observed in hybrid rats (10/sex) exposed to 11.6 mg/m^3 diazinon (nose-only) for 6 hours/day, 5 days/week for 3 weeks (Hartman 1990).

Renal Effects. Polyuria was observed in Sprague-Dawley rats exposed to 2,330 mg/m^3 diazinon for 4 hours (Holbert 1989). No gross or histological evidence of treatment-related damage to the kidney was observed in hybrid rats (10/sex) exposed to 11.6 mg/m^3 diazinon (nose-only) for 6 hours/day, 5 days/week for 3 weeks (Hartman 1990).

Endocrine Effects. No gross or histological evidence of treatment-related damage to the adrenal gland was observed in hybrid rats (10/sex) exposed to 11.6 mg/m^3 diazinon (nose-only) for 6 hours/day, 5 days/week for 3 weeks (Hartman 1990).

Ocular Effects. Ptosis was observed in Sprague-Dawley rats exposed to 2,330 mg/m^3 diazinon for 4 hours in an inhalation chamber (Holbert 1989). No evidence of treatment-related ophthalmoscopic lesions was observed in hybrid rats (10/sex/group) exposed to up to 11.6 mg/m^3 diazinon (nose-only) for 6 hours/day, 5 days/week for 3 weeks (Hartman 1990).

Body Weight Effects. No effect on body weight was observed in Sprague-Dawley rats (5/sex) exposed to 2,330 mg/m^3 diazinon for 4 hours and observed for 14 days (Holbert 1989) or in hybrid rats (10/sex/group) exposed to up to 11.6 mg/m^3 diazinon (nose-only) for 6 hours/day, 5 days/week for 3 weeks (Hartman 1990).

3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological or lymphoreticular effects in humans after inhalation exposure to diazinon.

No gross or histological evidence of treatment-related damage to the spleen was observed in hybrid rats (10/sex) exposed to 11.6 mg/m^3 diazinon (nose-only) for 6 hours/day, 5 days/week for 3 weeks (Hartman 1990).

The NOAEL for immunological and/or lymphoreticular end points in hybrid rats for intermediate-duration exposure is recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.4 Neurological Effects

Diazinon, an anticholinesterase organophosphate, inhibits AChE in the central and peripheral nervous system. Inhibition of AChE results in accumulation of acetylcholine at muscarinic and nicotinic receptors leading to peripheral and central nervous system effects. These effects usually appear within a few minutes to 24 hours after exposure, depending on the extent of exposure. Most of the located reports of incidents of human exposure to diazinon involved occupational exposure via the inhalation route, although it is possible that significant exposure also took place via the dermal route.

Cholinergic symptoms began within 15 minutes in 17 of 18 mushroom workers exposed to diazinon sprayed around the only entrance to a room in which they were working. The workers exhibited reduced plasma cholinesterase (ChE) and erythrocyte acetylcholinesterase (RBC AChE) levels (markers for diazinon exposure) within 48 hours; plasma ChE levels were inhibited 27–29% by diazinon exposure during 15 days postexposure (Coye et al. 1987). In another report, members of a family complained of signs and symptoms of insecticide poisoning (headache, vomiting, fatigue, chest heaviness) after moving into a house that had been treated with diazinon. Five months after the diazinon treatment, analysis of the family members' urine samples showed "very high urinary levels" (0.5–1.5 mg/L) of a diazinon metabolite, diethylphosphate (DEP), while plasma ChE levels were slightly depressed (79–94% of normal levels). Surface concentrations of diazinon in the home ranged from 126 to 1,051 $\mu g/m^2$, air concentrations were between 5 and 27 $\mu g/m^3$, and some clothing showed contamination (0.5–0.7 μg/g). After cleanup of the house, the signs and symptoms reported by family members promptly ceased, and the urinary excretion of DEP dropped to background levels (Richter et al. 1992). Another case study of 99 individuals who were occupationally exposed to diazinon granules 8 hours/day for 39 days during an insecticide application program reported only slight neurological functional deficits (postshift symbol-digit speed and pattern memory accuracy) as a result of the exposure. A dose of 0.02 mg/kg/day, considered a NOAEL, was estimated for the workers on the basis of measured diazinon concentration in passive dermal badges, hand rinses, and full-shift breathing-zone air samples. Thus, multiple exposure routes were implied, making it difficult to verify the dose calculated by the authors of the study. Adequate information regarding exposure time to onset and recovery (if any) from the slight neurological functional deficits described was not provided in the report (Maizlish et al. 1987). Other persons occupationally exposed to organophosphorus insecticides, including diazinon, showed no significant change in neurological function, although there was a reduction in plasma ChE levels indicating exposure (Stalberg et al. 1978). In contrast, organophosphate-induced increases in hyperreflexia were reported in

workers occupationally exposed to many insecticides, including diazinon. These workers, however, showed no overt signs of poisoning or of cholinergic signs and symptoms after spraying diazinon (Rayner et al. 1972). Two other insecticide sprayers developed cholinergic symptoms after spraying diazinon. Symptoms included nausea, vomiting, muscle twitching, difficulty breathing, and blurred vision. Plasma ChE and RBC AChE activities remained depressed for at least 18 days after exposure (Soliman et al. 1982). In all of these cases of occupational exposure (Rayner et al. 1972; Soliman et al. 1982; Stalberg et al. 1978), no estimate of the exposure level to diazinon was made.

A 42-year-old woman (26 weeks pregnant) in the country of Qatar was exposed when she used undiluted diazinon liquid insecticide (60EC) to clean a nonventilated bathroom (Kamha et al. 2005). Her symptoms included dizziness, vomiting, blurred vision, and increased salivation. Laboratory tests revealed plasma ChE activity of 161 U/L (normal range 5,400–13,200 U/L), which confirmed a clinical diagnosis of organophosphate poisoning. The patient was treated with the cholineaterase reactivators atropine and 2-PAM (pralidoxime) and the symptoms of diazinon poisoning subsided.

Decreased activity and salivation were noted in Sprague-Dawley rats exposed to 2,330 mg/m^3 diazinon for 4 hours in an inhalation chamber (Holbert 1989). No clinical signs of neurological effects except piloerection were observed in hybrid rats exposed to 0.05, 0.46, 1.57, or 11.6 mg/m^3 diazinon for 6 hours/day, 5 days/week for 3 weeks (Hartman 1990). At study termination, plasma ChE activity (a marker for diazinon exposure) was significantly decreased in a dose-related manner in females. Decreases of 20, 27, and 43% were seen at airborne diazinon levels of 0.46, 1.57, and 11.6 mg/m^3, respectively. No change was seen at 0.05 mg/m^3. In males, no change was seen at 0.05 or 0.46 mg/m^3, but decreases of 14 and 19% were seen at 1.57 and 11.6 mg/m^3, respectively. RBC AChE activity (a surrogate marker for neural AChE) was unaffected in females at 0.05 and 0.46 mg/m^3, but was decreased by 10 and 39% at 1.57 and 11.6 mg/m^3, respectively. In males, no change was seen at 0.05, 0.46, or 1.57 mg/m^3, while a decrease of 36% was observed at 11.6 mg/m^3. Brain AChE activity was unchanged in males at all exposure levels, but was decreased in females at 0.05 mg/m^3 (24%), 0.46 mg/m^3 (17%), 1.57 mg/m^3 (20%), and 11.6 mg/m^3 (37%). The decreases in the females at the two lowest exposures are unusual in that no accompanying decrease in RBC AChE activity was observed. Diazinon exposure had a consistently greater effect on cholinesterase activities in females than in males in this study, although clinical signs of neurological effects (other than piloerection) were not observed in either sex.

No studies were located regarding organophosphate-induced delayed neurotoxicity (OPIDN) in humans or in animals after inhalation exposure to diazinon.

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

3.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals after inhalation exposure to diazinon.

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after inhalation exposure to diazinon.

3.2.1.7 Cancer

Several epidemiological studies have reported increased incidence of cancers in humans who were concurrently or sequentially exposed to a number of insecticides, including diazinon. Some of the exposure is presumed to have occurred by the inhalation route. Because epidemiological studies typically involve exposure to multiple pesticides, the carcinogenicity of diazinon itself has not been determined.

A case-control study suggested a possible link between family gardening use of diazinon (and other insecticides) and increased incidence of childhood brain cancer (type unspecified). However, this report gave no indication of level, duration, or frequency of exposure to diazinon (or to other insecticides) (Davis et al. 1993). Another case-control study suggested a positive association between an increased incidence of non-Hodgkin's lymphoma in farmers as compared to nonfarmers. The report attributed the increased incidence of lymphomas to handling of organophosphorus insecticides, including diazinon (Cantor et al. 1992). A third case-control study suggested an association between an increased incidence of multiple myeloma and exposure to high concentrations of insecticides, including diazinon. Actual exposure to diazinon was reported in 2 (0.3%) of the cases and 5 (0.3%) of the controls (Morris et al. 1986).

No studies were located regarding cancer effects in animals after inhalation exposure to diazinon.

3.2.2 Oral Exposure

3.2.2.1 Death

In humans and animals, acute-duration oral exposure to high doses of diazinon induces cholinergic signs and symptoms. With sufficiently high doses of diazinon, extensive edema and hemorrhage in tissues and organs, as well as severe respiratory distress in the victims, have been reported. On some occasions, the respiratory effects progressed to respiratory failure and death preceded by coma. Treatment of test animals with anticholinesterase antagonists such as atropine and pralidoxime (2-PAM) significantly reduced the acute lethality of diazinon in rats, indicating that acute diazinon lethality is primarily attributable to AChE inhibition (Harris et al. 1969).

A summary of autopsy findings of 76 cases of acute diazinon poisoning described cholinergic signs that included: congested, swollen, edematous brain with prominent dural and surface vasculature; livid, congested face; cyanosis; soft flabby heart with conspicuous vasculature on the pericardium and epicardium; cloudy swelling and hyperemia (upon histopathological examination); occasional and scattered petechial and ecchymotic hemorrhage; and occasional brain or spinal hemorrhage. In addition, the victims died with congested respiratory tract, sweating and frothing at the mouth, pulmonary edema and hyperemia, hypostatic congestion, and pneumonia. Generally, the cause of death was respiratory failure and, occasionally, cardiac arrest (Limaye 1966). Other reports of human deaths from diazinon exposure include descriptions of petechial hemorrhages throughout the stomach and gastric mucosa in a diazinon-poisoned 54-year-old female suicide victim who had ingested an estimated 293 mg/kg diazinon (Poklis et al. 1980). Accidental ingestion of an insecticide mixture containing diazinon, parathion, and chlordane resulted in the death of an 8-year-old girl from cardiac and respiratory arrest (DePalma et al. 1970). The estimated dose of diazinon in this case was 20 mg/kg. The toxicity in this case may have been related to the additive effects of diazinon and parathion and/or a possible interaction with chlordane.

The diazinon dose that causes death of experimental animals depends on the form of the test compound (pure, technical, or formulated preparations) as well as on the animal species, sex, age, and other modifying factors such as diet. It is likely that earlier formulations were more toxic to experimental animals than current ones due to the formation of toxic breakdown products (e.g., sulfotepp) in unstabilized diazinon (Hayes 1982). This section summarizes lethality in animals exposed to technical-grade diazinon.

Single-dose oral (gavage) studies in rats identify lethality at dose levels ranging from approximately 75 to 600 mg (Boyd and Carsky 1969; Boyd et al. 1969; Chow and Richter 1994; Enan et al. 1982; Gaines 1960, 1969; Harris et al. 1969). Strain-specific differences in sensitivity to the lethal effects of diazinon are apparent. For example, acute oral LD_{50} values of 108 and 76 mg/kg were reported for male and female Sherman rats, respectively, whereas LD_{50} values of 415 and 466 mg/kg were noted in separate studies of male Wistar rats (Boyd and Carsky 1969; Boyd et al. 1969). A single 600 mg/kg oral dose of diazinon MG87% (88% purity; 528 mg diazinon/kg) to Sprague-Dawley rats resulted in 2/15 and 1/15 deaths in males and females, respectively (Chow and Richter 1994).

Death was noted in 6 of 8 pregnant New Zealand rabbits administered diazinon orally at a dose level of 30 mg/kg/day on gestation days 5–15 (Robens 1969). In a similar rabbit study, a dose level of 100 mg/kg/day on gestation days 6–16 resulted in 9/22 deaths (Harris and Holson 1981). No deaths were reported in pregnant CD-1 rats receiving 10, 20, or 100 mg/kg/day diazinon during gestation days 6–15 (Infurna and Arthur 1985).

Intermediate-duration oral administration of 10 or 20 mg/kg/day diazinon dissolved in corn oil in gelatin capsules for 8 months to Beagle dogs (3/sex/group) resulted in mortality (1/3 of each sex at the 20 mg/kg dose level). Toxic signs, which were not consistent in all the dogs at a given dose, did not show a dose-response relationship. Generally, female dogs were less sensitive to diazinon toxicity than male dogs (Earl et al. 1971). Daily oral administration of diazinon capsules to Hormel-Hanford miniature swine (3/sex) at a dose of 10 mg/kg/day resulted in the deaths of 3/3 males and 2/3 females between treatment days 13 and 38 of a scheduled 8-month treatment period; no deaths were observed at dose levels of 1.25, 2.5, or 5.0 mg/kg/day (Earl et al. 1971).

No deaths were reported in male or female Sprague-Dawley rats receiving up to 183.2 mg/kg/day diazinon in feed for 6 weeks or up to 212 mg/kg/day for 13 weeks (Singh 1988) or in Beagle dogs (4/sex/group) receiving up to 15.99 mg/kg/day diazinon from feed for 4 weeks or up to 11.6 mg/kg/day diazinon for 13 weeks (Barnes 1988; EPA 2000a). Survival rates were similar to controls in Sprague-Dawley rats receiving up to 12 mg/kg/day diazinon from feed for 98 weeks (Kirchner et al. 1991). Daily doses as high as 8–9 mg/kg were not lethal to male and female Beagle dogs receiving diazinon in the diet for 52 weeks (Rudzki et al. 1991).

The LD_{50} values and doses associated with death in each species and duration category are shown in Table 3-2 and plotted in Figure 3-2.

Table 3-2 Levels of Significant Exposure to Diazinon - 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	Human	once (IN)				293 F (death)	Poklis et al. 1980	
2	Rat (Wistar)	once (GO)				466 M (LD50)	Boyd and Carsky 1969	Diazinon (91.4% purity); dose adjustment for purity uncertain.
3	Rat (Wistar albino)	once (GO)				415 M (LD50)	Boyd et al. 1969	
4	Rat (Sprague-Dawley)	once (GO)				600 (2/15 males and 1/15 females died)	Chow and Richter 1994	Diazinon MG87% (D*Z*N, 88% purity); doses not adjusted for purity.
5	Rat (white)	once (GO)				300 M (LD50)	Enan et al. 1982	Diazinon (97.1% purity).
6	Rat (Sherman)	once (GO)				108 M (LD50) 76[b] F (LD50)	Gaines 1960	Technical grade diazinon (purity not specified).
7	Rat (Sherman)	once (GO)				250[b] M (LD50) 285 F (LD50)	Gaines 1969	Technical grade diazinon (purity not specified).

Table 3-2 Levels of Significant Exposure to Diazinon - 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
8	Rat (albino)	once (GO)				294 F (LD50)	Harris et al. 1969	Diazinon (91.9% purity).
9	Rabbit (New Zealand)	Gd 6-18 1 x/d (G)				100 F (9/22 died)	Harris and Holson 1981	Diazinon (89.2% purity) in epoxidized soybean oil; doses apparently not adjusted for purity.
10	Rabbit (New Zealand)	Gd 5-15 1 x/d (C)				30 F (6/8 died)	Robens 1969	Diazinon (technical grade, purity unspecified).
Systemic								
11	Human	once (IN)	Resp			240[b] M (tachypnea, cyanosis)	Klemmer et al. 1978	
						509 F (tachypnea, cyanosis)		
			Cardio			240[b] M (bradycardia, tachycardia)		
						509 F (bradycardia, tachycardia)		
			Hemato	240[b] M				
				509 F				
			Metab			240[b] M (metabolic acidosis)		
						509 F (metabolic acidosis)		

Table 3-2 Levels of Significant Exposure to Diazinon - 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
12	Human	once (IN)	Resp			293 F (heavily congested lungs)	Poklis et al. 1980	
			Gastro			293 F (petechial hemorrhages throughout the stomach and gastric mucosa)		
13	Rat (Sprague-Dawley)	once (GO)	Hemato	600			Chow and Richter 1994	Diazinon MG87% (D*Z*N, 88% purity); doses not adjusted for purity.
			Ocular		600 (chromodacryorrhea)			
			Bd Wt	150[b] M 600 F		300 M (25% decrease in weight gain)		
14	Rat (Wistar)	7 d ad lib (F)	Bd Wt	0.21			Davies and Holub 1980b	Diazinon (99.2% purity).
15	Rat (CD-1)	Gd 6-15 1 x/d (G)	Bd Wt	20 F	100 F (5.5-9.6% decrease in maternal weight, 26-30% decrease in feed consumption)		Infurna and Arthur 1985	Diazinon technical (purity unspecified).
16	Rat (Sprague-Dawley)	once (GW)	Hemato		4.4 M (reduced platelet count, altered coagulation factor activities)		Lox 1983	Diazinon (87.6% purity) dose apparently not adjusted for purity.

Table 3-2 Levels of Significant Exposure to Diazinon - 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
17	Rat (Sprague-Dawley)	14 d ad lib (W)	Hemato		52 F (reduced hematocrit, altered clotting factor activities)		Lox 1987	Diazinon (purity not specified).
			Bd Wt	52 F				
18	Rat (Sprague-Dawley)	once (G)	Hepatic		300 (reduced hepatic cytochrome P-450, aniline hydroxylase, aminopyrine N-demethylase)		Mihara et al. 1981	
19	Rabbit (New Zealand)	Gd 6-18 1 x/d (G)	Resp	100 F			Harris and Holson 1981	Diazinon (89.2% purity) in epoxidized soybean oil; doses apparently not adjusted for purity.
			Cardio	100 F				
			Gastro	25 F		100 F (7/9 stomach mucosal hemorrhage, congestion and erosion)		
			Hepatic	100 F				
			Renal	100 F				
			Bd Wt	100 F				

Table 3-2 Levels of Significant Exposure to Diazinon - 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								
20	Human	once (IN)				240[b] M (stupor, profuse diaphoresis, coma)	Klemmer et al. 1978	
						509 F (stupor, profuse diaphoresis, coma)		
21	Human	once (IN)				293 F (petechial hemorrhages throughout the brain)	Poklis et al. 1980	
22	Rat (Sprague-Dawley)	once (GO)		2.5		150 (82% decrease in erythrocyte AChE, ataxia, alterations in functional observation battery tests 9-11 hrs post-dosing)	Chow and Richter 1994	Diazinon MG87% (D*Z*N, 88% purity); doses not adjusted for purity.
23	Rat (Wistar)	12 d ad lib (F)		0.6[c] F	1 F (22% RBC AChE inhibition)		Davies and Holub 1980a	Diazinon (99.2% purity); effects were noted at treatment day 12 of a 92-day oral study.
24	Rat (Wistar)	7 d ad lib (F)		0.21			Davies and Holub 1980b	Diazinon (99.2% purity).

Table 3-2 Levels of Significant Exposure to Diazinon - 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
25	Rat (Sprague-Dawley)	once (GO)			2.5 F (40% RBC AChE inhibition)	300 M (clinical signs of neurotoxicity)	EPA 2000a	Diazinon MG87% (D*Z*N, 88% purity); adjustment for purity uncertain.
26	Rat (Sprague-Dawley)	once (GO)		2.5 F	25 F (35% RBC and brain AChE inhibition)		EPA 2000a	Diazinon MG87%; adjustment for purity uncertain.
27	Rat (albino)	once (GO)				235 F (78% brain AChE inhibition)	Harris et al. 1969	Diazinon (91.9% purity).
28	Rat (Long- Evans)	once (GO)		50 M	75 M (35% brain AChE inhibition)		Moser et al. 2005	Diazinon (99.3% purity).
29	Rat (Sprague-Dawley)	once (GO)			15 M (30% RBC AChE inhibition)	60 M (>60% RBC AChE inhibition)	Timchalk et al. 2005	Diazinon (98.5% purity).
30	Hamster (Golden Syrian)	Gd 6, 7 and/or 8 1 x/d (GO)			0.125 F (diarrhea, salivation, incoordination)		Robens 1969	Diazinon (technical grade, purity unspecified).
31	Rabbit (New Zealand)	Gd 6-18 1 x/d (G)		25 F		100 F (tremors, convulsion)	Harris and Holson 1981	Diazinon (89.2% purity) in epoxidized soybean oil; doses apparently not adjusted for purity.

Table 3-2 Levels of Significant Exposure to Diazinon - 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
32	Rabbit (New Zealand)	Gd 5-15 1 x/d (C)		7 F		30 F (ataxia)	Robens 1969	Diazinon (technical grade, purity unspecified).
Reproductive								
33	Rat (CD-1)	Gd 6-15 1 x/d (G)		100 F			Infurna and Arthur 1985	Diazinon technical (purity unspecified).
34	Rabbit (New Zealand)	Gd 6-18 1 x/d (G)		100 F			Harris and Holson 1981	Diazinon (89.2% purity) in epoxidized soybean oil; doses apparently not adjusted for purity.
Developmental								
35	Rat (CD-1)	Gd 6-15 1 x/d (G)		20 F		100 F (increased incidence of rudimentary ribs at T-14 in fetuses)	Infurna and Arthur 1985	Diazinon technical (purity unspecified).
36	Hamster (Golden Syrian)	Gd 6, 7 and/or 8 1 x/d (GO)		0.25 F			Robens 1969	Diazinon (technical grade, purity unspecified).
37	Rabbit (New Zealand)	Gd 6-18 1 x/d (G)		100 F			Harris and Holson 1981	Diazinon (89.2% purity) in epoxidized soybean oil; doses apparently not adjusted for purity.
38	Rabbit (New Zealand)	Gd 5-15 1 x/d (C)		30 F			Robens 1969	Diazinon (technical grade, purity unspecified).

Table 3-2 Levels of Significant Exposure to Diazinon - 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
INTERMEDIATE EXPOSURE								
Death								
39	Dog (Beagle)	8 mo 1 x/d (C)				10 (3/3 males and 2/3 females died)	Earl et al. 1971	Technical grade diazinon (purity not specified).
40	Pig (Hormel-Hanford)	8 mo 1 x/d (C)				10 (3/3 males and 2/3 females died)	Earl et al. 1971	Technical grade diazinon (purity not specified).
Systemic								
41	Human	28-31 d (C)	Hemato	0.03 M			EPA 2001	Diazinon (99.5% purity).
42	Rat (Wistar)	7-28 wk 2 x/wk (G)	Hepatic		0.5 M (lipid vacuolation)		Anthony et al. 1986	Diazinon (87% purity); dose apparently not adjusted for purity.
			Bd Wt		0.5 M (10% reduction in body weight gain)			
43	Rat (Wistar)	92 d ad lib (F)	Bd Wt	1.2 F			Davies and Holub 1980a	Diazinon (99.2% purity).
44	Rat (Wistar)	42 d ad lib (F)	Bd Wt	0.4 F			Davies and Holub 1980a	Diazinon (99.2% purity).
45	Rat (Wistar)	35 d ad lib (F)	Bd Wt	0.2 F			Davies and Holub 1980a	Diazinon (99.2% purity).

Table 3-2 Levels of Significant Exposure to Diazinon - 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
46	Rat (Wistar)	30 d ad lib (F)	Bd Wt	2.86			Davies and Holub 1980b	Diazinon (99.2% purity).
47	Rat (white)	4 wk ad lib (F)	Hepatic		30 M (reduced serum beta-lipoproteins, increased ALT, AST, GGT, LDH)		Enan et al. 1982	Diazinon (97.1% purity).
48	Rat (Sprague-Dawley)	90 d (F)	Bd Wt	18 M	180 M (20% reduced body weight gain)		EPA 1996	Diazinon (D*Z*N* MG87%, purity 88%); apparently not adjusted for purity.
49	Rat (Sprague-Dawley)	28 d (F)	Bd Wt	23		213 (muscle fasciculations in forefoot; 26 and 39% decreased body weight gain in males and females, respectively)	EPA 1996	Diazinon (D*Z*N* MG87%; purity 88%); dose adjustment for purity uncertain.
50	Rat (Wistar)	7 wk 1x/d (GO)	Hepatic		10 M (40% increased serum liver enzymes, hepatocellular mitochondrial swelling and breaking up of cristae)		Kalender et al. 2005	Diazinon (99% purity)

Table 3-2 Levels of Significant Exposure to Diazinon - 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
51	Rat (Wistar)	7 wk 1x/d (GO)	Hemato			10 M (significantly altered hemoglobin; hematocrit; RBC, WBC, and thrombocyte counts, mean corpuscular volume)	Kalender et al. 2006	Diazinon (99% purity)
52	Rat (Sprague-Dawley)	6 mo ad lib (W)	Hemato	0.18 F			Lox and Davis 1983	Diazinon (92.4% purity) dose apparently not adjusted for purity.
			Hepatic	0.18 F				
			Bd Wt	0.18 F				
53	Rat (Wistar)	7 d 1x/d (GO)	Bd Wt			10 M (22% lower mean body weight)	Ogutcu et al. 2006	Diazinon (99% purity)

Table 3-2 Levels of Significant Exposure to Diazinon - 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
54	Rat (Sprague-Dawley)	13 wk 7 d/wk ad lib (F)	Resp	168[b] M 212 F			Singh 1988	Diazinon MG-8 (purity 87.7%); concentrations in food adjusted for purity.
			Cardio	168[b] M 212 F				
			Gastro	19 M 15[b] F	168[b] M (soft stools) 212 F (soft stools)			
			Hemato	168 M 19[b] F		212 F (decreased hemoglobin and hematocrit; increase in reticulocytes)		
			Hepatic	168 M 19[b] F	212 F (increase in relative and absolute liver weight, minimal centrolobular hepatocellular hypertrophy)			
			Renal	168[b] M 212 F				
			Endocr	168[b] M 212 F				
			Ocular	168[b] M 212 F				
			Bd Wt	168[b] M 212 F				

Table 3-2 Levels of Significant Exposure to Diazinon - 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
55	Rat (Sprague-Dawley)	6 wk 7 d/wk ad lib (F)	Gastro	0.2[b] M 9.4 F	8.4[b] M (soft stools) 182.9 F (soft stools)		Singh 1988	Diazinon MG-8 (purity 87.7%); concentrations in food adjusted for purity.
			Bd Wt	8.4	150.8 M (15% decrease in body weight)			
56	Dog (Beagle)	13 wk 7 d/wk (F)	Resp	11.6			Barnes 1988	Diazinon MG-8 (purity 87.7%); concentrations in food adjusted for purity.
			Cardio	11.6				
			Gastro	11.6				
			Hemato	11.6				
			Hepatic	11.6				
			Renal	11.6				
			Endocr	5.6[b] M 11.6 F		10.9 M (atrophy of pancreatic acini)		
			Ocular	11.6				
			Bd Wt	5.9 M 0.21[b] F		10.9 M (34% decreased weight gain in males) 5.6[b] F (33% decreased weight gain in females)		

Table 3-2 Levels of Significant Exposure to Diazinon - 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)	4 wk 7 d/wk (F)	Hemato	15.99			Barnes 1988	Diazinon MG-8 (purity 87.7%); concentrations in food adjusted for purity.
			Hepatic	15.99				
			Renal	15.99				
			Bd Wt	0.8	14.68 M (weight loss)	15.99 F (emaciation- 20% weight loss)		

Table 3-2 Levels of Significant Exposure to Diazinon - 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
58	Dog (Beagle)	8 mo 1 x/d (C)	Cardio	5 M[b] 20 F	10 M (no pericardial fat, cord-like heart vessels)		Earl et al. 1971	Technical grade diazinon (purity not specified).
			Gastro	5	10 M (duodenal wall thickening)	20 (duodenal and stomach ruptures)		
			Hemato	10 F		10 M[b] (peripheral anemia; bone marrow hypocellularity, increased myeloid element content, reticulocytopenia)		
						20 F (peripheral anemia; bone marrow hypocellularity, increased myeloid element content, reticulocytopenia)		
			Hepatic	2.5	5 (markedly elevated serum AST and OCT)	10 M (yellow, fatty liver; parenchymal atrophy, hepatocyte dissociation; moderate cirrhosis focal necrosis, fibrous infiltration elevated serum LDH)		
			Renal	5 M[b] 10 F		10 M (localized chronic nephritis, tubular atrophy, glomeruli necrosis, fibrous infiltration, elevated serum LDH)		
			Endocr	5		10 M (pancreatic atrophy and interstitial fibrosis)		
			Bd Wt	5 M[b] 10 F	10 M[b] (significant weight loss) 20 F (significant weight loss)			

Table 3-2 Levels of Significant Exposure to Diazinon - 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
59	Pig (Hormel-Hanford)	8 mo 1 x/d (C)	Gastro	1.25	2.5	(edema and serosal seepage in the ileum)	10	(jejunal edema, localized mucosal erosion into intestinal muscle layers with marked serosal seepage; duodenal ulceration)	Earl et al. 1971	Technical grade diazinon (purity not specified).
			Hemato	2.5			5	(occasional transient peripheal anemia, reticulocytopenia, bone marrow hypocellulartiy, increased myeloid element content)		
			Hepatic		1.25	(slight inflammation, occasional lobular congestion)	5			
							2.5	(interlobular connective tissue thickening, degenerative hepatocytes, hepatic hemorrhage)		
Immuno/ Lymphoret										
60	Rat (Sprague-Dawley)	13 wk 7 d/wk ad lib (F)		168[b] M 212 F					Singh 1988	Diazinon MG-8 (purity 87.7%); concentrations in food adjusted for purity.

Table 3-2 Levels of Significant Exposure to Diazinon - 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
61	Dog (Beagle)	13 wk 7 d/wk (F)		11.6			Barnes 1988	Diazinon MG-8 (87.7% purity); concentrations in food adjusted for purity.
Neurological								
62	Human	28-31 d (C)		0.03 M			EPA 2001	Diazinon (99.5% purity).
63	Rat (Wistar)	42 d ad lib (F)		0.18 F[d]	0.27 F (20% RBC AChE inhibition)		Davies and Holub 1980a	Diazinon (99.2% purity).
64	Rat (Wistar)	35 d ad lib (F)		0.2 F			Davies and Holub 1980a	Diazinon (99.2% purity).
65	Rat (Wistar)	92 d ad lib (F)		0.4 F	0.8 F (40% RBC AChE inhibition)		Davies and Holub 1980a	Diazinon (99.2% purity).
66	Rat (Wistar)	30 d ad lib (F)			2.86 (58% RBC AChE inhibition)		Davies and Holub 1980b	Diazinon (99.2% purity).
67	Rat (Sprague-Dawley)	90 d (F)		0.018		1.8 F (greater than 79-86% RBC AChE inhibition)	EPA 1996	Diazinon (D*Z*N* MG87%, purity 88%); apparently not adjusted for purity.

Table 3-2 Levels of Significant Exposure to Diazinon - 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
68	Rat (Sprague-Dawley)	28 d (F)		0.02	2.4 (38-59% RBC AChE inhibition)		EPA 1996	Diazinon (D*Z*N* MG87%; purity 88%); dose adjustment for purity uncertain.
69	Rat (Sprague-Dawley)	133 d (F)		7.63 F		41.43 F (tremors in 3/30 and 4/30 F0 and F1 parental females)	Giknis 1989	Diazinon technical (94.9% purity); dose adjustment for purity uncertain.
70	Rat (Sprague-Dawley)	42 d (F)		0.17 M 0.19 F	1.68 M (29-35% RBC AChE inhibition) 1.82 F (16-35% RBC AChE inhibition)	8.6 M (>59% RBC AChE inhibition) 9.27 F (>59% RBC AChE inhibition)	Mahkteshim-Agan 1989	Diazinon (97.2% purity); no allowance was made for purity
71	Rat (Sprague-Dawley)	6 wk 7 d/wk (F)		0.2 M	8.4[b] M (21% RBC AChE inhibition) 9.4 F (24% brain AChE inhibition)	150.8[b] M (58% decrease in brain AChE in males, 61% decrease in females) 182.9 F (61% decrease in brain AChE in females)	Singh 1988	Diazinon MG-8 (purity 87.7%); concentrations in food adjusted for purity.

Table 3-2 Levels of Significant Exposure to Diazinon - 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
72	Rat (Sprague-Dawley)	13 wk 7 d/wk ad lib (F)		0.4	15 M (27% RBC AChE inhibition)		Singh 1988	Diazinon MG-8 (purity 87.7%); concentrations in food adjusted for purity.
73	Rat (Sprague-Dawley)	42 d (F)		0.19 M 0.2 F	1.81 M (46-55% RBC AChE inhibition)	9.08 M (>59% RBC AChE inhibition) 1.97 F (>59% RBC AChE inhibition)	Trutter 1991	Diazinon (87.4% purity); concentrations in food adjusted for purity
74	Dog (Beagle)	13 wk 7 d/wk (F)		0.021	5.9 (31% RBC and brain AChE inhibition)		Barnes 1988	Diazinon MG-8 (87.7% purity); concentrations in food adjusted for purity.
75	Dog (Beagle)	4 wk 7 d/wk (F)		0.082	14.68 (30% RBC AChE inhibition, 44% brain AChE inhibition, emesis)		Barnes 1988	Diazinon MG-8 (87.7% purity); concentrations in food adjusted for purity.
76	Dog (Beagle)	8 mo 1 x/d (C)		5	10 (fasciculation, diarrhea, emesis)		Earl et al. 1971	Technical grade diazinon (purity not specified).
77	Pig (Hormel-Hanford)	8 mo 1 x/d (C)		1.25	2.5 (emesis, diarrhea, fasciculations)		Earl et al. 1971	Technical grade diazinon (purity not specified).

Table 3-2 Levels of Significant Exposure to Diazinon - 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
Reproductive								
78	Rat (albino)	65 d (GW)				1.5 M (increased sperm abnormalities, decreased fertility)	Abd El-Aziz et al. 1994	Diazinon (purity unspecified)
79	Rat (Sprague-Dawley)	133 d (F)		41.43 F			Giknis 1989	Diazinon technical (94.9% purity); dose adjustment for purity uncertain.
80	Rat (Sprague-Dawley)	60 d ad lib (F)		0.05			Green 1970	Diazinon (purity unspecified).
81	Rat (Sprague-Dawley)	13 wk 7 d/wk ad lib (F)		168[b] M 212 F			Singh 1988	Diazinon MG-8 (purity 87.7%); concentrations in food adjusted for purity.
82	Mouse (Hybrid)	Gd 1-18 1 x/d (F)			0.18 F (14% reduced maternal weight gain, 20% reduced litter size)		Spyker and Avery 1977	Diazinon technical grade (purity not specified).
83	Dog (Beagle)	13 wk 7 d/wk (F)		11.6			Barnes 1988	Diazinon MG-8 (87.7% purity); concentrations in food adjusted for purity.

Table 3-2 Levels of Significant Exposure to Diazinon - 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
84	Dog (Beagle)	8 mo 1 x/d (C)		5 M		10 M (testicular atrophy, aspermatogenesis)	Earl et al. 1971	Technical grade diazinon (purity not specified).
Developmental								
85	Rat (Sprague-Dawley)	133 d (F)		0.77 F		7.63 F (decreased F1 pup survival)	Giknis 1989	Diazinon technical (94.9% purity); dose adjustment for purity uncertain.
86	Mouse (Hybrid)	Gd 1-18 1 x/d (F)		0.18		9 (significantly reduced early weight gain by pups, increased mortality at ppd 28)	Barnett et al. 1980	Diazinon (technical grade, purity unspecified).
87	Mouse (Hybrid)	Gd 1-18 1 x/d (F)				0.18 F (neuromuscular coordination deficits, reduced litter size, delayed contact placing and sexual maturity)	Spyker and Avery 1977	Diazinon technical grade (purity not specified).

Table 3-2 Levels of Significant Exposure to Diazinon - 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								
88	Rat (Sprague-Dawley)	98 wk or 52 wk (F)	Resp	11			Kirchner et al. 1991	Diazinon MG-8 (87.7% purity); concentrations in food adjusted for purity.
			Cardio	11				
			Gastro	11				
			Hemato	11				
			Musc/skel	11				
			Hepatic	11				
			Renal	11				
			Endocr	11				
			Dermal	11				
			Ocular	11				
			Bd Wt	11				
			Metab	11				
89	Dog (Beagle)	52 wk (F)	Hemato	8.4			Rudzki et al. 1991	Diazinon MG-6 (87.7% purity); dose adjustment for purity uncertain.
			Bd Wt	0.015 M		4.7 M (42% depressed body weight gain)		

Table 3-2 Levels of Significant Exposure to Diazinon - 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
Immuno/ Lymphoret								
90	Rat (Sprague-Dawley)	98 wk or 52 wk (F)		11			Kirchner et al. 1991	Diazinon MG-8 (87.7% purity); concentrations in food adjusted for purity.
Neurological								
91	Rat (Sprague-Dawley)	98 wk or 52 wk (F)		0.065[e]	5.5 (22-29% RBC and brain AChE inhibition)		Kirchner et al. 1991	Diazinon MG-8 (87.7% purity); concentrations in food adjusted for purity.
92	Dog (Beagle)	52 wk (F)		0.017	4.6 (21-35% RBC AChE inhibition)		Rudzki et al. 1991	Diazinon MG-6 (87.7% purity); dose adjustment for purity uncertain.

Table 3-2 Levels of Significant Exposure to Diazinon - 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
Reproductive								
93	Rat (Sprague-Dawley)	98 wk or 52 wk (F)		11			Kirchner et al. 1991	Diazinon MG-8 (87.7% purity); concentrations in food adjusted for purity.

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

b Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

c Used to derive an acute-duration oral minimal risk level (MRL) of 0.006 mg/kg/day for diazinon based on significant RBC AChE inhibition in female rats by treatment day 12 of the 92-day study. The NOAEL of 0.6 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

d Study results used to derive an intermediate-duration oral minimal risk level (MRL) of 0.002 mg/kg/day for diazinon, as described in detail in Appendix A. Benchmark dose (BMD) analysis was performed on RBC AChE activity to select a point of departure, which was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

e Used to derive a chronic-duration oral minimal risk level (MRL) of 0.0007 mg/kg/day for diazinon. The NOAEL of 0.065 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

AChE = acetylcholinesterase; ad lib = ad libitum; ALT = alanine aminotransferase; AST = aspartate aminotransferase; Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; d = day(s); Endocr = endocrine; (F) = feed; F = Female; (G) = gavage; Gastro = gastrointestinal; gd = gestational day; (GO) = gavage in oil; GGT = gamma-glutamyl-transferase; (GW) = gavage in water; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; (IN) = ingestion; LD50 = lethal dose, 50% kill; LDH = lactate dehydrogenase; LOAEL = lowest-observed-adverse-effect level; Metab = Metabolic; M = male; min = minute(s); mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; OCT = ornithine carbamyl transferase; ppd = post-parturition day; RBC = red blood cell; Resp = respiratory; x = time(s); (W) = drinking water; wk = week(s); yr = year(s)

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

Acute (≤14 days)

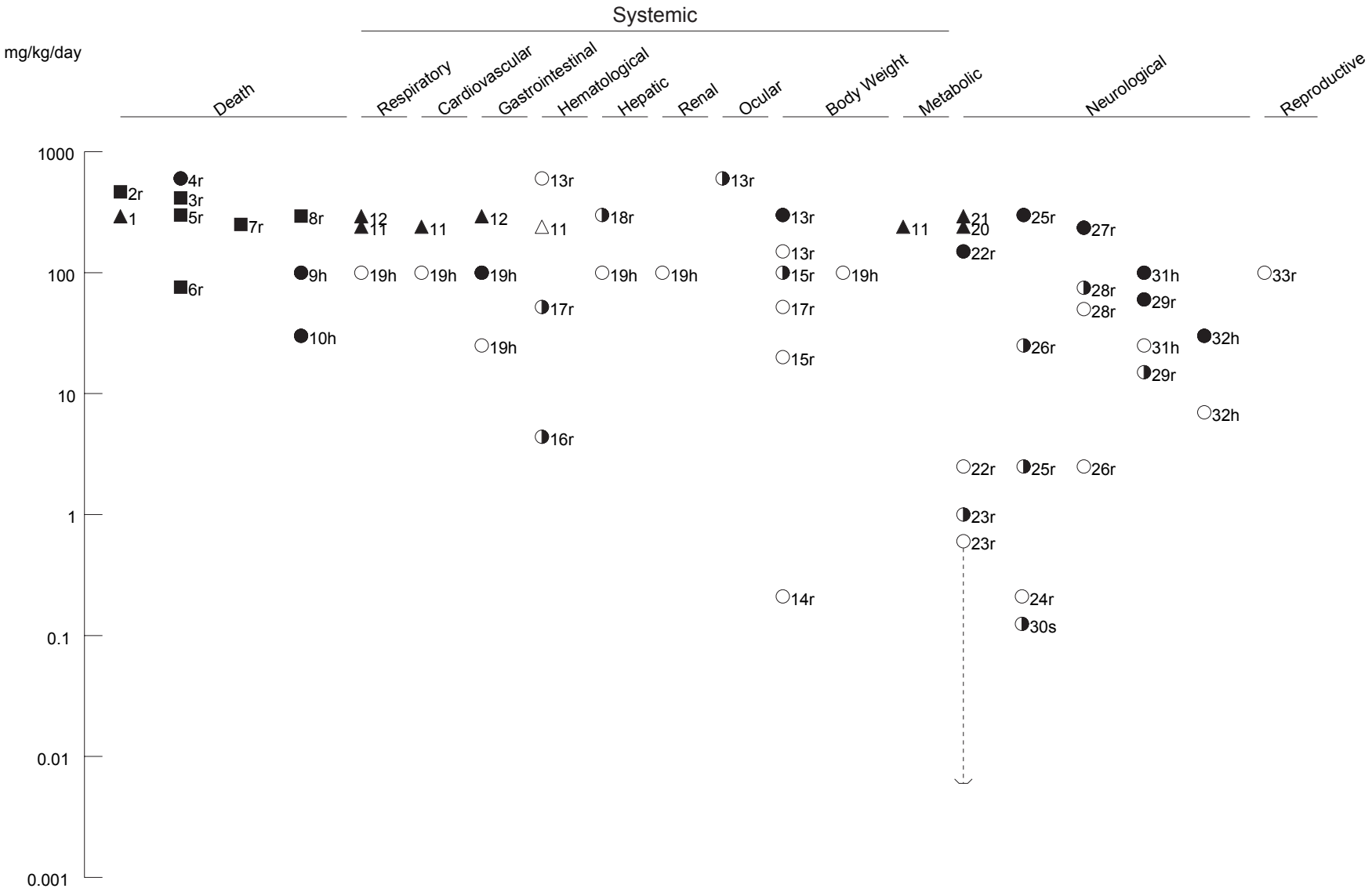


Figure 3-2 Levels of Significant Exposure to Diazinon - Oral *(Continued)*

Acute (≤14 days)

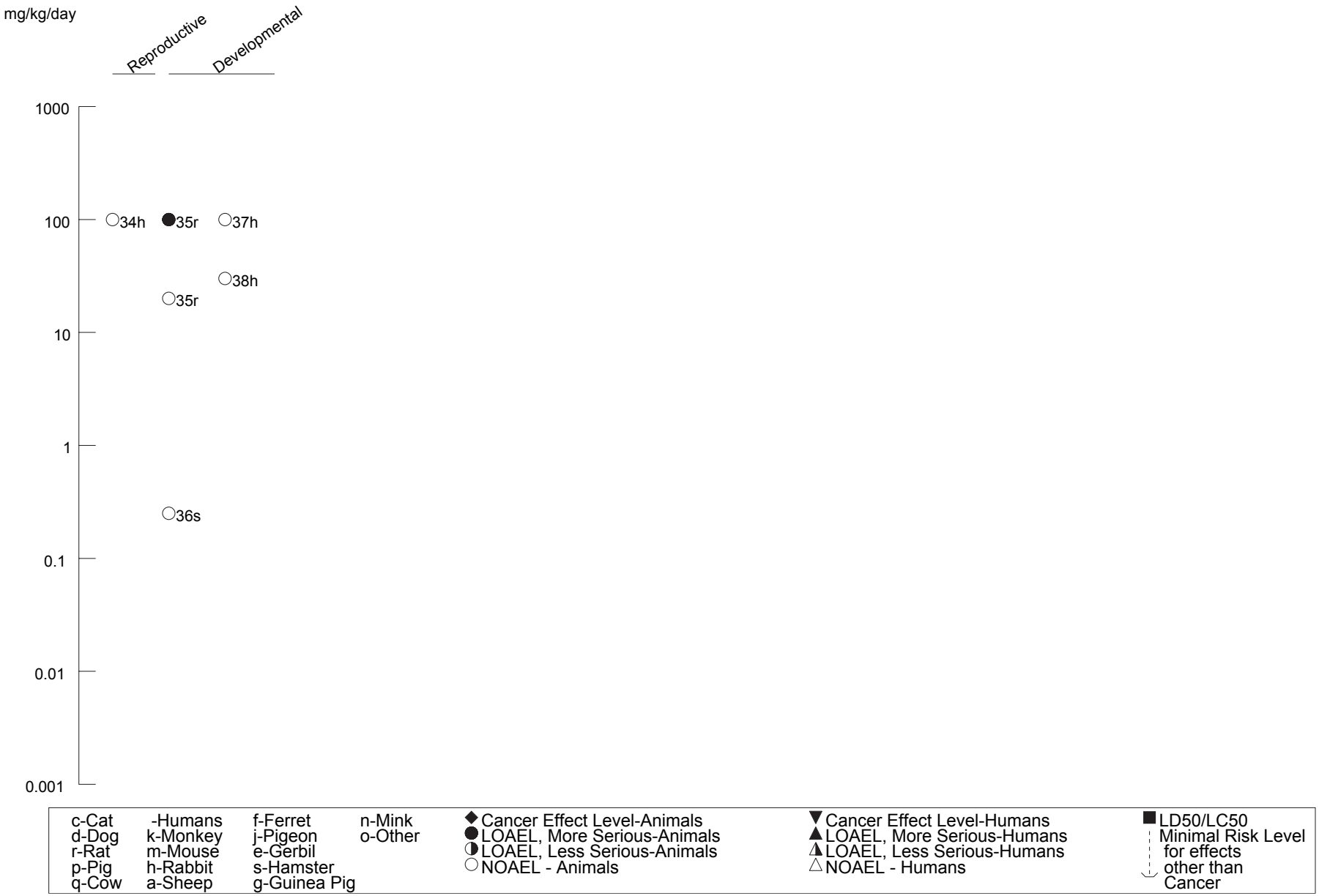


Figure 3-2 Levels of Significant Exposure to Diazinon - Oral *(Continued)*

Intermediate (15-364 days)

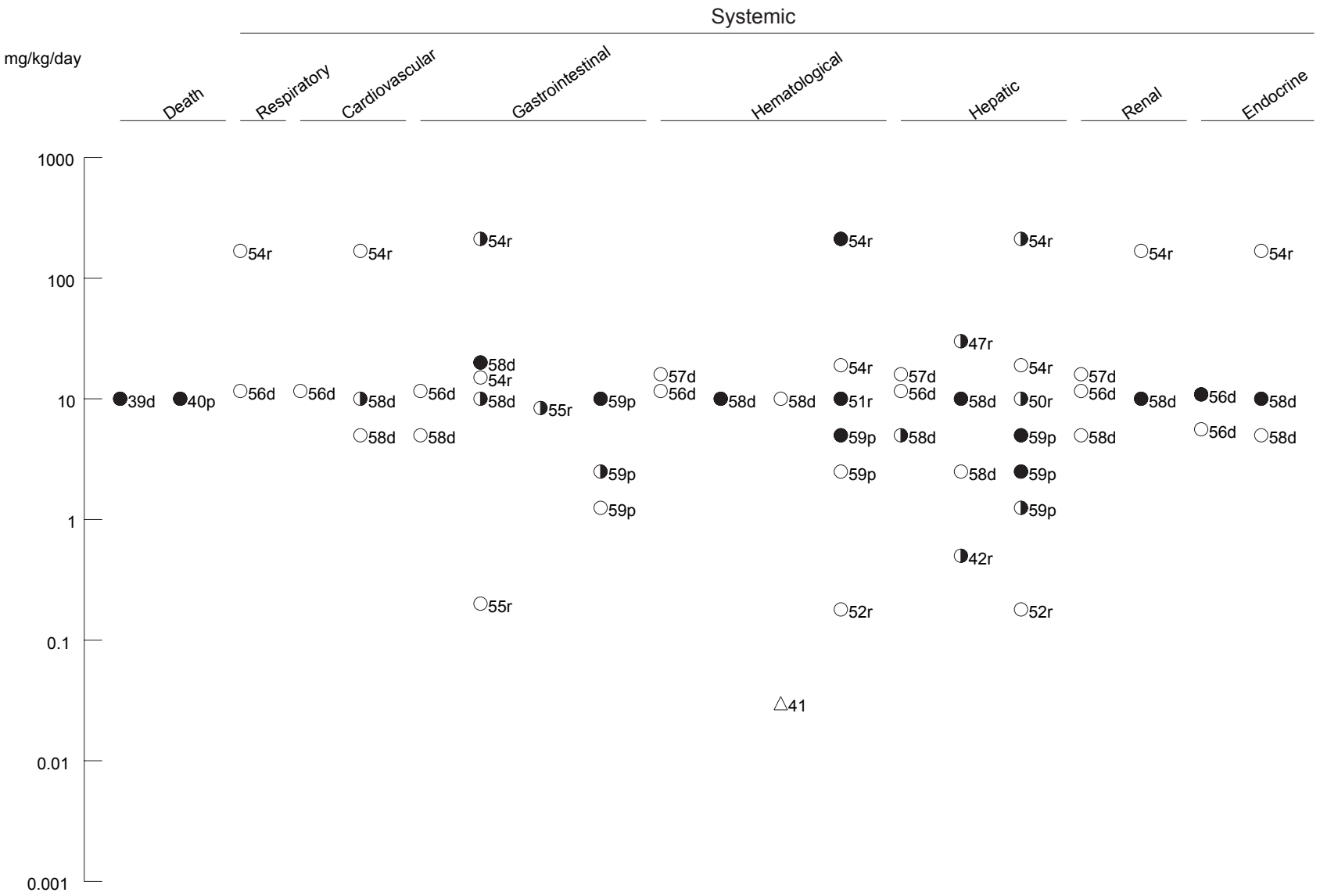


Figure 3-2 Levels of Significant Exposure to Diazinon - Oral *(Continued)*

Intermediate (15-364 days)

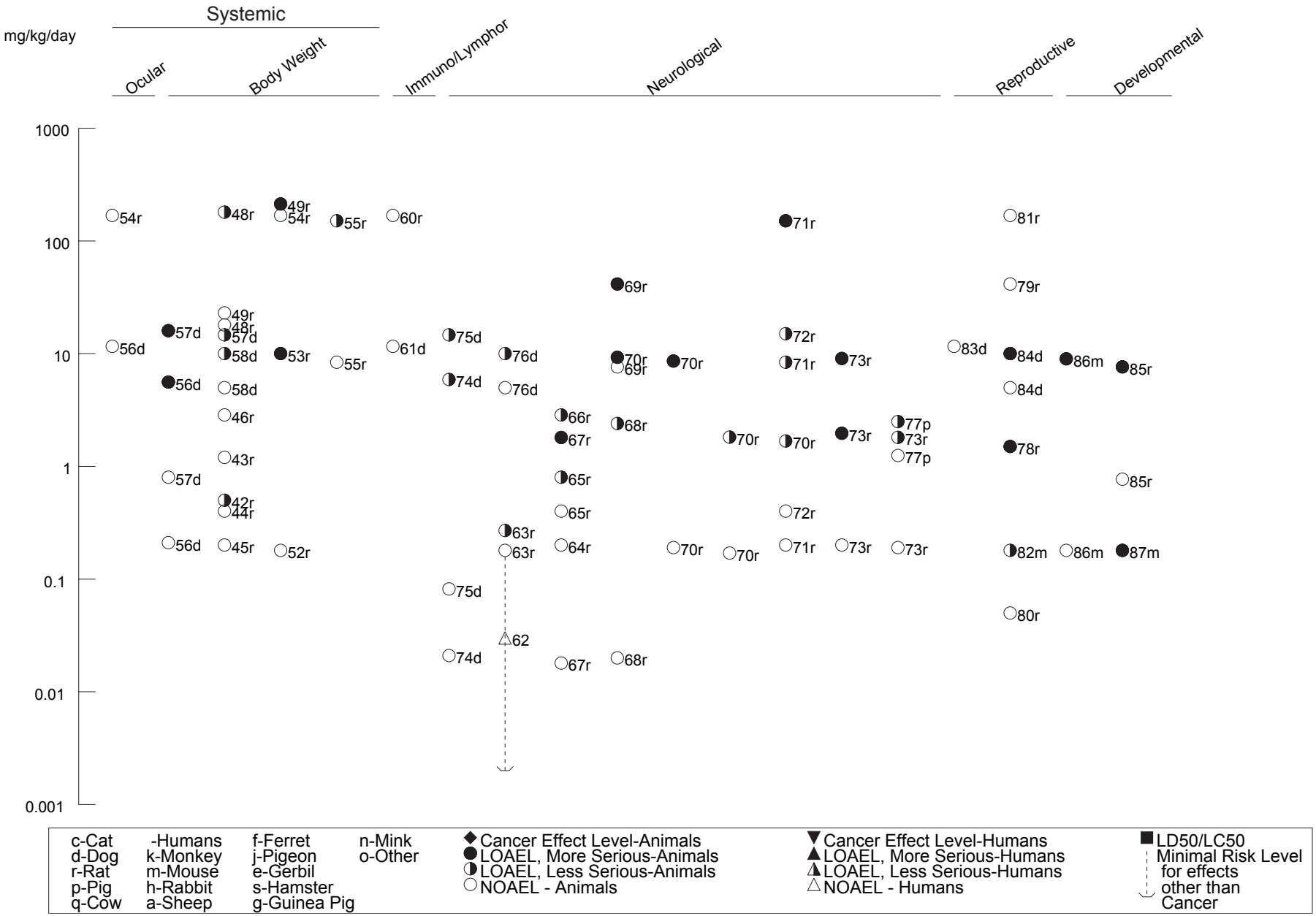
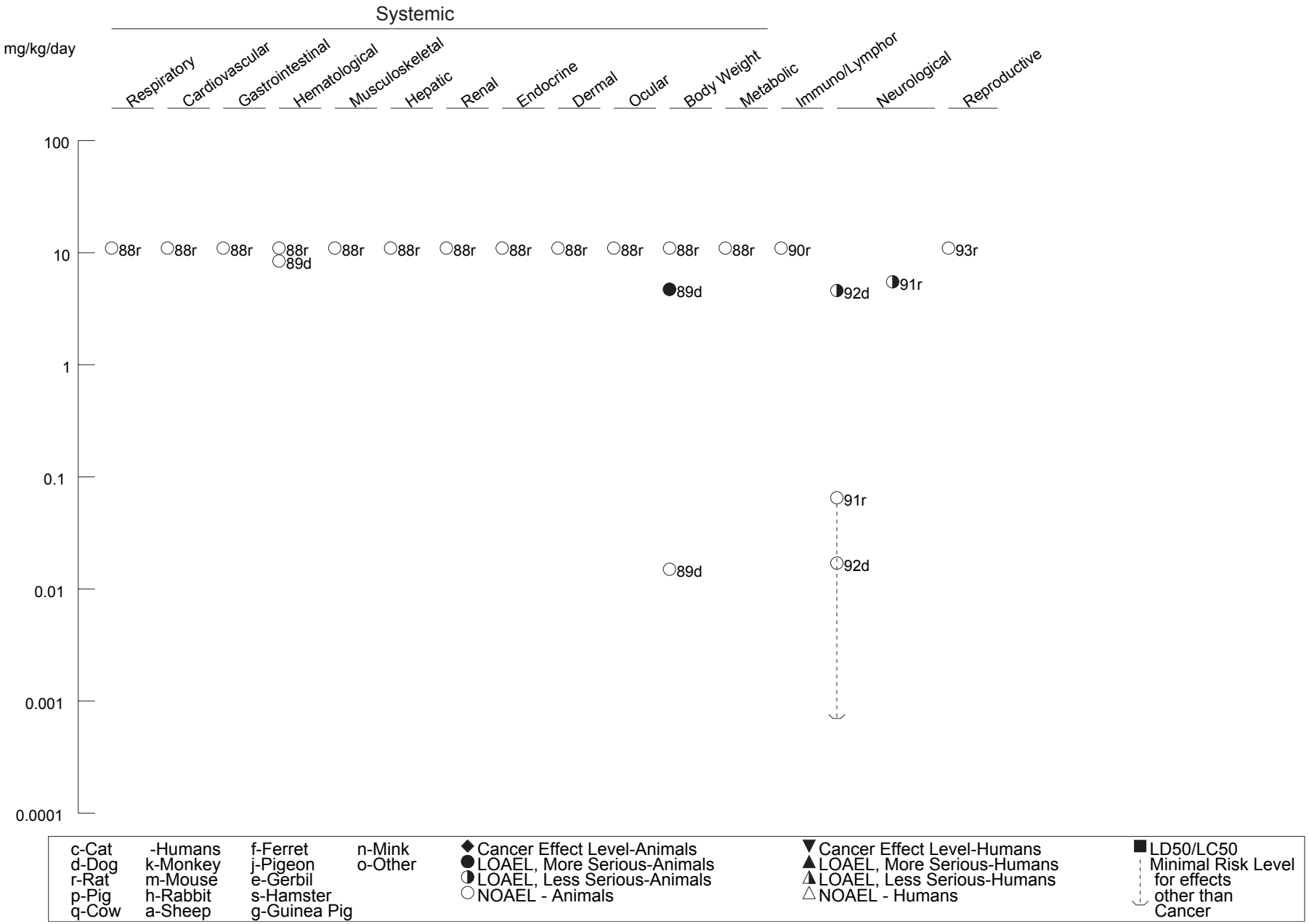


Figure 3-2 Levels of Significant Exposure to Diazinon - Oral *(Continued)*

Chronic (≤365 days)



3.2.2.2 Systemic Effects

No studies were located regarding musculoskeletal, dermal, or body weight effects in humans after oral diazinon exposure. No information was located regarding musculoskeletal or dermal effects in animals after oral exposure to diazinon. Autopsy findings in human acute diazinon poisonings and laboratory animal lethality studies, as well as findings from other human and laboratory animal nonlethal oral exposures, included respiratory impairment, cardiovascular, gastrointestinal, hematological, and endocrine (pancreas) effects. These effects were largely derived from cholinergic responses typical of high-level organophosphate poisoning.

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

Respiratory Effects. Respiratory distress, a typical cholinergic sign of AChE inhibition, was reported in several human acute poisoning incidents and laboratory animal evaluations following oral diazinon exposure. In humans, acute-duration oral exposure to high doses of diazinon causes pulmonary distress with signs that include congested respiratory tract, copious airway secretions, and pulmonary edema (Balani et al. 1968; Hata et al. 1986; Kabrawala et al. 1965). An 18% incidence of pulmonary edema was found in diazinon-poisoned patients (Limaye 1966; Shankar 1967). An autopsy report of a diazinon-poisoned 54-year-old female suicide victim described heavy and congested (edematous) lungs (Poklis et al. 1980). Tachypnea and cyanosis were observed in a male who intentionally ingested 240 mg/kg diazinon and in a female who ingested 509 mg/kg (Klemmer et al. 1978). Diazinon treatment also resulted in signs of respiratory effects in laboratory animals. Single oral diazinon doses of 50–700 mg/kg to rats resulted in respiratory distress from pulmonary inflammation, vascular congestion, venous stasis, and occasional extensive pneumonitis. Death generally resulted from respiratory failure that was usually preceded by coma (Boyd and Carsky 1969). Dyspnea was observed in male Sprague-Dawley rats given a single gavage dose of 264 mg/kg diazinon and impaired respiration was observed in females receiving a dose of 528 mg/kg (Chow and Richter 1994).

No gross or histological evidence of treatment-related damage to the lungs was observed in New Zealand rabbit dams receiving up to 100 mg/kg/day diazinon during gestation days 6–18 (Harris and Holson 1981), in male or female Sprague-Dawley rats receiving up to 212 mg/kg/day diazinon from feed for 13 weeks (Singh 1988) or up to 12 mg/kg/day for 98 weeks (Kirchner et al. 1991), or in male or female Beagle dogs receiving up to 11.6 mg/kg/day diazinon for 13 weeks (Barnes 1988).

Cardiovascular Effects. Acute-duration oral, lethal human exposure to diazinon resulted in extensive congestion of the heart and blood vessels as reported in a summary of autopsy findings of 76 cases of acute diazinon poisoning which described cardiovascular signs that included: livid, congested face; soft flabby heart with conspicuous vasculature on the pericardium and epicardium; occasional and scattered petechial/ecchymotic hemorrhage; and cloudy swelling and hyperemia (upon histopathological examination) (Limaye 1966). In a case study of 25 attempted suicides by diazinon ingestion, some patients showed hypertension and peripheral circulatory failure (Kabrawala et al. 1965). Other cardiovascular signs reported after acute oral exposure to high doses of diazinon in humans include tachycardia (Kabrawala et al. 1965; Klemmer et al. 1978; Shankar 1967), hypertension (Balani et al. 1968; Hata et al. 1986), and bradycardia (Hata et al. 1986; Klemmer et al. 1978).

One male dog given 10 mg/kg/day diazinon for 8 months exhibited an absence of pericardial fat on the heart, as well as a cord-like appearance of the heart vessels (Earl et al. 1971). Two other dogs, given 10 or 20 mg/kg/day diazinon, exhibited markedly elevated serum lactate dehydrogenase (LDH). This is a nonspecific response that may be suggestive of either cardiac or skeletal muscle damage or some other unknown pathology. Pallor was reported in male Sprague-Dawley rats receiving a single oral dose of 132 mg/kg diazinon (Chow and Richter 1994). Oral administration of 10 mg/kg/day diazinon to rats for 7 weeks resulted in significantly increased malondialdehyde levels in heart tissue and histopathologic evidence of vacuolization and swelling of mitochondria in myocardial cells (Ogutcu et al. 2006). The biological significance of these results is uncertain because the diazinon-treated rats exhibited 22% lower mean body weight than controls.

No gross or histological evidence of treatment-related damage to the heart was seen in New Zealand rabbit dams receiving up to 100 mg/kg/day diazinon during gestation days 6–18 (Harris and Holson 1981), in male or female Sprague-Dawley rats receiving up to 212 mg/kg/day diazinon in feed for 13 weeks (Singh 1988) or up to 12 mg/kg/day diazinon in feed for 98 weeks (Kirchner et al. 1991), or in male or female Beagle dogs receiving up to 11.6 mg/kg/day diazinon for 13 weeks (Barnes 1988).

Gastrointestinal Effects. A summary of autopsy findings from 76 cases of acute diazinon poisoning describes gastrointestinal signs that include: dark, blood-stained stomach contents; congested stomach mucosa with submucosal petechial hemorrhage; and occasional erosion and ulceration (Limaye 1966). Petechial hemorrhages throughout the stomach and gastric mucosa were revealed in the autopsy report of a diazinon-poisoned 54-year-old female suicide victim who had ingested an estimated 293 mg/kg

diazinon (Poklis et al. 1980). Other signs of gastrointestinal toxicity seen in humans after acute exposure to high doses of diazinon include nausea, diarrhea and vomiting (Balani et al. 1968; Klemmer et al. 1978), and abdominal pain (Balani et al. 1968). A 16-year-old female who drank an estimated 1.5 mg/kg of a diazinon formulation (Tik-20) developed pancreatitis after being treated for cholinergic manifestations. The pancreatic effects may well have been secondary to the diazinon-induced cholinergic manifestations (Dagli et al. 1981). Acute pancreatitis was also found in two children poisoned with diazinon (Weizman and Sofer 1992).

In male albino Wistar rats exposed to acute lethal doses of diazinon, lamina propria of the small intestine were congested, and occasional small areas of hemorrhage and necrosis at the mouth of gastric glands were observed. The digestive tract was dehydrated with small increases in organ wet weight except for the cecum, whose wet weight declined approximately 32%. Other effects included pyloric stomach ulceration and inflammation of the small intestine and cecum (Boyd and Carsky 1969). Similar effects were seen in an intermediate exposure of Beagle dogs given diazinon orally for 8 months. Marked edematous thickening of the intestinal wall was observed in 5/6 dogs at the lethal 20 mg/kg/day dose with one developing a duodenal rupture and subsequent peritonitis, and another rupture of the pyloric portion of the stomach. At the 10 mg/kg/day dose, the duodenal wall thickening was observed only in the solitary male dog that exhibited weight loss and other gross pathological changes. Elevated serum amylase levels were also found in dogs of both sexes at the 10 mg/kg/day dose, but apparently did not correlate with observable pancreatic pathology with the exception of one male dog. Either congestion or hemorrhage (or both) of the small intestines and colon was present in varying degrees among dogs receiving 5–100 mg/kg/day diazinon for various time periods in a preliminary dose-range study. Apparently, many of the effects described were not found uniformly in all of the dogs at a given dose, and a clear dose-response relationship was not always present (Earl et al. 1971). Treatment of Hormel-Hanford miniature pigs with daily diazinon doses of 1.25–10 mg/kg/day for 8 months resulted in injury to the gastrointestinal tract. At 10 mg/kg/day, 4/5 pigs which died had edematous thickening of the walls of the jejunum, 3/5 had ulcer formation in the duodenum, and one had localized mucosal erosion into the muscular layer with serosal seepage throughout the intestines. One pig at each of the 5.0 and 2.5 mg/kg/day dose levels displayed edema of the jejunum; serosal seepage of the ileum was noted at the lower dose. Histopathologically, slight thickening of the serosa, occasional focal hyperemia, and outer muscle hemorrhaging were observed in the intestines of swine exposed to 10 or 5 mg/kg/day diazinon. Abdominal ascites that clotted on exposure to air was reported without further description for one pig exposed to 2.5 mg/kg/day. This animal also suffered intestinal edema and serosal seepage, liver toxicity, and death on day 141 (Earl et al. 1971).

Stomach mucosal hemorrhage, congestion, and erosion were observed in 7/9 New Zealand rabbit dams that died while receiving 100 mg/kg/day diazinon during gestation days 6–18 (Harris and Holson 1981). No signs of gastrointestinal toxicity were seen in dams treated at 7 or 25 mg/kg/day. Diarrhea was observed in male Sprague-Dawley rats receiving a single oral dose of 528 mg/kg diazinon, but not in females receiving the same dose (Chow and Richter 1994). Soft stools were observed in male Sprague-Dawley rats receiving 8.4 mg/kg/day diazinon from feed for 6 weeks and in females receiving 183.2 mg/kg/day (Singh 1988), as well as males receiving 168 mg/kg/day diazinon from feed for 13 weeks and in females receiving 212 mg/kg/day (Singh 1988). Emesis was reported in male and female Beagle dogs receiving 14.68 mg/kg/day diazinon from feed for 4 weeks (EPA 2000a). Emesis, bloody feces, and diarrhea were observed in Beagle dogs receiving up to 11.6 mg/kg/day diazinon from feed for 13 weeks (Barnes 1988). These signs were not dose-related and were considered by the authors to be unrelated to treatment.

No histological evidence of treatment-related damage to gastrointestinal tissues was found in Sprague-Dawley rats receiving up to 12 mg/kg/day diazinon for 98 weeks (Kirchner et al. 1991), or up to 212 mg/kg/day for 13 weeks (Singh 1988). Similar results were reported in Beagle dogs receiving up to 11.6 mg/kg/day diazinon over a 13-week period (Barnes 1988).

Hematological Effects. A report on five individuals (three males, two females) who intentionally ingested 60–180 mL of 25% diazinon solution (estimated to deliver a dose of 240–400 mg/kg for males and 509–986 mg/kg for females) found that leucocyte counts (3,700, 95% polymorphonuclear), hemoglobin (16.3 g), and hematocrit (47) were all within normal ranges (Klemmer et al. 1978).

Diazinon-induced hematological effects have been reported in several animal studies. The hematological effects of a single oral dose of 4.4 mg/kg diazinon were studied in Sprague-Dawley rats 2 hours after treatment. Although diazinon exposure did not significantly alter hematocrit or factor VII activity, platelet count was significantly ($p<0.05$) reduced when compared with pre-exposure values ($694x10^3/mm^3$ as compared to $856x10^3/mm^3$). Similarly, small (6–14%) but significant ($p<0.05$) changes were observed in activities of the remaining clotting factors; fibrinogen activity was reduced, while prothrombin, partial thromboplastin, factor II, factor V, and factor X activities were increased. Since fibrinogen and factors II, V, VII, and X are synthesized in the liver, the associated alterations may reflect hepatic effects of diazinon exposure. The data indicate an overall diazinon-induced condition of hyper-coagulability that, considered together with observations from other studies of various haemorrhagia, may

suggest that diazinon might affect hemostasis in general (Lox 1983). Other rats received 52 mg/kg/day diazinon from drinking water for 14 days and were monitored for hematocrit and platelet count, and various clotting factor times (prothrombin, partial thromboplastin, fibrinogen, and factors II, V, VII, X, and XII). Immediately after treatment, increased times for prothrombin, partial thromboplastin, and fibrinogen suggest an overall state of hypocoagulability, despite no consistent pattern for the other factors and parameters (decreased for VII and XII, no changes for II, V, VII, and X, or in hematocrit and platelet count). One week after treatment, partial thromboplastin time was shortened (indicating intrinsic pathway activation), as were the clotting times for factors VIII, X, and XII, although that for II was lengthened. Overall, this suggests a hypercoagulability of the intrinsic pathway. Also, hematocrit was decreased. These alterations may reflect a time-course in hepatic damage (at least for II, VII, X, and fibrinogen which are of liver origin) (Lox 1987). A group of 24 rats receiving approximately 0.18 mg/kg/day diazinon from drinking water for 6 months showed no changes compared with controls in the clotting activities associated with prothrombin, partial thromboplastin, fibrinogen, or the coagulation factors II, V, VII, and X (Lox and Davis 1983). Significantly ($p<0.01$) decreased hematocrit, hemoglobin, and RBC and thrombocyte counts, and increased white blood cell (WBC) count and mean corpuscular volume were observed in rats orally administered 10 mg/kg/day diazinon for 7 weeks (Kalender et al. 2006). One of six dogs treated with 20 mg/kg/day diazinon exhibited marked reductions in peripheral red blood cells, hematocrit, and hemoglobin. All six dogs displayed greatly elevated myeloid/erythroid (M/E) bone marrow ratios (114–183/1 as opposed to 1.1–1.9/1 for controls) with slight to moderate bone marrow hypocellularity, and a pronounced reticulocytopenia in two dogs (one male, one female) (Earl et al. 1971). Three of six Hormel-Hanford miniature pigs orally administered 5.0 mg/kg/day diazinon showed a transient drop in red blood cells, hematocrit, and hemoglobin content, but no indication of peripheral anemia. No peripheral anemia was present in any of five pigs in the 10 mg/kg/day group, but all the pigs exhibited reticulocytopenia, with three displaying elevated M/E ratios (Earl et al. 1971).

Hematological parameters were normal in Sprague-Dawley rats (10–15/sex/group) receiving a single oral gavage dose of up to 528 mg/kg diazinon and examined 14 days later (Chow and Richter 1994). Decreased hemoglobin and hematocrit along with an increase in reticulocytes were observed in female Sprague-Dawley rats receiving 212 mg/kg/day diazinon from feed for 13 weeks. Hematological parameters were normal in female rats receiving up to 19 mg/kg/day and in males receiving up to 168 mg/kg/day (Singh 1988). No changes in hematological parameters were observed in Beagle dogs receiving up to 11.6 mg/kg/day diazinon from feed for 13 weeks (Barnes 1988) or up to 9 mg/kg/day for 52 weeks (Rudzki et al. 1991), or in Sprague-Dawley rats receiving up to 12 mg/kg/day for 98 weeks (Kirchner et al. 1991).

Hepatic Effects. A summary of autopsy reports from 76 human diazinon poisonings includes findings of congested liver (Limaye 1966).

In laboratory animals, single oral doses of 300 mg/kg diazinon given to male and female Sprague-Dawley rats were followed by significant ($p<0.001–0.05$) reductions in hepatic microsomal cytochrome P-450 content and aniline hydroxylase and aminopyrine N-demethylase activities, especially during the first 24 hours. These effects largely disappeared within 72 hours to 2 weeks, with values often exceeding those of controls. No significant changes in mitochondrial respiratory function (respiratory control ratio, ADP/O ratio, and ATPase activity) were observed (Mihara et al. 1981). Oral administration of 30 mg/kg/day diazinon for 4 weeks to white male rats reduced serum beta-lipoprotein, alanine amino-transferase, aspartate aminotransferase, and gamma-glutamyl transferase. Although elevated levels of these transaminases are generally associated with liver pathology, the toxicological implications of the significant reduction (13–67%) of these liver enzymes and its relevance to diazinon poisoning are unclear (Enan et al. 1982). In another rat study, normal lobular architecture was maintained in the livers, but small lipid droplets were observed in some hepatocytes after 7 weeks. In this study, male Wistar rats were treated with oral doses of 0.5 mg/kg twice a week for 28 weeks. Lipid accumulation became progressively more severe from 14 to over 28 weeks, but no cellular necrosis was observed (at least after 14 weeks). This lipid accumulation could result from disturbed metabolism in the hepatocellular rough endoplasmic reticulum, increased lipid mobilization from peripheral tissue, or impaired lipoprotein release from liver cells. Electron microscopic examination revealed fat droplets near mitochondria, with abundant rough and smooth endoplasmic reticulum, mitochondria, and glycogen present in liver cells from both treated and control rats. No changes were observed in hepatocyte nuclei or nucleoli. But in another study, groups of rats exposed to approximately 0.18 mg/kg/day diazinon in the drinking water for 6 months exhibited no adverse effects on the liver as determined by histopathological examination (Lox and Davis 1983). The autopsy of a male Beagle dog that died from exposure to 10 mg/kg/day diazinon for 8 months revealed fatty liver, markedly elevated serum aspartate aminotransferase, serum lactate dehydrogenase, and ornithine carbamyl transferase, parenchymal atrophy, and hepatocyte dissociation (Earl et al. 1971). Female dogs treated with 20 mg/kg/day diazinon showed moderate cirrhosis, focal necrosis, fibrous infiltration, and hepatocyte dissociation. In another study, hepatic effects noted in pigs treated with 1.25 mg/kg/day diazinon for 8 months included slight inflammation and occasional lobular congestion with degenerative hepatocytes (Earl et al. 1971). Animals treated with a daily dose of 2.5 mg/kg exhibited interlobular connective tissue thickening and lobular congestion. In addition to the noted hepatic effects, all livers from swine exposed to 10 mg/kg/day were very firm to the touch and hard

to cut, and one liver from a pig treated with 5 mg/kg/day diazinon was described as “friable” and very gritty, with focal subscapular hemorrhages.

An increase in relative and absolute liver weight was observed in female Sprague-Dawley rats receiving 212 mg/kg/day diazinon from feed for 13 weeks (Singh 1988). This was accompanied by histological evidence of minimal centrilobular hepatocellular hypertrophy. Kalender et al. (2005) noted serum biochemical and hepatocellular structural changes in male Wistar rats administered diazinon by gavage at a dose of 10 mg/kg/day for 7 weeks. The observed effects included significantly ($p<0.01$) increased hepatic enzyme activity (ALP, ALB, AST), total protein, and albumin levels, increased total cholesterol and decreased low density lipoprotein cholesterol and triglycerides, and histopathologic evidence of pronounced mitochondrial swelling, structural changes in mitochondrial cristae, swelling of endoplasmic reticulum, and changes in the density of nuclear chromatin.

No gross or histological evidence of treatment-related damage to the liver after oral exposure to diazinon was observed in Sprague-Dawley rats receiving up to 12 mg/kg/day diazinon for 98 weeks (Kirchner et al. 1991), in New Zealand rabbit dams receiving up to 100 mg/kg/day diazinon during gestation days 6–18 (Harris and Holson 1981), or in Beagle dogs (4/sex/group) receiving up to 11.6 mg/kg/day diazinon for 13 weeks (Barnes 1988) or up to 9 mg/kg/day diazinon for 52 weeks (Rudzki et al. 1991).

Renal Effects. A summary of autopsy findings in 76 cases of acute diazinon poisoning described renal signs that included congested kidney and rare renal tract and kidney cortex submucosal petechiae and ecchymoses (Limaye 1966).

A single oral dose of diazinon ranging from 50 to 700 mg/kg produced dose-dependent renal effects in rats. These effects were observed to varying degrees during the first 72 hours following diazinon exposure. Substituting a purified protein diet for Purina lab chow resulted in additional oliguria, in aciduria rather than alkalinuria, and in somewhat more severe hematuria. A low-protein purified diet exacerbated the aciduria. Other renal effects included tubular swelling, capillary loop congestion, glycosuria, proteinuria, and hematuria (Boyd and Carsky 1969). Beagle dogs treated with 5 mg/kg for 8 months showed kidney corticomedullary congestion and capsular adhesions. One dog that died from exposure to 10 mg/kg/day diazinon exhibited localized chronic nephritis, tubular atrophy, and glomeruli with fibrous infiltrations (Earl et al. 1971).

No gross or histological evidence of treatment-related damage to the kidneys after oral exposure to diazinon was observed in New Zealand rabbit dams receiving up to 100 mg/kg/day diazinon during gestation days 6–18 (Harris and Holson 1981), in Sprague-Dawley rats receiving up to 212 mg/kg/day diazinon in feed for 13 weeks (Singh 1988), or up to 12 mg/kg/day for 98 weeks (Kirchner et al. 1991), or in Beagle dogs receiving up to 11.6 mg/kg/day diazinon for 13 weeks (Barnes 1988) or up to 9 mg/kg/day for 52 weeks (Rudzki et al. 1991).

Endocrine Effects. A 16-year-old female who drank an estimated 10 mL of a diazinon formulation (Tik-20) developed pancreatitis after being treated for cholinergic manifestations. A dose could not be calculated because the concentration of diazinon in the liquid was not reported. The pancreatic effects may well have been secondary to the diazinon-induced cholinergic manifestations (Dagli et al. 1981). Acute pancreatitis was also found in two children poisoned with diazinon (Weizman and Sofer 1992).

Pancreatic atrophy and interstitial fibrosis was reported in male Beagle dogs receiving 10 mg/kg/day diazinon in capsule form for 8 months (Earl et al. 1971), but not in females. Atrophy of the pancreatic acini was observed in male Beagle dogs receiving 10.9 mg/kg/day of diazinon from feed for 13 weeks, but not in similarly treated female dogs receiving 11.6 mg/kg/day diazinon (Barnes 1988).

No gross or histological evidence of treatment-related damage to the adrenals after oral exposure to diazinon was observed in Sprague-Dawley rats (15/sex/group) receiving up to 212 mg/kg/day diazinon from feed for 13 weeks (Singh 1988). No gross or histological evidence of treatment-related damage to the adrenals, pituitary, or thyroid glands was observed in Sprague-Dawley rats receiving up to 12 mg/kg/day diazinon for 98 weeks (Kirchner et al. 1991), or in Beagle dogs receiving up to 11.6 mg/kg/day diazinon for 13 weeks (Barnes 1988).

Ocular Effects. Miosis has been reported in humans admitted to the hospital with diazinon poisoning (Shankar 1967).

Exophthalmos has been reported in male Wistar rats receiving single doses of 50–700 mg/kg diazinon by gavage (Boyd et al. 1969; Boyd and Carsky 1969). No ocular effects were reported in Sprague-Dawley rats receiving a single dose of up to 528 mg/kg diazinon and observed for a further 14 days (Chow and Richter 1994); in Sprague-Dawley rats receiving up to 212 mg/kg/day diazinon from feed for 13 weeks (Singh 1988), or up to 12 mg/kg/day for 98 weeks (Kirchner et al. 1991); or in Beagle dogs receiving up

to 11.6 mg/kg/day diazinon from feed for 13 weeks (Barnes 1988) or up to 9 mg/kg/day for 52 weeks (Rudzki et al. 1991).

Body Weight Effects. Dogs administered diazinon by the oral route in an intermediate-duration study exhibited significant weight loss at doses >10 mg/kg/day. Reduced food intake, diarrhea, and emesis were also reported in this study (Earl et al. 1971). The body weight effects are probably a result of the emesis, diarrhea, generalized emaciation, and anorexia reported in the study. Significant ($p<0.05$) reductions in body weight gain were also found in male Wistar rats treated orally with 0.5 mg/kg diazinon twice a week for 28 weeks. Body weight was significantly greater in 28-week controls (602.5 g) than in diazinon-treated rats (542.0 g) despite the absence of significant deviations in average daily food intake (Anthony et al. 1986). Other male Wistar rats receiving 10 mg/kg/day diazinon by oral gavage for 7 weeks exhibited 22% lower terminal mean body weight than vehicle controls (Ogutcu et al. 2006).

Significant reductions in maternal weight (5.5–9.6%) and weight gain were seen in CD-1 rats receiving 100 mg/kg/day diazinon by gavage during gestation days 6–15 (Infurna and Arthur 1985). This effect was most striking during gestation days 6–10 when the 100 mg/kg/day group lost on average 11 grams while the control group gained 14 grams. A 25% decrease in body weight gain was seen in male Sprague-Dawley rats receiving a single gavage dose of 264 mg/kg diazinon and observed for a period of 14 days (Chow and Richter 1994). Male Sprague-Dawley rats receiving 150.8 mg/kg/day diazinon from feed had a 15% decrease in body weight compared to controls after 6 weeks (Singh 1988). Weight gain in females was unaffected. Emaciation was observed in female Beagle dogs receiving 15.99 mg/kg/day diazinon from feed for 4 weeks; less severe, but still significant, weight loss was observed in male Beagle dogs receiving 14.68 mg/kg/day (EPA 2000a). Significantly reduced rates of body weight gain were observed in male Beagle dogs receiving 10.9 mg/kg/day diazinon from feed (34%) and in females receiving 5.6 mg/kg/day (33%) for 13 weeks (Barnes 1988). Reduced body weight gain was also noted in male Beagle dogs receiving 4.7 mg/kg/day diazinon for 52 weeks (Rudzki et al. 1991). A clear dose-response for body weight gain was not detected in similarly-treated female dogs, but the highest dose level (9.1 mg/kg/day) resulted in a 19% reduction in body weight gain (Rudzki et al. 1991).

No effects on body weight were observed in New Zealand rabbit dams receiving 100 mg/kg/day diazinon by gavage during gestation days 6–18 (Harris and Holson 1981). No effect on body weight was observed in female Wistar rats receiving up to 1.35 mg/kg/day from feed for 92 days (Davies and Holub 1980a); in Wistar rats of both sexes receiving 0.21 mg/kg/day from feed for 7 days or 2.86 mg/kg/day for 30 days (Davies and Holub 1980b); and in Sprague-Dawley rats receiving up to 212 mg/kg/day from feed for

13 weeks (Singh 1988), 0.18 mg/kg/day from drinking water for 6 months (Lox and Davis 1983), or up to 12 mg/kg/day for 98 weeks (Kirchner et al. 1991). No effects on body weight were observed in male Beagle dogs receiving 5 mg/kg/day and females receiving 10 mg/kg/day in capsules daily for 8 months (Earl et al. 1971).

Metabolic Effects. Metabolic acidosis was reported in patients who had ingested 240–916 mg/kg diazinon (Klemmer et al. 1978).

No effect on blood electrolytes was observed in Sprague-Dawley rats receiving up to 12 mg/kg/day diazinon orally for 98 weeks (Kirchner et al. 1991).

3.2.2.3 Immunological and Lymphoreticular Effects

A summary of autopsy findings of 76 cases of acute diazinon poisoning described signs that included congested spleen (Limaye 1966).

Single oral administration of 50–700 mg/kg diazinon to male albino Wistar rats resulted in a reduction in spleen weight (35%) and splenic red pulp contraction, reduced thymus weight, and thymic atrophy ranging from minor to near total loss of thymocytes (Boyd and Carsky 1969). In an intermediate-duration study of dogs administrated oral doses of 2.5–20 mg/kg/day diazinon, the spleen of an anorexic and emaciated male dog given 10 mg/kg/day diazinon was markedly shrunken and pale in appearance with moderate atrophy in the splenic pulp prior to death after 232 days of exposure (Earl et al. 1971). The splenic atrophy reported in this study may be a result of the generalized emaciated condition of the dog due to diarrhea, emesis, and anorexia, as reported in the study.

Repeated oral administration of diazinon to mice (50 mg/kg/day for 30 days) resulted in significantly increased levels of interleukin-10 in splenic lymphocyte subpopulations CD4+, CD8+, and B cells and significantly decreased levels of interferon-γ in B cells (Alluwaimi and Hussein 2007). These results indicate a diazinon-induced effect on cytokines involved in the regulation of cellular and humoral responses.

No gross or histological evidence of treatment-related damage to the spleen or thymus was observed in Sprague-Dawley rats receiving up to 212 mg/kg/day diazinon from feed for 13 weeks (Singh 1988), or up

to 12 mg/kg/day for 98 weeks (Kirchner et al. 1991), or in Beagle dogs receiving up to 11.6 mg/kg/day diazinon for 13 weeks (Barnes 1988) or up to 9 mg/kg/day for 52 weeks (Rudzki et al. 1991).

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

3.2.2.4 Neurological Effects

In humans, typical signs and symptoms of cholinesterase poisoning have been widely reported following intentional or accidental ingestion of diazinon (Balani et al. 1968; Bichile et al. 1983; Dagli et al. 1981; Hata et al. 1986; Jaksa and Palahniuk 1995; Kabrawala et al. 1965; Klemmer et al. 1978; Poklis et al. 1980; Reichert et al. 1977; Wadia et al. 1974; Wedin et al. 1984). Reported signs and symptoms of diazinon poisoning included vomiting, abdominal pain, giddiness, excessive sweating, diarrhea, brachycardia, tachycardia, muscle fasciculations, hyperreflexia, restlessness, constricted pupils, miosis, clonus, weakness, bronchospasm, stupor, and coma. In a few of these reports, oral diazinon dose estimates ranged from approximately 200 to 1,000 mg/kg (Klemmer et al. 1978; Poklis et al. 1980). In one case, a neurological examination showed lateral nystagmus and gross incoordination (Bichile et al. 1983). In some cases, measurements of plasma and blood ChE activities indicated significant reduction (Klemmer et al. 1978). Diazinon-poisoned patients responded well to ChE-reactivating agents such as atropine and pralidoxime (Dagli et al. 1981; Kamha et al. 2005; Klemmer et al. 1978). Autopsy findings in patients who died following acute diazinon poisoning include spinal hemorrhage and congestion, swelling, and hemorrhage of the brain (Limaye 1966; Poklis et al. 1980).

As discussed in detail in Section 3.5. Mechanisms of Action, diazinon poisoning is characterized by the inhibition of AChE in the central and peripheral nervous system. AChE is also present in erythrocytes (RBCs). In *in vitro* assays, roughly equivalent inhibition of AChE in RBCs and neural tissues is produced by a given concentration of organophosphates such as diazinon (Iyaniwura 1991). Therefore, inhibition of RBC AChE can be used as a surrogate indicator of the extent of inhibition of neural AChE. Blood plasma also contains other cholinesterases. In humans, plasma ChE is almost exclusive composed of butyrylcholinesterase, which is capable of hydrolyzing acetylcholine and butyrylcholine *in vitro*. The *in vivo* substrate of plasma ChE is unknown. In general, plasma ChE can be inhibited by diazinon at lower levels of exposure than those required to inhibit neural or RBC AChE (Barnes 1988; Singh 1988). Plasma ChE activity is considered to be a sensitive indicator of exposure to organophosphates such as diazinon, but not an indicator of a neurologic effect.

Results of a few controlled studies of human subjects were submitted to the U.S. EPA Office of Prevention, Pesticides and Toxic Substances (OPPT) (EPA 2001). Inhibition of plasma ChE was observed following ingestion of gelatin capsules containing diazinon (EPA 2001). Single doses of 0.12 or 0.2 mg diazinon/kg (purity not specified in the data evaluation record available to ATSDR) resulted in approximately 40 and 60 % inhibition of plasma ChE, respectively. Approximately 90% inhibition was observed in a single volunteer given a 0.3 mg/kg dose of diazinon. Maximum inhibition of plasma ChE in these volunteers was achieved between 4 and 8 hours postdosing. Recovery began 24 hours postdosing, but was only about 70% complete in some volunteers at 15 days postdosing. Although plasma ChE inhibition was noted, RBC AChE was not inhibited and there were no clinical signs of treatment-related neurotoxicity even at the highest dose tested.

In a repeated-dose oral investigation, four male volunteers were administered 0.03 mg diazinon/kg/day (purity 99.5%) in gelatin capsules at breakfast for 28–31 days (EPA 2001). Maximum plasma ChE inhibition reached 47–56% near the end of the treatment period. Recovery was 86–92% by day 28 following the cessation of treatment. There was no apparent treatment-related effect on RBC AChE activity.

In another controlled human study, diazinon (purity not specified in the EPA summary review available to ATSDR) was administered to male volunteers (apparently three males per dose level) at doses of 0.02, 0.025, or 0.05 mg/kg/day (EPA 2001). There was no indication of treatment-related effects on plasma ChE activity following dosing at 0.02 mg/kg for up to 37 days. At 0.025 mg/kg/day, a 23% plasma ChE inhibition was noted from day 12 through day 43. The 0.05 mg/kg/day dose level resulted in 40% plasma ChE inhibition after 5 days of treatment. No treatment-related effects on RBC AChE activity were seen at any of the dose levels.

Results of animal studies support the findings in humans of diazinon-induced neurotoxicity. For example, single oral gavage doses in the range of 75–300 mg/kg, administered to rats (Boyd and Carsky 1969; Chow and Richter 1994; EPA 1996, 2000a; Moser 1995; Moser et al. 2005) or rabbits (Harris and Holson 1981), resulted in signs of cholinergic stimulation such as muscle fasciculations, tremors, miosis, lacrimation, diarrhea, gait changes, and hypoactivity. Robens (1969) reported cholinergic signs in pregnant rabbits administered technical diazinon (purity unspecified) by daily oral gavage on gestation days 5–15 at a dose level of 30 mg/kg/day and in hamsters dosed on gestation days 6–8 at a level of 0.125 mg/kg/day. However, no supporting studies were located to confirm clinical signs of diazinon-

induced neurotoxicity at these relatively low acute dose levels. In a 92-day feeding study in rats, daily doses of 168 mg/kg/day (females) or 212 mg/kg/day (males) resulted in signs of cholinergic stimulation, but doses of 15 and 19 mg/kg/day, respectively, did not elicit clinical signs of neurotoxicity (Singh 1988). Cholinergic signs (muscle fasciculations, emesis, and/or diarrhea) were reported in 3/3 male and 3/3 female dogs administered diazinon in the food at a concentration resulting in a calculated dose of 20 mg diazinon/kg/day for 8 months; only 1/6 dogs in the next lower dose group (10 mg/kg/day) exhibited cholinergic signs (Earl et al. 1971). Similarly-treated swine exhibited clinical signs of neurotoxicity at a dose level of 10 mg/kg/day, but not at 5 mg/kg/day (Earl et al. 1971). In a chronic-duration rat study, 98 weeks of diazinon treatment in the food resulted in no treatment-related clinical signs of neurotoxicity at doses up to approximately 12 mg/kg/day (the highest exposure level) (Kirchner et al. 1991). No clinical signs of neurotoxicity were observed in dogs administered diazinon in the diet for up to 52 weeks at concentrations resulting in doses as high as 9 mg/kg/day (Kirchner et al. 1991).

In an extensive study of diazinon-induced neurological effects following single oral dosing, Sprague-Dawley rats of both sexes were treated by gavage with diazinon (88% purity) at doses of 0, 2.5, 150, 300, or 600 mg/kg and observed in a functional observation battery (FOB) of tests (Chow and Richter 1994). Signs of neurotoxicity were seen only at the expected time of peak effect (9–11 hours postdosing) and not at weeks 1 or 2 posttreatment. At the 150 mg/kg dose level, decreased rearing in a 2-minute period and suppressed maze activity (females only), repetitive opening and closing of the mouth, ataxia, and abnormal gait were noted. Additional treatment-related effects at the next higher dose (300 mg/kg) included altered fecal consistency, soiled fur, stained nose, impaired righting reflex and hindlimb extensor reflex, decreased rearing in a 2-minute period and suppressed maze activity (males), tremors, body twitch, and lowered arousal (females only). Treatment at 600 mg/kg resulted in impaired respiration, lacrimation, reduced forelimb and hindlimb grip strength, decreased hindlimb foot splay, abnormal hindlimb positioning, decreased tail pinch response, lowered arousal level (males), and reduced touch response (females). No treatment-related gross or histopathologic lesions were observed in brain, spinal cord, peripheral nerves, skeletal muscle, eyes, or optic nerve at doses up to and including the highest dose tested (600 mg/kg).

Most available oral animal studies include assessments of diazinon-induced changes in RBC and/or neural AChE activity. Many of these studies were particularly designed to assess AChE activity at diazinon doses below those eliciting clinical signs of neurotoxicity. Diazinon-induced decreased AChE activity may be a sensitive indicator of neurotoxicity, and a 20–59% inhibition of neural or RBC AChE is considered a less serious effect in the absence of more serious indicators of neurotoxicity. Results of

several rat and dog studies identified diazinon-induced RBC and/or brain AChE inhibition of 20% or more following acute-, intermediate-, and chronic-duration oral exposure at doses in the range of 2–15 mg/kg/day (Barnes 1988; EPA 1996, 2000a; Makhteshim-Agan 1989; Trutter 1991). For example, single oral gavage dosing of rats at 2.5 mg/kg resulted in 40% RBC AChE inhibition (EPA 2000a). In a 28-day feeding study, male and female rats receiving diazinon at 2.4 mg/kg/day exhibited 38% RBC AChE inhibition by the end of the first week of treatment (EPA 1996). Davies and Holub (1980a) reported 9, 20, and 22% RBC AChE inhibition in female rats receiving diazinon (99.2% purity) in the diet for 42 days at concentrations of 2, 3, and 4 ppm (0.18, 0.27, and 0.36 mg/kg/day), respectively. This study was specifically designed to assess low-dose cholinesterase responses to oral diazinon and serves as the principal study for deriving an intermediate-duration oral MRL for diazinon (see Appendix A). Administration of diazinon in the diet of male and female rats for 98 weeks at a concentration resulting in a dose level of approximately 5 mg/kg/day caused 26–28% RBC AChE inhibition and 24–29% brain AChE inhibition (Kirchner et al. 1991). The next lower dose level (0.07 mg/kg/day in the female rats) represented a NOAEL and serves as the basis for deriving a chronic-duration oral MRL for diazinon (see Appendix A).

Available single and repeated-dose oral studies in animals demonstrate that significant diazinon-induced plasma ChE inhibition occurs at doses lower than those required to produce significant RBC AChE inhibition and that RBC AChE inhibition is a somewhat more sensitive indicator of effect than brain AChE inhibition (Barnes 1988; Davies and Holub 1980a; Kirchner et al. 1991; Rudzki et al. 1991; Singh 1988; Timchalk et al. 2005). Peak cholinesterase inhibition is typically observed between 6 and 12 hours following single oral dosing (Chow and Richter 1994; Timchalk et al. 2005). In a longer-term (90-day) dietary rat study, diazinon-induced plasma ChE and RBC AChE inhibition increased in severity with exposure duration to a peak at approximately 35 days, after which the severity of the inhibition remained relatively constant (Davies and Holub 1980a). Available animal data indicate that females may be more sensitive than males to diazinon-induced cholinesterase inhibition, particularly with respect to brain AChE inhibition (Barnes 1988; Davies and Holub 1980b; EPA 1996, 2000a; Singh 1988; Trutter 1991).

Limited animal data were located regarding potential for diazinon to elicit other neurophysiologic or neurohistopathologic effects. There were no indications of histopathologic lesions in central and peripheral nervous tissue samples from rats administered diazinon once by oral gavage at doses up to 600 mg/kg (Chow and Richter 1994) or in rats administered diazinon in the diet for 90 days at concentrations resulting in doses as high as 212 mg/kg/day (Singh 1988). No clinical signs or

histopathologic evidence of diazinon-induced delayed neuropathy were seen in hens following oral administration of 11.3 mg diazinon/kg on 2 days (21 days apart) (Jenkins 1988).

Refer to Section 3.2.2.6, Developmental Effects, for information regarding diazinon-induced neurodevelopmental effects.

The highest NOAEL values and all reliable LOAEL values for neurological effects in each species and duration category are presented in Table 3-2 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to diazinon. Limited information is available regarding the reproductive toxicity of diazinon in orally-exposed laboratory animals. No adverse effects on reproduction were reported for four generations of female Sprague-Dawley rats fed diazinon in the diet at a concentration resulting in a dose of 0.05 mg/kg/day during gestation and lactation for 60 days prior to weaning (Green 1970). No adverse effect on fertility was observed, as all females became pregnant. Apparently, diazinon exposure increased the average number of pups per litter compared to undosed controls (9.7–11.1 as opposed to 6.2–8) for all five generations (F_0–F_4). Oral administration of diazinon to male albino rats at dose levels of 1.5 or 3 mg/kg/day for 65 days resulted in significantly decreased reproductive tissue weights, increased percentage of dead and morphologically abnormal spermatozoa, decreased plasma testosterone levels, and decreased fertility as assessed by conception rates of untreated females mated to diazinon-treated males (Abd El-Aziz et al. 1994). In a study of hybrid mice, litter size was reduced by 20% at oral maternal diazinon doses of 0.18, but not at 9 mg/kg/day relative to controls (Spyker and Avery 1977). A 14% reduction in maternal weight gain was observed at both doses. Male and female Beagle dogs were given daily capsules containing diazinon in corn oil at doses ranging from 2.5 to 20 mg/kg/day for 8 months (Earl et al. 1971). Testicular atrophy with completely arrested spermatogenesis was observed in the one male dog of the 10 mg/kg/day group that lost weight and evidenced other gross pathological changes. All three male dogs in the 20 mg/kg/day group suffered similar effects (testicular atrophy observed in 2/3, arrested spermatogenesis observed in the other dog).

Administration of diazinon at 10, 20, or 100 mg/kg/day by oral gavage during gestation days 6–15 in CD-1 rats had no significant effect on litter sizes or numbers of viable fetuses (Infurna and Arthur 1985). Maternal toxicity was noted at 100 mg/kg/day with a significant reduction in feed consumption and body

weight gain. In New Zealand rabbits dosed by gavage at 7, 25, or 100 mg/kg/day diazinon during gestation days 6–18, no treatment-related effects were seen in number of implantations, proportion of live, dead or resorbed fetuses, fetal weights, or fetal sex ratios (Harris and Holson 1981). The highest dose level (100 mg/kg/day) resulted in the death of 9/22 dams.

No gross or histological evidence of treatment-related damage to reproductive tissues (ovaries, uterus, vagina, epididymides, seminal vesicles, testes) was observed in Sprague-Dawley rats exposed to up to 168 mg/kg/day diazinon (males) or 212 mg/kg/day (females) for 13 weeks via feed (Singh 1988), or up to 10 mg/kg/day (males), or 12 mg/kg/day (females) for 98 weeks (Kirchner et al. 1991), or in Beagle dogs exposed to up to 10.9 mg/kg/day (males) or 11.6 mg/kg/day (females) for 13 weeks (Barnes 1988).

The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species and duration category are presented in Table 3-2 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

No studies were located regarding developmental effects of diazinon in orally-exposed humans. Mouse studies provide evidence that lactational exposure to diazinon does not cause developmental toxicity. Results of other studies in rats, mice, hamsters, and rabbits also have not demonstrated dose-response effects on the developing mammalian fetus or neonate. The adverse effects reported for pups have been suggested to derive from diazinon impairment of placental transport of nutrients or maternal regulation of fetal growth, or directly via antagonism to cholinergic development of the fetus. No significant effects were seen in rabbit offspring at maternally lethal doses.

In a teratology study, mouse dams were administered doses of 0, 0.18, or 9 mg/kg/day diazinon (technical grade, purity not specified) in peanut butter throughout gestation. The study found no maternal toxicity at any of the doses tested. Significantly elevated ($p<0.05$) mortality (12%, 18 of 150) was observed in the high dose group at weaning (postpartum day 28), but not in the low dose group (2%, 3 of 134), when compared with controls (6%, 19 of 311). Histological examination indicated that the majority of these pups died from pulmonary congestion and mucosal infiltration consistent with acute bronchitis. Diazinon treatment did not adversely affect postweaning mortality. Lactational exposure to diazinon did not have any adverse effect (Barnett et al. 1980). A previous study using the same protocol and dose regimen exposed dams throughout gestation to doses of 0, 0.18, or 9 mg/kg/day diazinon in peanut butter (Spyker and Avery 1977). Dams exposed to either diazinon dose experienced reduced weight gain (86% that of

controls, $p<0.05$) during pregnancy, but gestation length was not significantly affected. Pup weight gain during the first 14 weeks after parturition was significantly ($p<0.05$) less at the 9 mg/kg/day dose than at the 0 and 0.18 mg/kg/day doses. With the exception of contact placing and sexual maturity, which were delayed with respect to controls ($p<0.05$) in the low dose pups, developmental ontogeny as measured by numerous parameters was not significantly affected by diazinon exposure. No teratological effects were evident. However, both diazinon groups displayed endurance and coordination deficits during neuromuscular function tests (rod cling and inclined plane), and 9 mg/kg/day offspring also displayed slower running speed in a Lashley III maze and reduced swimming endurance. Morphologically, the brains of 9 (but not 0.18) mg/kg/day offspring had focal abnormalities in the forebrain area, including dense aggregations of atypical chromatin-containing cells. Among the offspring of hybrid mouse dams exposed to 0.18 or 9 mg/kg/day diazinon during gestation days 1–18, females from the 9 mg/kg/day group showed a 33% decrease of serum IgG_1 levels 101 days after birth (Barnett et al. 1980). These levels were normal at 400 and 800 days after birth, and no effects on serum Ig levels were observed in male offspring at either dose. Fetal exposure to low levels of diazinon may result in functional deficits in otherwise normal animals that can only be detected by systemic behavioral evaluation. These neural dysfunctions and pathologies might occur either indirectly through diazinon impairment of placental transport of nutrients or maternal regulation of fetal growth, or directly via antagonism to cholinergic development of the fetus (Spyker and Avery 1977).

Pregnant Golden Syrian hamsters were orally exposed by gavage to diazinon during organogenesis (0.125 mg/kg/day to eight dams on gestation day 6, 7, and 8; 0.25 mg/kg/day to five dams on gestation day 7 or 8). All dams survived, but displayed cholinergic signs of diarrhea, salivation, and ataxia. No terata were observed at either dose, nor were average number of fetuses per litter, fetal mortality, or average fetal weight adversely affected. Thus, at maternally toxic doses, diazinon was not feto- or developmentally toxic to hamsters (Robens 1969).

Diazinon was not fetotoxic or developmentally toxic to rabbits at maternally lethal doses. When pregnant New Zealand white rabbits were orally exposed by gel capsules to 7 or 30 mg/kg/day diazinon on day 15 of gestation, 6/8 of the dams in the high dose group died. The dams in this dose group also exhibited severe cholinergic signs. However, no terata or dose-related embryotoxic effects (average number of fetuses per litter, fetal mortality, average fetal weight) were observed even at maternally toxic doses (Robens 1969). In a study of New Zealand rabbit does exposed by gavage to 7, 25, or 100 mg/kg/day diazinon during gestation days 6–18 and sacrificed on gestation day 25, no significant treatment-related fetal malformations or skeletal malformations were observed in the offspring (Harris and Holson 1981).

Nine of the 22 dams in the 100 mg/kg/day group died during the study, indicating that significant maternal toxicity occurred at this dose.

An increased incidence of rudimentary ribs at T-14 was observed in CD-1 rats receiving 100 mg/kg/day diazinon during gestation days 6–15 (Infurna and Arthur 1985). This finding was accompanied by severe weight loss in the dams and this developmental effect was considered by the authors of this study to be secondary to maternal toxicity.

The highest NOAEL values and all reliable LOAEL values for developmental effects in each species and duration category are presented in Table 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

Several epidemiological studies have reported increased incidence of cancers in humans who were concurrently or sequentially exposed to a number of insecticides, including diazinon. Some degree of oral exposure is presumed to have occurred. However, it is not possible to attribute the increased cancer incidence exclusively to diazinon exposure.

A case-control study suggested a possible link between family gardening use of diazinon (and other insecticides) and increased incidence of childhood brain cancer (type unspecified). However, this report gave no indication of level, duration, or frequency of exposure to diazinon (or to other insecticides) (Davis et al. 1993). Another case-control study suggested a positive association between an increased incidence of non-Hodgkin's lymphoma in farmers compared to nonfarmers. The report attributed the increased incidence of lymphomas to handling of organophosphorus insecticides, including diazinon (Cantor et al. 1992). A third case-control study suggested an association between an increased incidence of multiple myelomas and high exposure to insecticides, including diazinon. Actual exposure to diazinon was reported in 2/698 (0.3%) of the cases and 5/1,683 (0.3%) of the controls (Morris et al. 1986).

A cancer bioassay was conducted with groups of Fischer 344 rats (50/sex/group) exposed *ad libitum* to estimated dietary doses of 20 or 40 mg/kg/day diazinon for 103 weeks; groups of 25 rats/sex served as unexposed controls. Tissue masses were noted especially in high-dose males and low-dose females, and tachypnea incidence was elevated in exposed groups. A variety of neoplastic and nonneoplastic lesions was observed with approximately equal frequency in the control and dosed groups in both sexes. An increase in the common lesion of endometrial stromal polyps observed in female rats (control=2/23, low

dose=8/43, high dose=11/49) was considered unrelated to diazinon exposure. In male rats, lymphomas and leukemias were significantly ($p<0.011$) elevated in the low dose group (25/50), but not in the high dose group (12/50), relative to controls (5/25). The study concluded that diazinon was not carcinogenic under the conditions of this assay for either sex of Fischer 344 rats (NCI 1979). In another cancer bioassay, groups of $B6C3F_1$ mice (50/sex/group) were exposed for 103 weeks to estimated dietary doses of 13 or 26 mg/kg/day diazinon; groups of 25 mice/sex served as unexposed controls. A number of neoplastic and nonneoplastic lesions, essentially considered nontreatment-related, were observed in both the control and treated mice. An elevation in hepatocellular adenomas and carcinomas was observed in low-dose (20/46), but not high-dose (13/48) male mice, relative to controls (5/21). The study concluded that diazinon was not carcinogenic under the conditions of this assay for either sex of $B6C3F_1$ mice (NCI 1979).

3.2.3 Dermal Exposure

3.2.3.1 Death

No studies were located regarding death in humans after dermal exposure to diazinon.

In laboratory animal studies, the acute dermal toxicity of diazinon and its formulations varies profoundly, largely as the result of sample aging and differences in purity of formulation, particular solvent, and area of exposed skin. In general, aged diazinon samples that contained more impurities were more toxic (Gaines 1960). The use of an occlusive dressing after dermal application usually increases dermal toxicity because it enhances sweating and dermal absorption. Dermal LD_{50} values were determined in Sherman rats of both sexes (Gaines 1960). Diazinon was applied to a shaved dermal area of approximately 13.5 cm^2. LD_{50} values were 900 and 455 mg/kg for males and females, respectively. Among New Zealand rabbits dermally exposed for 24 hours to 2,020 mg/kg diazinon, 2/5 females and 0/5 males died within 2 days after exposure ceased (EPA 1990).

The LD_{50} values and doses associated with death in each species and duration category are shown in Table 3-3.

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

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
ACUTE EXPOSURE							
Death							
Rat (Sherman)	once				900 M mg/kg (LD50)	Gaines 1960	Technical grade diazinon (purity not specified).
					455 F mg/kg (LD50)		
Systemic							
Human	72 hr	Dermal	1 Percent (%)			Lisi et al. 1987	
Gn Pig (Hartley)	24 hr	Dermal	5 F Percent (%)	10 F Percent (%) (erythema)		Matsushita et al. 1985	Diazinon (purity not specified).
Rabbit (New Zealand)	4 hr	Dermal		0.5 B colonies per 100 milliters (erythema, slight edema)		EPA 1990	
Immuno/ Lymphoret							
Human	once		1 Percent (%)			Lisi et al. 1987	
Neurological							
Rat (Sprague-Dawley)	once (C)			65 F mg/kg (52% RBC AChE inhibition)		Abu-Qare and Abou-Donia 2001	

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

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
INTERMEDIATE EXPOSURE							
Systemic							
Rat (dark agouti)	12 wk 7 d/wk 1 x/d	Hepatic		114 F mg/kg (elevated fecal porphyrin)		Bleakley et al. 1979	
Immuno/ Lymphoret							
Gn Pig (Hartley)	24 hr			0.05 F Percent (%) (moderate delayed contact sensitivity)		Matsushita et al. 1985	

AChE = acetylcholinesterase; (C) = capsule; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = Female; Gn pig = guinea pig; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); mo = month(s); NOAEL = no-observed-adverse-effect level; RBC = red blood cell; x = time(s); wk = week(s)

3.2.3.2 Systemic Effects

No studies were located regarding musculoskeletal, renal, ocular, or body weight effects in humans after dermal exposure to diazinon. No studies were located regarding cardiovascular, hematological, musculoskeletal, renal, endocrine, or ocular effects in animals after dermal exposure to diazinon.

Respiratory Effects. A 56-year-old female gardener, dermally exposed to spilled diazinon of unknown purity, developed respiratory distress as one of the cholinergic symptoms of AChE inhibition. The victim exhibited pulmonary edema with bilateral lung crepitations and tachypnea (Lee 1989).

Nasal discharge was observed in New Zealand rabbits of both sexes following dermal exposure for 24 hours to 2,020 mg/kg diazinon (EPA 1990).

Cardiovascular Effects. A 56-year-old female gardener, dermally exposed to spilled diazinon of unknown purity, developed sinus tachycardia with no evidence of infarction and showed increased cardiac enzyme (serum glutamate oxalate transaminase, total lactate dehydrogenase creatine phosphokinase) levels. The victim was diagnosed on discharge with acute left ventricular failure (Lee 1989).

Gastrointestinal Effects. Two female gardeners, dermally exposed to spilled diazinon of unknown purity, developed signs of acute pancreatitis which included abdominal colic, diarrhea, nausea, vomiting, and epigastric pain, as well as elevated serum amylase and urinary diastase levels (Lee 1989).

Both decreased defecation and diarrhea were observed in New Zealand rabbits of both sexes following dermal exposure for 24 hours to 2,020 mg/kg diazinon (EPA 1990).

Hematological Effects. Two female gardeners, dermally exposed to spilled diazinon of unknown purity, developed hypokalemia and leucocytosis (Lee 1989).

Hepatic Effects. Female dark Agouti rats received daily cutaneous doses of either 114 or 229 mg/kg/day diazinon. Significant elevations in total fecal porphyrin excretion were observed at the 114 mg/kg/day dose after 8–12 weeks (3–5-fold), and at the 229 mg/kg/day dose at least by week 12 (4-fold). No concomitant rises in urinary porphyrin excretion were observed. Electrophoretic analysis revealed the presence of isocoporphyrin in the feces. Except for the unexplained lack of urinary

porphyrin, these findings were noted to be biochemically characteristic of human porphyria cutanea tarda, and indicative of disturbed hepatic porphyrin metabolism. However, oral administration of 46 mg/kg/day to another group of rats was without this effect (Bleakley et al. 1979).

Endocrine Effects. Two female gardeners, 56 and 48 years old, dermally exposed to spilled diazinon of unknown purity, developed signs of acute pancreatitis, which included abdominal colic, diarrhea, nausea, vomiting, and epigastric pain, as well as elevated serum amylase and urinary diastase levels. One of the victims was diagnosed on discharge with organophosphate poisoning and diabetes mellitus. The authors of this study noted that acute pancreatitis is frequently a component of organophosphate intoxication, although it is often not recognized as such in the medical literature or by treating physicians (Lee 1989).

Dermal Effects. Dermal exposure to diazinon resulted in contact dermatitis in farm workers (Matsushita et al. 1985). But, according to another report, a 1% diazinon solution in a skin patch did not elicit an irritation or cause sensitization in humans (Lisi et al. 1987).

Skin erythema was noted in guinea pigs dermally exposed to 10 and 20% diazinon, but not at lower concentrations of 0.5–5.0% (Matsushita et al. 1985). Well defined erythema and slight edema were observed in New Zealand rabbits of both sexes following dermal exposure for 4 hours to 0.5 mL of diazinon (EPA 1990).

Body Weight Effects. Body weight was unaffected in New Zealand rabbits dermally exposed to up to 2,020 mg/kg diazinon for 24 hours and observed for 14 days (EPA 1990).

3.2.3.3 Immunological and Lymphoreticular Effects

One percent diazinon in "pet" (presumably petroleum ether) has been tested for allergic reactions by patch tests in 294 volunteers examined after 48 and 72 hours of dermal contact (Lisi et al. 1987). The 1% diazinon solution on a skin patch did not elicit allergic reactions in any of the volunteers studied.

Diazinon has also been tested for delayed contact hypersensitivity following skin application to guinea pigs. Induction concentrations of diazinon were reported as 5% (intradermal) and 25% (topical). At both 24 and 48 hours after challenge in the guinea pig maximization test, response to a 0.05% diazinon challenge was scored as grade III (moderate, 30% sensitization rate), and to 0.5% diazinon as grade V

(extreme, 100% rate). When cross-sensitization was tested using a challenge of 0.2 or 2% benomyl, allergenicities were grade I (0%) and grade III (30%), respectively (Matsushita et al. 1985). Skin sensitization did not occur in Hartley guinea pigs treated 11 times over a 36-day period with 0.5 mL of diazinon (Kuhn 1989b).

3.2.3.4 Neurological Effects

Two female gardeners, 56 and 48 years old, dermally exposed to spilled diazinon of unknown purity, developed cholinergic organophosphate poisoning symptoms. The victims exhibited signs and symptoms which included cyanosis, frothing at the mouth, drowsiness, nausea, vomiting, abdominal colic, diarrhea, tachypnea, miosis, and sinus tachycardia with no evidence of infarction. One victim showed significantly depressed plasma ChE levels (Lee 1989).

Tremors were reported in female (but not male) New Zealand rabbits after 24 hours of dermal exposure to 2,020 mg/kg diazinon (EPA 1990).

No studies were located regarding OPIDN in humans or in animals after dermal exposure to diazinon.

3.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals following dermal exposure to diazinon.

3.2.3.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after dermal exposure to diazinon.

3.2.3.7 Cancer

Several epidemiological studies have reported increased incidence of cancers in humans who were concurrently or sequentially exposed to a number of insecticides, including diazinon. Some degree of dermal exposure is presumed to have occurred. However, it is not possible to attribute the increased cancer incidence exclusively to diazinon exposure.

A case-control study suggested a possible link between family gardening use of diazinon (and other insecticides) and increased incidence of childhood brain cancer (type unspecified). However, this report gave no indication of level, duration, or frequency of exposure to diazinon, or to other insecticides (Davis et al. 1993). Another case-control study suggested a positive association between an increased incidence of non-Hodgkin's lymphoma in farmers compared to nonfarmers. The report attributed the increased incidence of lymphomas to handling of organophosphorus insecticides, including diazinon (Cantor et al. 1992). A third case-control study suggested an association between an increased incidence of multiple myelomas and high exposure to insecticides, including diazinon. Actual exposure to diazinon was reported in 2/698 (0.3%) of the cases and 5/1,683 (0.3%) of the controls (Morris et al. 1986).

No studies were located regarding cancer in animals after dermal exposure to diazinon.

3.2.4 Other Routes of Exposure

This section contains diazinon toxicity data from injection studies that reported effects not observed in studies using natural (inhalation, oral, or dermal) exposure routes. *In vitro* diazinon toxicity data are included as well.

Slotkin and coworkers (Jameson et al. 2007; Slotkin et al. 2006a, 2006b, 2007) reported evidence of diazinon-induced neurodevelopmental effects in the forebrain and brainstem of neonatal rats at dose levels near or below those eliciting significant cholinesterase inhibition, the most commonly-observed indicator of diazinon toxicity. In these studies, newborn rats were subcutaneously injected with diazinon on postnatal days 1–4 at doses ranging from 0.5 to 2 mg/kg/day. Slotkin et al. (2006a) noted impaired neuritic outgrowth, evidenced by treatment-related decreased ratio of membrane protein to total protein, and a dose-dependent deficit in choline acetyltransferase activity (a constitutive marker of cholinergic projections) in the absence of an effect on hemicholinium-3 binding to the presynaptic choline transporter (an index of cholinergic neuronal activity). Diazinon-induced up-regulation of 5HT receptors and 5HT transporter was reported by Slotkin et al. (2006b). Diazinon-induced regional suppression of selected fibroblast growth factors, neurotrophic factors that play critical roles in neuronal development and recovery from injury, were reported by Slotkin et al. (2007).

Diazinon inhibited the outgrowth of axon-like processes in mouse N2a neuroblastoma cells *in vitro* at a concentration (1 μM) causing slight AChE inhibition but not affecting cell viability (Flaskos et al. 2007).

3.3 GENOTOXICITY

Chronic occupational exposure to multiple insecticides, including diazinon, has been associated with an increased incidence of chromosomal aberrations and increased sister chromatid exchanges in peripheral blood lymphocytes as compared with nonexposed populations (De Ferrari et al. 1991; Kiraly et al. 1979; See et al. 1990). Some of these exposures are presumed to be by inhalation. However, it is not possible to attribute the results of these studies to diazinon alone, as workers were exposed to up to 80 different insecticides in unknown amounts for variable durations.

Limited information is available regarding the *in vivo* genotoxicity of diazinon. Significantly increased sister chromatid exchanges were noted in peripheral blood lymphocytes from a group of volunteers following exposure to diazinon in a sheep-dip formulation (approximately 45% diazinon); the magnitude of the increase in sister chromatid exchanges was approximately 2-fold greater than the pre-exposure sister chromatic exchange rate (Hatjian et al. 2000). However, the specific role of diazinon in the observed effect could not be determined because the sheep-dip formulation contained other ingredients as well. Diazinon (95% purity) induced mutations in a wing somatic mutation and recombination test (SMART) of *Drosophila melanogaster* (Çakir and Sarikaya 2005). Diazinon did not induce sister chromatid exchanges in the bone marrow of mice administered diazinon (88% purity) in single 100 mg/kg gavage dose (EPA 1990).

Results of *in vitro* laboratory testing for diazinon-induced genotoxicity in mammalian cells and microorganisms are equivocal (see Table 3-4). Diazinon induced gene mutations in one Ames assay of *Salmonella typhimurium* in the presence (but not the absence) of metabolic activation (Wong et al. 1989). The chemical was not mutagenic in other Ames assays either with (Kubo et al. 2002) or without (Kubo et al. 2002; Marshall et al. 1976) metabolic activation. Diazinon did not induce gene mutation in the rec-assay utilizing strains of *Bacillus subtilis* tested without metabolic activation (Shirasu et al. 1976). In one mouse lymphoma mutagenicity assay, diazinon elicited a mutagenic response in the absence of metabolic activation (McGregor et al. 1988). However, mutagenicity was not indicated in a similar assay of mouse lymphoma cells either with or without metabolic activation (EPA 1988). Diazinon induced chromosomal aberrations in Chinese hamster cells with metabolic activation (Matsuoka et al. 1979), but tested negative for chromosomal aberrations in human peripheral blood lymphocytes (Lopez et al. 1986). Negative results were obtained in a test for sister chromatid exchanges in Chinese hamster V79 cells, both with and without metabolic activation (Chen et al. 1982) and in a test for micronuclei in cultured rat hepatocytes (Frölichstahl and Piatti 1996). A weakly positive result was obtained for micronuclei in

Table 3-4. Genotoxicity of Diazinon *In Vitro*

Species (test system)	End point	Results		References
		With activation	Without activation	
Prokaryotic organisms:				
Bacillus subtilis (rec assay)	Gene mutation	Not done	–	Shirasu et al. 1976
Salmonella typhimurium	Gene mutation	+	–	Wong et al. 1989
S. typhimurium	Gene mutation	Not done	–	Marshall et al.1976
S. typhimurium	Gene mutation	–	–	Kubo et al. 2002
Eukaryotic organisms:				
Mammalian cells:				
Mouse lymphoma cells	Gene mutation	Not done	+	McGregor et al. 1988
Mouse lymphoma cells	Gene mutation	–	–	EPA 1988
Human peripheral blood lymphocytes	Chromosomal aberration	Not done	–	Lopez et al. 1986
Chinese hamster cells	Chromosomal aberrations	+	–	Matsuoka et al. 1979
Chinese hamster cells	Sister chromatid exchange	–	–	Chen et al. 1982
Human peripheral blood lymphocytes	Micronuclei	Not done	(+)	Bianchi-Santamaria et al. 1997
Rat hepatocytes	Micronuclei	–	Not done	Frölichstahl and Piatti 1996
Human primary nasal mucosal cells	DNA damage	Not done	+	Tisch et al. 2002
Transformed PC12 pheochromocytoma cells	Inhibition of DNA synthesis	Not done	+	Qiao et al. 2001
Transformed C6 glioma cells	Inhibition of DNA synthesis	Not done	+	Qiao et al. 2001
Human 1321N1 astrocytoma cells	Inhibition of DNA synthesis	Not done	+	Guizzetti et al. 2005
Fetal rat astrocytes	Inhibition of DNA synthesis	Not done	+	Guizzetti et al. 2005

– = negative result; + = positive result; (+) = weakly positive; DNA = deoxyribonucleic acid

cultured human peripheral blood lymphocytes exposed to diazinon at concentrations ranging from 0.04 to 4 μg/mL (Bianchi-Santamaria et al. 1997). Diazinon-induced deoxyribonucleic acid (DNA) damage was reported in a Comet assay using human primary nasal mucosal cells (Tisch et al. 2002). Diazinon inhibited DNA synthesis in transformed PC12 pheochromocytoma and C6 glioma cells (Qiao et al. 2001), as well as fetal rat astrocytes and human 1321N1 astrocytoma cells (Guizzetti et al. 2005).

3.4 TOXICOKINETICS

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

No studies were located regarding absorption after inhalation exposure of diazinon in humans or animals. However, efficient absorption of inhaled diazinon is expected.

3.4.1.2 Oral Exposure

Diazinon is readily absorbed by the oral exposure route. Rapid and extensive absorption was noted following the ingestion of a 0.011 mg/kg dose of diazinon (94% purity) by a group of five volunteers, as evidenced by the excretion of approximately 60% of the administered dose as dialkyl phosphate metabolites in the urine, most (90%) of which was recovered within 14 hours postadministration (Garfitt et al. 2002). Diazinon was detected in several tissues from a woman who had ingested a lethal amount of an estimated 293 mg/kg diazinon formulation ("FERTI-LOME" bagworm spray) containing 10% diazinon suggesting rapid absorption from the gastrointestinal tract (Poklis et al. 1980).

Animal studies also demonstrate rapid absorption of diazinon following oral administration. Wistar WU rats of both sexes were given either a single oral dose of 4 mg/kg or daily doses of 8.0 mg/kg [^{14}C]diazinon for 10 consecutive days. The rapid absorption of diazinon was indicated by the early excretion of radioactivity (Mücke et al. 1970). Similar results were obtained following a single oral dose of 4.0 mg/kg [^{14}C]diazinon to female Beagle dogs where absorption was determined to be at least 85% (Iverson et al. 1975). Within 30 minutes following the oral administration of an 80 mg/kg dose of diazinon (99.8% purity) to eight male Wistar rats, the mean plasma concentration of diazinon exceeded 0.6 mg/L; by 2 hours postadministration, a peak plasma concentration of 1.22 mg/L was achieved (Wu et al. 1996a). In goats given daily oral doses of 0.5 or 5.0 mg/kg/day diazinon for 7 days or a single 150 or 700 mg/kg dose, diazinon was detected in blood from the first day of treatment (Mount 1984). Other

studies demonstrated rapid absorption of orally administered diazinon in sheep (Janes et al. 1973; Machin et al. 1971, 1974) and cows (Abdelsalam and Ford 1986).

3.4.1.3 Dermal Exposure

Absorption of diazinon following dermal exposure has been demonstrated in humans. Volunteers were exposed for 24 hours to [^{14}C]diazinon applied to either the forearm or abdomen in acetone or lanolin wool grease (Wester et al. 1993). Based on the excretion of ^{14}C in the urine, absorption was determined to be 3–4% of the applied dose with no difference related to vehicle or the area applied. In another study, 8-hour occluded dermal application of 100 mg of diazinon (94% purity) on an 80 cm^2 area of the forearm of four volunteers resulted in approximately 0.5% absorption based on the recovery of urinary dialkyl phosphate metabolites (Garfitt et al. 2002). Greater than 90% of the administered dose was recovered from the application site.

No studies were located regarding absorption of diazinon after dermal exposure in animals.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

No studies were located regarding distribution of diazinon after inhalation exposure in humans or animals.

3.4.2.2 Oral Exposure

Samples of stomach contents, blood, bile, adipose tissue, liver, brain, and kidney were collected at autopsy of a woman who ingested a lethal dose estimated at 293 mg/kg of a diazinon formulation ("FERTI-LOME" bagworm spray) containing 10% diazinon (Poklis et al. 1980). The highest concentrations of diazinon were found in the blood, followed by stomach contents and the bile. Lowest concentrations were found in the kidney, followed by adipose tissue and bile. Animal studies support the human data and demonstrate that diazinon is widely distributed in all analyzed tissues in rats (Mücke et al. 1970), sheep (Janes et al. 1973; Machin et al. 1971, 1974), and cows (Abdelsalam and Ford 1986). Although widely distributed via the circulation, it is generally understood that absorbed diazinon is rapidly metabolized and does not accumulate significantly in body tissues. However, Garcia-Repetto et al. (1996) reported detectable levels of diazinon in blood, adipose tissue, muscle, liver, and brain of rats following oral dosing at approximately 23 mg/kg. In blood, adipose tissue, and brain, the levels

decreased with time from 4 to 20 days postdosing. Levels in muscle and liver increased up to 12 and 8 days postdosing, respectively, and decreased thereafter. By 30 days postdosing, detectable levels were no longer observed in blood, adipose tissue, muscle, liver, or brain.

3.4.2.3 Dermal Exposure

No studies were located regarding distribution of diazinon after dermal exposure in humans or animals.

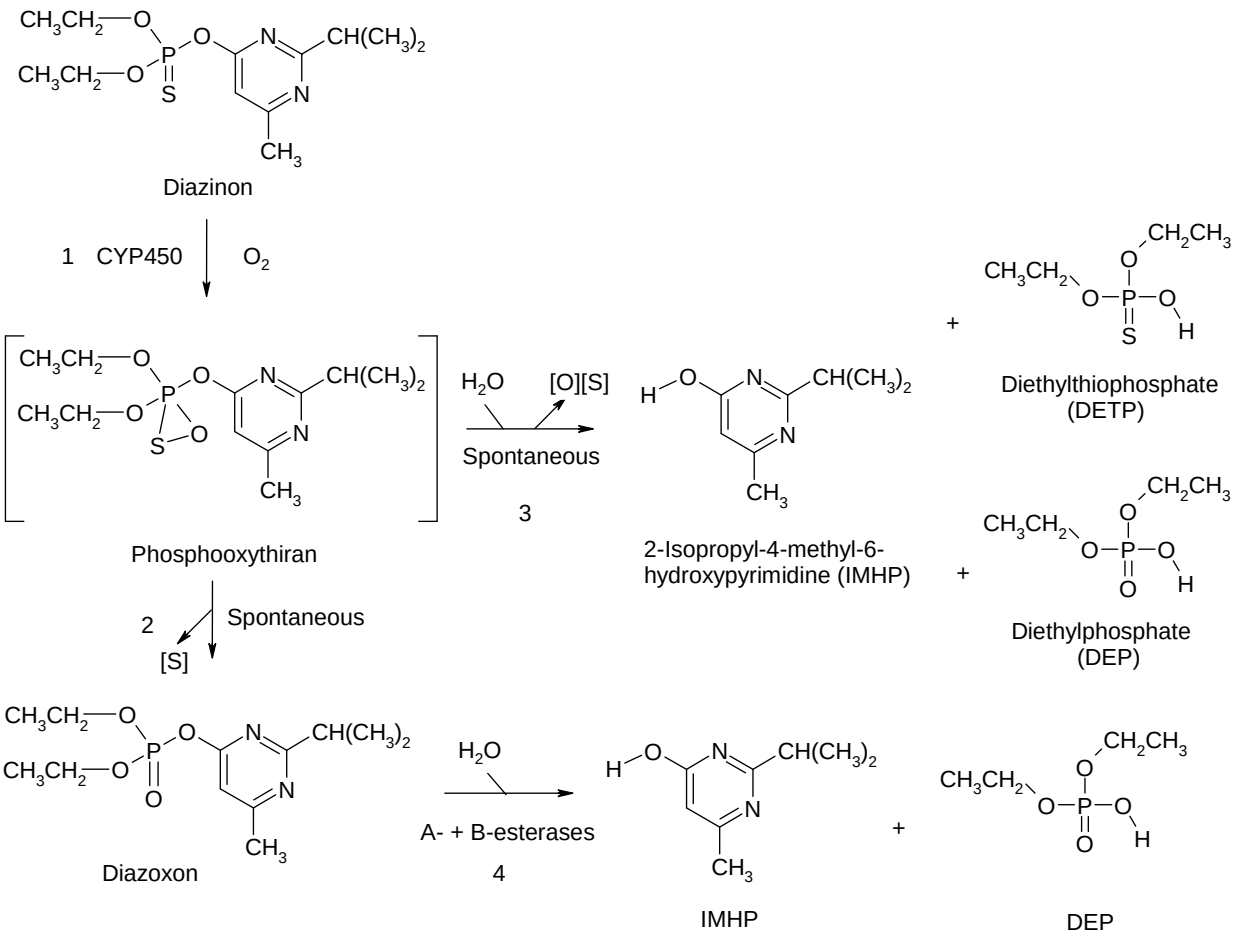
3.4.3 Metabolism

Although diazinon (as parent compound) can elicit mild cholinergic stimulation, its oxygenated metabolite (diazoxon) is mainly responsible for these neurotoxic signs (Wilson 2001). A proposed metabolic scheme for diazinon is presented in Figure 3-3. The CYP450-catalyzed oxidation of diazinon (reaction 1 in Figure 3-3) results in an intermediate (phosphooxythiran), which in turn undergoes spontaneous desulfuration (reaction 2) to form diazoxon. Alternatively, phosphooxythiran may be deactivated via hydrolysis, desulfuration, and deoxygenation (reaction 3) to form metabolites 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP), diethylthiophosphate (DETP), and DEP, all of which are excreted in the urine. Detoxification of diazoxon (reaction 4) to IMHP and DEP occurs via hydrolysis catalyzed by hepatic and extrahepatic A-esterases (paraoxonase or PON1) and B-esterases (carboxylesterases) (Fabrizi et al. 1999; Poet et al. 2003; Yang et al. 1971). Results of several *in vitro* assays using human liver cells implicate CYP2C19 as a major P-450 isozyme involved in the formation of diazoxon from diazinon; other P-450 isozymes (e.g., CYP1A2, CYP2B6, CYP3A4, CYP3A5, CYP2D6) are also implicated (Buratti et al. 2003; Kappers et al. 2001; Mutch and Williams 2006; Sams et al. 2004). In rat liver, CYP2C11, CYPA2, and CYP2B1/2 appear to be the major catalyzing agents in the formation of diazoxon from the parent compound, diazinon.

Available data indicate that absorbed diazinon is rapidly metabolized. Wu et al. (1996a) administered diazinon to male Wistar rats at a dose of 80 mg/kg and followed the timecourse of measurable plasma concentrations. Based on the rate of disappearance of diazinon from the plasma, an elimination half-time of 2.86 hours was estimated. These results provide suggestive evidence for the rapid metabolism of absorbed diazinon.

Age-related differences in the detoxification of diazinon and its active metabolite, diazoxon, are apparent, as demonstrated in an *in vitro* assay of rat liver and plasma (Padilla et al. 2004). The results indicated a much lower degree of detoxification by liver and plasma from young rats compared to adult tissues.

Figure 3-3. Putative Pathways of Diazinon Biotransformation



IMHP, DEP, and DETP are urinary metabolites of diazinon

Sources: Kappers et al. 2001; Poet et al. 2004

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

No studies were located regarding excretion of diazinon after inhalation exposure in humans or animals.

3.4.4.2 Oral Exposure

Following oral dosing of diazinon (0.011 mg/kg) to four volunteers, rapid absorption, metabolism, and elimination was indicated as evidenced by the excretion of approximately 60% of the administered dose as dialkyl phosphate metabolites (DEP and DETP) in the urine, most (90%) of which was recovered within 14 hours postadministration (Garfitt et al. 2002). Unmetabolized diazinon was not detected. Following a single oral dose of 4.0 mg/kg 2-pyrimidinyl ring-labeled and 4-pyrimidinyl ring-labeled [^{14}C]diazinon to rats, approximately 50% of the dose was excreted within 12 hours of dosing (Mücke et al. 1970). Sixty-nine to 80% of the radioactivity was recovered in the urine and 18–25% was excreted in the feces. Only 5.6% of an ethyl-[^{14}C]diazinon dose was recovered as $^{14}CO_2$ in expired air. No $^{14}CO_2$ was expired from rats given an oral dose of 2-[^{14}C] or 4-[^{14}C]pyrimidine diazinon, indicating that complete degradation of the pyrimidine ring did not take place. Traces of unchanged diazinon were recovered in the feces. Three of the unidentified metabolites recovered in the urine and feces of treated rats accounted for 70% of the total administered dose. The half-life of the ^{14}C-ring labeled diazinon was 12 hours while that of [ethyl-^{14}C]diazinon was 7 hours (Mücke et al. 1970). Recovery of radioactivity in the urine of female Beagle dogs 24 hours after receiving a single oral dose of [^{14}C]diazinon was 85% (53% water-soluble fraction, and two metabolites that no longer had a phosphorothioate group, comprising 10 and 23%). No diazinon was detected in the feces (Iverson et al. 1975). Following oral administration of diazinon to lactating goats, DETP was detected in the urine but not in the milk (Mount 1984).

3.4.4.3 Dermal Exposure

Diazinon urinary metabolites (DEP and DETP) were recovered from volunteers treated by occluded dermal application of 100 mg of diazinon (94% purity) on an 80 cm^2 area of the forearm for 8 hours (Garfitt et al. 2002). Most (90%) of the administered dose was recovered from the application site. Approximately 0.5% was recovered as urinary metabolites. In another human study, volunteers were exposed for 24 hours to 2-pyrimidinyl ring-labeled [^{14}C]diazinon applied to either the forearm or abdomen in either an acetone solution or a lanolin wool grease at doses of approximately 15–20 µg for each application method to test the percutaneous absorption of diazinon (Wester et al. 1993). Daily

complete void urine samples were collected and analyzed for levels of radioactivity for 7 days after dosing. The percentage of the administered dose excreted in the urine was approximately 3–4%.

3.4.4.4 Other Routes of Exposure

Following an intravenous injection of [ethyl-^{14}C]diazinon to female Beagle dogs, approximately 58% of the radioactivity was recovered in the urine within 24 hours as DETP (42%) and DEP (16%). No unchanged diazinon was excreted (Iverson et al. 1975).

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.

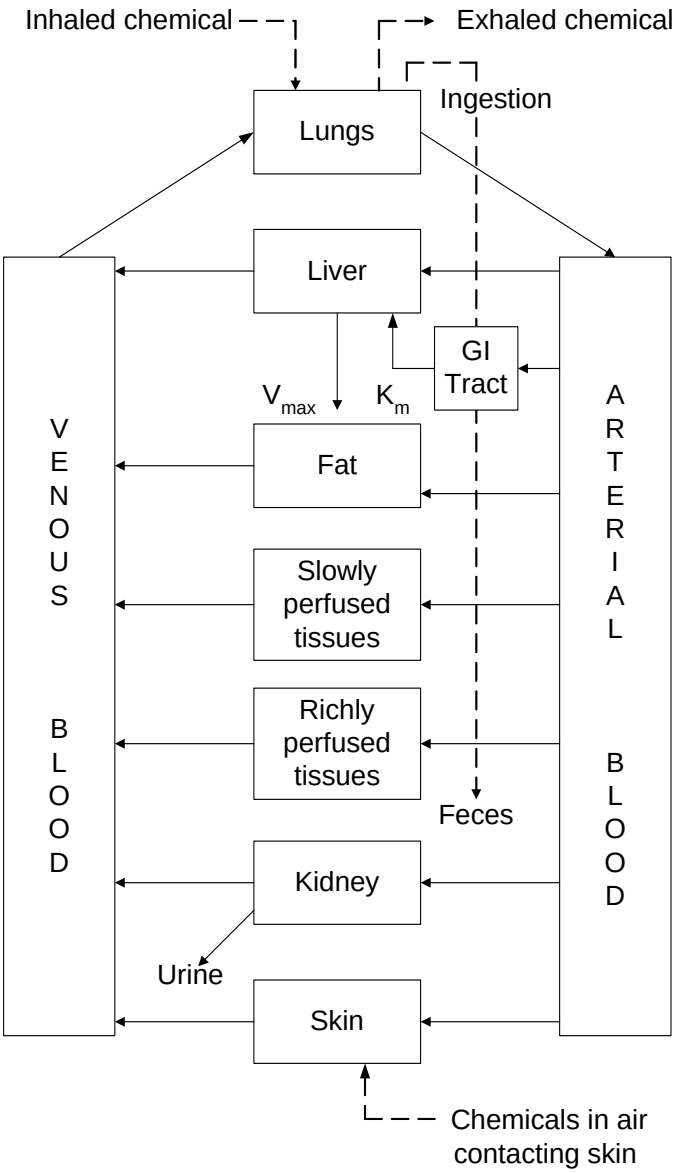
PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in 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 diazinon 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.

A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model has been developed for predicting the absorption, distribution, metabolism, and elimination of diazinon and its metabolites, diazoxon and IMHP, in rats and humans (Poet et al. 2004). The model also quantifies the inhibition of B-esterases (AChE, butylcholinesterase [BuChE], ChE, and carboxylesterase) activities in blood, RBCs, liver, diaphragm, and brain.

Description of the Model. The PBPK/PD model for diazinon (Poet et al. 2004) is based on the PBPK/PD model for chlorpyrifos in rats and humans (Timchalk et al. 2002). Represented tissues include the blood, liver, brain, diaphragm, fat, skin, and other rapidly and slowly perfused tissues connected via arterial and venous blood flows. Portals of entry include oral absorption from a two-compartment model of the gut (for gavage dosing) or zero-order absorption directly into the liver (for dietary exposure), first-order dermal absorption into the skin compartment, and intravenous, and intraperitoneal injection.

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



Source: adapted from Krishnan et al. 1994

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.

Competing metabolism of diazinon to diazoxon or IMHP occurs exclusively in the liver via a CYP450 Michaelis-Menten process. Elimination of parent diazinon is strictly by hepatic metabolism. Detoxification metabolism of diazoxon by the A-esterase, PON, occurs in the blood and liver compartments. A fraction of diazoxon is also eliminated as IMHP as part of the B-esterase inhibition pathway. IMHP is eliminated to urine via first-order transfer to a single compartment. Parameter estimates for physiological volumes and flow rates were taken from the literature (Brown et al. 1997). Metabolic constants were determined using *in vitro* rat data previously published (Poet et al. 2003). Physiological flow rates and metabolic constants were scaled to the 0.74 power of body weight. Diffusion of diazinon and diazoxon across tissues was based on the algorithm of Poulin and Krishnan (1996). Oral absorption and IMHP elimination parameter values were derived from fitting the model to rat and human blood and urine data.

In the liver, brain, diaphragm, and blood, free esterase (resulting from net new esterase synthesized minus esterase degraded) may bind to free diazoxon to form an oxon-esterase complex that may be aged or reversed to yield free esterase and IMHP. The parameters governing these processes were taken from the PBPK/PD model for chlorpyrifos (Timchalk et al. 2002), with subsequent optimization to esterase inhibition data in rats (Poet et al. 2004).

Risk Assessment. The model has not been used in risk assessment. It represents both pharmacokinetic disposition of diazinon and metabolites and pharmacodynamic effect (esterase activity inhibition) for diazoxon in target tissues of rats and humans for multiple routes of exposure.

Validation of the Model. The model was calibrated against rat plasma levels of diazinon, plasma and urine levels of IMHP, ChE activity in blood and diaphragm, AChE activity in brain and RBCs, and BuChE levels in blood and diaphragm of rats given oral bolus doses of 15, 50, or 100 mg diazinon/kg in corn oil vehicle (Poet et al. 2004). The model was validated against observations of plasma, brain, and liver levels and inhibition of plasma ChE and RBC AChE in rats (Tomokuni et al. 1985; Wu et al. 1996a). The sole available human data set was for urinary metabolite levels in humans following single gavage or dermal exposures (Garfitt et al. 2002). For both oral and dermal routes of exposure, parameter values governing urinary excretion rate and dermal absorption, respectively, were modified to achieve visual fit of the model output to the data. The effect of these changes to blood and other target tissue levels was not reported.

Species Extrapolation. The model has been applied to rats and humans. The model structure for both species is identical, with species-specific parameter values used for physiological volumes and flow rates and model-optimized values used for oral absorption. Extrapolation to other species would require data for physiological parameters and oral absorption rates.

High-Low Dose Extrapolation. The model has been evaluated for simulating oral, intravenous, and intraperitoneal doses in rats ranging from 15 to 100 mg/kg. For humans, it has been evaluated for a single oral dose level of 11 μg/kg and a dermal dose of 4 mg/kg.

Interroute Extrapolation. The model is structured to simulate exposures from oral gavage and diet, dermal absorption, and intravenous and intraperitoneal injection.

Strengths and Limitations. The model has been shown to make predictions that are quite similar to observations of blood and tissue levels of diazinon and metabolites from multiple routes of exposure in rats from multiple studies (Poet et al. 2004). The human model also makes predictions of blood, red blood cell, and tissue esterase inhibition, which are important toxicodynamic end points. In humans, the model predicts urine levels of diazinon metabolites from oral and dermal exposures that are very similar to observations; however, the ability of the model to accurately simulate levels of diazinon and diazoxon in human target tissues is unknown. Limitations include the lack of validation of model performance for human blood and tissue levels due to the absence of human data for these end points. An associated limitation is uncertainty in the model to accurately describe esterase inhibition in blood, RBCs, or tissues, including the peripheral and central nervous systems.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

No studies were located in which mechanisms of absorption were assessed for diazinon. It is expected that absorption is accomplished via passive diffusion. Limited information was located regarding mechanisms of distribution of absorbed diazinon. Results of *in vitro* assays indicate that plasma diazinon is predominantly (90%) bound to plasma proteins (Wu et al. 1996a). It is generally understood that diazinon does not appreciably accumulate in any specific body tissues and that absorbed diazinon is rapidly metabolized and eliminated. As discussed in Section 3.4.3, detoxification of diazoxon, the diazinon metabolite responsible for the cholinergic response, is catalyzed by A- and B-esterases. The efficacy of diazinon as an effective insecticide is attributed to deficiencies in these esterases, particularly

among target insects. No information was located regarding mechanisms of elimination and excretion of parent compound or metabolites of diazinon.

3.5.2 Mechanisms of Toxicity

Diazinon toxicity results predominantly from the inhibition of AChE in the central and peripheral nervous system. AChE is responsible for terminating the action of the neurotransmitter, acetylcholine, in the synapse of the pre- and postsynaptic nerve endings and in the neuromuscular junction. The action of acetylcholine does not persist long as it is hydrolyzed by AChE and rapidly removed. As an anticholinesterase organophosphate, diazinon inhibits AChE by reacting with the active site to form a stable phosphorylated complex incapable of destroying acetylcholine at the synaptic gutter between the pre- and postsynaptic nerve endings or neuromuscular junctions of skeletal muscles resulting in accumulation of acetylcholine at these sites. This leads to continuous or excessive stimulation of cholinergic fibers in the postganglionic parasympathetic nerve endings, neuromuscular junctions of the skeletal muscles, and cells of the central nervous system that results in hyperpolarization and receptor desensitization. These cholinergic actions involving end organs (heart, blood vessels, secretory glands) innervated by fibers in the postganglionic parasympathetic nerves result in muscarinic effects, which are manifested as miosis, excessive glandular secretions (salivation, lacrimation, rhinitis), nausea, urinary incontinence, vomiting, abdominal pain, diarrhea, bronchoconstriction or bronchospasm, increased bronchosecretion, vasodilation, bradycardia, and hypotension. Nicotinic effects are due to accumulation of acetylcholine at the skeletal muscle junctions and sympathetic preganglionic nerve endings. Nicotinic effects are manifested as muscular fasciculations, weakness, mydriasis, tachycardia, and hypertension. The central nervous system effects are due to accumulation of acetylcholine at various cortical, subcortical, and spinal levels (primarily in the cerebral cortex, hippocampus, and extrapyramidal motor system). The central nervous system effects are manifested as respiratory depression, anxiety, insomnia, headache, restlessness, tension, mental confusion, loss of concentration, apathy, drowsiness, ataxia, tremor, convulsion, and coma (Klaassen et al. 1986; Williams and Burson 1985). Although diazinon directly inhibits AChE, its oxidation product, diazoxon (Iverson et al. 1975; Yang et al. 1971) formed in the liver, is an even more potent inhibitor of the enzyme (Davies and Holub 1980a, 1980b; Enan et al. 1982; Harris et al. 1969; Rajendra et al. 1986; Takahashi et al. 1991).

The primary cause of death in acute diazinon poisoning is a depression of the neurons in the brainstem (medulla), collectively known as the respiratory center, resulting in loss of respiratory drive or, in the case of managed treatment, cardiac failure due to electrical impulse or beat conduction abnormalities in

cardiac muscles (fatal arrhythmias). Other effects, such as bronchoconstriction, excessive bronchial secretions, and paralysis of the respiratory muscles (intercostal muscles and diaphragm) may also contribute to respiratory insufficiency and death. Thus, death results from loss of respiratory drive and paralysis of the respiratory muscles, or cardiac failure, or both, with attendant asphyxia or cardiac arrest (Klaassen et al. 1986; Shankar 1967, 1978; Williams and Burson 1985).

Oxidative stress has been proposed as an additional mechanism of action for organophosphorus pesticides such as diazinon, particularly with respect to chronic effects on the central nervous system (Ray 1998) and developmental toxicity (Garry 2004; Roy et al. 2005). Akturk et al. (2006) noted increased malondialdehyde levels and increased activities of superoxide dismutase and catalase, indicators of increased lipid peroxidation, in myocardial cells of rats administered diazinon in a single 235 mg/kg oral dose. Giordano et al. (2007) assessed the role of oxidative stress in the neurotoxicity of diazinon and its oxygen analog (diazoxon) to cerebellar granule neurons from wild type and glutathione-deficient mice. Glutathione-deficient cells exhibit increased sensitivity to agents that increase oxidative stress. Cytotoxicity was significantly higher in neurons from glutathione-deficient mice and was antagonized by a variety of antioxidants. Manipulated depletion of glutathione in neurons from wild type mice resulted in increased cytotoxicity. Diazinon caused increased intracellular levels of reactive oxygen species and lipid peroxidation, the magnitudes of which were greatest in neurons from the glutathione-deficient mice. Diazinon increased cellular levels of oxidized glutathione without altering levels of reduced glutathione. Whereas diazinon-induced cytotoxicity was not altered by cholinergic antagonists, it was decreased by the calcium chelator BAPTA-AM. Collectively, these results indicate that diazinon cytotoxicity includes glutathione-modulated generation of reactive oxygen species and may include intracellular homeostasis of calcium.

3.5.3 Animal-to-Human Extrapolations

The general pharmacokinetic behavior of diazinon is similar in humans and laboratory animals. Available comparative data derive mainly from oral exposure. Following oral exposure, diazinon is rapidly absorbed, widely distributed, and metabolized to reactive intermediates and other metabolites, which are primarily quickly eliminated in the urine (see Section 3.4). Although animals and humans share these similarities, potential differences in pharmacokinetic behavior and biotransformation in blood and target tissues, particularly at exposure levels of toxicity concern, have not been extensively studied. Therefore, extrapolation from animals to humans includes an appreciable degree of uncertainty.

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).

Information regarding the potential for diazinon-induced neuroendocrine effects is limited. No data are available regarding diazinon-induced hormonal effects. Results of available animal studies indicate that the reproductive system is not particularly sensitive to diazinon toxicity. No treatment-related morphological or functional effects on reproductive systems were seen in rats, mice, or rabbits administered diazinon orally at doses up to and including those eliciting maternal toxicity (Giknis 1989;

Green 1970; Harris and Holson 1981; Infurna and Arthur 1985; Spyker and Avery 1977). There was no histopathological evidence of diazinon-induced effects on reproductive organs of male or female rats or dogs chronically exposed to diazinon in the diet at doses up to and including those eliciting neurotoxic effects (Barnes 1988; Kirchner et al. 1991; Rudzki et al. 1991; Singh 1988). One study reported testicular atrophy and arrested spermatogenesis in 3 male dogs administered encapsulated diazinon at a dose level of 20 mg/kg/day for 8 months (Earl et al. 1971); however, one of these dogs died and there was significant weight loss, indicating that the testicular effects were likely secondary to primary neurotoxic effects. In another study, oral administration of diazinon to male albino rats at dose levels of 1.5 or 3 mg/kg/day for 65 days resulted in significantly decreased reproductive tissue weights, increased percentage of dead and morphologically abnormal spermatozoa, decreased plasma testosterone levels, and decreased fertility as assessed by conception rates of untreated females mated to diazinon-treated males (Abd El-Aziz et al. 1994).

Results of available *in vitro* assessments of diazinon estrogenicity indicate a potentially weak estrogenic effect at best. A positive estrogenic response was not elicited in a yeast two-hybrid assay at diazinon concentrations up to and including the highest concentration tested ($1x10^{-4}$ M) (Nishihara et al. 2000). Results of the E-CALUX assay indicated a weakly positive response at a diazinon concentration of $4.6x10^{-4}$ M (Kojima et al. (2005).

Collectively, these limited data indicate that the endocrine system may not be particularly sensitive to diazinon toxicity.

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 infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). 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).

It is not known whether children are more susceptible than adults to diazinon toxicity, although available human and animal data provide suggestive evidence of increased sensitivity during critical periods of development.

A single human study reported neurophysiological and neuropsychological deficits and delayed bone growth in young children exposed at home to a formulation of diazinon that was misused to control an infestation of fleas (Dahlgren et al. 2004). These results provide suggestive evidence that children may be particularly susceptible to diazinon toxicity during critical periods of neural and skeletal development.

Results of one animal study indicate that fetal exposure to low levels of diazinon may result in functional deficits that can only be detected by systemic behavioral evaluation (Spyker and Avery 1977). In the study, pregnant mice were exposed to diazinon (technical grade, purity not specified) in peanut butter at doses of 0, 0.18, or 9 mg/kg/day throughout gestation. When subjected to neuromuscular function tests (rod cling and inclined plane) as adults, the pups of both groups of diazinon-exposed dams exhibited endurance and coordination deficits. Offspring of the high-dose dams also displayed slower running speed in a Lashley III maze and reduced swimming endurance. Morphologically, focal abnormalities in the forebrain area, including dense aggregations of atypical chromatin-containing cells, were observed in the high-dose offspring. These neural dysfunctions and pathologies might occur either indirectly through diazinon impairment of placental transport of nutrients or maternal regulation of fetal growth, or directly via antagonism to cholinergic development of the fetus (Spyker and Avery 1977).

Results of subcutaneous injection studies indicate that critical periods of neurological development may be particularly sensitive to diazinon toxicity. Slotkin and coworkers (Jameson et al. 2007; Slotkin et al. 2006a, 2006b, 2007) reported evidence of diazinon-induced neurodevelopmental effects in the forebrain and brainstem of neonatal rats at dose levels near or below those eliciting significant cholinesterase inhibition, the most commonly-observed indicator of diazinon toxicity. In these studies, newborn rats were subcutaneously injected with diazinon on postnatal days 1–4 at doses ranging from 0.5 to 2 mg/kg/day.

The neurotoxicity of diazinon is dependent on its bioactivation via a cytochrome P-450 mediated desulfuration to the oxon form (Buratti et al. 2003). Age-related differences in production and regulation of enzymes involved in metabolism could conceivably result in age-related differences in susceptibility to diazinon toxicity. Age-related differences in regulation of selected P-450 isozymes have been demonstrated (Leeder and Kearns 1997). Age-related differences in relative amounts of plasma ChE,

RBC AChE, and neural AChE could potentially play roles in susceptibility to diazinon toxicity. Because plasma ChE binds diazinon, lesser amounts of plasma ChE would result in greater amounts of diazinon and its oxon available to interact with RBC and neural AChE, which could result in increased susceptibility to diazinon neurotoxicity. The same reasoning is plausible for RBC AChE concentrations. Decreased levels of RBC AChE could result in increased sensitivity to diazinon and its oxon via increased binding of neural AChE. Garcia-Lopez and Monteoliva (1988) demonstrated that RBC AChE activity in humans increases with age, starting at birth and exceeding 60 years of age.

In an *in vitro* assay designed to test the efficacy of rat liver and plasma to detoxify diazinon and its active metabolite, diazoxon, Padilla et al. (2004) demonstrated that liver and plasma from young rats possessed much less detoxification capability than adult tissues. Padilla et al. (2004) further demonstrated that oral administration of 75 mg diazinon/kg to 17-day-old rats resulted in 75% brain AChE inhibition, whereas the same dose to adult rats resulted in only 38% brain AChE inhibition. These results indicate that young rats may be more susceptible to the neurotoxic effects of diazinon and that age-related susceptibility may be at least partially associated with age-related differences in metabolic processes involved in detoxification.

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).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. 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 diazinon 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 diazinon 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 Diazinon

Diazinon is rapidly absorbed from the gastrointestinal tract and widely distributed throughout the body in both humans (Poklis et al. 1980) and animals (Janes et al. 1973; Mücke et al. 1970). Detection of diazinon in the blood and urine of occupationally-exposed workers may serve as a useful biomarker of very recent exposure to diazinon (Lu et al. 2006). No human or animal studies have reported the presence of unchanged diazinon in the urine following exposure. Traces of unchanged diazinon have been detected in animal feces following exposure (Mücke et al. 1970). Diazinon undergoes biotransformation to a variety of polar metabolites which have been detected in the urine and feces of animals. Urinary and fecal excretion of IMHP, DEP, and DETP have been reported following exposure of animals to diazinon (Iverson et al. 1975; Machin et al. 1975; Mount 1984; Mücke et al. 1970; Seiber et al. 1993; Yang et al. 1971). Both DEP and DETP have been detected in the urine of exposed insecticide applicators (Maizlish et al. 1987) and volunteers administered diazinon orally or dermally (Garfitt et al. 2002). Analysis of blood samples for the presence of these metabolites represents a potential means of assessing exposure; however, only IMHP is specific for diazinon. Analysis of urine samples for metabolic products provides

a noninvasive method for detecting exposure. As diazinon is rapidly metabolized and excreted from the body, urinary and fecal metabolite analysis is useful only in the evaluation of recent exposures. There are no reports of quantitative associations between metabolite levels and exposure to diazinon in humans. Therefore, these biomarkers are only indicative of exposure and are not useful for dosimetric analysis.

3.8.2 Biomarkers Used to Characterize Effects Caused by Diazinon

The major action resulting from human exposure to diazinon is the inhibition of cholinesterase activity (see Section 3.5 for discussion). Two pools of cholinesterases are present in human blood; RBC AChE and ChE. RBC AChE is identical to AChE present in neural tissue (the target of diazinon action) while plasma ChE has no known physiological function. Inhibition of both forms of cholinesterase has been associated with exposure to diazinon in humans (Coye et al. 1987; Soliman et al. 1982) and animals (Barnes 1988; Davies and Holub 1980a; EPA 1996, 2000a; Kirchner et al. 1991; Makhteshim-Agan 1989; Rudzki et al. 1991; Trutter 1991). Inhibition of plasma, RBC, or whole blood ChE may be used as a marker of exposure to diazinon. However, cholinesterase inhibition is a common action of anticholinesterase compounds such as organophosphates (which include diazinon) and carbamates. In addition, a wide variation in normal cholinesterase values exists in the general population, and there are no studies which report a quantitative association between cholinesterase activity levels and exposure to diazinon in humans. Thus, cholinesterase inhibition is not a specific biomarker of effect for diazinon exposure, but is indicative only of effect, and not useful for dosimetric analysis.

It should be noted that plasma ChE activity has been reported to be a more sensitive marker for diazinon exposure than RBC AChE activity (Endo et al. 1988; Hayes et al. 1980). In light of this, it has been suggested that in the absence of baseline values for cholinesterase activity, sequential postexposure cholinesterase analyses be used to confirm a diagnosis of organophosphate poisoning (Coye et al. 1987).

In combination with analysis of reductions in the level of cholinesterase activity, the manifestations of severe diazinon poisoning, clinically characterized by a collection of cholinergic signs and symptoms (which may include dizziness, fatigue, tachycardia or bradycardia, miosis, and vomiting) (Bichile et al. 1983; Dagli et al. 1981; Hata et al. 1986; Kabrawala et al. 1965; Klemmer et al. 1978; Reichert et al. 1977; Wadia et al. 1974; Wedin et al. 1984) are useful biomarkers of effect for identifying poisoned victims of diazinon. These manifestations are also not specific to diazinon but to anticholinesterase compounds (such as organophosphates and carbamates) in general.

3.9 INTERACTIONS WITH OTHER CHEMICALS

Diazinon is one of many pesticides (organophosphates and carbamates) designed to act as AChE inhibitors. Significant occupational exposure to diazinon often occurs in workers who are exposed to other similarly-acting compounds. Neurotoxic effects in such individuals are the result of the cumulative dose and relative potency of each individual compound.

Although no studies were located that specifically assessed dermal absorption of diazinon in the presence of other chemicals, it is generally understood that a variety of solvents influence the rate and extent of absorption of organophosphate pesticides following dermal exposure.

A variety of chemicals may interfere with the toxicity of diazinon indirectly by influencing its metabolism through their actions on drug metabolizing enzymes. The duration and intensity of action of diazinon are largely determined by the speed at which it is metabolized in the body by the oxidative and hydrolytic liver enzymes. More than 200 drugs, insecticides, carcinogens, and other chemicals are known to induce the activity of liver microsomal drug-metabolizing enzymes. The characteristic biological actions of these chemicals are highly varied. Although there is no relationship between their actions or structures and their ability to induce enzymes, most of the inducers are lipid soluble at physiological pH. These inducers of the MFO system include the following classes of drugs: hypnotic and sedatives (barbiturates, ethanol); anesthetic gases (methoxyflurane, halothane); central nervous system stimulators (amphetamine); anticonvulsants (diphenylhydantoin); tranquilizers (meprobamate); antipsychotics (trifluprom azine); hypoglycemic agents (carbutamide); anti-inflammatory agents (phenylbutazone); muscle relaxants (orphenadrine); analgesics (aspirin, morphine); antihistaminics (diphenhydramine); alkaloids (nicotine); insecticides (chlordane, DDT, BHC, aldrin, dieldrin, heptachlor epoxide, pyrethrins); steroid hormones (testosterone, progesterone, cortisone); and carcinogenic polycyclic aromatic hydrocarbons (3-methyl cholanthrene, 3,4-benzpyrene) (Klaassen et al. 1986; Williams and Burson 1985).

Thus, exposure to any of these enzyme inducers concurrent with or after exposure to diazinon may result in accelerated bioactivation to the more potent anticholinesterase diazoxon. The extent of toxicity mediated by this phenomenon is dependent on how fast diazoxon is hydrolyzed to less toxic metabolites, a process that is also accelerated by enzyme induction. Similarly, concurrent exposure to diazinon and MFO enzyme-inhibiting substances (e.g., carbon monoxide; ethylisocyanide; SKF 525A, halogenated alkanes, such as CCl_4; alkenes, such as vinyl chloride; and allelic and acetylenic derivatives) may increase

the toxicity of diazinon by decreasing the rate of the hydrolytic dealkylation and hydrolysis of both parent diazinon and activated diazinon (diazoxon) (Williams and Burson 1985). The balance between activation and detoxification determines the biological significance of these chemical interactions with diazinon.

Cimetidine, a histamine H_2 receptor agonist used to treat peptic ulcers and other gastric acid-related disorders, has been shown to potentiate the toxicity of diazinon. In a series of studies, Wu et al. (1996b, 1996c) demonstrated enhanced cholinergic signs, as well as increased brain AChE and carboxylesterase inhibition in diazinon-treated rats that had been pretreated with cimetidine. Significant decreases in total body clearance of diazinon and marked increases in the area under the plasma concentration-time curves following cimetidine treatment were also noted. *In vitro* assays demonstrated that cimetidine significantly decreased the hepatic metabolism of diazinon.

Diazinon exposure may interfere with the short-acting muscle relaxant, succinylcholine, used concurrently with anesthetics. The action of succinylcholine is terminated by means of its hydrolysis by plasma ChE (Klaassen et al. 1986). Since plasma ChE is strongly inhibited by diazinon (Davies and Holub 1980b; Klemmer et al. 1978), it is possible that concurrent exposure to diazinon may result in the prolongation of the action of succinylcholine leading to prolonged muscular paralysis.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to diazinon than will most persons exposed to the same level of diazinon 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 diazinon, or compromised function of organs affected by diazinon. Populations who are at greater risk due to their unusually high exposure to diazinon are discussed in Section 6.7, Populations with Potentially High Exposures.

The magnitude of diazinon toxicity, like the toxicity of any xenobiotic, is affected by the rate of its metabolic biotransformation to both more and less toxic substances (Klaassen et al. 1986). The newborn of several animal species, including humans, have a reduced ability to metabolize xenobiotics. Available animal data indicate that developing animals may be particularly sensitive to diazinon neurotoxicity (Spyker and Avery 1977). However, the effect of decreased metabolism on diazinon-induced neurotoxicity has not been demonstrated.

Studies on experimental animals showed that starvation depressed liver microsomal enzyme (P-450) activity due to actual loss of the enzyme protein (Boyd and Carsky 1969). Thus, dietary protein deficiency could potentially alter diazinon toxicity by diminishing its metabolism in the liver. Hereditary factors may also contribute to population sensitivity to diazinon. Atypical plasma ChE with low activity is present in a small percentage of the human population. This altered enzyme is the result of a hereditary factor with 0.04% occurrence in the population. Since plasma ChE is strongly inhibited by diazinon (Davies and Holub 1980b; Klemmer et al. 1978), it is expected that individuals who have atypical ChE (or low plasma ChE activity) will be unusually sensitive to the muscle relaxant succinylcholine (Klaassen et al. 1986) and may suffer prolonged muscle paralysis if administered succinylcholine while exposed to diazinon. Congenital low plasma ChE activity may also increase subpopulation sensitivity to diazinon exposure. This is because, after exposure, plasma ChE acts as a depot for diazinon due to its strong affinity for the substance (Davies and Holub 1980b; Klemmer et al. 1978), thus decreasing the availability of the diazinon dose to the target (neuromuscular tissue) of diazinon toxicity in the population with normal plasma ChE levels. In individuals with congenital low plasma ChE activity, less diazinon is bound in the blood and more unbound diazinon is in circulation to reach the target of diazinon toxicity (neuromuscular tissue). Ueyama et al. (2007) demonstrated significantly increased ChE and RBC and brain AChE inhibition in streptozotocin-induced diabetic rats compared to normal rats, an indication that diabetics may be more susceptible to OP-induced neurotoxicity.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to diazinon. 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 diazinon. 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 diazinon:

Clark RF. 2002. Insecticides: Organic phosphorus compounds and carbamates. In: Goldfrank LR, Flomenbaum NE, Lewin NA, et al. eds. Goldfrank's toxicologic emergencies. 7th ed. New York, NY: Mc-Graw-Hill Medical Publishing Division, 1346-1360.

Carlton FB, Simpson WM, Haddad LM. 1998. The organophosphates and other insecticides. In: Haddad LM, Shannon MW, Winchester JF, eds. Clinical management of poisoning and drug overdose. 3rd ed. Philadelphia, PA: WB Saunders Company, 836-845.

Osmundson M. 1998. Insecticides and pesticides. In: Viccellio P, Bania T, Brent J, et al., eds. Emergency toxicology. 2nd ed. Philadelphia, PA: Lippincott-Raven Publishers, 401-413.

3.11.1 Reducing Peak Absorption Following Exposure

The following information was extracted from the texts listed above; specific chapters were written by Clark (2002), Carlton et al. (1998), and Osmundson (1998), respectively. Following dermal contamination with organophosphates, most texts recommend washing the skin with copious amounts of soap and water, which may be followed by a second washing with ethyl alcohol to remove the contaminant from the skin. However, it should be noted that ethyl alcohol may also enhance the dermal absorption of some chemicals as evidenced by its function as an enhancer in some transdermal patches. Contaminated clothing, including leather garments, should be destroyed. After oral ingestion, activated charcoal is recommended for many organophosphates, although Carlton et al. (1998) note that it may lack efficiency with some organophosphates. Osmundson (1998) points out that Ipecac should not be used for organophosphate poisoning. Cathartics may be unnecessary as intestinal motility is greatly increased. Gastric lavage may be performed with care to prevent aspiration, as organic solvent vehicles may precipitate pneumonitis. Treatment of inhaled organophosphates is mostly supportive as respiratory distress is a common effect of poisoning; intubation may be necessary to facilitate control of secretions.

3.11.2 Reducing Body Burden

Diazinon is rapidly metabolized, with an estimated mammalian biological half-life of 12–15 hours (Iverson et al. 1975; Mücke et al. 1970). Consequently, efforts at reducing body burdens of poisoned persons may not be critical to the outcome. Dialysis and hemoperfusion are not indicated in organophosphate poisonings because of the extensive tissue distribution of the absorbed doses (Mücke et al. 1970; Poklis et al. 1980).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

The following information has been extracted from the texts listed above. Administration of atropine and pralidoxime (2-PAM) seems to be a universally accepted treatment for organophosphate poisoning. It should be mentioned, however, that glycopyrrolate, a quaternary ammonium compound, has also been used instead of atropine (Bardin and Van Eeden 1990). Unlike atropine, glycopyrrolate does not cross the blood-brain barrier and, therefore, has fewer central nervous system effects. 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. Atropine is particularly helpful in drying excessive secretions especially from the

tracheobronchial tree. Atropine does not antagonize nicotinic effects; therefore, 2-PAM is needed for treatment of muscle weakness and respiratory depression. Most texts recommend an initial dose of 1–2 mg for an adult and 0.05 mg/kg for children, preferably by the intravenous route. This may be repeated every 15–30 minutes until signs of atropinization occur. 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 (Carlton et al. 1998). 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 normally 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 higher doses or multiple doses, as enzyme regeneration depends on plasma levels of the organophosphate. A 2-PAM serum level of 4 μg/L is suggested as the minimum therapeutic threshold. 2-PAM is considered a very safe drug with few side effects.

3.12 ADEQUACY OF THE DATABASE

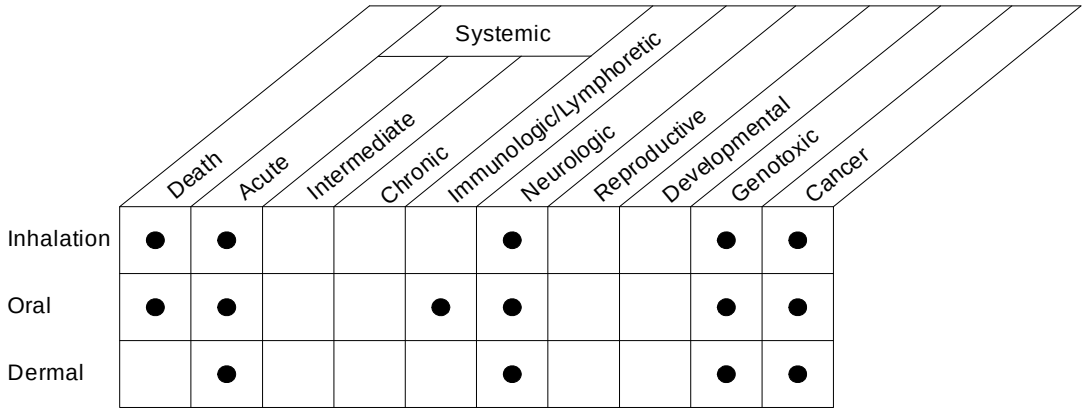
Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of diazinon is available. Where adequate information is not available, 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 diazinon.

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.

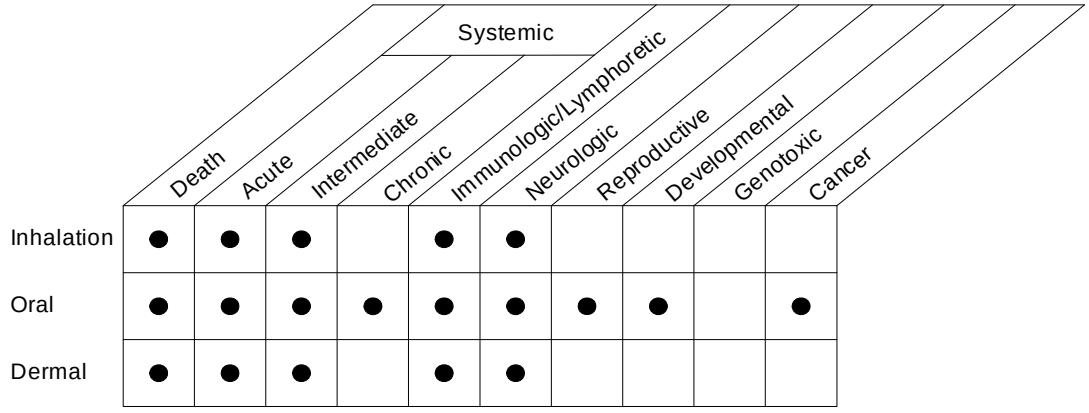
3.12.1 Existing Information on Health Effects of Diazinon

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to diazinon are summarized in Figure 3-5. The purpose of this figure is to illustrate the existing information concerning the health effects of diazinon. Each dot in the figure indicates that one or more studies

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



Human



Animal

● Existing 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 diazinon in humans described case reports of individuals or groups of individuals exposed either occupationally or in the home following intentional poisoning attempts or otherwise accidental misuse of diazinon or diazinon-containing solutions. The predominant route of occupational exposure is believed to be dermal while that for accidental or intentional exposure in the home is oral, although some inhalation exposures were reported. Thus, Figure 3-5 reflects that information exists for all three routes of exposure. However, all of these reports are limited because of the possibility of concurrent or sequential exposure to other potentially toxic substances present in the environment (workplace or home), such as other insecticides, or present as components of diazinon-containing formulations. In all cases, accurate information regarding levels and duration of exposure were not presented in these reports. Further, the health effects of human acute exposure to diazinon are much more fully characterized than those associated with intermediate and chronic exposures.

Information regarding the health effects of diazinon following ingestion in laboratory animals is substantial, but less information is available on the effects of inhalation and dermal exposures (see Figure 3-5). Furthermore, the health effects of acute- and intermediate-duration exposures to diazinon are more fully characterized than those associated with chronic-duration exposures. The available information indicates that diazinon is a toxic substance to all species of experimental animals, deriving its toxicity from AChE inhibition.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. Information is available on the effects of acute-duration exposures in humans and experimental animals (rats and mice). The available human data consist primarily of studies of cholinergic (neurological) reactions resulting from AChE inhibition. Effects noted include respiratory, cardiovascular, hematological, kidney, liver, gastrointestinal tract, endocrine, neurological, and

immunologic/lymphoreticular system toxicity (Balani et al. 1968; Bichile et al. 1983; Dagli et al. 1981; DePalma et al. 1970; Hata et al. 1986; Kabrawala et al. 1965; Klemmer et al. 1978; Lee 1989; Limaye 1966; Lisi et al. 1987; Matsushita and Aoyama 1981; Poklis et al. 1980; Shankar 1967; Wadia et al. 1974; Wecker et al. 1985; Wedin et al. 1984; Weizman and Sofer 1992). The type of information available in animals includes LD_{50} values (Boyd and Carsky 1969; Enan et al. 1982; Gaines 1960, 1969; Harris et al. 1969) and cholinergic (neurological) reactions resulting from AChE inhibition. Effects noted include respiratory, gastrointestinal, hematological, liver, kidney, immunologic/lymphoreticular, and neurological toxicity (Boyd and Carsky 1969; Enan et al. 1982; Lox 1983; Mihara et al. 1981). Thus, while the acute effects of diazinon inhalation and oral exposure in humans are well-characterized and stem principally from AChE inhibition, the diazinon exposure levels at which these effects begin to occur are usually not known. Available animal studies provide adequate insight into the AChE inhibiting action of diazinon in acute oral exposures. Results of one study (Davies and Holub 1980a) serve as the basis for deriving an acute-duration oral MRL for diazinon. Available acute-duration inhalation data in animals are restricted to a single report of nasal discharge, polyuria, decreased activity, and salivation in a group of five rats exposed to a diazinon aerosol at a concentration of 2,330 mg/m^3. This study was not suitable for MRL derivation because it included a single exposure level at which serious effects were observed and no supporting data were available. Quantitative acute-duration inhalation toxicity data for humans and laboratory animals are needed to assist in the derivation of an acute-duration inhalation MRL for the protection of populations, especially those surrounding hazardous waste sites or establishments where wastes containing diazinon are released into the air or water, and those that are occupationally exposed to high levels of diazinon for brief periods.

Intermediate-Duration Exposure. Information is available on the effects of intermediate-duration exposures in humans and experimental animals (rats, dogs, pigs). The type of information available includes studies of cardiovascular, gastrointestinal, hematological, hepatic, musculoskeletal, renal, body weight, immunologic/lymphoreticular, and neurological effects (Alluwaimi and Hussein 2007; Anthony et al. 1986; Davies and Holub 1980a, 1980b; Earl et al. 1971; Enan et al. 1982; Kalender et al. 2005, 2006; Lox and Davis 1983; Ogutcu et al. 2006). Data from these studies sufficiently demonstrate the cholinergic effects of diazinon. The adverse effects reported in humans and laboratory animals following exposure via inhalation, oral, or dermal routes are predominately cholinergic responses deriving from inhibition of AChE. An intermediate-duration inhalation MRL was derived for diazinon based on RBC AChE inhibition in rats (Hartman 1990). An intermediate-duration oral MRL was derived based on RBC AChE inhibition in orally-exposed rats (Davies and Holub 1980a). Further information on the dermal toxicity and toxicokinetics for all routes in both humans and laboratory animals would be helpful for use

in additional assessment of intermediate-duration exposure, especially for persons near hazardous waste sites or establishments where wastes containing diazinon are released, or near agricultural establishments where diazinon is used regularly.

Chronic-Duration Exposure and Cancer. No adequate epidemiological studies are available regarding the potential carcinogenicity or systemic toxicity of diazinon resulting from chronic exposure in humans. Two adequate studies have been conducted with rats and mice orally exposed to diazinon (NCI 1979). While not designed as a cancer bioassay, in a study where rats (groups of 20–30) were orally exposed to diazinon for 98 weeks, histopathology of some 30–40 different tissues showed no treatment-related increase in neoplasms (Kirchner et al. 1991). This rat study included sufficient information regarding AChE inhibition to justify the derivation of a chronic-duration oral MRL for diazinon. A 52-week oral toxicity study in dogs is available as well (Rudzki et al. 1991). No chronic inhalation MRL was calculated for diazinon because no studies for this route are available. Toxicity and toxicokinetic data from well-conducted inhalation studies in both humans and laboratory animals would be helpful in developing a chronic-duration inhalation MRL for the protection of populations, especially those surrounding hazardous waste sites or establishments where wastes containing diazinon are released into the air or water, and those occupationally exposed to diazinon for long periods of time.

Epidemiological studies available on diazinon are inadequate for assessing the carcinogenic potential of this chemical substance. The results from these studies are confounded by either concurrent or sequential (or both) exposures to other potentially toxic substances, mainly other insecticides (Cantor et al. 1992; Davis et al. 1993; Morris et al. 1986), although cancers in several tissue types (unspecified type of childhood brain cancer, non-Hodgkin's lymphoma, multiple myeloma) were identified in these chronic human exposure studies(presumed to involve multiple concurrent routes of exposure). In adequate cancer oral bioassays conducted in rats and mice, the NCI (1979) concluded that diazinon is not carcinogenic in these species under the conditions of the bioassays. Chronic inhalation and dermal bioassays would be helpful to determine whether long-term inhalation or dermal exposures in populations, especially those surrounding hazardous waste sites or establishments where wastes containing diazinon are released into the air or water, and those occupationally exposed to diazinon for long periods of time, are at risk of developing cancers.

Genotoxicity. Chronic occupational exposure to multiple insecticides, including diazinon, has been associated with an increased incidence of chromosomal aberration and increased sister chromatid exchange in peripheral blood lymphocytes of these individuals (de Ferrari et al. 1991; Kiraly et al. 1979;

See et al. 1990). The results from these studies are confounded by either concurrent or sequential (or both) exposures to other unknown toxic substances, mainly other insecticides, that may be genotoxic. Significantly increased sister chromatid exchanges were noted in peripheral blood lymphocytes from a group of volunteers following exposure to diazinon in a sheep-dip formulation (Hatjian et al. 2000). However, the specific role of diazinon in the observed effect could not be determined because the sheep-dip formulation contained other ingredients as well.

Limited information is available regarding the genotoxicity of diazinon in nonhuman species *in vivo*. Diazinon did not induce sister chromatid exchanges in the bone marrow of mice administered 100 mg/kg diazinon by gavage (EPA 1990). Diazinon induced mutations in a wing SMART of *Drosophila melanogaster* (Çakir and Sarikaya 2005).

The results of *in vitro* tests in a variety of test systems (predominantly microbial assays) are equivocal. Diazinon was positive for gene mutations in one test using the *S. typhimurium* mutagenicity or reverse mutation assay with metabolic activation (Wong et al. 1989) and in the mouse lymphoma cell forward mutation assay without metabolic activation (McGregor et al. 1988). The compound was also positive for chromosomal aberrations in Chinese hamster cells with metabolic activation (Matsuoka et al. 1979). In contrast, evaluations for genetic mutation activity in the *S. typhimurium* mutagenicity or reverse mutation assay (Marshall et al. 1976) and in the *rec*-assay utilizing strains of *B. subtilis* (Shirasu et al. 1976) without metabolic activation, and in tests for sister chromatid exchange in Chinese hamster cells, both with and without metabolic activation (Chen et al. 1982), and for chromosomal aberrations in human peripheral blood lymphocytes (Lopez et al. 1986), were all negative. A full battery of *in vivo* tests in animals and additional *in vitro* tests in microbial systems for all genetic end points is necessary for the determination of the genetic toxicity potential of diazinon.

Reproductive Toxicity. No information was located on the reproductive effects of diazinon exposure in humans. Limited data are available regarding diazinon-induced reproductive effects in animals. Increased litter size was reported in one study of diazinon-treated rats (Green 1970), although a second rat study reported significant reduction in litter size at oral maternal diazinon doses of 0.18 and 9 mg/kg/day (Spyker and Avery 1977). Diazinon-induced adverse effects on reproductive tissue weights, sperm quality, and fertility were noted in orally-exposed male rats (Abd El-Aziz et al. 1994). Testicular atrophy and arrested spermatogenesis were noted in dogs administered diazinon orally at doses ≥10 mg/kg/day for up to 8 months (Earl et al. 1971). No adverse effects on reproduction were observed in four generations of rats following oral administration of diazinon to female rats from each generation for 60 days prior to

weaning (Green 1970). No gross or histological evidence of treatment-related damage to reproductive tissues was observed in rats receiving diazinon in the diet for 13 weeks (Singh 1988)or 98 weeks (Kirchner et al. 1991), or in dogs exposed for 13 weeks (Barnes 1988). A well-designed multigenerational reproductive toxicity rat or mouse study is needed to more adequately assess the potential for diazinon to cause reproductive toxicity in humans.

Developmental Toxicity. Information regarding the developmental effects in humans from exposure to diazinon was not located. Four of the located studies in laboratory animals did not find any significant developmental effects in the rats, mice, hamsters, and rabbits tested (Barnett et al. 1980; Green 1970; Robens 1969; Spyker and Avery 1977). In two of these studies, marked reduction in rat pup birth weight and continued significant retardation in growth rate (Green 1970), or significantly elevated mortality in rat pups at weaning (Barnett et al. 1980) were reported. It has been suggested that the effects reported for pups derive from diazinon impairment of placental transport of nutrients or maternal regulation of fetal growth, or directly via antagonism to cholinergic development of the fetus (Spyker and Avery 1977). Collectively, the results of available studies for diazinon indicate that the compound is not of particular developmental toxicity concern at exposure levels lower than those resulting in maternal neurotoxicity, although additional neurodevelopmental toxicity studies could be designed to more critically test the neurodevelopmental toxicity potential.

Immunotoxicity. Autopsy reports in which the victims were exposed to high acute doses of diazinon described damage to lymphoreticular organs (spleen, thymus) (Limaye 1966; Poklis et al. 1980). One human study reported allergic interaction between the fungicide benomyl and diazinon from prolonged dermal contact with diazinon (Matsushita and Aoyama 1981). Several oral animal studies also reported damage to immune structures in rats and dogs. Rats exhibited reduced spleen weight, splenic red pulp contraction, reduced thymus weight, and thymic atrophy ranging from minor to near total loss of thymocytes following acute exposure to moderate doses of diazinon (Boyd and Carsky 1969). Dose-related splenic degeneration after 232 days of diazinon exposure was also reported in 1/3 diazinon-treated dogs (Earl et al. 1971). The splenic atrophy reported in this study may be a result of the generalized emaciated condition of the dog due to diarrhea, emesis, and anorexia. Exposure of guinea pigs in a dermal sensitization study resulted in allergic interaction between the fungicide benomyl and diazinon (Matsushita and Aoyama 1981). Dermal application of diazinon induced delayed contact hypersensitivity at both 24 and 48 hours after challenge in the guinea pig maximization test (Matsushita et al. 1985). Oral administration of diazinon to mice resulted in increased levels of interleukin-10 in selected splenic lymphocyte subpopulations and decreased levels of interferon-γ in B cells (Alluwaimi and Hussein 2007).

These results indicate a diazinon-induced effect on cytokines involved in the regulation of cellular and humoral responses. No gross or histological evidence of treatment-related damage to the spleen or thymus was observed in rats receiving diazinon from feed for 13 weeks (Singh 1988) or 98 weeks (Kirchner et al. 1991), or dogs receiving diazinon in the diet for 13 weeks (Barnes 1988) or 52 weeks (Rudzki et al. 1991). Based on equivocal results from available animal studies, additional human and animal data would be helpful in defining the immunologic/lymphoreticular injury potential of diazinon in humans.

Neurotoxicity. Available evidence shows that diazinon exposure in humans results in the inhibition of neural AChE (Coye et al. 1987; Davies and Holub 1980a, 1980b; Enan et al. 1982; Harris et al. 1969; Rajendra et al. 1986; Takahashi et al. 1991; Wecker et al. 1985). Severe inhibition of this enzyme results in accumulation of acetylcholine at its sites of action and excessive or interminable stimulation of both sympathetic and parasympathetic cholinergic receptors leading to muscarinic and nicotinic effects. Clinical signs of diazinon-induced neurotoxicity include muscular fasciculations, weakness, and paralysis; mydriasis; tachycardia; hypertension; miosis; excessive glandular secretions (salivation, lacrimation, rhinitis); nausea; urinary incontinence; vomiting; abdominal pain; diarrhea; bronchoconstriction or bronchospasm; increased bronchosecretion; vasodilation; bradycardia; hypotension; respiratory depression; anxiety; insomnia; headache; restlessness; tension; mental confusion; loss of concentration; apathy; drowsiness; ataxia; tremor; convulsion; and coma (Adlakha et al. 1988; Bichile et al. 1983; Coye et al. 1987; Kabrawala et al. 1965; Klaassen et al. 1986; Klemmer et al. 1978; Maizlish et al. 1987; Rayner et al. 1972; Shankar 1967, 1978; Williams and Burson 1985). These neurological effects have also been reported in diazinon-treated rats (Boyd and Carsky 1969; Earl et al. 1971). The current information from human and laboratory animal studies provides sufficient demonstration that the nervous system is the primary target of diazinon poisoning. The database of animal information for acute-, intermediate-, and chronic-duration oral exposure to diazinon is sufficiently characterized to allow the derivation of acute-, intermediate-, and chronic-duration oral MRLs. Health effects following intermediate-duration inhalation exposure to diazinon have been sufficiently characterized to allow the derivation of an intermediate-duration inhalation MRL. However data are lacking for acute- and chronic-duration inhalation exposure. Additional animal studies to assess inhalation exposure for acute- and chronic-duration exposure to diazinon should be designed to allow for the derivation of inhalation MRLs for these exposure durations as well. Information regarding health effects following dermal exposure are limited (EPA 1990; Lee 1989), but indicate that dermal exposure to relatively high doses of diazinon would result in neurological effects similar to those elicited from oral or inhalation exposure.

Epidemiological and Human Dosimetry Studies. Information on the health effects of diazinon in humans is derived from case reports of accidental or intentional exposure to diazinon, epidemiological studies, and controlled exposure studies (Adlakha et al. 1988; Alavanja et al. 2004; Beane Freeman et al. 2005; Bichile et al. 1983; Cantor et al. 1992; Dagli et al. 1981; Dahlgren et al. 2004; Davis et al. 1993; EPA 2000a, 2001; Hata et al. 1986; Kabrawala et al. 1965; Klemmer et al. 1978; Maizlish et al. 1987; Morris et al. 1986; Rayner et al. 1972; Reichert et al. 1977; Richter et al. 1992; Schenker et al. 1992; Shankar 1967, 1978; Soliman et al. 1982; Wadia et al. 1974; Wedin et al. 1984). The most likely identifiable subpopulations exposed to diazinon are pesticide applicators, farm workers, and individuals involved in the production of diazinon, since diazinon is no longer registered for use in residential pesticides in the United States (EPA 2004b). Well-designed epidemiological studies of exposed workers are needed. The nervous system is a known target of acute exposure, but little is known regarding possible long-term effects of acute exposure to high levels of diazinon or longer-term exposure at relatively low exposure levels. Additional epidemiological studies should assess the potential effects of such exposure scenarios.

Biomarkers of Exposure and Effect.

Exposure. Diazinon is rapidly absorbed from the gastrointestinal tract and widely distributed throughout the body in both humans (Poklis et al. 1980) and animals (Janes et al. 1973; Mücke et al. 1970). Potential biomarkers of exposure to diazinon include parent compound and metabolites of diazinon such as IMHP, DEP, and DETP. However neither parent compound nor metabolites of diazinon have been demonstrated to represent quantitative indicators of diazinon exposure levels. No human or animal studies have reported the presence of unchanged diazinon in the urine following exposure, although traces of unchanged diazinon have been detected in animal feces following exposure (Mücke et al. 1970). Urinary and fecal excretion of IMHP, DEP, and DETP have been reported following oral exposure of animals to diazinon (Iverson et al. 1975; Machin et al. 1975; Mount 1984; Mücke et al. 1970; Seiber et al. 1993; Yang et al. 1971). Both DEP and DETP have been detected in the urine of exposed insecticide applicators (Maizlish et al. 1987) and volunteers administered diazinon orally or dermally (Garfitt et al. 2002). Although analysis of urine samples for the presence of these metabolites represents a potential means of assessing recent human exposure to diazinon, DEP and DETP can originate from exposure to other organophosphorus compounds and, therefore, are not specific for diazinon exposure. Further studies designed to refine the identification of metabolites specific to diazinon and provide dosimetric data will be useful in the search for a more dependable biomarker of diazinon exposure.

The development of recent analytical techniques allows for the simultaneous detection of numerous biomolecules, thus facilitating complete description of the genome for a particular organism (genomics). These techniques can be applied to analysis of multiple gene transcripts (transcriptomics), proteins (proteomics), and metabolites (metabolomics). The application of these techniques to conventional toxicology is known as toxicogenomics. Although toxicogenomic data are not presently available for diazinon, such data could eventually lead to a more complete understanding of pharmacokinetic pathways involved in diazinon toxicity and might possibly elucidate particular biomarkers of exposure.

Effect. The major action resulting from human exposure to diazinon is the inhibition of AChE (Coye et al. 1987; Davies and Holub 1980a, 1980b; Enan et al. 1982; Harris et al. 1969; Rajendra et al. 1986; Takahashi et al. 1991; Wecker et al. 1985). Two pools of cholinesterases are present in human blood: RBC AChE and plasma ChE. RBC AChE is identical to AChE present in neuromuscular tissue (the target of diazinon action). Inhibition of both forms of cholinesterase has been associated with exposure to diazinon in humans (Coye et al. 1987; Soliman et al. 1982) and animals. While plasma ChE has no known physiological function, available data indicate that plasma ChE activity is a more sensitive marker for diazinon exposure than RBC AChE activity (Endo et al. 1988; Hayes et al. 1980). Therefore, future studies that provide qualitative and dosimetric information regarding diazinon exposure and plasma ChE inhibition may provide a useful biomarker of effect for diazinon (or other anticholinesterase compounds) exposure. Currently, no effect specific to diazinon exposure has been identified by any study. Future studies designed to provide such information would be useful in identifying exposure to diazinon.

Absorption, Distribution, Metabolism, and Excretion. No studies were located regarding distribution and metabolism of diazinon after inhalation or dermal exposure in humans or animals, or regarding the excretion of diazinon after dermal exposure in animals. Diazinon was detected in several tissues from a woman who had ingested a lethal amount of a diazinon formulation, indicating rapid gastrointestinal tract absorption (Poklis et al. 1980). Rapid and extensive absorption was noted following the ingestion of a 0.011 mg/kg dose of diazinon by a group of five volunteers (Garfitt et al. 2002). Results of animal studies confirm the rapid absorption of diazinon following oral administration (Abdelsalam and Ford 1986; Iverson et al. 1975; Janes et al. 1973; Machin et al. 1971, 1974; Mücke et al. 1970; Wu et al. 1996a). Dermal absorption of diazinon has also been demonstrated in humans (Garfitt et al. 2002; Wester et al. 1993). Animal studies confirm the observation of rapid, widespread distribution of absorbed diazinon (Abdelsalam and Ford 1986; Janes et al. 1973; Machin et al. 1971, 1974; Mücke et al. 1970). Both human and animal data demonstrate rapid metabolism of diazinon and its oxon to DEP, DETP, and IMHP, which are predominantly excreted in the urine (Garfitt et al. 2002; Klemmer et al.

1978; Mücke et al. 1970; Poklis et al. 1980; Wester et al. 1993; Wu et al. 1996a). Additional studies designed to quantify the toxicokinetics of diazinon following inhalation, oral, and dermal exposure in humans and animals would be useful. Dermal exposure studies could be designed to assess the extent of diazinon degradation on the skin prior to absorption and the relative dermal penetrability of diazinon breakdown products.

Comparative Toxicokinetics. A PBPK/PD model has been developed to predict both pharmacokinetic disposition of diazinon and metabolites and pharmacodynamic effect (esterase activity inhibition) for diazoxon in target tissues of rats and humans for multiple routes of exposure (Poet et al. 2004). The model structure is identical for both rats and humans, with species-specific parameter values used for physiological volumes and flow rates and model-optimized values used for oral absorption. The model was evaluated for simulating oral, intravenous, and intraperitoneal doses in rats ranging from 15 to 100 mg/kg. For humans, it was evaluated for a single oral dose level of 11 μg/kg and a dermal dose of 4 mg/kg. In humans, the model predicts urine levels of diazinon metabolites from oral and dermal exposures that are very similar to observations; however, the ability of the model to accurately simulate levels of diazinon and diazoxon and levels of esterase inhibition in human target tissues is uncertain due to the lack of human data to validate these endpoints. Therefore, the model was not used for MRL derivation. Additional human data regarding blood and RBC diazinon and diazoxon levels would serve to reduce uncertainty of the human model predictions. Comparative human and rat pharmacokinetic studies of diazinon could also provide valuable species-specific pharmacokinetic data and reduce uncertainty of PBPK model predictions.

Methods for Reducing Toxic Effects. Procedures used to limit absorption and to interfere with the mechanism of action of organophosphates, including diazinon, following acute exposure have been adequately described (Carlton et al. 1998; Clark 2002; Osmundson 1998). However, methods for reducing toxicity following long-term, low-level exposure are lacking, and would be needed if potential health effects from long-term, low-level exposure to diazinon are identified.

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.

It is not known whether children are more susceptible than adults to diazinon toxicity. A single human study reported neurophysiological and neuropsychological deficits and delayed bone growth in young

children exposed at home to a formulation of diazinon that was misused to control an infestation of fleas (Dahlgren et al. 2004). These results provide suggestive evidence that children may be particularly susceptible to diazinon toxicity during critical periods of neural and skeletal development. Results of one animal study indicated that fetal exposure to low levels of diazinon may result in functional deficits that can only be detected by systemic behavioral evaluation (Spyker and Avery 1977). Results of subcutaneous injection studies indicate that critical periods of neurological development may be particularly sensitive to diazinon toxicity (Jameson et al. 2007; Slotkin et al. 2006a, 2006b, 2007). Young rats appear to be more susceptible than adult rats to diazinon-induced brain AChE inhibition, which may be at least partially due to decreased detoxification capability in the young rats (Padilla et al. 2004). Additional animal studies should be designed to support initial findings of age-related differences in susceptibility to diazinon toxicity.

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

Three ongoing studies pertaining to diazinon were located in a search of the Federal Research in Progress database (FEDRIP 2006).

Dr. L. Costa of Fred Hutchinson Cancer Research Center, Seattle, Washington is investigating relationships between PON1 polymorphism and diazinon and diazoxon metabolism using a physiologically-based kinetic model.

Dr. J. Seifert of the University of Hawaii, Honolulu, Hawaii is searching for changes in rat liver proteins that may be linked to diazinon-induced alterations in blood glucose concentrations and the metabolism of l-tryptophan at intraperitoneally-injected doses of diazinon that are clearly neurotoxic.

Dr. B. Wilson of the University of California, Davis, California is using organophosphates, including diazinon, to develop biomarkers of exposure and to study the molecular and cellular mechanisms of toxicity as part of a more wide-ranging project.