2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO DIAZINON IN THE UNITED STATES

Diazinon is an organophosphorus insecticide primarily used for agricultural purposes and is released to the environment through spraying on a wide variety of agricultural crops and at agricultural sites. Once diazinon is introduced into the environment, it may be activated by atmospheric photooxidation or degraded by hydrolysis or biodegradation mediated by microorganisms found in most sediment, soils, and water. Diazinon and diazoxon can be transported from the site of application by precipitation, fog, and wind to other areas. Since diazinon is moderately mobile in soils under certain conditions, it has the potential to migrate through the soil and into groundwater. Volatilization of diazinon from ground surfaces following aerial applications has been observed. Data from limited studies suggest that bioconcentration of diazinon does not occur to a significant extent in most aquatic organisms tested, and that it is rapidly metabolized when it is accumulated.

Significant exposure of the general population to diazinon is not likely at present, due to the ban on residential uses. Diazinon was formerly used in household and garden products for pest control. However, manufacturing of indoor use products was discontinued on June 30, 2001 and production of non-agricultural outdoor use products containing diazinon was discontinued on June 30, 2003. As of December 31, 2004, sales of diazinon-containing products for residential use were discontinued, although numerous restricted-use commercial products that contain diazinon are still available. Because diazinon-containing products are no longer sold for residential use, potential for significant exposure of the general population is expected to decrease as supplies that were obtained and stored before discontinuation are expended. General population exposure to diazinon may occur through ingestion of contaminated food or drinking water and inhalation. Ingestion of foods contaminated with small residues of diazinon is the most likely route of exposure for the general population not living in areas where diazinon is extensively used. The general population may also be exposed to diazinon through inhalation of contaminated ambient (outdoor) air.

Populations living within or very near areas of heavy agricultural diazinon use would have an increased risk of exposure to relatively larger amounts of diazinon through dermal contact with contaminated plants, soils, surface waters, or artificial surfaces such as playground equipment and pavements; by

inhalation of the mist formed from the applied insecticide; or by ingestion of water or food-borne residues. Those likely to receive the highest levels of exposure are those who are involved in the production, formulation, handling, and application of diazinon, farm workers who enter treated fields prior to the passage of the appropriate restricted entry intervals, and workers involved in the disposal of diazinon or diazinon-containing wastes. Dermal contact appears to be the major route of exposure for workers. Inhalation of diazinon in occupational settings depends on its volatility, the type of formulation used, and the application technique employed.

Children are expected to be exposed to diazinon by the same routes that affect adults. Small children are more likely to come into contact with diazinon residues that may be present in soil and dust, due to increased hand-to-mouth activity and playing habits. Diazinon has been detected in foods found in infant and toddler diets at concentrations of up to 0.46 mg/kg food. No data were located regarding diazinon in breast milk; therefore, an adequate determination of the importance of this route of child exposure has not been made.

See Chapter 6 for more detailed information regarding concentrations of diazinon in environmental media.

2.2 SUMMARY OF HEALTH EFFECTS

Diazinon is considered to be of moderate toxicity compared to other organophosphates. 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.

High-level acute exposure to diazinon causes severe AChE inhibition that often leads to cholinergic signs and symptoms, manifest as reversible neuromuscular dysfunction when treated or when exposure is terminated. These manifestations include muscarinic effects (bronchoconstriction, increased bronchosecretion, nausea and vomiting, diarrhea, bradycardia, hypotension, miosis, urinary incontinence), nicotinic effects (tachycardia, hypertension, muscular twitching and weakness, fasciculation, cramping), and central nervous system effects (anxiety, apathy, depression, giddiness, drowsiness, insomnia, nightmares, headaches, confusion, ataxia, depressed reflex, seizure, respiratory depression, coma). In sufficiently high exposures (accidental or intentional), respiratory and cardiac failure and death may result

without timely treatment intervention. The cholinergic manifestations of high acute exposure to diazinon have also been reported in animals and include anorexia, ataxia, epistaxis, tremors, listlessness, gasping, convulsions, tachypnea, dyspnea, prostration, fasciculations, twitches, exophthalmos, diarrhea, salivation, diuresis, lacrimation, prostration, Straub tail reflex, and hypothermia. Clinical signs of diazinon neurotoxicity following repeated oral exposure in animals have been reported at doses ranging from 30 to 300 mg/kg/day. Limited information is available regarding clinical signs of neurotoxicity in animals exposed to diazinon by inhalation. One study reported decreased activity and salivation responses in rats exposed to an aerosol of diazinon for 4 hours at an exposure level of 2,330 mg/m^3.

As previously noted, the systemic toxicity of diazinon is mainly attributable to its action on the nervous system. Although AChE is intimately associated with neurotransmission within the central and peripheral nervous system, AChE is also found in red blood cells (RBCs). The blood plasma of humans and animals contains cholinesterases as well. Plasma cholinesterase (ChE) in humans is comprised almost entirely of butyrylcholinesterase (also known as pseudocholinesterase), whereas AChE constitutes a portion of the plasma ChE of animals, the relative amount of which is species dependent. In both humans and animals, measures of plasma ChE and RBC AChE activities have been used as indicators of exposure to cholinesterase inhibitors such as diazinon. Plasma ChE inhibition may often be observed at exposure levels lower than those inducing measurable RBC AChE inhibition. However, decreased activity of RBC AChE is more indicative of a potential neurotoxic effect because RBC AChE is identical to neural AChE, whereas butyrylcholinesterase has not been demonstrated to play a role in the development or function of the central or peripheral nervous system.

Numerous animal studies and limited controlled human studies identify levels of exposure to diazinon resulting in plasma ChE, RBC AChE, and/or brain AChE inhibition. RBC and/or brain AChE inhibition of 20–59% is considered to represent a less serious adverse neurological effect in the absence of more serious indicators of neurotoxicity. In this Toxicological Profile, “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. The animal studies identified exposure levels at which diazinon caused RBC AChE inhibition in the absence of more serious indicators of neurotoxicity, which indicates that RBC AChE inhibition at such exposure levels may represent the most sensitive effect for diazinon toxicity. For example, inhibition of RBC and/or brain AChE at magnitudes ranging from 20 to 60% (in the absence of clinical signs) was observed following repeated oral dosing in the range of 0.3–75 mg/kg/day. In a repeated-exposure inhalation study, exposure to an airborne concentration of 1.57 mg/m^3, 6 hours/day, 5 days/week for 3 weeks resulted in 36–39% RBC AChE inhibition in rats.

Plasma ChE inhibition can typically be observed at doses lower than those required to produce significant RBC and/or brain AChE inhibition; RBC AChE appears to be more sensitive than brain AChE to diazinon toxicity. Following single oral dosing, peak cholinesterase inhibition is typically observed at 6–12 hours. Results of longer-term oral studies indicate that diazinon-induced plasma ChE and RBC AChE inhibition increases in severity with exposure duration to a peak at approximately 35 days; after which the severity of the inhibition remains relatively constant. Rat and dog studies indicate that females may be more sensitive than males to diazinon-induced cholinesterase inhibition, particularly with respect to brain AChE inhibition. Diazinon-induced neurohistopathological effects have not been demonstrated.

Diazinon does not appear to be a reproductive or developmental toxicant at exposure levels that do not result in maternal toxicity. There is limited evidence of morphological changes in spleen, thymus, and lymph nodes of animals following oral exposure to relatively high doses of diazinon, but no studies have demonstrated compromised immunological function. Predominantly negative results have been reported in testing of diazinon for genotoxicity. Two epidemiological studies reported weak associations between exposure to diazinon and lung cancer. Results of a few case-control studies have suggested possible links between diazinon exposure and non-Hodgkin's lymphoma, multiple myeloma, and childhood brain cancer. However, all of these studies involved exposure to other pesticides as well. A 2-year oral cancer bioassay in rats and mice did not find evidence for diazinon-induced carcinogenicity.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for diazinon. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1994b), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an

example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

Diazinon is one member of a class of organophosphates that share a common mechanism of action, namely inhibition of acetylcholinesterase (AChE). Although the Toxicological Profile for Diazinon presents MRLs derived for diazinon in particular, human exposure scenarios may include simultaneous exposure to multiple organophosphate AChE inhibitors. MRLs derived specifically for diazinon may not be adequately protective for exposure scenarios that include exposure to multiple similarly-acting organophosphate AChE inhibitors.

Inhalation MRLs

An acute-duration inhalation MRL for diazinon was not derived due to the lack of suitable acute-duration human or animal data. Available reports of neurotoxicity indicators in humans exposed to diazinon by the inhalation route of exposure do not include quantitative data regarding exposure levels (Coye et al. 1987; Dahlgren et al. 2004; Kamha et al. 2005; Maizlish et al. 1987; Rayner et al. 1972; Richter et al. 1992; Soliman et al. 1982; Stalberg et al. 1978). Some of these exposures included multiple exposure routes and exposures to other pesticides as well. 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.

- An MRL of 0.01 mg/m^3 has been derived for intermediate-duration inhalation exposure (15–364 days) to diazinon.

This MRL is based on a no-observed-adverse-effect level (NOAEL) of 1.57 mg diazinon/m^3 for RBC AChE inhibition (a critical target of diazinon toxicity) observed in a 21-day study in hybrid rats (Hartman 1990).

No human reports were located regarding intermediate-duration inhalation exposure to diazinon. A single animal study (Hartman 1990) was located in which toxic effects of intermittent exposure to aerosols of diazinon for 21 days were assessed. This study served as the principal study for deriving an intermediate-

duration inhalation MRL for diazinon. Support for the selection of diazinon-induced RBC AChE inhibition as the critical effect is provided by the results of numerous animal studies that employed the oral exposure route. In the principal inhalation study (Hartman 1990), groups of rats (10 of each sex) were exposed to control air or air containing four different concentrations of aerosolized diazinon (0.05, 0.46, 1.57, or 11.6 mg/m^3) for 6 hours/day, 5 days/week for 3 weeks. No clinical signs of organophosphate neurotoxicity or effects on survival or body weight were observed. Histopathology of the nasal tract and lungs was normal in all groups, as was the spleen, heart, liver, kidney, and adrenal gland (examined only in the 11.6 mg/m^3 groups). Plasma ChE activity and RBC and brain AChE activity in the male and female rats are shown in Table 2-1. Significant reductions in plasma ChE (marker for exposure) were seen in males at exposure levels ≥1.57 mg/m^3 and in females at exposure levels ≥0.46 mg/m^3. Organophosphate-induced plasma ChE inhibition is typically observed at exposure levels lower than those inducing measurable RBC or brain AChE inhibition. Plasma ChE inhibition is used as an indicator of exposure, but does not serve as a reliable indicator of a neurotoxic effect. However, inhibition of RBC AChE and brain AChE represents a relevant neurological effect. In the principal study (Hartman 1990), significant reductions in RBC AChE activity (surrogate marker for neural AChE activity) were seen in male rats at 11.6 mg/m^3 and in female rats at 1.57 and 11.6 mg/m^3 (Table 2-1). Treatment-related 20–59% RBC or brain AChE inhibition is considered to represent a less serious adverse effect in the absence of more clear indicators of neurotoxicity (Chou and Williams-Johnson 1998). The 10% RBC AChE inhibition observed in the 1.57 mg/m^3 group of female rats is below the level of inhibition considered to represent an adverse effect. Therefore, the 1.57 mg/m^3 exposure level is a NOAEL and the highest exposure level (11.6 mg/m^3) is the lowest-observed-adverse-effect level (LOAEL) for 36 and 39% RBC AChE inhibition in the male and female rats, respectively. There was no significant difference between brain AChE activity in any of the exposure groups of male rats and that of vehicle controls. All diazinon-exposed groups of female rats exhibited significantly decreased brain AChE activity, relative to vehicle controls. The report of significantly increased brain AChE inhibition in the female rats of all exposure levels is indicative of an inherent problem with the brain data set, perhaps related to tissue collection or quantitative analysis of enzymatic activity in the brain tissue of the female rats. Furthermore, results of repeated oral dosing (Singh 1988) indicate that the male and female rats are comparably sensitive to diazinon-induced effects on both RBC and brain AChE activity. Therefore, the report (Hartman 1990) of significant brain AChE inhibition in the female rats exposed to diazinon by inhalation at levels much lower than the LOAEL of 11.6 mg/m^3 for RBC AChE inhibition is questionable, and a clear LOAEL for brain AChE inhibition cannot be determined.

Table 2-1. Effect of Aerosol Diazinon on Plasma ChE and RBC and Brain AChE Activity in Male and Female Rats Exposed for 6 Hours/Day, 5 Days/Week for 3 Weeks

	Mean plasma ChE activity in U/L (percent change from controls)	Mean RBC AChE activity in U/L (percent change from controls)	Mean brain AChE activity in U/g (percent change from controls)
Males			
Vehicle controls	368.9	894.2	3.893
0.05 mg/m^3	401.5 (+9%)[a]	908.6 (+2%)	3.855 (-1%)
0.46 mg/m^3	351.4 (-5%)	849.5 (-5%)	3.898 (0%)
1.57 mg/m^3	316.6 (-14%)[b]	840.1 (-6%)	3.737 (-4%)
11.6 mg/m^3	297.9 (-19%)[b]	573.0 (-36%)[a]	3.883 (0%)
Females			
Vehicle controls	631.8	887.7	3.742
0.05 mg/m^3	615.5 (-3%)	882.7 (-1%)	2.838 (-24%)[a]
0.46 mg/m^3	506.0 (-20%)[b]	943.0 (+6%)	3.106 (-17%)[b]
1.57 mg/m^3	459.0 (-27%)[a]	798.6 (-10%)[b]	2.983 (-20%)[b]
11.6 mg/m^3	361.0 (-43%)[a]	545.0 (-39%)[a]	2.369 (-37%)[a]

[a]statistically significantly different from control ($p \leq 0.01$)
[b]statistically significantly different from control ($p \leq 0.05$)

AChE = acetylcholinesterase; ChE = cholinesterase; RBC = red blood cell

Source: Hartman 1990

Benchmark dose (BMD) analysis of the critical effect data sets for AChE inhibition from the principal study of Hartman (1990) was not possible. Although mean values for RBC and brain AChE activity were reported, measures of variance (standard deviation or standard error) were not included in the report. The NOAEL of 1.57 mg/m^3 for RBC AChE activity in the male and female rats of the principal study (Hartman 1990) served as the point of departure for deriving an intermediate-duration inhalation MRL for diazinon. The NOAEL was adjusted for intermittent exposure as follows:

$NOAEL_{ADJ}$ = 1.57 mg diazinon/m^3 x 6 hours/24 hours x 5 days/7 days = 0.28 mg diazinon/m^3

A regional deposited dose ratio ($RDDR_{ER}$) of 1.558 for extrarespiratory effects was used to extrapolate from rats to humans. The $RDDR_{ER}$ was calculated using EPA's software (Version 2.3) (EPA 1994b) for calculating RDDRs and the parameters listed in Table 2-2.

The human equivalent concentration was calculated using Equation 4-5 (EPA 1994b) as follows:

$NOAEL_{HEC}$ = $NOAEL_{ADJ}$ x $RDDR_{ER}$ = 0.28 mg diazinon/m^3 x 1.558 = 0.44 mg diazinon/m^3

The $NOAEL_{HEC}$ of 0.44 mg/m^3 was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans using dosimetric adjustment and 10 for human variability) resulting in an intermediate-duration inhalation MRL of 0.01 mg/m^3.

No human or animal data were located regarding health effects from chronic-duration inhalation exposure to diazinon, precluding the derivation of a chronic-duration inhalation MRL for diazinon.

Oral MRLs

- An MRL of 0.006 mg/kg/day has been derived for acute-duration oral exposure (1–14 days) to diazinon.

The acute-duration oral MRL is based on a NOAEL of 0.6 mg/kg/day and a LOAEL of 1.2 mg/kg/day for >20% RBC AChE inhibition in rats exposed to diazinon in the diet (Davies and Holub 1980a).

Available information regarding health effects in humans following acute-duration oral exposure to diazinon is restricted to individual case reports of serious effects and death, which precludes derivation of an acute-duration oral MRL based on human data. Most acute-duration oral studies in animals involved

Table 2-2. Parameters Used to Calculate the Regional Deposited Dose Ratio ($RDDR_{ER}$) for Diazinon-induced Extrarespiratory Effects Using EPA's Software (Version 2.3)

Biological parameters[a]	Rat	Human
Surface area		
Extrathoracic	15 cm^2	200 cm^2
Tracheobronchial	22.5 cm^2	3,200 cm^2
Pulmonary	0.34 m^2	54 m^2
Minute ventilation	147.24 mL	13.8 L
Body weight	196 g	70 kg

[a]Parameters are default values for rats and humans from the EPA software, except for the rat body weight, which was the mean body weight for the 1.57 mg/m^3 exposure group of female rats.

Mass Median Aerodynamic Diameter (MMAD) = 0.85 µm from lower limit of 0.8 µm and upper limit of 0.9 µm for the 1.57 mg/m^3 exposure group of female rats reported by Hartman (1990).

Geometric Standard Deviation (GSD) = 1.3 µm from lower limit of 1.2 µm and upper limit of 1.4 µm reported by Hartman (1990).

Source: Hartman 1990

diazinon doses that caused serious neurological effects. One single-dose oral gavage study (unpublished) identified a NOAEL of 2.5 mg/kg and a LOAEL of 25 mg/kg for 35% RBC AChE inhibition and 36% brain AChE inhibition (EPA 2000a). However, the single-dose gavage level of 2.5 mg/kg was the lowest dose tested in a similar study (unpublished) and represented a LOAEL for 40% RBC AChE inhibition (EPA 2000a). In yet another unpublished study (EPA 1996), rats were administered diazinon in the diet for 28 days and assessed for cholinesterase inhibition at weeks 1, 2, and 4. An estimated dose of 2.4 mg/kg/day resulted in 38–59% RBC AChE inhibition, which was observed as early as week 1 and peaked at week 2. The next lower dose (0.02 mg/kg/day) represented a NOAEL.

Results of repeated-dose oral animal studies indicate that diazinon-induced AChE inhibition progressively increases in magnitude with time. For example, in female Wistar rats administered diazinon (99.2% purity) in the diet for 92 days, RBC AChE activity was significantly depressed as early as day 8 (Davies and Holub 1980a). By day 12, the magnitude of RBC AChE inhibition was approximately 5 and 22% at dietary concentrations resulting in doses of approximately 0.6 mg/kg/day (NOAEL) and 1.2 mg/kg/day (LOAEL), respectively. The study of Davies and Holub (1980a) identified the lowest LOAEL for the critical effect (22% RBC AChE inhibition) associated with the highest NOAEL for acute-duration oral exposure to diazinon and was therefore selected as the principal study for deriving an acute-duration oral MRL for diazinon.

In the principal study (Davies and Holub 1980a), female Wistar rats were administered diazinon (99.2% purity) in the diet at concentrations of 0, 5, 10, or 15 ppm for 92 days. Blood samples were collected on treatment days 3, 8, and 12 from 10 rats/group for assessment of plasma ChE and RBC AChE activity. Other groups of similarly-treated rats were sacrificed (n=6) for assessment of brain AChE activity. All rats were assessed daily for clinical signs of neurotoxicity and body weights and food intake were monitored throughout the treatment period. Based on reported food consumption and body weight data, calculated doses for the first 12 days of exposure were 0.6, 1.2, and 1.8 mg/kg/day for the 5- 10-, and 15-ppm exposure groups, respectively.

No clinical signs of toxicity were observed in any of the treated groups. At treatment day 12, treatment-related effects included 43, 70, and 73% plasma ChE inhibition and 5, 22, and 33% RBC AChE inhibition in the 0.6, 1.2, and 1.8 mg/kg/day dose groups, respectively. There was no significant effect on brain AChE activity. Plasma ChE inhibition is used as an indicator of exposure, but does not serve as a reliable indicator of a neurotoxic effect. Therefore, plasma ChE inhibition was not considered relevant to the selection of the critical effect for diazinon. However, inhibition of RBC AChE and brain AChE

represents a relevant neurological effect. Treatment-related 20–59% RBC or brain AChE inhibition is considered to represent a less serious adverse effect in the absence of more clear indicators of neurotoxicity (Chou and Williams-Johnson 1998). The principal study (Davies and Holub 1980a) identified a NOAEL of 0.6 mg/kg/day and a LOAEL of 1.2 mg/kg/day for 22% RBC AChE inhibition at interim day 12 assessment of female rats administered diazinon in the diet for 92 days.

BMD analysis of the critical effect data (RBC AChE inhibition) was not possible because quantitative statistical data (mean and standard error or standard deviation) for the critical effect were not included in the study report. 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) resulting in an intermediate-duration inhalation MRL of 0.006 mg/kg/day.

- An MRL of 0.002 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to diazinon.

The intermediate-duration MRL is based on RBC AChE inhibition in female rats exposed to diazinon in the diet (Davies and Holub 1980a).

Available human information regarding intermediate-duration oral exposure to diazinon is restricted to a controlled study in which four male volunteers were administered diazinon in gelatin capsules at a dose level of 0.03 mg/kg/day for up to 31 days (EPA 2001). There were no treatment-related clinical signs. Approximately 22–42% plasma ChE inhibition was noted as early as treatment day 8 and reached a maximum of 47–55% by day 20 or the end of treatment. Because there was no indication of treatment-related effects on RBC AChE activity or clinical signs of neurotoxicity, the 0.03 mg/kg/day dose level represents a free-standing NOAEL.

Results of numerous oral studies in animals identify AChE inhibition as the most sensitive effect of diazinon toxicity following oral exposure. Table 2-3 presents a summary of NOAELs and LOAELs for RBC and brain AChE inhibition following intermediate-duration oral exposure to diazinon. These values were identified from publicly-available studies and unpublished studies submitted to EPA's Office of Prevention, Pesticides, and Toxic Substances. Although dose spacing among these intermediate-duration oral studies is variable, and in some studies may be in excess of 100-fold for levels at or below identified LOAELs for AChE inhibition, these studies collectively indicate that the threshold for less serious AChE inhibition occurs in rats and dogs at repeated oral dose levels between 0.2 and 2 mg/kg/day.

Table 2-3. NOAELs and LOAELs for RBC and Brain AChE Inhibition Following Intermediate-duration Dietary Exposure to Diazinon

Study type estimated doses (mg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day) AChE inhibition	Reference
28-Day rat study	0.02 (M, F)	2.4; M, F: 38–59% RBC	EPA 1996
M, F: 0, 0.02, 2.4, 23, 213			
30-Day rat study	ND	2.86; 58% RBC	Davies and Holub 1980b
F: 0, 2.86			
35-Day rat study	0.2	ND	Davies and Holub 1980a
F: 0, 0.009, 0.05, 0.09, 0.2			
42-Day rat study	0.2	0.3; 20% RBC	Davies and Holub 1980a
F: 0, 0.09, 0.18, 0.27, 0.36			
6-Week rat study	0.2 (M, F)	2.0; M, F: 46–61% RBC	EPA 2000a
0, 0.02, 0.05, 0.2, 2, 9.5, 28			
6-Week rat study			EPA 2000a
M: 0, 0.02, 0.04, 0.17, 1.68, 8.6, 25.8	0.17 (M)	1.68; M: 29–35% RBC	
F: 0, 0.02, 0.05, 0.19, 1.82, 9.27, 29	0.19 (F)	1.82; F: 16–35% RBC	
6-Week rat study	0.2 (M, F)	8.4; M: 21% RBC	Singh 1988
M: 0, 0.04, 0.2, 8.4, 165			
F: 0, 0.05, 0.2, 9.4, 198		9.4; F: 21% RBC and 24% brain	
90-Day rat study			Singh 1988
M: 0, 0.03, 0.3, 15, 168	0.3 (M)	15; M: 27% RBC	
F: 0, 0.04, 0.4, 19, 212	0.4 (F)	19; F: 41% RBC	
90-Day rat study	0.018 (M, F)	1.8; M, F: 37–75% RBC	EPA 1996
0, 0.018, 1.8, 18, 180			
92-Day rat study	0.4	0.7; 40% RBC	Davies and Holub 1980a
F: 0, 0.4, 0.7, 1			
4-Week dog study			Barnes 1988
M: 0, 0.02, 0.073, 0.8, 14.68	0.8 (M)	14.68; M: 25% RBC; 31% brain	
F: 0, 0.023, 0.082, 0.75, 15.99	0.75 (F)	5.6; F: 31% RBC; 30% brain	
13-Week dog study	0.02 (M, F)		Barnes 1988
M: 0, 0.0034, 0.02, 5.9, 10.9		5.9; M: 26% RBC; 31% brain	
F: 0, 0.0037, 0.02, 5.6, 11.6		5.6; F: 31% RBC ; 30% brain	

AChE = acetylcholinesterase; F = female; LOAEL = lowest-observed-adverse-effect level; M = male; ND = not determined; NOAEL = no-observed-adverse-effect level; RBC = red blood cell

In selecting the principal study for deriving an intermediate-duration oral MRL for diazinon, two 6-week oral rat studies (EPA 2000a) employed relatively narrow dose spacing in the region of 0.2–2 mg/kg/day and were initially considered as candidates for deriving an intermediate-duration oral MRL for diazinon. However, the results of these studies are available only in summary form. The 90-day oral rat study of Singh (1988) was considered as a candidate for the principal study because (1) the full study was available to Agency for Toxic Substances and Disease Registry, (2) the study identified the highest NOAEL (0.4 mg/kg/day) below the lowest LOAELs identified in other repeated-dose oral studies, and (3) quantitative dose-response data for the critical effect (RBC AChE inhibition) were available for BMD analysis. The 42-day oral rat study of Davies and Holub (1980a) was considered because the effects of diazinon on RBC AChE inhibition were assessed at several doses within the low-dose range (0.1–0.4 mg/kg/day). This study identified a NOAEL of 0.18 mg/kg/day and a LOAEL of 0.27 mg/kg/day for 20% RBC AChE inhibition.

In the study of Singh (1988), groups of male and female Sprague-Dawley rats (15/sex) were administered diazinon MG-8 (purity 87.7%) in the diet at concentrations of 0, 0.5, 5, 250, or 2,500 ppm (after adjusting for purity) for 90 days. The corresponding doses were calculated by the study authors to be 0.03, 0.3, 15, and 168 mg/kg in males and 0.04, 0.4, 19, and 212 mg/kg in females. Clinical observations were made daily and body weight and food consumption were recorded weekly. Clinical laboratory measurements and physical, auditory, and ophthalmoscopic exams were performed prior to termination. Prior to necropsy, blood and urine samples were collected for hematology, clinical chemistry, and urinalysis. At necropsy, organ weights were recorded and comprehensive gross and microscopic examinations were performed on all rats. A portion of each brain was processed for assessment of AChE activity. All animals survived the 90-day dosing period. Treatment-related clinical symptoms were observed at the highest dose level and included hyperactivity and hypersensitivity to touch and sound in males and females and aggressive behavior in males. No treatment-related gross or microscopic abnormalities were seen in any of the treatment groups. Hematology and urinalysis were unremarkable, with the exception of decreased mean hemoglobin and hematocrit accompanied by an increase in reticulocytes in high-dose females. Statistically-significant ($p<0.01$) effects on ChE and AChE included decreased plasma ChE activity in males and females at doses ≥0.3 mg/kg/day, decreased RBC AChE activity at doses ≥15 mg/kg/day in males and ≥0.4 mg/kg/day in females, and decreased brain AChE activity at the highest dose in males and doses ≥19 mg/kg/day in females. As discussed earlier, a 20–59% inhibition (reduction in measured activity) of neural or RBC AChE may be considered a less serious effect in the absence of more serious indicators of neurotoxicity (Chou and Williams-Johnson 1998). Treatment-related decreased RBC activity was noted at doses lower than those resulting in decreased brain AChE activity

and was therefore selected as the critical effect for BMD analysis. Table 2-4 contains the data that were modeled.

The linear model in the EPA Benchmark Dose Software (Version 1.3.2) was initially fit to the male rat data for RBC AChE activity shown in Table 2-4. A benchmark response (BMR) of 20% below the control mean RBC AChE activity was selected because 20–59% RBC or brain AChE inhibition is considered to represent a less serious effect in the absence of more serious indicators of neurotoxicity (Chou and Williams-Johnson 1998).

Inadequate fit was provided by the linear model, as indicated by a p value <0.0001 for the test of mean fit (according to BMD technical guidance, a p value ≥0.1 indicates an adequate goodness-of-fit). The same result was obtained using the BMD software for the polynomial and power models. The BMD software for the Hill model calculated a BMD_{20} of 0.38 mg/kg/day for the male rat data, but failed to identify the lower 95% confidence limit ($BMDL_{20}$) value (presumably due to a bad completion code in an optimization routine). Under the presumption that the highest dose may have been a major influence in the model output, the data from the highest dose were eliminated and the linear model was fit to the remaining data. In this case, a near-adequate fit was obtained, as indicated by a p value of 0.09294 for the test of mean fit. The polynomial and power model outputs from the male rat data set (minus the highest dose) were found to be clearly inadequate, based on p values of 0.02919 and 0.02928 for the test of mean fit. Without the high-dose data, the data set for RBC AChE activity in the male rats of the Singh (1988) study contained insufficient dose groups to accommodate the requirements for the Hill model. In summary, the results of BMD analysis of RBC AChE activity in the male rats of the Singh (1988) study were rejected due to inadequate fit from all available continuous data models in the EPA Benchmark Dose Software (Version 1.3.2).

For the female rat data (see Table 2-4), inadequate fit was provided by the linear, polynomial, and power models, as indicated by p values <0.0001 for the test of mean fit. The Hill model provided the only adequate fit of the female data and resulted in a BMD_{20} of 0.56 mg/kg/day and a $BMDL_{20}$ of 0.38 mg/kg/day. Elimination of the RBC AChE activity data for the highest-dose female rats did not result in adequate fit for the linear, polynomial, or power models. Furthermore, this elimination resulted in insufficient dose groups to accommodate the requirements of the Hill model. In summary, BMD analysis of RBC AChE activity in the female rats of the Singh (1988) study resulted in a single adequate fit (Hill model output using all dose groups) and resulting BMD_{20} of 0.56 mg/kg/day and a $BMDL_{20}$ of 0.38 mg/kg/day.

Table 2-4. RBC AChE Data From Male and Female Rats Exposed to Diazinon in the Diet for 90 Days

Dose group (mg/kg/day)	Number of rats	RBC AChE activity (mU/mL)[a]	Percent RBC AChE inhibition
Males			
0	15	2093.333±44.150	
0.03	15	2186.667±68.220	–
0.3	15	2000.000±59.362	4
15	15	1526.667±58.119	27
168	15	1540.000±60.788	26
Females			
0	14	2300.000±58.366	
0.04	15	2213.333±46.667	4
0.4	15	1913.333±45.635	17
19	15	1353.333±40.079	41
212	15	1346.667±33.618	41

[a]Mean±standard error

Source: Singh 1988

In the study of Davies and Holub (1980a), groups of female Wistar rats (16/group) were exposed to diazinon (99.2% purity) in the diet at concentrations of 0, 1, 2, 3, or 4 ppm for 42 days. Blood samples were collected periodically from 10 rats/group for assessment of plasma ChE and RBC AChE activity. Six rats per group were sacrificed on day 35 for assessment of brain AChE activity. All rats were assessed daily for clinical signs of neurotoxicity and body weights and food intake were monitored throughout the treatment period. Based on reported food consumption and body weight data, the doses to the 1-, 2-, 3-, and 4-ppm exposure groups were calculated to be 0.09, 0.18, 0.27, and 0.36 mg/kg/day (rounded to one significant figure). No clinical signs of toxicity were observed in any of the treated groups. Significant plasma ChE inhibition was observed at most timepoints in all diazinon-treated groups, relative to controls. The magnitude of inhibition in all treatment groups increased with time and appeared to peak around day 35, remaining near the peak level for the remaining 7 treatment days. Maximum plasma ChE inhibition in the 0.09, 0.18, 0.27, and 0.36 mg/kg/day treatment groups was approximately 35, 50, 55, and >60%, respectively. Plasma ChE inhibition is used as an indicator of exposure, but does not serve as a reliable indicator of a neurotoxic effect. Therefore, plasma ChE inhibition was not considered relevant to the selection of the critical effect for diazinon. Through treatment day 35, there was no significant treatment-related effect on RBC AChE activity in any of the treatment groups. However, on treatment day 42, significant RBC AChE inhibition was observed at treatment levels of 0.18, 0.27, and 0.36 mg/kg/day (magnitude 9, 20, and 22%, respectively). There were no indications of treatment-related significant brain AChE inhibition at any timepoint during the 42 days of treatment. The results of RBC AChE activity in the female rats of the principal study (Davies and Holub 1980a) are presented in Table 2-5. Inhibition of RBC AChE and brain AChE represents a relevant neurological effect. Treatment-related 20–59% RBC or brain AChE inhibition is considered to represent a less serious adverse effect in the absence of more clear indicators of neurotoxicity (Chou and Williams-Johnson 1998). The principal study (Davies and Holub 1980a) identified a NOAEL of 0.18 mg/kg/day and a LOAEL of 0.27 mg/kg/day for 20% RBC AChE inhibition in female rats administered diazinon in the diet for 42 days.

The linear model in the EPA Benchmark Dose Software (Version 1.3.2) was fit to the female rat data. As discussed previously, a BMR of 20% below the control mean RBC AChE activity was selected because 20–59% RBC or brain AChE inhibition is considered to represent a less serious effect in the absence of more serious indicators of neurotoxicity (Chou and Williams-Johnson 1998). Initial BMD analysis using the linear model was performed using constant variance as one of the selected parameters. The model output indicated that a nonhomogeneous variance was more appropriate for the data set. Using a

Table 2-5. RBC AChE Data From Female Rats Exposed to Diazinon in the Diet for 42 Days

Dose group (mg/kg/day)	Number of rats	RBC AChE activity (µmole/mL packed cells/minute)[a]	Percent RBC AChE inhibition
0	10	0.74±0.05	
0.09	10	0.68±0.07	8
0.18	10	0.67±0.06	9
0.27	10	0.59±0.04	20
0.36	10	0.58±0.02	22

[a]Mean±standard error

AChE = acetylcholinesterase; RBC = red blood cell

Source: Davies and Holub 1980a

nonhomogeneous variance, the linear model provided adequate fit to the data from Table 2-5, as indicated by acceptable p values for tests for (1) differences in response and/or variances among dose levels, (2) homogeneous or nonhomogeneous variance, and (3) model mean fit. The resulting BMD_{20} was 0.36 mg/kg/day and the $BMDL_{20}$ was 0.25 mg/kg/day. Because the simplest model, the linear model, provided adequate fit to the RBC AChE data from the 42-day rat study of Davies and Holub (1980a), the application of more complex continuous variable models was not considered necessary.

BMD analysis identified two potential points of departure for deriving an intermediate-duration oral MRL for diazinon, the $BMDL_{20}$ of 0.3267 mg/kg/day for RBC AChE activity in the female rats from the 90-day oral rat study of Singh (1988) and the $BMDL_{20}$ of 0.2238 mg/kg/day for RBC AChE activity in the female rats from the 42-day oral study of Davies and Holub (1980a). The 42-day oral rat study of Davies and Holub (1980a) was selected as the principal study based on the fact that this study employed several dose groups in the low-dose region of threshold effect. Therefore, the $BMDL_{20}$ of 0.2238 mg/kg/day from the RBC AChE data of Davies and Holub (1980a) was selected as the point of departure for deriving an intermediate-duration oral MRL for diazinon. The $BMDL_{20}$ of 0.2238 mg/kg/day was divided by an uncertainty factor (UF) of 100 (10 for extrapolation from animals to humans and 10 for human variability) resulting in an intermediate-duration oral MRL of 0.002 mg/kg/day.

- An MRL of 0.0007 mg/kg/day has been derived for chronic-duration oral exposure (365 days or more) to diazinon.

The chronic-duration oral MRL is based on a NOAEL of 0.065 mg/kg/day and a LOAEL of 5.5 mg/kg/day for >20% RBC AChE inhibition in female rats administered diazinon in the diet for 98 weeks (Kirchner et al. 1991).

No human data are available from which to evaluate the health effects associated with chronic-duration oral exposure to diazinon. The available chronic-duration oral database in animals consists of two unpublished studies, a 98-week feeding study in rats and a 52-week feeding study in dogs. These studies identified RBC AChE inhibition as the most sensitive effect of diazinon toxicity.

The 52-week dog study (Rudzki et al. 1991) identified a NOAEL of 0.5 ppm (0.017 mg/kg/day) and a LOAEL of 150 ppm (4.6 mg/kg/day) for RBC AChE inhibition of 20% or more in both males and females. The 98-week rat study (Kirchner et al. 1991) identified a NOAEL of 0.065 mg/kg/day and a LOAEL of 5.5 mg/kg/day for RBC AChE inhibition of 20% or more in both males and females. The 98-week rat study (Kirchner et al. 1991) was selected as the principal study for deriving a chronic-

duration oral MRL for diazinon because it identified a slightly higher NOAEL (0.065 mg/kg/day) than the NOAEL (0.017 mg/kg/day) identified in the 52-week dog study (Rudzki et al. 1991).

In the 98-week rat study (Kirchner et al. 1991), diazinon MG-8 (purity 87.7%) was dissolved in acetone vehicle and added to the diet of male and female Sprague-Dawley rats at concentrations of 0, 0.1, 1.5, 125, or 250 ppm for up to 98 weeks. The study included both untreated and vehicle control groups. According to the study authors, the corresponding diazinon doses (adjusted for purity) were 0, 0.004, 0.06, 5, and 10 mg/kg/day for males and 0, 0.005, 0.07, 6, and 12 mg/kg/day for females), which result in averaged doses of 0, 0.0045, 0.065, 5.5, and 11 mg/kg/day for both males and females. Twenty rats/sex/group were treated for the full 98 weeks. Ten rats/sex/group were treated for 52 weeks and sacrificed for interim assessment. Additional groups of 10 rats/sex were assigned to the untreated control, vehicle control, and 250 ppm groups and assessed for recovery 45 days following 52 weeks of treatment. Animals were observed daily for clinical signs of toxicity. Food consumption, water intake, and body weights were monitored. Ophthalmoscopic examinations were performed during weeks 2, 51, and 97 or 98. Blood was collected on at several timepoints between days 88 and 684. Ten animals/sex/group from the 98-week treatment groups received clinical chemistry evaluation at treatment days 88, 181, 356, 390, 552, and 684. Urinalysis was performed on all surviving rats of the 98-week treatment groups at treatment days 81, 189, 350, 545, and 679. All rats were subjected to comprehensive gross and microscopic pathologic examination at death or sacrifice. There were no apparent treatment-related effects on survival, food or water consumption, body weights, or hematological or urinalysis parameters examined. Due to mortality in all groups, including controls, the study was terminated at 97 weeks. Ophthalmoscopic and gross and microscopic examinations did not reveal evidence of dose-related effects. The major findings of this study were those of dose related decreased plasma ChE and RBC and brain AChE activity in both male and female rats. Significantly decreased plasma ChE activity (28–51% lower than controls) was noted in 0.065 mg/kg/day male rats at treatment days 88 and 684, but not at treatment days 181, 356, or 552 and in 0.065 mg/kg/day female rats (approximately 50% lower than controls) at most timepoints. High-dose male and female rats consistently exhibited significantly decreased plasma ChE activity, ranging from 80 to 97% lower than controls. In 0.065 mg/kg/day groups, RBC and brain AChE activity was not significantly decreased at any timepoint. The 5.5 mg/kg/day groups exhibited significantly decreased RBC AChE activity at all timepoints, ranging in magnitude from 15 to 28% and from 22 to 25% in males and females, respectively. At the 5.5 and 11 mg/kg/day levels, the magnitude of the effect did not appear to increase with either duration of treatment or increased dose. Following 52 weeks of treatment and 45 days of recovery, RBC AChE activity had returned to control levels in high-dose male rats and to within 7% of control levels in high-dose female rats. Brain AChE activity was

significantly decreased in 5.5 and 11 mg/kg/day male and female rats. In 5.5 and 11 mg/kg/day males, the magnitude of the effect was effect was 24 and 42%, respectively, after 684 days of treatment, but not significantly different from controls at 370 days. In 5.5 and 11mg/kg/day female rats, the effect was noted at both 370 and 684 day timepoints; the magnitude of the effect was >24% at 5.5 mg/kg/day and >40% at 11 mg/kg/day.

All available continuous variable models in the EPA Benchmark Dose Software (Version 1.3.2) were fit to the male and female rat data for RBC AChE activity reported in the principal study (Kirchner et al. 1991). A benchmark response (BMR) of 20% below the control mean RBC AChE activity was selected because 20–59% RBC or brain AChE inhibition is considered to represent a less serious effect in the absence of more serious indicators of neurotoxicity (Chou and Williams-Johnson 1998). Inadequate mean fit was provided by all models, as indicated by p values <0.05 for tests of mean fit (according to BMD technical guidance, a p value ≥0.1 indicates an adequate goodness-of-fit). Because BMD analysis provided inadequate mean fit to the RBC AChE data sets, a NOAEL/LOAEL approach was taken to derive a chronic-duration oral MRL for diazinon. The principal study (Kirchner et al. 1991) identified a NOAEL of 0.065 mg/kg/day (1.5 ppm of diazinon in the diet) and a LOAEL of 5.5 mg/kg/day (125 ppm of diazinon in the diet) for 22–28% decreased RBC AChE activity in male and female rats, which is considered the critical effect. The effect was observed as early as day 88 of treatment and did not appear to increase in magnitude with duration of treatment. The NOAEL of 0.065 mg/kg/day was divided by a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) resulting in a chronic-duration oral MRL of 0.0007 mg/kg/day.