2. HEALTH EFFECTS

2.1 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 of the toxicology of dichlorvos. 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.

2.2 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 lowestobserved-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 (LOAEL) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of dichlorvos are indicated in Table 2-2 and Figure 2-2. Because cancer effects could occur at lower exposure levels, Figure 2-2 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for dichlorvos. 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 1990), 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.

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.

2.2.1 Inhalation Exposure

All of the studies discussed in this section were conducted in exposure chambers into which air containing dichlorvos was introduced. Thus, some exposure may also have occurred by the oral and/or dermal route, since dichlorvos vapor came into contact with chamber surfaces and the bodies of the subjects. In some studies, food may have been in the chambers during the exposure.

Air concentrations of dichlorvos in all of the studies discussed below were expressed in units of either μg/L or mg/m^3. Since inhalation exposure to dichlorvos is more likely to be to the vapor phase than to aerosols, air concentrations are also presented as the equivalent parts per million (ppm). All concentrations in the inhalation exposure sections of the Levels of Significant Exposure (LSE) table (Table 2-l) and figure (Figure 2-l) are expressed as ppm, as are those for the Minimal Risk Levels derived for this profile (Section 2.5). The conversion calculation is described in the footnote to Table 2-l and in Appendix A.

Effective insecticidal air concentrations for dichlorvos are in the range of 0.15-0.25 mg/m^3 (0.017-0.028 ppm) (Hayes 1982). Insects are particularly sensitive to dichlorvos because of a lack of organophosphate metabolizing enzymes compared to mammals. Their open gas-exchange system (a network of tubules penetrating the body) also allows high concentrations of dichlorvos to reach target tissues in the nervous system.

2.2.1.1 Death

No studies were located regarding death in humans after inhalation exposure to dichlorvos.

Deaths have been reported in animals after acute-duration inhalation exposure to dichlorvos. In an early toxicity study (Durham et al. 1957), male and female Sherman rats were exposed to an

atmosphere derived from air bubbled through dichlorvos before entering the chamber. The air was essentially saturated with dichiorvos. Dichlorvos concentrations in the incoming air were measured in one trial: 8 separate determinations averaged 306 mg dichlorvos/m^3 (34 ppm) (range = 230-341 mg/m^3 or 25-38 ppm). The time until death depended on how long the chamber had been pre-equilibrated with the atmosphere containing dichlorvos. The time to death was shorter (as little as 4.5 hours) in chambers pre-equilibrated for the longest times. Rats were exposed until death occurred, which took from 6.9 to 61.9 hours on average. Clinical signs reported before death included slow, labored respiration, sialorrhea, and paleness in the extremities.

In an experiment designed to investigate the toxicity of dichlorvos after spraying in an enclosed area, Sherman rats and Rhesus monkeys were placed in a chamber whose walls and ceilings had been sprayed with a xylene emulsion containing 2.5% dichlorvos by weight and applied to give 1.08 g/m^2 of surface (Durham et al. 1957). The initial dichlorvos concentration in the air of the chamber was about 6 mg/m^3 (0.66 ppm); it fell to about 1 mg/m^3 (0.11 ppm) after 3 days and then to 0.1 mg/m^3 (0.01 ppm) for the rest of the 2-week exposure. No deaths occurred during this experiment. Dichlorvos was determined in this study by a total phosphate method. This method also detects the breakdown products of dichlorvos, so these concentrations may be overestimated.

No deaths were reported in male Swiss CF-1 mice exposed for 16 hours to atmospheres containing 30 or 55 mg dichlorvos/m^3 (3.3 or 6.1 ppm) (Dean and Thorpe 1972) or in male Sprague-Dawley rats exposed for 3, 7, or 14 days at levels up to 56 mg dichlorvos/m^3 (6.3 ppm) (Schmidt et al. 1979). No deaths were reported in pregnant CF-1 mice exposed to 4 mg dichlorvos/m^3 (0.44 ppm) for 7 hours a day for 10 days (gestation days 6-15) or in pregnant New Zealand rabbits exposed to the same concentration for 7 hours a day for 13 days (gestation days 6-18) (Schwetz et al. 1979).

Deaths were reported in rabbits from inhalation exposure to dichlorvos in an intermediate-duration study (Thorpe et al. 1972). In this experiment, groups of 20 pregnant Dutch rabbits were exposed to dichlorvos for 23 hours a day at concentrations of 0, 0.25, 1.25, or 6.25 mg/m^3 (0, 0.03, 0.14, or 0.69 ppm, respectively) for the 28 days of gestation. Sixteen of the 20 rabbits died at the 6.25 mg/m^3 concentration. Nine of these deaths may have been related to an unintentional increase to a level of 8 mg/m^3 (0.88 ppm) for one day during the experiment. Before death, the animals were anorexic, lethargic, showed tremors and torticollis, and had nasal discharges and diarrhea; these are all signs of dichlorvos neurotoxicity. Some animals in a state of advanced toxicosis were killed. An additional

group of 20 pregnant rabbits was exposed to 4 mg/m^3 (0.44 ppm) over the 8-day gestational period. Six of these rabbits died during the study, and clinical signs were similar to those observed in the 6.25 mg/m^3 (0.69 ppm) treatment group. Five of these animals died following an increase in dichlorvos concentration due to a filter failure. The maximum concentration due to system failure was not given. None of the rabbits exposed at levels of 0.25 or 1.25 mg/m^3 (0.03 or 0.14 ppm) died over the 28 days of exposure.

No deaths were reported in pregnant Carworth E rats exposed to atmospheres containing 0, 0.25, 1.25, or 6.25 mg/m^3 (0, 0.03, 0.14, and 0.69 ppm, respectively) over their 20-day gestation period (Thorpe et al. 1972), in male CF-1 mice exposed to 2.1 or 5.8 mg/m^3 (0.23 and 0.64 ppm, respectively) for 4 weeks (Dean and Thorpe 1972), or in 20-kg Yorkshire-Landrace pigs exposed for a 24-day period to concentrations of 0.092-0.114 mg/m^3 (0.01-0.013 ppm) (Loeffler et al. 1976).

In the only chronic-duration inhalation study available (Blair et al. 1976), groups of 50 Carworth E rats of each sex were exposed 23 hours a day to atmospheres containing dichlorvos at levels of 0, 0.05, 0.5, or 5 mg/m^3 (0, 0.006, 0.06, or 0.6 ppm, respectively) for 2 years. Survival was slightly increased in the rats exposed to the higher levels of dichlorvos, compared with controls. Clinical signs of intoxication were not observed in the 0.05 and 0.5 mg/m^3 groups. Rats in the 5 mg/m^3 group had necrosis of the tips of their tails, which was not seen in the control or other treated groups. However, because more than half of both sexes of the control rats died in this study, conclusions on the lethality of dichlorvos after chronic inhalation exposure cannot be drawn.

All reliable LOAELs for death in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

2.2.1.2 Systemic Effects

No studies regarding systemic effects in humans after inhalation exposure to dichlorvos were located. No studies regarding gastrointestinal, renal, musculoskeletal, endocrine, dermal, or ocular effects in animals after inhalation exposure to dichlorvos were located. Most of the systemic effects observed after inhalation exposure to dichlorvos are the result of the neurotoxicity of this chemical.

Table 2-1. Levels of Significant Exposure to Dichlorvos - Inhalation

Key to figure[a]	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
	ACUTE EXPOSURE						
	Neurological						
1	Human	20 or 10 hr		0.08 M			Blair et al. 1975
2	Rat (Sprague-Dawley)	3-14 d		0.20[b] M		0.48 M (62% inhibition of erythrocyte AChE)	Schmidt et al. 1979
	Reproductive						
3	Mouse (CF-1)	16 hr		6.1 M			Dean and Thorpe 1972
	Developmental						
4	Mouse (CF-1)	10 d Gd 6-15 7 hr/d		0.44			Schwetz et al. 1979
5	Rabbit (New Zealand)	13 d Gd 6-18 7 hr/d		0.44			Schwetz et al. 1979
	INTERMEDIATE EXPOSURE						
	Death						
6	Rabbit (Dutch)	Gd 1-28 23 hr/d				0.44 F (6 of 20 dams died)	Thorpe et al. 1972
	Neurological						
7	Rat (Carworth E)	Gd 1-20 23 hr/d		0.03[c] F	0.14 F (erythrocyte and brain AChE inhibition 29% and 28% respectively)	0.69 F (erythrocyte and brain AChE inhibition 88% and 83% respectively)	Thorpe et al. 1972
8	Rabbit (Dutch)	Gd 1-28 23 hr/d		0.03 F		0.14 F (68% and 56% inhibition of erythocyte AChE and brain AChE respectively in dams)	Thorpe et al. 1972

Table 2-1. Levels of Significant Exposure to Dichlorvos - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
9	Pig (Yorkshire Landrace)	24 d 23 hr/d		0.013			Loeffler et al. 1976
	Reproductive						
10	Rat (Carworth E)	20 d Gd 1-20 23 hr/d		0.69 F			Thorpe et al. 1972
11	Mouse (CF-1)	4 wk 23 hr/d		0.64			Dean and Thorpe 1972
12	Rabbit (Dutch)	28 d Gd 1-28 23 hr/d		0.44 F			Thorpe et al. 1972
	Developmental						
13	Rat (Carworth E)	20 d Gd 1-20 23 hr/d		0.69			Thorpe et al. 1972
14	Rabbit (Dutch)	28 d Gd 1-28 23 hr/d		0.44			Thorpe et al. 1972
	CHRONIC EXPOSURE						
	Systemic						
15	Rat (Carworth E)	2 yr 23 hr/d	Hemato	0.6			Blair et al. 1976
			Hepatic	0.06	0.6 M (increased SGOT and SGPT levels)		
			Bd Wt	0.06		0.6 M (greater than 20% reduction in body weight)	
			Metabolic	0.06	0.6 M (decreased serum chloride)		

Table 2-1. Levels of Significant Exposure to Dichlorvos - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
Neurological							
16	Rat (Carworth E)	2 yr 23 hr/d		0.006 [d]	0.06 F (31% inhibition of erythrocyte AChE)	0.6 (79-81% inhibition of brain AChE, 95-96% inhibition of erythrocyte AChE)	Blair et al. 1976

[a]The number corresponds to entries in Figure 2-1.

[b]Used to derive an acute-duration inhalation minimal risk level (MRL) of 0.002 ppm. Concentration divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

[c]Used to derive an intermediate-duration inhalation MRL of 0.0003 ppm. Concentration divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

[d]Used to derive a chronic-duration inhalation MRL of 0.00006 ppm. Concentration divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Note: Dichlorvos air levels reported as µg/L were converted to the equivalent parts per million dichlorvos by the following equation:

(µg dichlorvos/L x (24.45 L/mole) x (220.98 g dichlorvos/mole) = µg/g = parts per million where 24.45 is the volume of 1 mole of vapor at 25 degrees Centigrade 760 mm Hg and 220.98 is the molecular weight of dichlorvos in grams/mole.

AChE = acetylcholinesterase; Bd Wt = body weight; d = day(s); F = female; Gd = gestational day; Hemato = hematological; hr = hour(s); LOAEL = lowest-observable-adverse-effect level; M = male; NOAEL = no-observable-adverse-effect level; SGOT = serum glutamic oxaloacetate transaminase; SGPT = serum glutamic pyruvic transaminase; wk = week(s).

Figure 2-1. Levels of Significant Exposure to Dichlorvos - Inhalation

Acute (≤14 days)

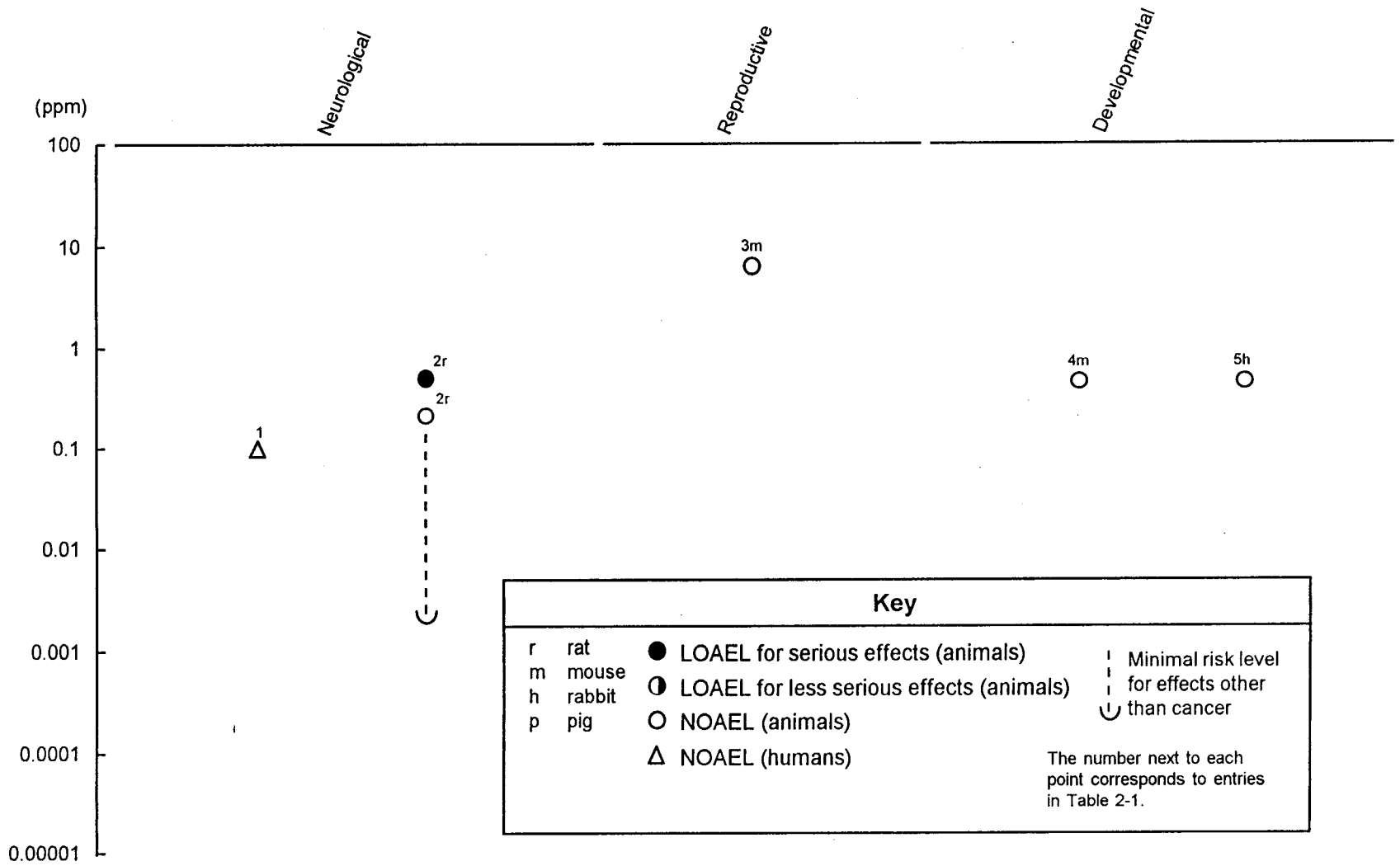


Figure 2-1. Levels of Significant Exposure to Dichlorvos - Inhalation (cont.)

Intermediate (15-364 days)

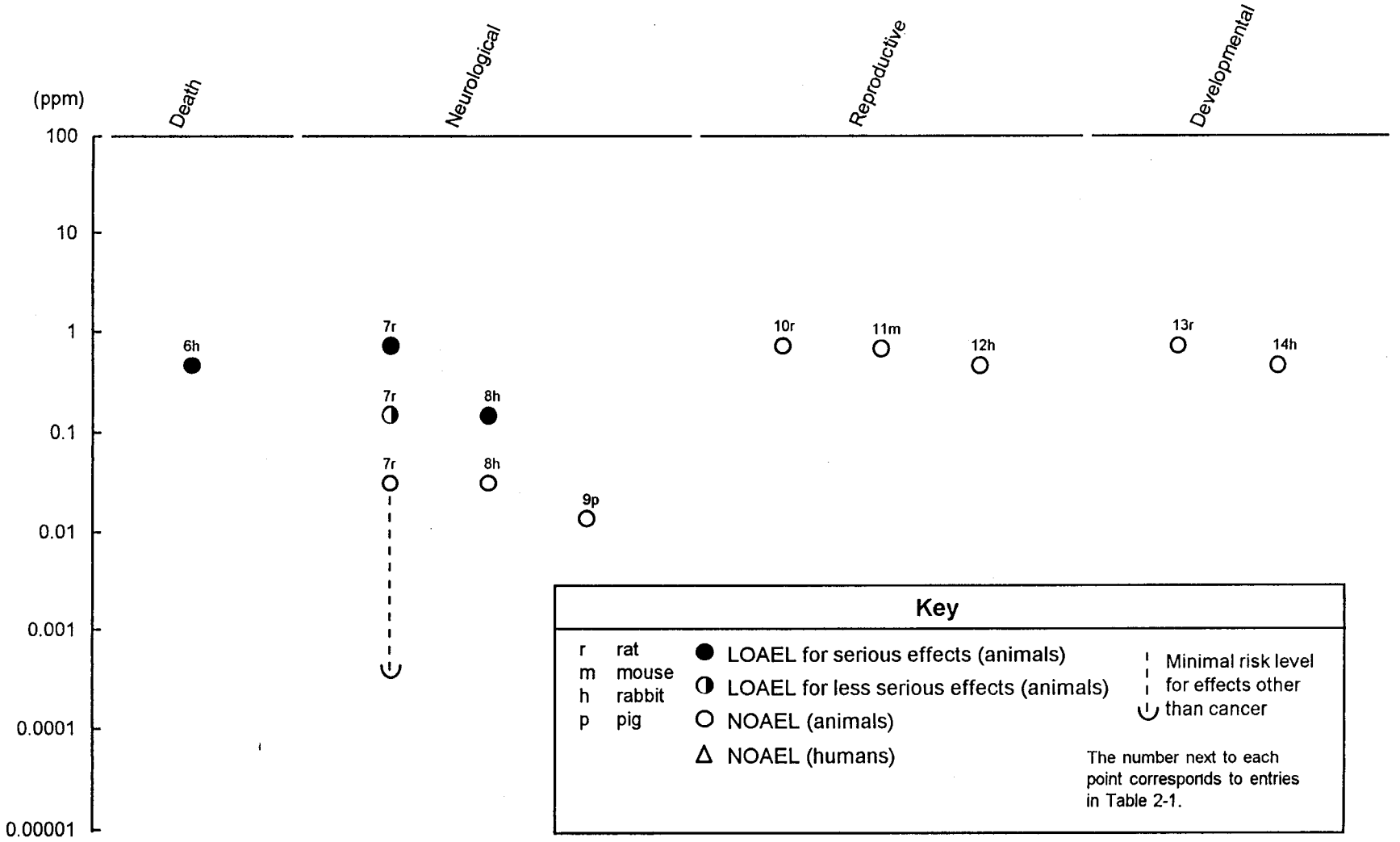
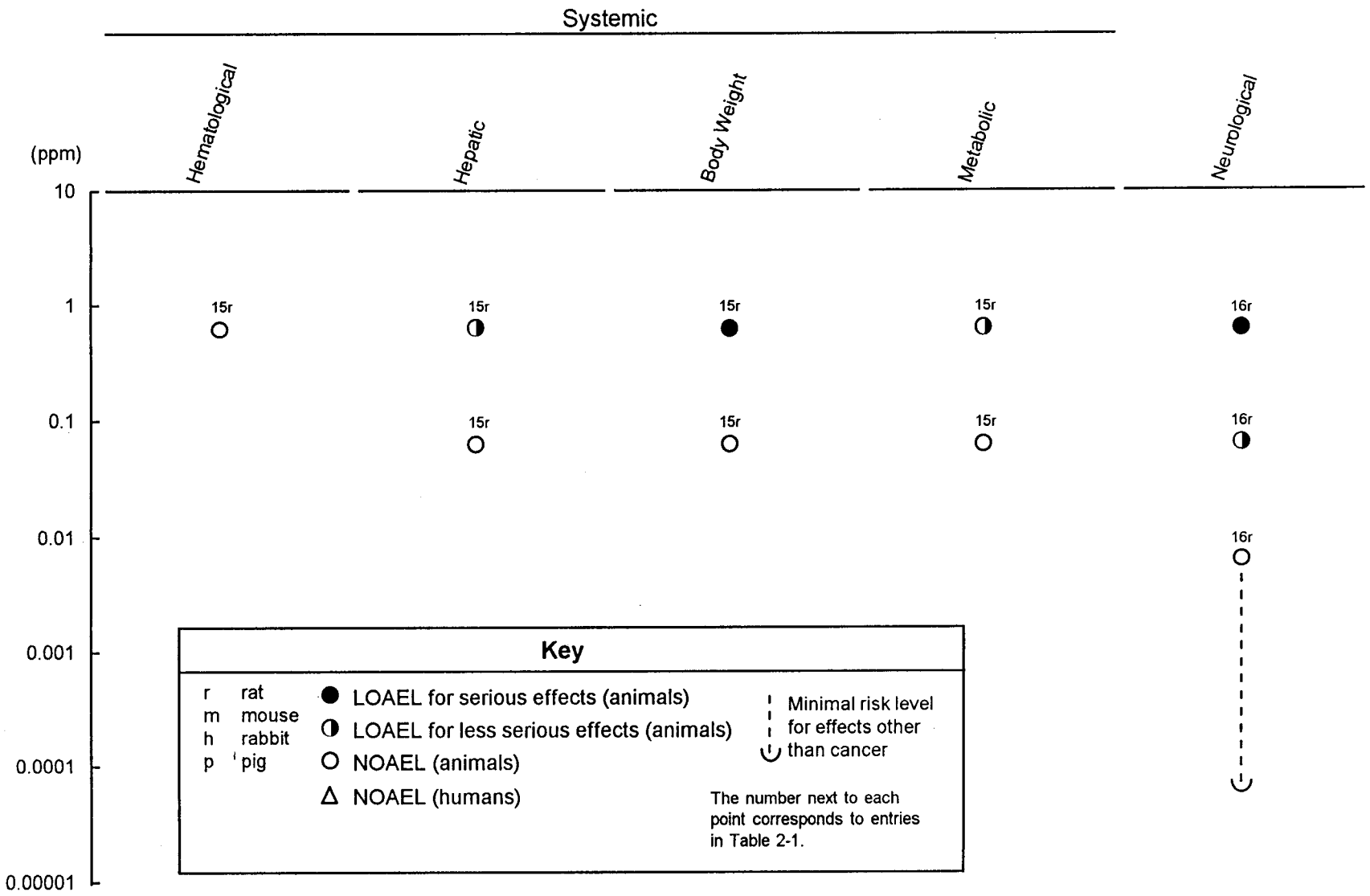


Figure 2-1. Levels of Significant Exposure to Dichlorvos - Inhalation (cont.)

Chronic (≥365 days)



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

Respiratory Effects. Prior to death, slow, labored respiration was seen in Sherman rats exposed to dichlorvos in chambers where the incoming air contained 306 mg/m^3 (34 ppm) on average (Durham et al. 1957). These signs were seen within two hours of exposure in chambers pre-equilibrated with dichlorvos.

Cardiovascular Effects. Paleness of the extremities, suggesting inadequate perfusion, was seen in Sherman rats within 2 hours of exposure in chambers until death occurred (4.5-61.9 hours). The incoming air contained, on average, 306 mg/m^3 (34 ppm), with a range of 230-341 mg/m^3 (25-38 ppm) (Durham et al. 1957).

Hematological Effects. Hematological parameters (hemoglobin concentration, erythrocyte numbers, total and differential leucocyte numbers, prothrombin time, and kaolin-cephalin coagulation time) for rats exposed to atmospheres containing up to 5 mg dichlorvos/m^3 (0.6 ppm) for 2 years were not significantly different from controls (Blair et al. 1976).

Hepatic Effects. Increased serum levels of serum glutamic oxaloacetic transaminase (SGOT, now identified as aspartate aminotransferase [AST]) and serum glutamic pyruvic transaminase (SGPT, now identified as alanine aminotransferase [ALT]), possibly indicating hepatic damage, were observed in male Carworth E rats exposed to 5 mg dichlorvos/m^3 (0.6 ppm) in a 2-year inhalation study (Blair et al. 1976). No changes in SGOT or SGPT were reported in rats of either sex exposed to 0.06 ppm dichlorvos.

Body Weight Effects. The body weight of male Cat-worth E rats exposed to atmospheres containing 5 mg dichlorvos/m^3 (0.6 ppm) for 2 years was consistently 20% or more below the body weight of control rats from the tenth week of treatment (Blair et al. 1976). Body weights of female rats exposed under the same conditions were not significantly different from controls.

Metabolic Effects. Decreased serum chloride was reported in male Carworth E rats exposed to 5 mg/m^3 dichlorvos (0.6 ppm) in a 2-year inhalation study (Blair et al. 1976). The magnitude of this

decrease was not reported. No changes in serum chloride were reported in rats of either sex exposed to 0.06 ppm dichlorvos.

2.2.1.3 Immunological and Lymphoreticular Effects

No studies regarding immunological and lymphoreticular effects in humans or animals after inhalation exposure to dichlorvos were located. However, total and differential leucocyte numbers were unchanged compared to controls in Carworth E rats exposed to atmospheres containing up to 5 mg/m^3 (0.6 ppm) for 2 years (Blair et al. 1976).

2.2.1.4 Neurological Effects

Dichlorvos exerts its toxic effects in humans and animals by inhibiting neural acetylcholinesterase. This enzyme is present at cholinergic synapses throughout the central and peripheral nervous systems, and is responsible for hydrolyzing acetylcholine released from the pre-synaptic terminal. If this enzyme is inhibited, acetylcholine accumulates in the synapse, resulting in increased firing of the postsynaptic neuron or increased neuroeffector activity. The consequences of increased cholinergic activity in the parasympathetic autonomic nervous system (muscarinic receptors) can include increased salivation, lacrimation, perspiration, miosis, nausea, vomiting, diarrhea, excessive bronchial secretions, bradycardia, frequent micturition, and incontinence. The effects of increased neuroeffector activity on skeletal muscles (nicotinic receptors) can include muscle fasciculations, cramps, muscle weakness, and depolarization-type paralysis. Effects on cholinergic synapses in the central nervous system (predominantly muscarinic) can result in drowsiness, fatigue, mental confusion, headache, convulsions, and coma. These classical symptoms of organophosphate neurotoxicity increase in severity and rapidity of onset in a dose-dependent manner (Ecobichon 1991).

Acetylcholinesterase is also present in erythrocytes where it is known as erythrocyte acetylcholin esterase. Both forms of acetylcholinesterase are produced by the same gene (Taylor ek al. 1993). In *in vitro* assays, erythrocyte and neural acetylcholinesterase are inhibited to roughly the same extent by exposure to dichlorvos and many other organophosphorus compounds with insecticide activity (Hayes 1982). Measurement of erythrocyte acetylcholinesterase is used as a surrogate of the inhibition of neural acetylcholinesterase. A cholinesterase capable of hydrolyzing acetylcholine is also produced by the liver and circulates in the blood. This enzyme, called serum cholinesterase, is also inhibited by

dichlorvos and is often used as a marker for exposure. The endogenous substrate of this enzyme is unknown. Usually, this enzyme is inhibited by dichlorvos at lower levels of exposure than required to inhibit neural or erythrocyte acetylcholinesterase (Hayes 1982).

Male volunteers exposed to atmospheres containing 0.25 or 0.7 mg/m^3 (0.03 or 0.08 ppm) showed no signs of neurological toxicity (Blair et al. 1975). One volunteer was exposed to 0.25 mg/m^3 for 10 hours and another was exposed to 0.7 mg/m^3 for 20 hours. Blood samples were not assayed for erythrocyte acetylcholinesterase, which would have suggested whether neural acetylcholinesterase was affected by the exposure to dichlorvos. Another group of seven male volunteers was exposed to dichlorvos-containing atmospheres in a simulated aircraft cabin to learn safe levels for aircraft insect control (Witter et al. 1961). In this study, the volunteers were exposed to dichlorvos on 4 consecutive days for either one or 2 hours. The average dichlorvos concentration was 0.49 mg/m^3 (range, 0.26-0.88 mg/m^3) (0.055 ppm, range 0.029-0.097 ppm) in the first experiment and 2.1 mg/m^3 (range, 0.9-3.5 mg/m^3) (0.23 ppm, range 0.10-0.38 ppm) in a second experiment with the same group. General physical examinations were performed and blood samples drawn three times before the beginning of the experiment to establish baseline rates of serum cholinesterase and erythrocyte acetylcholinesterase activity. Other samples were taken daily before exposure, thus enzyme activity was determined 24 hours after exposure. In the first experiment, no changes were observed in serum cholinesterase or erythrocyte acetylcholinesterase in any of the men whether they had been exposed for one or 2 hours a day to 0.49 mg/m3 over the 4-day exposure period. Serum cholinesterase was slightly inhibited (about 20%) in 2 of 3 volunteers exposed for 2 hours a day for 4 consecutive days at 2.1 mg/m^3. No changes were seen in erythrocyte acetylcholinesterase in any of the men exposed to 2.1 mg/m^3 for either one or 2 hours a day over the 4-day period.

In the same study , groups of 2 rhesus monkeys (one of each sex) were exposed to atmospheres containing 0.48, 2.3, 2.6, or 12.9 mg/m^3 (0.053, 0.25, 0.29, or 1.43 ppm, respectively) for 2 hours a day on 4 consecutive days (Witter et al. 1961). The blood sampling procedures were the same as those used on the humans in this study. At the 0.48 and 2.3 mg/m^3 level, the monkeys were exposed with the humans. Exposure for 2 hours a day on 4 consecutive days at 0.48 mg/m^3 did not affect serum cholinesterase or erythrocyte acetylcholinesterase. Similar exposure at 2.3 and 2.6 mg/m^3 did not affect erythrocyte acetylcholinesterase, but caused about a 30% inhibition of serum cholinesterase. Exposure at 12.9 mg/m^3 (1.43 ppm) had visible effects on both cholinesterases and produced miosis, a clinical sign of organophosphate neurotoxicity. The monkeys exposed at this level showed substantial

inhibition of both cholinesterases from the first day of exposure. Activity fell throughout the 4-day exposure period; after the last day of exposure, serum cholinesterase was inhibited about 40-50% in both monkeys, and erythrocyte acetylcholinesterase fell about 40% in one monkey and 67% in the other. Pronounced miosis was also noted in both monkeys at the end of each 2-hour exposure period, but was not observed 24 hours later. No other clinical signs were noted. Serum cholinesterase and erythrocyte acetylcholinesterase determinations after exposure was terminated suggested that 40-50 days were required for a return to pre-exposure levels.

Rhesus monkeys housed in a chamber whose walls and ceiling had been sprayed with a xylene emulsion of dichlorvos were observed for two weeks (Durham et al. 1957). The original concentration in the chamber was approximately 6 mg/m^3 (0.66 ppm) and decreased to about 1 mg/m^3 (0.11 ppm) after 3 days and was about 0.1 mg/m^3 (0.01 ppm) for the remainder of the 2-week exposure. Blood samples were taken before exposure, after 1 week, after 2 weeks, and after exposure ceased, until serum cholinesterase and erythrocyte acetylcholinesterase had returned to pre-exposure values. Signs of neurological toxicity were not observed. By the end of the first week, both serum cholinesterase and erythrocyte acetylcholinesterase had fallen from their pre-exposure levels. Inspection of a graph in this report shows that levels of both blood cholinesterases fell about 50-60% during the first week of the study. Serum cholinesterase recovered partially in the third week, but erythrocyte acetylcholin-esterase did not. After exposure was ended, the activities of both enzymes returned to pretreatment values in about five weeks.

Ten Sherman rats of each sex housed in the same chamber as the monkeys were also monitored in this experiment (Durham et al. 1957). No clinical signs of neurological toxicity were observed in the rats over the 2-week exposure period. There was a slight decrease in serum cholinesterase and erythrocyte acetylcholinesterase at the end of the first week (about 10% for each enzyme). At the end of 2 weeks, no difference was observed between exposed rats and controls. Bronchial and erythrocyte acetyl-cholinesterase were measured in male Sprague-Dawley rats exposed to atmospheres ranging from 0 to 56.64 mg/m^3 (0-6.26 ppm) over a 3-day period (Schmidt et al. 1979). A dose-dependent reduction in both bronchial and erythrocyte acetylcholinesterase was observed. Bronchial tissue acetylcholin-esterase measured in homogenates from treated rats at 0.83 and 1.82 mg/m^3 (0.09 and 0.20 ppm, respectively) was lower than in control rats; bronchial tissue acetylcholinesterase was inhibited by 50% at 1.82 mg/m^3, a dose that did not affect erythrocyte acetylcholinesterase. Erythrocyte acetylcholin-esterase was inhibited by 62% at 4.32 mg/m^3 (0.48 ppm) and was more than 80% inhibited at

8.2 mg/m^3 (0.91 ppm) after 3 days exposure. The authors reported that "similar" results were found in animals exposed for 7- and l4-day periods, but the data were not presented. Because clinical signs or pulmonary function parameters were not reported in this study, the toxicological significance of this level of bronchial enzyme inhibition in the male Sprague-Dawley rats cannot be assessed.

Several studies in animals have addressed neurological effects after intermediate-duration inhalation exposure to dichlorvos. In a study of pregnant Carworth E rats exposed over their gestation period (20 days), some dams exposed to atmospheres containing 6.25 mg/m^3 (0.69 ppm) were less active than controls (Thorpe et al. 1972). Exposure at 0.25 mg/m^3 (0.03 ppm) did not affect erythrocyte or brain acetylcholinesterase. Exposure at 1.25 mg/m^3 (0.14 ppm) resulted in a 29% inhibition of erythrocyte and a 28% inhibition of brain acetylcholinesterase, while exposure at 6.25 mg/m^3 resulted in 88% inhibition of erythrocyte acetylcholinesterase and an 83% inhibition of brain acetylcholinesterase. In the same exposure atmosphere, pregnant Dutch rabbits showed inhibition of 14 and 10% in erythrocyte and brain acetylcholinesterase, respectively, at an exposure of 0.25 mg/m^3 (0.03 ppm) over a period of 28 days (Thorpe et al. 1972). At exposures of 1.25 mg/m^3 (0.14 ppm) erythrocyte acetylcholinesterase was inhibited 68% and brain acetylcholinesterase was inhibited 56% compared to controls. Exposure of Yorkshire pigs for 24 days to atmospheres containing 0.09-0.11 mg/m^3 (0.010-0.012 ppm) had no effect on serum cholinesterase or erythrocyte acetylcholinesterase (Loeffler et al. 1976).

In a 2-year chronic inhalation study with dichlorvos (Blair et al. 1976), 50 Carworth E rats of each sex were exposed to atmospheres containing 0, 0.05, 0.5, or 5 mg/m^3 (0, 0.006, 0.06, or 0.6 ppm). No clinical signs of neurological toxicity were seen in any of the groups. Acetylcholinesterase activity was measured in brain and erythrocytes, as was serum cholinesterase, at the end of this study. In male animals exposed to 0.05 mg/m^3 (0.006 ppm), no significant differences with control animals were seen for any of the cholinesterases. Female animals at this exposure level had a statistically significant decrease of 12% in erythrocyte acetylcholinesterase. At 0.5 mg/m^3 (0.06 ppm), brain cholinesterase was 10% lower compared to controls in both male and female rats. Females at this exposure level also showed erythrocyte acetylcholinesterase inhibition of 31%, while the males were unaffected. At 5 mg/m^3 (0.6 ppm), brain acetylcholinesterase was inhibited by 79% and 81% in male and female rats, respectively. Erythrocyte acetylcholinesterase inhibition at this dose was 96% in the male rats and 95% in the female rats.

No studies were located in humans or animals describing organophosphate-induced delayed neuropathy (OPIDN) after inhalation exposure to dichlorvos. This is a syndrome observed in humans and some animal models after recovery from the acute cholinergic effects of certain organophosphorus compounds, for example tri-o-cresyl phosphate (Coppock 1995; Johnson 1981). The characteristic signs are disturbances of gait, and a "dying-back" type degeneration of motor fibers. Studies on the relationship between dichlorvos and OPIDN for parenteral routes of exposure are discussed in Section 2.5.

All reliable NOAELs and LOAELs for neurological effects in each species and duration category are recorded in Table 2- 1 and plotted in Figure 2-l.

2.2.1.5 Reproductive Effects

No studies were located regarding reproductive toxicity in humans after inhalation exposure to dichlorvos.

In a reproductive toxicity study involving male CF-1 mice, groups of 16 mice were exposed to atmospheres containing dichlorvos at 0, 30, or 55 mg/m^3 for 16 hours (0, 3.3, or 6.1 ppm) (Dean and Thorpe 1972). Following dosing, each male mouse was caged with 3 randomly selected females for 7 days; this procedure was repeated weekly for a total of 8 weeks. Thirteen days after the presumed mating (which occurred by the middle of the week), the female mice were sacrificed and the uteri removed for examination. There were no differences between control and treated mice in the number of early fetal deaths, late fetal deaths, or live fetuses found in the pregnant females. The percentages of pregnancies for females mated to males exposed to 30 or 55 mg/m^3 (3.3 or 6.1 ppm) for 16 hours ranged from 67 to 88% and 63-92%, respectively; for controls, the percentages ranged from 73 to 88%. Under these exposure conditions, dichlorvos did not appear to affect the fertility of male CF-1 mice.

In another experiment in this study, similar results were obtained for male mice exposed for 4 weeks to atmospheres containing 2.1 or 5.8 mg/m3 (0.23 or 0.64, respectively) dichlorvos for 23 hours a day (Dean and Thorpe 1972).

Maternal toxicity was reported in Dutch rabbits exposed throughout gestation to atmospheres containing dichlorvos at 4 or 6.25 mg/m^3 (0.44 or 0.69 ppm). Sixteen of 20 dams died at the higher dose; when the concentration was reduced to 4 mg/m^3, 6 of 20 dams died (Thorpe et al. 1972). Exposure spikes occurred at both exposure concentrations and may have contributed to the observed toxicity. Maternal toxicity was not observed in Carworth E strain rats exposed to atmospheres containing 0, 0.25, 1.25, and 6.25 ug/L dichlorvos (0, 0.03, 0.14, and 0.69 ppm) through day 20 of pregnancy (Thorpe et al. 1972).

The NOAELs for reproductive effects are recorded in Table 2- 1 and plotted in Figure 2- 1.

2.2.1.6 Developmental Effects

No studies were located regarding developmental toxicity in humans after inhalation exposure to dichlorvos.

Several animal studies examining developmental toxicity during continuous inhalation exposure to dichlorvos are available. A study in which pregnant mice and rabbits were exposed to dichlorvos only during the organogenesis period of gestation showed no significant effect on development (Schwetz et al. 1979). In this study, pregnant CF-1 mice were exposed to 4 mg/m^3 (0.44 ppm) dichlorvos for 7 hours a day during gestation days 6-15. At sacrifice on day 18, no significant effects were seen on the mean number of fetuses per litter, the incidence or distribution of resorptions, or on fetal body measurements. Twenty control litters and 15 litters from treated animals were examined in this study. There was no difference between the litters from controls and dichlorvos-treated dams. Pregnant New Zealand rabbits exposed to 4 mg/m^3 (0.44 ppm) for 7 hours a day during gestation days 6-18 also showed no evidence of developmental toxicity (Schwetz et al. 1979). Mean number of fetuses per litter, incidence or distribution of resorptions and fetal body measurements were similar in 14 control litters and 19 treated litters.

Groups of 15 pregnant Cat-worth E rats were exposed to atmospheres containing 0, 0.25, 1.25, or 6.25 mg/m^3 (0, 0.03, 0.14, or 0.69 ppm) throughout their 20-day gestation period (Thorpe et al. 1972). At the end of 20 days, the rats were sacrificed and the uteri removed for examination. The number of live fetuses, late fetal deaths, and resorption sites were noted, and live fetuses were weighed and examined for external malformations. Approximately half the fetuses in each litter were processed for

alizarin-stained preparations of the skeleton, and the other half were fixed in Bouin's fluid and examined for structural abnormalities of the viscera by transverse sections. Exposure of dams to all three concentrations of dichlorvos did not affect the number of fetal resorptions, late fetal deaths, litter size, or mean weight per fetus. One fetus in the litter of dams in the 0.25 mg/m^3 group had skeletal defects and gastroschisis. Because no other fetuses from dams exposed to the same or higher concentrations had these defects, the authors concluded that they were not exposure-related. Brain and erythrocyte acetylcholinesterase activities were inhibited 83 and 88%, respectively, in dams in the high-exposure (6.25 mg/m^3) group, suggesting that acetylcholinesterase inhibition is not associated with teratogenicity. Measurement of acetylcholinesterase activities in the pups was not performed.

In a parallel experiment conducted on groups of 20 pregnant Dutch rabbits (Thorpe et al. 1972), similar results were seen. Dams exposed to dichlorvos at 6.25 mg/m^3 (0.69 ppm), as in the previously described rat study, had high mortality (16 of 20 died). Consequently, the doses used in this experiment were 0, 0.25, 1.25, 2, and 4 mg/m^3 (0, 0.03, 0.14, 0.22, and 0.44 ppm) over the 28-day rabbit gestational period. Six of the 20 rabbits exposed to 4 mg/m^3 died. In both the 4 and 6.25 mg/m^3 exposure groups, spiking of the exposure concentration occurred. Sizes of litters, fetal resorptions, and late fetal deaths were unaffected by inhalation exposure to dichlorvos. Mean fetal weights were significantly depressed (23.1 ± 0.98 g for controls and 20.2 ± 0.98 g for the 4 mg/m^3 exposure group), but the authors ascribed this to maternal toxicity. Clinical signs were similar to signs for dams exposed to 6.25 mg/m^3; 6 dams out of 20 in this group died during the study. Three fetuses from groups that had not been exposed to dichlorvos had gastroschisis. Two dead fetuses from one litter in the 4 group had cleft palates, but this may also be a result of maternal toxicity rather than a developmental effect.

The NOAELS for developmental toxicity are recorded in Table 2-l and plotted, in Figure 2-l.

2.2.1.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

No studies were located regarding cancer in humans after inhalation exposure to dichlorvos.

In a 2-year carcinogenicity study of dichlorvos by inhalation exposure (Blair et al. 1976). groups of 50 Carworth E rats of each sex were exposed to atmospheres containing dichlorvos at 0, 0.05, 0.5, or 5 mg/m^3 (0, 0.006, 0.06, or 0.6 ppm) for 23 hours a day. Necropsies were carried out on animals that died or were sacrificed because of ill health during the study. At the end of the study, the surviving rats were sacrificed, and blood samples taken, necropsies performed, and major organs weighed. Major viscera, macroscopic tumors, and blocks of tongue, nasal cavity, trachea, skeletal muscle, eye, and lachrymal gland were fixed in formalin and processed for paraffin section. Respiratory tissue from a small number (not stated) of control and high-dose animals was examined by electron microscope. Only 11 of the unexposed male controls and 25 of the unexposed female controls survived to the end of the study. Survival was highest in the rats exposed to the highest concentration of dichlorvos (32 of 50 males and 34 of 50 females). Microscopical examination revealed a wide range of lesions in all groups; the authors stated that these are commonly seen in old rats of this strain. There was a high incidence in control and treated groups of chronic nephrosis, focal myocardial fibrosis, degenerative artery disease, lymphoid hyperplasia of the spleen, and testicular atrophy. Common tumors in all groups were adenomas of the anterior pituitary gland, parafollicular cell adenomas and carcinomas of the thyroid gland, adrenal pheochromocytomas, and mammary fibroadenomas in the females. Examination of the lungs (presumably the tissue receiving the highest dose) revealed minor changes in all groups. Peribronchial and perivascular lymphoid aggregates, mild degrees of bronchiolitis, and focal alveolar thickening were noted. Electron microscopic examination of bronchi, bronchioli, and alveoli of a small number of control and high-dose group animals showed no differences between the groups. None of the lesions in the study was associated with dichlorvos exposure.

The high mortality of the control animals in this study makes interpretation of the carcinogenicity data problematic. The possibility also exists that exposure by the oral and dermal routes occurred since the animals received whole-body exposure to dichlorvos vapor in cages rather than nose-only exposure. However, no significant increase in neoplastic or non-neoplastic lesions was found in the nasal and respiratory tract tissues that presumably received the highest dose of dichlorvos.

2.2.2 Oral Exposure

2.2.2.1 Death

Two deaths in humans have been associated with oral exposure to dichlorvos (Hayes 1982). In one case, a young woman who drank an undetermined amount of dichlorvos died despite prompt medical treatment. In another case, a 19-month-old girl who ate part of a cake-like bait that contained dichlorvos died after 4 days (Hayes 1982). The actual bait she consumed was not recovered for analysis, but because similar baits were found to contain both dichlorvos and malathion, it is possible that an interaction with malathion may have occurred. Malathion had much lower acute toxicity than dichlorvos when measured in an LD 50 (lethal dose, 50% kill) study in male Sherman rats; the LD_{50} for malathion was 1,375 mg/kg, while that for dichlorvos was 80 mg/kg (Durham et al. 1957). Severe cerebral edema was considered the cause of death.

A number of oral exposure LD_{50} studies have been done with dichlorvos in rats and mice. Based on reported LD_{50} values, dichlorvos is considered to be of moderate to high acute toxicity (WHO 1989). In the most extensive study of this type, dichlorvos was one of 5 chemicals used in a study designed to investigate how well an experimentally derived LD50 value would predict the level that would result in 1% lethality (LD_l value) for CD-l mice (Haley et al. 1975). The LD50 for male mice in this study was calculated to be 139 mg/kg, and the LD50 for females was 133 mg/kg. In a subsequent study at lower doses, the LD_l for the male mice was 84 mgkg (predicted LD_1 was 81 mg/kg). In the female mice, the LD_1 was 95 mg/kg (predicted LD_1 was 106 mg/kg). The authors further estimated an $LD_{0.1}$ of 70 mg/kg for male mice and 82 mg/kg for female mice. An LD_{50} of 110 mg/kg was reported in male ICR mice (Takahashi et al. 1987). Dichlorvos administered by gavage in water at a dose of 150 mg/kg to male Swiss mice caused 100% lethality within 9 minutes (Mohammad et al. 1989).

In Sherman rats, oral LD_{50} values of 80 mg/kg in males and 56 mg/kg in females were reported (Durham et al. 1957). The LD_{50} for female Wistar rats was reported to be 58.8 mg/kg (Gajewski and Katkiewicz 1981) and in male Fischer 344 rats 97.5 mg/kg (Ikeda et al. 1990). Crossbred pigs weighing 12-27 kg treated with dichlorvos in gelatin capsules were reported to have an LD_{50} of 157 mg/kg (Stanton et al. 1979).

Other studies have reported deaths in animals after acute-duration oral exposure to dichlorvos. Three of 12 greyhound dogs receiving 22 mg/kg in a gelatin capsule died (Snow and Watson 1973). Two of the dogs died within 20 minutes of dosing; cyanosis was severe and progressive, and sounds of gas passage in the respiratory tract ceased in spite of continued respiratory effort. This was followed soon afterwards by respiratory arrest and then cardiac arrest. Another dog dosed at 22 mg/kg was comatose for a long period, had repeated convulsive episodes, and died 15.5 minutes after dosing. In castrated male (surgery at 10 days of age) and female crossbred pigs treated with dichlorvos in gelatin capsules, deaths occurred 15-30 minutes after dosing (Stanton et al. 1979) in a single pig dosed with 560 mg/kg, in 7 of 8 at 320 mg/kg, in 5 of 8 at 150 mg/kg, and in 2 of 8 at 100 mg/kg. Clinical signs observed in these animals included hypoactivity, vomiting, ataxia, muscle fasciculations, uncoordinated movements, frothy salivation, and defecation. One white Leghorn hen treated on 2 consecutive days with 7.1 mg/kg in gelatin capsules also died after the second treatment (Francis et al. 1985). Clinical signs were staggering gait, salivation and convulsions. Birds are generally more susceptible to organophosphorus compounds due to lack of detoxification pathways.

Intermediate-duration exposure to dichlorvos has also caused death in experimental animals. In a dose-finding study where Osborne-Mendel rats (5 of each sex) were exposed to dichlorvos-containing feed at dosages of 0-360 mg/kg/day for a 6-week period, all rats consuming 2180 mg/kg/day died (NCI 1977). Exposure to 90 mg/kg/day did not cause any deaths. In a dose-finding study for a 2-year carcinogenicity experiment, Fischer 344 rats (10 of each sex) were treated by corn oil gavage 5 days a week for 13 weeks over a dosage range of 0-64 mg/kg/day (NTP 1989). All rats receiving 32 and 64 mg/kg died before the end of the study. Some animals that died were trembling and inactive immediately before death. Of the male rats receiving 64 mg/kg, 90% died by the first week of the study, while 90% of the male rats receiving 32 mg/kg died in the seventh week of the study. One of the 10 male rats receiving 16 mg/kg died, but the authors stated that this was gavage-related. Male rats at dosages of 2, 4, and 8 mg/kg survived for 13 weeks. All female rats receiving 32 or 64 mg/kg died in the first week. Four of 10 females receiving 16 mg/kg died by the seventh week. All female rats receiving 2, 4, and 8 mg/kg survived for 13 weeks.

In similar intermediate-duration studies in mice, groups of 5 $B6C3F_1$ mice were exposed by feed to dosages ranging from 0 to 1,080 mg/kg/day for 6 weeks (NCI 1977). Four of 5 females died at the 720 mg/kg/day dose; all mice consuming 1,080 mg/kg/day died. No deaths were reported in mice consuming 360 mg/kg/day or less. In another study (NTP 1989), groups of 10 $B6C3F_1$ mice of each

sex were dosed by corn oil gavage at levels ranging from 0 to 160 mg/kg for 5 days a week for 13 weeks. All 10 male mice and 9 of 10 female mice who received 160 mg/kg died. Eight of the male mice died in the first week, as did 3 female mice. Five of 10 male mice died after receiving 80 mg/kg.

In a chronic-duration study (2 years) where Osborne-Mendel rats of both sexes were exposed through feed to doses of 0, 13.5, and 29.3 mg/kg/day, survival was higher in the treated groups than in the matched control animals (NCI 1977); 76% of the high-dose and 64% of the low-dose males survived, as did 84% of the high-dose and 80% of the low-dose females. The authors stated the rats were in poor condition in both In a similar study using Fischer 344 rats (NTP 1989), no significant differences were observed in survival among groups of either sex treated by corn oil gavage at doses of 0, 4, or 8 mg/kg for 5 days a week for 103 weeks. In a 2-year study of $B6C3F_1$ mice, no significant differences in survival were noted between groups of either sex exposed through feed to doses of 0, 57.2, or 114.3 mg/kg/day (NCI 1977). In another 2-year study in $B6C3F_1$ mice (NTP 1989), no significant differences in survival were noted among groups treated by corn oil gavage at doses of 0, 10, or 20 mg/kg for 5 days a week in male mice or at 0, 20, or 40 mg/kg for 5 days a week in female mice.

No deaths were reported in Beagle dogs (groups of 4 of each sex) receiving up to 3 mg/kg/day dichlorvos by capsule in corn oil for 52 weeks (AMVAC Chemical Corp. 1990).

All reliable LD_{50} values and LOAELs for death in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

No studies regarding systemic effects in humans after oral exposure to dichlorvos were located. No studies regarding musculoskeletal or metabolic effects in animals after oral exposure to dichlorvos were located. Most of the systemic effects of dichlorvos after oral exposure are secondary to the neurotoxicity of this chemical.

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

Table 2-2. Levels of Significant Exposure to Dichlorvos - Oral

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	ACUTE EXPOSURE						
	Death						
1	Rat (Sherman)	once (GO)				80 M (LD_{50}) 56 F (LD_{50})	Durham et al. 1957
2	Rat (Wistar)	once (GO)				58.8 F (LD_{50})	Gajewski and Katkiewicz 1981
3	Rat (Fischer- 344)	once (GO)				97.5 M (LD_{50})	Ikeda et al. 1990
4	Mouse (CD-1)	once (GO)				139 M (LD_{50}) 133 F (LD_{50})	Haley et al. 1975
5	Mouse (ICR)	once (G)				110 M (LD_{50})	Takahashi et al. 1987
6	Dog (greyhound)	once (C)				22 (3 of 12 died)	Snow and Watson 1973
7	Pig (Hybrid)	once (C)				157 (LD_{50})	Stanton et al. 1979
8	Chicken (white leghorn)	2 d 1 x/d (C)				7.1 F (1 of 1 died)	Francis et al. 1985

Table 2-2. Levels of Significant Exposure to Dichlorvos - Oral (continued)

Key to figure[a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	Systemic						
9	Dog (greyhound)	once (C)	Hemato		11 (transient increase in hematocrit > 10%)		Snow and Watson 1973
			Musc/skel		11 (increase in serum creatine phosphokinase)		
			Hepatic		11 (increase in SGOT)		
	Immunological/Lymphoreticular						
10	Mouse (C57BL/6N)	1 x or 4 x (GO)		40M	120M (reduced IgM response, 11% decrease in relative spleen weight)		Casale et al. 1983
	Neurological						
11	Rat (Fischer- 344)	10 d 5 x/wk (GO)		16			NTP 1989
12	Rat (Albino)	once (NS)				40 M (83% inhibition of brain AChE 15 min after administration)	Pachecka et al. 1977
13	Rat (Sprague-Dawley)	once (GO)				40 M (brain AChE inhibited 70%)	Teichert et al. 1976
14	Rat (Sprague-Dawley)	14 d 1 x/d (GO)			4[b] M (brain AChE inhibited 45%)		Teichert et al. 1976
15	Mouse (C57BL/6N)	1x or 4 x (GO)			40M (30 and 31% reductions in brain and erythrocyte AChE respectively)	120 M (75% reduction in brain AChE 1 hour after dosing, tremor)	Casale et al. 1983

Table 2-2. Levels of Significant Exposure to Dichlorvos - Oral (continued)

Key to figure[a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
16	Mouse (B6C3F1)	11 d 5 x/wk (GO)		40			NTP 1989
17	Dog (greyhound)	once (C)				11 (ataxia, fasciculations, 75% decrease in erythrocyte AChE)	Snow and Watson 1973
18	Pig (NS)	once (C)				18 (ataxia, fasciculations)	Stanton et al. 1979
	Reproductive						
19	Mouse (CF-1)	10 d Gd 6-15 1 x/d (GO)		60			Schwetz et al. 1979
	Developmental						
20	Mouse (CF-1)	10 d Gd 6-15 1 x/d (GO)		60			Schwetz et al. 1979
21	Rabbit (New Zealand)	13 d Gd 6-18 1 x/d (GO)		5			Schwetz et al. 1979

Table 2-2. Levels of Significant Exposure to Dichlorvos - Oral (continued)

Key to figure[a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	INTERMEDIATE EXPOSURE						
	Death						
22	Rat (Osborne-Mendel)	6 wk (F)				180 (10 of 10 died)	NCI 1977
23	Rat (Fischer- 344)	13 wk 5 d/wk (GO)				16 F (4 of 10 died in the 7th week)	NTP 1989
24	Mouse (B6C3F1)	6 wk (F)				720 F (4 of 5 died)	NCI 1977
25	Mouse (B6C3F1)	13 wk 5 d/wk (GO)				80 M (5 of 10 died) 160 F (9 of 10 died)	NTP 1989
	Neurological						
26	Human	21 d 3 x/d (F)		0.033[c] M			Boyer et al. 1977
27	Rat (Sherman)	90 d (F)		3.5 F	14.2 F (30% inhibition of erythrocyte AChE)	35.7 F (80% inhibition of erythrocyte AChE)	Durham et al. 1957
28	Rat (Fischer- 344)	32 d 5 x/wk (GO)		8 M 16 F	16M (22% inhibition of erythrocyte AChE on day 24)		NTP 1989
29	Mouse (B6C3F1)	25 or 33 d 5 x/wk (GO)		40			NTP 1989

Table 2-2. Levels of Significant Exposure to Dichlorvos - Oral (continued)

Key to figure[a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
30	Chicken (white leghorn)	35-90 d 1 x/d (C)		4.4 F		6.1 F (staggering gait after 35 days)	Francis et al. 1985
	CHRONIC EXPOSURE						
	Systemic						
31	Rat (Fischer- 344)	103 wk 5 d/wk (GO)	Cardio	8			NTP 1989
			Gastro	8			
			Musc/skel	8			
			Hepatic	8 F	4M (cytoplasmic vacuolization in liver cells)		
			Renal	8			
			Endocr		4 (cytoplasmic vacuolization in adrenal cortical cells)		
			Dermal	8			
			Bd Wt	8			

Table 2-2. Levels of Significant Exposure to Dichlorvos - Oral (continued)

Key to figure[a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
32	Mouse (B6C3F1)	103 wk 5 d/wk (GO)	Resp	20 M 40 F			NTP 1989
			Cardio	20 M 40 F			
			Hemato	20 M 40 F			
			Musc/skel	20 M 40 F			
			Hepatic	20 M 40 F			
			Renal	20 M 40 F			
			Endocr	20 M 40 F			
			Dermal	20 M 40 F			
			Bd Wt	20 M 40 F			

Table 2-2. Levels of Significant Exposure to Dichlorvos - Oral (continued)

Key to figure[a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
33	Dog (Beagle)	52 wk 1 x/d (C)	Resp	3.0			AMVAC Chemical Corp. 1990
			Cardio	3.0			
			Gastro	3.0			
			Hemato	3.0			
			Musc/skel	3.0			
			Hepatic	3.0			
			Renal	3.0			
			Endocr	3.0			
			Dermal	3.0			
			Ocular	3.0			
			Bd Wt	3.0			
			Metab	3.0			
	Immunological/Lymphoreticular						
34	Dog (Beagle)	52 wk 1 x/d (C)		3.0			AMVAC Chemical Corp. 1990
	Neurological						
35	Rat (Fischer- 344)	103 wk 5 d/wk (GO)			4 (mild diarrhea)		NTP 1989
36	Mouse (B6C3F1)	103 wk 5 d/wk (GO)		20 M 40 F			NTP 1989

Table 2-2. Levels of Significant Exposure to Dichlorvos - Oral (continued)

Key to figure[a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
37	Dog (Beagle)	52 wk 1 x/d (C)		0.05 d	1.0 M (22% inhibition of brain AChE, 53% inhibition of erythrocyte AChE) 1.0 F (45% inhibition of erythrocyte AChE)	3.0 M (47% inhibition of brain AChE, 85% inhibition of erythrocyte AChE) 3.0 F (81% inhibition of erythrocyte AChE)	AMVAC Chemical Corp. 1990
Reproductive							
38	Rat (Fischer- 344)	103 wk 5 d/wk (GO)		8			NTP 1989
39	Mouse (B6C3F1)	103 wk 5 d/wk (GO)		20 M 40 F			NTP 1989
40	Dog (Beagle)	52 wk 1 x/d (C)		3.0			AMVAC Chemical Corp. 1990

Table 2-2. Levels of Significant Exposure to Dichlorvos - Oral (continued)

Key to figure[a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	Cancer						
41	Mouse (B6C3F1)	103 wk 5 d/wk (GO)				40 F CEL: (forestomach squamous cell papillomas and carcinomas)	NTP 1989

[a]The number corresponds to entries in Figure 2-2.

[b]Used to derive an acute-duration minimal risk level (MRL) of 0.004 mg/kg/day; dose divided by an uncertainty factor of 100 (10 for human variability and 10 for extrapolation from animals to humans)

[c]Used to derive an intermediate-duration MRL of 0.003 mg/kg/day; dose divided by an uncertainty factor of 10 for human variability.

[d]Used to derive a chronic-duration MRL of 0.0005 mg/kg/day; dose divided by an uncertainty factor of 100 (10 for human variability and 10 for extrapolation from animals to humans).

AChE = acetylcholinesterase; Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; CEL = cander effect level; d = day(s); F = female; (F) = food; (G) = gavage; Gastro = gastrointestinal; (GO) = gavage in oil; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observable-adverse-effect level; M = male; Musc/skel = musculoskeletal; NOAEL = no-observable-adverse-effect level; NS = not specified; SGO = serum glutamic oxaloacetic transaminase; (W) = water; wk = week(s); x = times.

Figure 2-2. Levels of Significant Exposure to Dichlorvos - Oral

Acute (≤14 days)

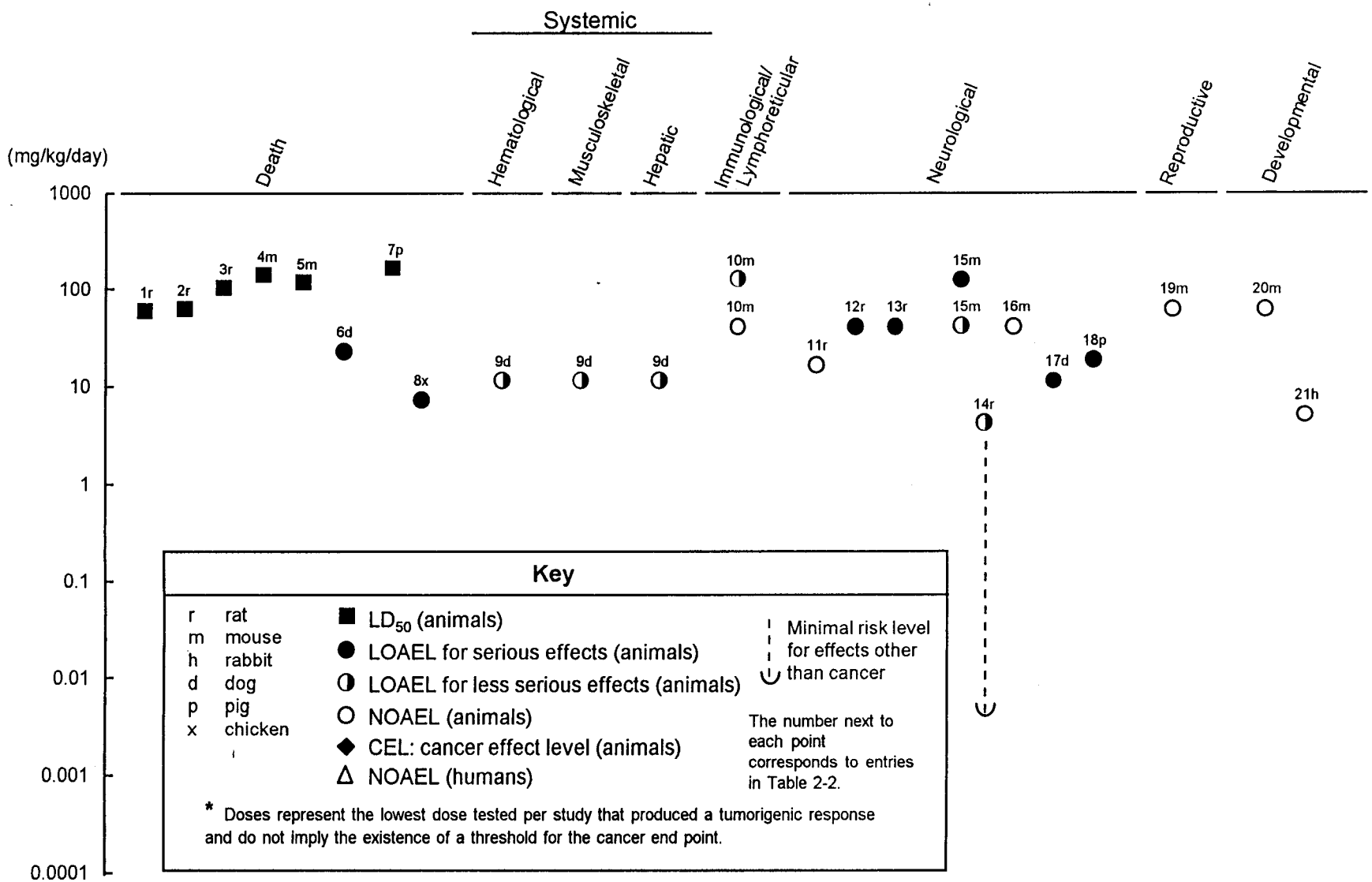


Figure 2-2. Levels of Significant Exposure to Dichlorvos - Oral (cont.)

Intermediate (15-364 days)

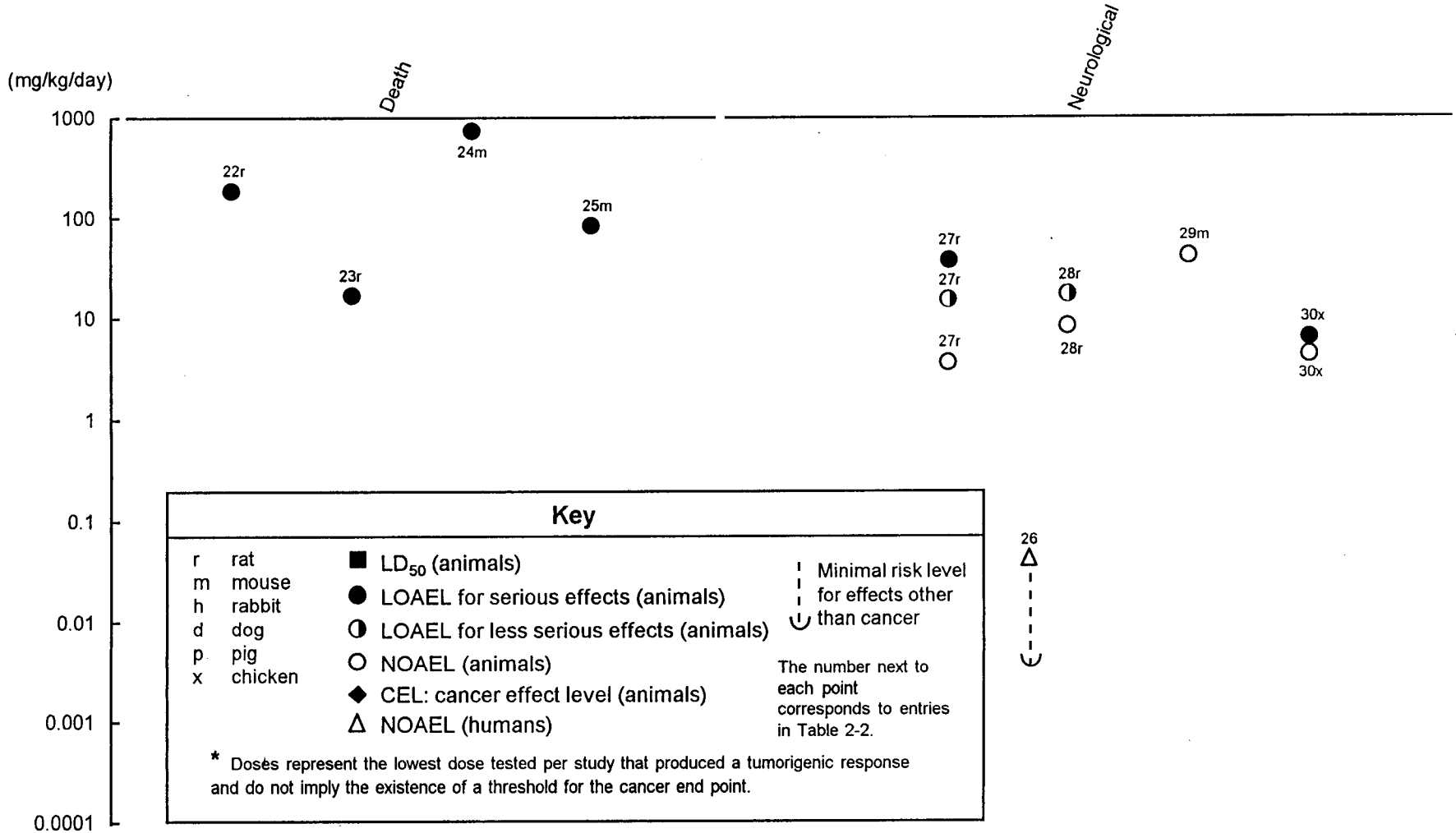
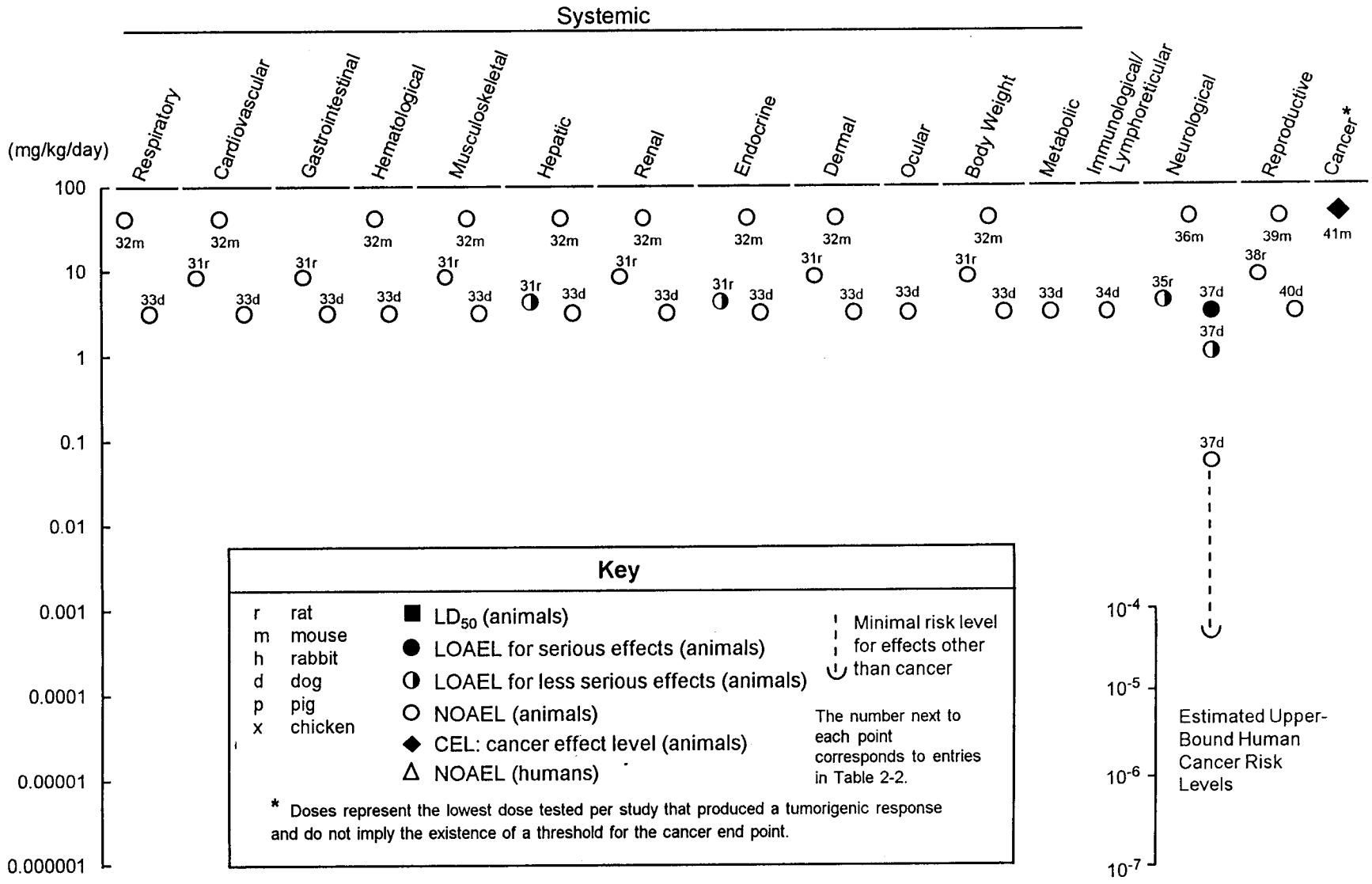


Figure 2-2. Levels of Significant Exposure to Dichlorvos - Oral (cont.)

Chronic (≥365 days)



Respiratory Effects. No gross or histological evidence of treatment-related damage to the lungs, mainstream bronchi, or trachea was observed in B6C3F, mice treated by oral gavage with dichlorvos at 20 mg/kg/day (males) or 40 mg/kg/day (females) for 5 days a week for 2 years (NTP 1989) or in Beagle dogs (groups of 4 of each sex) receiving up to 3 mg/kg/day by capsule for 52 weeks (AMVAC Chemical Corp. 1990).

Cardiovascular Effects. No gross or histological evidence of treatment-related damage to the heart was seen in Fischer 344 rats treated with up to 8 mg/kg/day dichlorvos for 5 days a week for 2 years. Similar results were seen in B6C3Fl mice treated in the same study with dichlorvos at 20 mg/kg/day (males) or 40 mg/kg/day (females) for 5 days a week (NTP 1989) or in Beagle dogs (groups of 4 of each sex) receiving up to 3 mg/kg/day by capsule for 52 weeks (AMVAC Chemical Corp. 1990).

Gastrointestinal Effects. Diarrhea, sometimes bloody, and vomiting were observed in greyhound dogs exposed orally to dichlorvos in gelatin capsules at a single dose of 11 mg/kg (Snow and Watson 1973). Vomiting and defecation were also reported in crossbred pigs dosed at 18 mg/kg in gelatin capsules (Stanton et al. 1979). Excessive salivation and defecation were observed before death in male Swiss mice receiving 150 mg dichlorvos emulsion/kg by water gavage (Mohammad et al. 1989). Diarrhea was reported in Osborne-Mendel rats receiving 90 mg dichlorvos kg/day over a 3-week period (NCI 1977). This diarrhea was so severe that the dichlorvos dose was reduced to 29 mg/kg/day for the remainder of this 2-year study. Similar signs were seen in B6C3F, mice receiving 360 mg/kg/day dichlorvos in the same study (NCI 1977), and dosages were reduced to 114 mg/kg/day in these mice. Mild diarrhea was reported throughout a 2-year study in male rats receiving 4 or 8 mg/kg/day dichlorvos (NTP 1989). Diarrhea and vomiting may reflect a local irritant action of dichlorvos on the gastrointestinal tract; however, these signs may also be caused by muscarinic cholinergic stimulation (Ecobichon 1991).

No gross or histological evidence of treatment-related damage to gastrointestinal tissues (cecum, colon, duodenum, esophagus, ileum, jejunum, rectum and stomach) was found in Fischer 344 rats treated with up to 8 mg/kg/day dichlorvos by gavage 5 days a week for 2 years (NTP 1989) or in Beagle dogs (groups of 4 of each sex) receiving up to 3 mg/kg/day by capsule for 52 weeks (AMVAC Chemical Corp. 1990). Significantly increased neoplastic lesions were found in the forestomach of female mice treated in the NTP study at doses of 20 and 40 mg/kg/day (see Section 2.2.2.8).

Hematological Effects. A 10% increase in hematocrit was seen in greyhound dogs exposed to a single dose of 11 mg/kg and showing moderate to severe signs of toxicity (Snow and Watson 1973). Total plasma proteins increased in all but one dog that received 22 mg/kg. Diarrhea was also observed, thus the increase in hematocrit and total plasma proteins may have been caused by dehydration. In dogs dosed with 11 or 22 mg/kg, a slight leukocytosis was observed in animals showing moderate to severe signs of toxicity (Snow and Watson 1973).

No gross or histological evidence of treatment-related damage to bone marrow or spleen was observed in $B6C3F_1$ mice treated with up to 40 mg/kg/day dichlorvos by gavage for 5 days a week for 2 years (NTP 1989) or in Beagle dogs (groups of 4 of each sex) receiving up to 3 mg/kg/day by capsule for 52 weeks (AMVAC Chemical Corp. 1990). Significantly increased neoplastic lesions (leukemia) were observed in Fischer 344 male rats in the NTP study at doses of 4 and 8 mg/kg/day (See Section 2.2.2.8)

Musculoskeletal Effects. A l0-fold increase in serum creatine phosphokinase, suggestive of muscle damage, was observed in a greyhound dog treated once with 11 mg/kg dichlorvos in capsules (Snow and Watson 1973). No gross or histological evidence of treatment-related damage to skeletal muscle was observed in Fischer 344 rats treated with up to 8 mg/kg/day dichlorvos by gavage 5 days a week for 2 years, $B6C3F_1$ mice treated with up to 40 mg/kg/day dichlorvos by gavage for 5 days a week for 2 years (NTP 1989) or in Beagle dogs (groups of 4 of each sex) receiving up to 3 mg/kg/day by capsule for 52 weeks (AMVAC Chemical Corp. 1990).

Hepatic Effects. A 2-fold increase in SGFT, indicating liver damage, was observed in a greyhound dog treated once with 11 mg/kg dichlorvos in capsules (Snow and Watson 1973). Cytoplasmic vacuolization of liver cells was noted in male Fischer 344 rats receiving 4 or 8 mg/kg/day dichlorvos by gavage for 5 days a week for 2 years (NTP 1989). The authors stated that these changes were minor in extent but have been associated with lipid accumulation in cells. No gross or histological evidence of treatment-related damage was observed in livers from female rats or in $B6C3F_1$ mice receiving 20 mg/kg/day (males) or 40 mg/kg/day (females) for 2 years in the same study (NTP 1989) or in Beagle dogs (groups of 4 of each sex) receiving up to 3 mg/kg/day by capsule for 52 weeks (AMVAC Chemical Corp. 1990).

Renal Effects. No gross or histological evidence of treatment-related damage to the kidneys was observed in Fischer 344 rats or B6C3Ft mice treated with dichlorvos by gavage 5 days a week for 2 years at doses of 8 mg/kg/day (rats), 20 mg/kg/day (male mice) or 40 mg/kg/day (female mice) (NTP 1989) or in Beagle dogs (groups of 4 of each sex) receiving up to 3 mg/kg/day by capsule for 52 weeks (AMVAC Chemical Corp. 1990).

Endocrine Effects. Cytoplasmic vacuolization of adrenal cortical cells was noted in male Fischer 344 rats receiving 4 or 8 mg/kg/day dichlorvos by gavage for 5 days a week for 2 years (NTP 1989). The authors stated that these changes were minor in extent but have been associated with lipid accumulation in cells. Significant neoplastic lesions of the pancreas (adenomas) were also observed in the male rats (see Section 2.2.2.8). No increase in lesions was observed for male or female rats in the parathyroid, pituitary, thyroid or thymus glands. No treatment-related lesions were observed for any endocrine tissue in B6C3F$_I$ mice treated for 2 years at 20 mg/kg/day (males) or 40 mg/kg/day (females) (NTP 1989) or in Beagle dogs (groups of 4 of each sex) receiving up to 3 mg/kg/day by capsule for 52 weeks (AMVAC Chemical Corp. 1990).

Dermal Effects. No gross or histological evidence of treatment-related damage to the skin was observed in Fischer 344 rats or B6C3F$_1$, mice treated with dichlorvos by gavage 5 days a week for 2 years at doses of 8 mg/kg/day (rats), 20 mg/kg/day (male mice), or 40 mg/kg/day (female mice) (NTP 1989) or in Beagle dogs (groups of 4 of each sex) receiving up to 3 mg/kg/day by capsule for 52 weeks (AMVAC Chemical Corp. 1990).

Ocular Effects. The effect of dichlorvos on pupillary response to light has been studied in Beagles (Wagstaff and Winston 1980). The dogs received 0, 13.5, 27, or 40.5 mg/kg in a PVC-resin formulation. Dichlorvos did not affect the resting pupil and no miosis was observed. However, in response to a strobe light flash, dogs receiving 27 or 40.5 mg/kg had a greater contraction and a slower recdvery to baseline pupillary diameter. No histopathological evidence of ocular damage related to dichlorvos treatment was found in Beagle dogs (groups of 4 of each sex) receiving up to 3 mg/kg/day by capsule for 52 weeks (AMVAC Chemical Corp. 1990).

Body Weight Effects. No effects on body weight were observed in Fischer 344 rats or B6C3F$_1$, mice treated with dichlorvos by gavage 5 days a week for 2 years at doses of 8 mg/kg/day (rats),

20 mg/kg/day (male mice) or 40 mg/kg/day (female mice) (NTP 1989), or in Beagle dogs (groups of 4 of each sex) receiving up to 3 mg/kg/day by capsule for 52 weeks (AMVAC Chemical Corp. 1990).

2.2.2.3 Immunological and Lymphoreticular Effects

No studies regarding immunological and lymphoreticular effects were located in humans after oral exposure to dichlorvos.

Immunosuppression after oral exposure to dichlorvos has been reported (Desi et al. 1978). A doserelated suppression of the humoral immune response induced by *Salmonella typhimurium* was observed in rabbits administered 0.3-2.5 mg/kg dichlorvos 5 days a week for 6 weeks (Desi et al. 1978). A single oral dose of 120 mg/kg dichlorvos in male C57B l/6 mice inoculated with sheep erythrocytes 2 days earlier suppressed the primary IgM response observed 48 hours later (Casale et al. 1983). A decrease in relative spleen weight was also noted in this study. Severe signs of dichlorvos neurotoxicity were noted, and the authors stated that the immunosuppression observed in this study may have been mediated indirectly by toxic chemical stress.

In Beagle dogs treated with dichlorvos at up to 3 mg/kg/day for 52 weeks (AMVAC Chemical Corp. 1990) no histopathologic treatment-related changes were seen in the mesenteric lymph node, spleen, sternum with bone marrow, and thymus. Leukocyte and reticulocyte counts were also unchanged compared to control, as was the leukocyte differential count.

2.2.2.4 Neurological Effects

Dichlorvos exerts its toxic effects in humans and animals by inhibiting neural acetylcholinesterase. This enzyme is present at cholinergic synapses throughout the central and peripheral nervous systems, and is responsible for hydrolyzing acetylcholine released from the pre-synaptic terminal. If this enzyme is inhibited, acetylcholine accumulates in the synaptic cleft, resulting in increased depolarization of the post-synaptic membrane. The consequences of this increased cholinergic activity in the parasympathetic autonomic nervous system can include lacrimation, perspiration, miosis, nausea, vomiting, diarrhea, excessive bronchial secretions, bradycardia, increased salivation, and increased urinary frequency and incontinence. Effects on motor nerve fibers in the skeletal muscles can include muscle fasciculations, cramps, muscle weakness, and paralysis. Effects on cholinergic synapses in the

central nervous system result in drowsiness, fatigue, mental confusion, headache, convulsions. and coma. These classical symptoms of organophosphate neurotoxicity increase in severity and rapidity of onset in a dose-dependent manner (Ecobichon 1991).

This same enzyme is present in erythrocytes where it is known as erythrocyte acetylcholinesterase. Both forms of acetylcholinesterase are produced by the same gene (Taylor et al. 1993). Erythrocyte and neural acetylcholinesterase are inhibited to roughly the same extent by exposure to dichlorvos and other organophosphorus compounds (Hayes 1982); measurement of erythrocyte acetylcholin-esterase is used as a surrogate of the inhibition of neural acetylcholinesterase.

Neurological effects have been seen in many studies in animals after acute oral exposure to dichlorvos. Male Fischer 344 rats exposed to dichlorvos by olive oil gavage during an LD_{50} study had signs of excessive cholinergic stimulation including salivation, tremors, lacrimation, fasciculations, irregular respiration, and prostration (Ikeda et al. 1990). The authors did not report the dose at which these signs appeared; the LD_{50} calculated from the study was 97.5 mg/kg. In 2 other studies, in which clinical signs were not reported, single oral doses of 40 mg/kg dichlorvos in male rats resulted in a 70% inhibition of brain acetylcholinesterase after one hour (Teichert et al. 1976) and an 83% inhibition after 15 minutes (Pachecka et al. 1977). Brain acetylcholinesterase activity was inhibited 45% in rats receiving 4 mg/kg/day dichlorvos for 14 consecutive days (Teichert et al. 1976). Dichlorvos given to Fischer 344 rats by corn oil gavage 5 days a week over a l0-day period did not affect erythrocyte acetylcholinesterase at doses of up to 16 mg/kg/day (NTP 1989).

Whole-body tremor in male Swiss mice was evident within 3.6 minutes after a single dose of 150 mg/kg dichlorvos by gavage (Mohammad et al. 1989). Tremor was reported in male C57BL/6N mice receiving a single dose of 120 mg/kg dichlorvos by gavage (Casale et al. 1983). This clinical sign was accompanied by a 75% reduction in brain acetylcholinesterase activity one hour after dosing. In mice receiving 40 mg/kg on days 1, 3, 5, and 7, and sacrificed on day 8, significant reductions in acetylcholinesterase were observed in brain (30%) and erythrocytes (31%). Erythrocyte acetylcholin-esterase was not affected by administration of dichlorvos by gavage for 5 days a week over an 11-day period at doses up to 40 mg/kg in B6C3FI mice (NTP 1989).

In greyhound dogs receiving 11 or 22 mg/kg in a single dose, signs of neurological toxicity appeared within 7-15 minutes of dosing (Snow and Watson 1973). Restlessness was seen initially, followed by

increased salivation. muscle fasciculations, involuntary urination, diarrhea, sometimes bloody, and tenesmus. There was no apparent difference in severity of clinical signs between dogs given 11 or 22 mg/kg. Erythrocyte acetylcholinesterase was determined in 11 dogs, and the values ranged from 69 to 97% inhibition. The dog with 97% inhibition of erythrocyte acetylcholinesterase (dosed at 11 mg/kg) had severe clinical signs of intoxication but survived. Crossbred pigs receiving single doses from 18 to 560 mg/kg had clinical signs of neurological toxicity including hypoactivity, vomiting, ataxia, muscle fasciculations, uncoordinated movements, frothy salivation, and defecation (Stanton et al. 1979).

In an intermediate-duration 21-day study in which volunteers were given dichlorvos orally, no signs of neurological toxicity were seen at doses of 0.033 mg/kg/day (Boyer et al. 1977). Twenty-four male volunteers had their serum cholinesterase and erythrocyte acetylcholinesterase determined twice a week for 3 weeks to establish their baseline levels. They were then given 0.9 mg dichlorvos 3 times a day for 21 days in either a pre-meal capsule or a 3-ounce container of gelatin. Serum cholinesterase and erythrocyte acetylcholinesterase were measured twice a week during the exposure period. Once a week, each volunteer had his vital signs measured, and was examined for tremor, pupillary response to light, and skin moisture. Following.the end of the study, serum cholinesterase and erythrocyte acetyl-cholinesterase were measured weekly for the next seven weeks. No clinical signs of neurological toxicity were observed in any of the volunteers. Erythrocyte acetylcholinesterase was not inhibited at 0.033 mg/kg/day in either the gelatin or capsule formulation. Serum cholinesterase was inhibited, on average, 38% in the group given the pre-meal capsule and 28% in the gelatin group. Measurements after the dosing period showed that the half-life for regeneration of serum cholinesterase was 13.7 days.

In a 90-day study in female Sherman rats, groups of 10 animals were exposed to doses ranging from 0 to 69.9 mg/kg/day in their feed (Durham et al. 1957). Two animals from each group were bled on days 3, 11, 60, and 90, and serum cholinesterase and erythrocyte acetylcholinesterase were determined. Clinical signs of neurological toxicity were not noted in any dosage group. Cholinesterase data was presented graphically so the percentage inhibition of the cholinesterases can only be estimated. For serum cholinesterase, doses of 0.4 and 1.5 mg/kg/day appeared to have no effect. Doses of 3.5 and 14.2 mg/kg/day appeared to produce 25-40% inhibition of enzyme activity compared to control values by the third day of feeding; activity remained depressed up to 60 days, and rose to near control values by the end of the experiment at 90 days. Serum cholinesterase in rats consuming

35.7 and 69.9 mg/kg/day fell by 50% after 3 days and remained at this level throughout the experiment. Erythrocyte acetylcholinesterase was unaffected at doses up to 3.5 mg/kg/day. Acetylcholinesterase activity was inhibited by 30% after 3 days at 14.2 mg/kg/day and remained depressed until the end of the experiment. At 35.7 and 69.9 mg/kg/day, erythrocyte acetylcholinesterase was inhibited about 50% after 3 days and 80% after 10 days. There appeared to be some recovery to about 50% of control by the end of the experiment. Female Fischer 344 rats treated with dichlorvos by oral gavage 5 days a week for up to 32 days had no significant changes in erythrocyte acetylcholinesterase activity at doses up to 16 mg/kg/day (NTP 1989). Male rats at the same dosage levels had a 22% decrease in erythrocyte acetylcholinesterase at day 24 at 16 mg/kg/day.

In a similar study with $B6C3F_1$ mice, gavage doses of dichlorvos 5 days a week up to 40 mg/kg/day did not affect erythrocyte acetylcholinesterase after 32 days of treatment (NTP 1989).

Staggering gait was observed in a white Leghorn chicken receiving 6.1 mg/kg/day dichlorvos by oral gavage after 35 days (Francis et al. 1985). In 5 hens treated in this study with doses ranging from 3.1 to 4.4 mg/kg/day, no adverse neurological effects were observed.

Mild diarrhea was reported in Fischer 344 rats receiving dichlorvos at 4 and 8 mg/kg/day, 5 days a week for 103 weeks (NIT 1989). There were no adverse neurological effects reported in a similar study in male B6C3FI mice receiving up to 20 mg/kg/day or females receiving up to 40 mg/kg/day (NTP 1989). No gross or histopathological changes were observed in brain or sciatic nerve in any of the rats or mice at the end of this study (NTP 1989).

Inhibition of erythrocyte and brain acetylcholinesterase, but no neurological signs, were reported in Beagles receiving 0.05, 1.0, or 3 mg/kg/day dichlorvos in capsules for 52 weeks (AMVAC Chemical Corp. 1990). No changes were noted at 0.05 mgkglday, but erythrocyte acetylcholinesterase was inhibited 45-53% in dogs receiving 1 mg/kg/day and 81-85% in dogs receiving 3 mg/kg/day. Brain acetylcholinesterase was inhibited 22% in male dogs receiving 1 mg/kg/day and 47% and 29%, respectively, in male and female dogs receiving 3 mg/kg/day. No treatment-related changes were seen upon histopathology review of the following neural tissues: brain with brainstem, cervical, thoracic or lumbar spinal cord, optic nerve, and sciatic nerve.

No studies were located in humans or animals describing OPIDN after oral exposure to dichlorvos. This is a syndrome observed in humans and some animal models after recovery from the acute effects of certain organophosphorus compounds (for example tri-o-cresyl phosphate) (Coppock et al. 1995; Johnson 1981). The characteristic signs are disturbances of gait, an axonalpathy or "dying-back" type degeneration of motor fibers. Studies on the relationship between dichlorvos and OPIDN during parenteral routes of exposure are discussed in Section 2.5.

All reliable NOAELs and LOAELs for neurological effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive toxicity in humans after oral exposure to dichlorvos. Maternal toxicity was not reported in CF-1 mice treated by gavage with 60 mg/kg/day dichlorvos during gestation days 6-15 or in New Zealand rabbits treated by gavage with 5 mg/kg/day (Schwetz et al. 1979). Microscopic examination of male reproductive tissues (prostate, testes, epididymis) and female reproductive tissues (ovaries, uterus) revealed no changes attributable to oral dichlorvos exposure during 2-year studies in Fischer 344/N rats treated at 4 or 8 mg/kg/day for 5 days a week or $B6C3F_1$ mice (males treated at 10 or 20 mg/kg/day, females at 20 or 40 mg/kg/day) (NTP 1989). Similar results were obtained in Beagle dogs receiving dichlorvos by capsule for 52 weeks at up to 3 mg/kg/day (AMVAC Chemical Corp. 1990). Male reproductive tissues examined were the testes, prostate, and epididymides; female tissues examined were the cervix, ovaries, uterus, and vagina.

2.2.2.6 Developmental Effects

No studies were located regarding developmental toxicity in humans after oral exposure to dichlorvos.

No adverse developmental effects were observed in CF-1 mice treated by gavage with 60 mg/kg/day dichlorvos during gestation days 6-15 (Schwetz et al. 1979). There was no significant effect on implantations, mean number of fetuses per litter, incidence or distribution of resorptions, or on fetal body measurements. Similar results were observed in New Zealand rabbits treated by gavage with 5 mg/kg/day over gestation days 6-18 (Schwetz et al. 1979).

Pregnant crossbred sows given PVC-resin formulations of dichlorvos in the diet at a daily dose of 0, 5, or 25 mg/kg during the last 30 days of pregnancy had 100% live births and birth weights similar to control animals (Stanton et al. 1979). There are a number of reports on dichlorvos treatment of pregnant sows causing a dose-related increase in percentage of live births, mean birth weights and weaning weights. The mechanism of action for this phenomenon has not been established (Gallo and Lawryk 1991).

2.2.2.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

No studies were located regarding cancer in humans after oral exposure to dichlorvos.

In a carcinogenicity study in Osborne-Mendel rats, groups of 50 animals of each sex were originally dosed through feed at levels of 45 and 90 mg/kg/day. Ten animals of each sex were used as controls and were pooled with 50 other control rats of each sex from studies conducted concurrently on 5 other compounds (NCI 1977). During the initial 3 weeks of dosage, acute signs of toxicity were observed, including tremor and diarrhea in the 90-mg/kg/day group. Therefore, the dosages were then lowered to 30% of the original. The time-weighted average (TWA) doses over the 80-week period of dosing were 13.5 and 29.3 mg/kg/day. After the go-week dosing period, the animals were observed for a further 30 weeks until sacrifice. Gross necropsy was done on all animals that died during the study and at termination. Microscopic examination was done on sections of brain, pituitary, adrenal, thyroid, parathyroid, trachea, esophagus, thymus, salivary gland, lymph nodes, heart, lung, spleen, liver, kidney, stomach, pancreas, small intestine, large intestine, urinary bladder, prostate or uterus, testis or ovary, mammary gland, skin, and bone, including marrow. Adverse clinical signs (hematuria, rough coats, epistaxis) were noted in control and dosed animals gradually increasing during the second year of the study. The authors stated that at the end of the study, the rats (i.e., both treated and control) were in generally poor condition. The matched control groups had significantly lower survival than the treated groups at the end of the study, mainly due to deaths during the 30-week observation period after treatment (NCI 1977). At the end of the study, only 2 of 10 male rats and 5 of 10 female rats survived in the matched control groups. For this reason, these control rats were pooled with control

rats from concurrent studies for comparison with the treated groups. Of the male rats, 76% of the high-dose and 64% of the low-dose group survived to the end of the study, as did 84% of the highdose and 80% of the low-dose females.

Many inflammatory, degenerative, and proliferative lesions commonly seen in aged rats occurred with approximately equal frequency in treated and the pooled control rats (NCI 1977). Several nonneoplastic lesions occurred more frequently in the treated rats than in the controls. These included aggregates of alveolar macrophages in the lungs, interstitial fibrosis of the myocardium, and focal follicular cell hyperplasia in the male rats. Benign endocrine neoplasms occurred frequently in both test and control rats. There was a high incidence of benign mammary neoplasms in both control and treated rats. Because of the low survival of the matched control rats, control animals from other concurrent studies were pooled for statistical analysis. The authors stated that on the basis of variability of both the incidence and type of spontaneous lesions, and the lack of significant proportions of tumors in the dosed groups compared to the controls, no statistical significance could be attached to the incidence of tumors seen in the dichlorvos-treated rats in this study. Because of the poor survival of control animals in this study, the results are difficult to interpret. Dichlorvos was subsequently re-tested by the National Toxicology Program (NTP 1989).

In another carcinogenicity study in rats, groups of 50 Fischer 344 rats of each sex were dosed with dichlorvos in corn oil by oral gavage at levels of 0, 4, or 8 mg dichlorvos for 5 days a week for 103 weeks (NTP 1989). Necropsy and histopathologic examinations were performed on all animals at the end of the study or at the time of death. Histopathology was done on the following tissues: adrenal gland, brain, cecum, colon, duodenum, esophagus, femur including marrow, heart, ileum, jejunum, kidneys, liver, lungs and mainstem bronchi, mammary glands, mandibular and mesenteric lymph nodes, nasal cavity and turbinates, pancreas, parathyroid glands, pituitary gland, preputial or clitoral gland, prostate/testes/epididymis or ovaries/uterus, rectum, salivary glands, sciatic nerve, skin, spleen, stomach, thymus, thyroid gland, tissue masses, trachea, and urinary bladder. No significant differences in survival were noted between any groups. Survival rates were: 31 of 50-for male and female controls; 25 of 50 males and 26 of 50 females in the low-dose group; and 24 of 50 males and 26 of 50 females in the high-dose group.

Statistically significant increases in neoplasms compared to control animals were observed in the pancreas and hematopoietic system in male rats and in the mammary gland in female rats in this study

(NTP 1989). Adenoma of the exocrine pancreas exhibited a significant positive trend, and the incidences were greater in treated as compared to control groups (16 of 50 in vehicle control, 25 of 49 in the low-dose group and 30 of 50 in the high-dose group). When horizontal sections of the pancreas were examined, additional adenomas were observed. When the data from both methods were combined, the incidences of pancreatic adenoma were 25 of 50 in the controls, 30 of 49 in the low-dose group, and 33 of 50 in the high-dose group. The incidence for the treated groups was statistically significant compared to the vehicle controls.

A significant positive trend (i.e., not dose related) for mononuclear cell leukemia was also observed in the male rats. This neoplasm was found in 11 of 50 controls, 20 of 50 in the low-dose group, and 21 of 50 in the high-dose group. A significant positive trend also occurred for mammary gland tumors in female rats. Fibroadenoma, adenoma, or carcinoma occurred in 9 of 50 control rats, 19 of 50 in the low-dose group, and 17 of 50 in the high-dose group. Peer review panels characterized these results as “some evidence” of carcinogenic activity in male rats and “equivocal evidence” in female rats.

Dichlorvos has also been tested for carcinogenicity in two long-term studies on $B6C3F_1$ mice (NCI 1977; NTP 1989). Using the same protocol as the NCI (1977) study described above for Osbome-Mendel rats, groups of 50 male and 50 female $B6C3F_1$ rats were fed dichlorvos in the diet at doses of 57 or 114 mg/kg/day for 80 weeks followed by a 14-week observation period before sacrifice (NCI 1977). These doses were converted from the originally reported dichlorvos-in-feed doses (318 and 635 ppm) by conversion factors for average mouse daily feed consumption (EPA 1988). Groups of 10 male and 10 female mice served as matched controls. The same tissues were examined histologically as in the Osborne-Mendel rat study described above. Two squamous-cell carcinomas of the esophagus (in one low-dose male and one high-dose female) and one papilloma of the esophagus in a high-dose female were seen. The authors stated that these were historically very rare lesions in this strain of mice. Focal hyperplasia of the esophageal epithelium was also seen in three low-dose males. However, no statistically significant differences in neoplastic lesions were seen due to treatment.

Dichlorvos was also tested for carcinogenicity in male and female B6C3Fl mice by a protocol similar to that used on the Fischer 344 rats described above (NTP 1989). Because of higher toxicity in male mice during the dose-finding study, groups of 50 male mice were dosed by corn oil gavage at 0, 10, or 20 mg/kg for 5 days a week, while the female groups were dosed at 0, 20, or 40 mg/kg for 5 days a

week. Necropsy and histopathologic examinations were performed on all animals at the end of the study (102 weeks) or at the time of death. The same tissues were examined as in the Fischer 344 rat study described above.

The only neoplasms that occurred with a significant positive trend in treated compared to control mice were squamous cell papilloma and carcinomas of the forestomach. The overall incidences of this lesion in male mice were 1 of 50 in the controls, 1 of 50 in the low-dose group, and 5 of 50 in the high-dose group. In the females, overall incidences were 5 of 49 in the control group, 6 of 49 in the low-dose group and 18 of 50 in the high-dose group. Incidence was near the historical incidence of 1% in male controls, but was higher in the female controls (10% compared to 1%). Peer review panels characterized the level of carcinogenic activity as “some evidence” in male mice and “clear evidence” in female mice.

2.2.3 Dermal Exposure

2.2.3.1 Death

Two workers in Costa Rica died after splashing a concentrated formulation of dichlorvos on their bare arms and failing to wash it off promptly (Hayes 1982). Information on the concentration of dichlorvos in the concentrate was not available.

Three cynomolgus monkeys were given daily dermal doses of dichlorvos in xylene on a shaved area between the shoulder blades (Durham et al. 1957). A monkey receiving 100 mg/kg/day died after 4 days. A monkey given 50 mg/kgl/day died after 8 doses over 10 days and a monkey given 75 mg/kg/day died after 10 doses over 12 days. Clinical signs occurred within 10-20 minutes after dosing. Signs in their order of appearance were nervousness, gritting of teeth, incoordination, muscle fasciculations, excessive salivation, labored breathing, miosis, and flaccidity.

In an LD_{50} study in Sherman rats where dichlorvos in xylene was applied to an area of clipped skin between the shoulder blades (Durham et al. 1957), the dermal LD_{50} was 107 mg/kg for male rats and 75 mg/kg for females. All rats killed by a single dermal dose died within 20 minutes of dosage, except a male dosed at 110 mg/kg that survived for 40 minutes and another male dosed at 125 mg/kg that survived for 17 days. The symptoms of poisoning observed were bulging eyes, lacrimation,

sialorrhea, muscle fasciculations, and tremors. Some animals had convulsions just before death. Rats that survived appeared to make a full recovery. In another LD_{50}, study where dichlorvos was applied to female Wistar rats in ethanol-water on depilated skin, an LD_{50} of 70.4 mg dichlorvos was found (Gajewski and Katkiewicz 1981). Two white Leghorn chickens dosed daily on the ventral wing surface at the humerus with 14.4 and 15.7 mg/kg in an emulsion containing xylene and 2% Triton X-100 died after 2 and 3 doses, respectively (Francis et al. 1985). Five of 9 hens in this study receiving 0.54-3.8 mg/kg/day dichlorvos died between 30 and 45 days after the beginning of dosing. The lowest dose resulting in death was 1.7 mg/kg/day after 36 days.

All reliable LOAELs for death are recorded in Table 2-3.

2.2.3.2 Systemic Effects

No studies regarding systemic effects in humans after dermal exposure were located except for dermal effects. No studies regarding systemic effects in animals were located except for respiratory, dermal and ocular effects.

The LOAELs for systemic effects are recorded in Table 2-3.

Respiratory Effects. Labored breathing was observed in 3 cynomolgus monkeys receiving daily dermal doses of 50, 75, or 100 mg/kg/day (Durham et al. 1957). The authors stated that these and other cholinergic signs occurred within 10-20 minutes of dosing.

Dermal Effects. A 52-year-old male truck driver who had been hauling pesticide containers presented with dermatitis of his neck, anterior chest, dorsal hands, and forearms (Mathias 1983). On the previous day, several containers spilled in his truck, and he apparently had direct dermal contact with a pesticide containing 5% dichlorvos, 15% petroleum distillates, and 80% trichloroethane. A faint papular dermatitis was present over the dorsal arms, hands, and V of the neck. .Vertical erythematous, slightly scaling streaks were present over the lateral and posterior neck, a pattern suggesting that liquid droplets had produced the dermatitis. The dermatitis was treated with 1% hydrocortisone ointment. Follow-up examination six weeks later showed persistent vertical, mildly erythematous streaks over the posterior and lateral neck; the arms and anterior chest had cleared.

Table 2-3. Levels of Significant Exposure to Dichlorvos - Dermal

Species/ (Strain)	Exposure/ Duration/ Frequency/ (Specific Route)	System	NOAEL	LOAEL			Reference
				Less Serious	Serious		
ACUTE EXPOSURE							
Death							
Monkey (Cyno-molgus)	12 d 5 d/wk 1 x/d (NS)				50 mg/kg/d	(death after 8 doses over 10 days)	Durham et al. 1957
Rat (Sherman)	once				107 M mg/kg 75 mg/kg F	(LD_{50}) (LD_{50})	Durham et al. 1957
Rat (Wistar)	once				70.4 F mg/kg	(LD_{50})	Gajewski and Katkiewicz 1981
Chicken (white leghorn)	2-3 d 1 x/d				14.4 mg/kg/d	(1 of 1 died in 2 days)	Francis et al. 1985
Systemic							
Monkey (Cyno-molgus)	4-12 d 5 d/wk 1 x/d (NS)	Resp			50 mg/kg/d	(labored breathing)	Durham et al. 1957
Neurological							
Monkey (Cyno-molgus)	4-12 d 5 d/wk 1 x/d (NS)				50 mg/kg/d	(muscle fasciculations, erythrocyte AChE activity decreased by 80%)	Durham et al. 1957

Table 2-3. Levels of Significant Exposure to Dichlorvos - Dermal (continued)

Species/ (Strain)	Exposure/ Duration/ Frequency/ (Specific Route)	System	NOAEL	LOAEL		Reference
				Less Serious	Serious	
INTERMEDIATE EXPOSURE						
Death						
Chicken (white leghorn)	28-90 d 1 x/d				1.7 F (death after 36 days) mg/kg/d	Francis et al. 1985
Neurological						
Chicken (white leghorn)	28-90 d 1 x/d		0.71 F		1.8 F (staggering gait) mg/kg/d	Francis et al. 1985

AChE = acetylcholinesterase; d = day(s); F = female; LOAEL = lowest-observable-adverse-effect level; M = male; LD_{50} = lethal dose, 50% kill; NOAEL = no-observable-adverse-effect level; NS = not specified; Resp = respiratory; wk = week(s); x = time(s)

Negative patch test results suggested that the dermatitis resulted from a primary irritant effect of dichlorvos on the skin. Dermatitis resolved completely approximately 10 weeks after onset. The persistence of dermatitis 2 months after exposure is very unusual, and the author suggested that this may be related to some unique local toxic effect of dichlorvos, but did not speculate further.
As part of a study on the ability of dichlorvos to cause sensitization in a guinea pig maximization test (Ueda et al. 1994), the threshold irritation concentration of dichlorvos on guinea pig skin was reported to be 1%. No further details were given.

Ocular Effects. Miosis was observed in cynomolgus monkeys receiving daily dermal doses of 50, 75, or 100 mg/kg/day (Durham et al. 1957). It was not reported whether this resolved before the next daily dose. This sign is related to the cholinergic overstimulation caused by dichlorvos.

2.2.3.3 Immunological and Lymphoreticular Effects

Six of 59 males and 9 of 48 females in an occupational study of flower growers showed positive reactions on patch testing to dichlorvos for an overall rate of 14%. Twelve of 18 subjects who had positive skin patch test reactions to triforine (1,4-bis [2,2,2-trichlor- 1-formamidoethyl] piperazine) also showed positive reactions to dichlorvos (Ueda et al. 1994). These subjects may have also been occupationally exposed to dichlorvos.

In a guinea pig maximization test conducted in this study, induction with dichlorvos by intradermal injection and topical application and subsequent challenge with topical dichlorvos solutions showed sensitization (Ueda et al. 1994). Cross-reactivity with dichlorvos was demonstrated in animals induced with triforine.

2.2.3.4 Neurological Effects

Dichlorvos exerts its toxic effects in humans and animals by inhibiting neural acetylcholinesterase. This enzyme is present at cholinergic synapses throughout the central and peripheral nervous systems and is responsible for hydrolyzing acetylcholine released from the pre-synaptic terminal. If this enzyme is inhibited, acetylcholine accumulates in the synapse, resulting in increased firing of the postsynaptic neuron or increased contractions in muscle. The consequences of this increased cholinergic

activity in the parasympathetic autonomic nervous system can include lacrimation, perspiration. miosis, nausea, vomiting, diarrhea, excessive bronchial secretions, bradycardia, increased salivation, and increased urinary frequency and incontinence. Effects on motor nerve fibers in the skeletal muscles can include muscle fasciculations, cramps, muscle weakness and flaccidity. Effects on cholinergic synapses in the central nervous system result in drowsiness, fatigue, mental confusion, headache, convulsions and coma (Ecobichon 1991).

This same enzyme is present in erythrocytes where it is known as erythrocyte acetylcholinesterase. Both forms of acetylcholinesterase are produced by the same gene (Taylor et al. 1993). Erythrocyte and neural acetylcholinesterase are inhibited to roughly the same extent by exposure to dichlorvos and other organophosphorus compounds; measurement of erythrocyte acetylcholinesterase is used as an indicator of inhibition of neural acetylcholinesterase (Hayes 1982).

A truck driver who had direct skin contact with a pesticide solution containing dichlorvos developed dermatitis and also complained of headache, mild rhinorrhea, a burning sensation on his tongue and a bitter taste in his mouth the day after exposure occurred (Mathias 1983). He was exposed to a solution containing 5% dichlorvos, 15% petroleum distillates, and 80% trichloroethane. Initial blood cholinesterase determination (specific enzyme not specified) were in the low-normal range and had risen to the high-normal range two weeks later.

Three cynomolgus monkeys were exposed to dichlorvos dissolved in xylene by daily derrnal doses on a shaved area between the shoulder blades (Durham et al. 1957); cholinergic signs appeared within 10-20 minutes of dosage. Signs of toxicity in order of appearance were: nervousness, incoordination, muscle fasciculations, excessive salivation, labored breathing, miosis, and inability to move. The authors stated that at a given dose, the cholinergic signs tended to become more severe with subsequent doses. Serum cholinesterase and erythrocyte acetylcholinesterase were measured in one monkey that received 75 mg/kg/day. After 2 doses, erythrocyte acetylcholinesterase had declined about 67%, while serum cholinesterase was unchanged. When these values were measured shortly after the next day’s dosage, the serum cholinesterase had fallen about 33%, while erythrocyte acetyl-cholinesterase remained inhibited about 67%. The serum cholinesterase recovered after 2 days without dosing, but the erythrocyte acetylcholinesterase did not. After 5 doses, the erythrocyte acetyl-cholinesterase had fallen by 90% and stayed there until death occurred after 12 days, during which 10 doses were administered.

Signs of neurological toxicity were also observed in Sherman rats dermally exposed to dichlorvos during an LD_{50} experiment (Durham et al. 1957). Rats that survived exhibited bulging eyes, excessive lacrimation, and generalized muscle fasciculation and tremors. Surviving rats appeared to completely recover after 24 hours.

In a study involving daily dermal administration of dichlorvos in white Leghorn chickens, 3 hens receiving 2.8-3.8 mg/kg/day exhibited a staggering gait after 14 days of treatment (Francis et al. 1985). Three hens receiving doses between 0.54 and 0.71 mg/kg/day showed no signs of abnormal gait over 90 days of dosing.

In a study designed to determine the optimal configuration of skin patches for delivery of dichlorvos as a potential therapy for Alzheimer's disease (Moriearty et al. 1993), dichlorvos was dissolved in mineral oil or olive oil and applied in 10-mg doses (34 $mg/cm^2/kg$). Groups of three male Sprague-Dawley rats received patches of different materials taped in place on the shaved intracapsular area. At one and 7 days after administration, rats were sacrificed and brain acetylcholinesterase, serum cholinesterase and erythrocyte acetylcholinesterase were measured. Brain acetylcholinesterase inhibition after 24 hours ranged from 47.5% in the olive oil patches to 67.4% in the mineral oil patches. Brain acetylcholinesterase inhibition persisted for seven days. An experiment delivering 4 different doses of dichlorvos over a l0-fold range showed a linear log-dose relationship for inhibition of brain acetylcholinesterase. Erythrocyte acetylcholinesterase was not reported, although the authors said it was "similar" to the inhibition seen in brain. Serum cholinesterase inhibition peaked on the first day (approximately 60%), and returned to normal by the seventh day. No clinical signs of toxicity were reported.

No studies were located in humans or animals describing OPIDN after dermal exposure to dichlorvos. This is a syndrome observed in humans and some animal models after recovery from the acute effects of certain organophosphorus compounds, for example tri-o-cresyl phosphate (Coppock et al. 1995; Johnson 1981). The characteristic signs are disturbances of gait, a "dying-back" type degeneration of motor fibers. Studies on the relationship between dichlorvos and OPIDN during parenteral routes of exposure are discussed in Section 2.5.

The LOAELs for neurological effects are recorded in Table 2-3.

No studies were located regarding the following effects in humans or animals after dermal exposure to dichlorvos:

2.2.3.5 Reproductive Effects

2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

No studies regarding cancer in humans or animals after dermal exposure to dichlorvos were located.

2.3 TOXICOKINETICS

Dichlorvos is a small, lipid-soluble molecule that can be absorbed by passive diffusion through the lungs, gastrointestinal tract, or skin. Little information is available on how rapidly dichlorvos is absorbed during inhalation exposure, but it appears to be rapidly absorbed by the oral and dermal routes of exposure. Because of the difficulty in assaying dichlorvos in biological tissues, this rapid rate of absorption is inferred from the time to onset of clinical signs and/or cholinesterase inhibition. This is due to the rapid degradation of dichlorvos by tissue esterases, particularly in the liver and the serum. The half-life of dichlorvos in human blood in vitro is about 10 minutes (Blair et al. 1975). Distribution is also difficult to study because of rapid metabolism, but there does not appear to be preferential distribution to particular tissues. Dichlorvos does not appear to be stored or concentrated in any tissue. The products of the esterase-catalyzed degradation of dichlorvos are dimethyl phosphate and dichloroacetaldehyde. Dimethyl phosphate is excreted in the urine, while dichloroacetaldehyde can be reduced to dichloroethanol or dehalogenated to glyoxal, which enters 2-carbon metabolism. Dichloroethanol is either conjugated to glucuronic acid and excreted in the urine or dehalogenated and further metabolized. There is also evidence that dichlorvos can be demethylated in a glutathione-dependent reaction. The target for the toxicity of dichlorvos is neural acetylcholinesterase in the central and peripheral nervous systems. Dichlorvos chemically reacts with this enzyme’s active site

and inhibits enzyme activity. None of the metabolites of dichlorvos inhibit neural acetylcholinesterase activity. Reported kinetic parameters for dichlorvos are listed in Table 2-4.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

Indirect evidence for absorption of dichlorvos following inhalation in humans was obtained by measuring dichloroethanol, a specific dichlorvos metabolite, in the urine of a male volunteer (Hutson and Hoadley 1972b). This individual was exposed at the extremely high level of 38 mg/m3 (4.2 ppm) for 105 minutes. The first urine sample obtained after exposure ended was analyzed by gas-liquid chromatography and 0.42 μg dichloroethanol/mL urine was detected (Hutson and Hoadley 1972b). The dichlorvos metabolite dimethyl phosphate was found in the urine of 3 of 13 male volunteer pesticide applicators who applied dichlorvos during an 8hour workday (Das et al. 1983). During the application, they wore goggles, caps, respirators, coats, gloves, and shoes. Each applicator sprayed 4 homes using 10-14 aerosol cans (230-330 g dichlorvos) and 18-22 pints of 0.5% emulsion spray (40-50 g dichlorvos). A range of 0.32-l .39 μg of dimethyl phosphate was measured in the urine of 3 workers. No effect was seen in clinical parameters or plasma cholinesterase activities. Dichlorvos was not detected (detection limit was 1 μg/g) in the blood of 2 male volunteers immediately after exposure to air concentrations of 0.25 mg/m^3 (0.03 ppm) for 10 hours or to 0.7 mg/m^3 (0.08 ppm) for 20 hours (Blair et al. 1975). Low-level exposure and breakdown by esterases may account for nondetection of dichlorvos.

In Sherman rats exposed to air saturated with dichlorvos (approximately 33 ppm), clinical signs of cholinergic stimulation were observed within 2 hours, showing that absorption had taken place (Durham et al. 1957). Intact dichlorvos was detected in rats after inhalation only at very high atmospheric levels. Thus, 0.07 and 0.08 μg/g could only be detected in the kidney of 2 of 3 male rats exposed for 4 hours to 10 mg/m^3 (1.1 ppm) (Blair et al. 1975). Similarly, dichlorvos was detected in all the tissues tested in the male mice and in blood, fat, and lungs of 3 female mice exposed for 4 hours to 90 mg/m^3 (10 ppm) (Blair et al. 1975). Neither dichlorvos nor desmethyl dichlorvos was detected in tissues of 3 young pigs (20 kg; 1 female, 2 males) that were exposed to 1-^{14}C-vinylldichlorvos (0.092 mg/m^3 for the female, and 0.114 mg/m^3 for the males) for 23 hours a day for

Table 2-4. Kinetic Parameters for Dichlorvos

Parameter	Value	Tissue (species)	Description	Reference
Absorption rate	3.8 mg/min/cm^2	skin (rabbit)	Percutaneous absorption rate for dichlorvos	Shellenberger et al. 1965
K_m	7.1 mM	serum (human)	K_m for degradation of dichlorvos	Traverso et al. 1989
K_m	4.0 mM	serum (human)	K_m for degradation of dichlorvos	Reiner er al. 1980
V_{max}	143 nmol/min/mL	serum (human)	V_{max} for degradation of dichlorvos	Traverso et al. 1989
$t_{1/2}$	8.1 min	whole blood (human male)	half-life for degradation of dichlorvos	Blair et al. 1975
$t_{1/2}$	11.2 min	whole blood (human female)	half-life for degradation of dichlorvos	Blair et al. 1975
$t_{1/2}$	18.0 min	serum (human)	half-life for degradation of dichlorvos	Blair et al. 1975
$t_{1/2}$	2.0 min	whole blood (rabbit male)	half-life for degradation of dichlorvos	Blair et al. 1975
$t_{1/2}$	3.6 min	whole blood (rabbit female)	half-life for degradation of dichlorvos	Blair et al. 1975
$t_{1/2}$	19.9 min	whole blood (rat male)	half-life for degradation of dichlorvos	Blair et al. 1975
k_i	$1.4x10^3$ M^{-1} min^{-1}	brain AChE (rat)	rate constant for inhibition of brain AChE	Maxwell 1992
IC_{50}	0.95 µm	brain AChE (human)	50% inhibition concentration for brain AChE	Lotti and Johnson 1978
IC_{50}	10 µm	brain NTE (human)	50% inhibition concentration for brain NTE	Lotti and Johnson 1978

AChE = acetylcholinesterase; IC = inhibitory concentration; NTE = neurotoxic esterase

24 days (detection limit 3 ng/g) (Loeffler et al. 1976). Total radioactivity expressed as ppm dichlorvos equivalent in tissues ranged from 0.2 to 0.4 ppm in brain and subcutaneous fat to 2.4-2.6 ppm in liver.

2.3.1.2 Oral Exposure

When 5 mg [vinyl-^{14}C]dichlorvos was ingested in orange juice by a male volunteer, 27% of the dose was recovered from expired air as ^{14}CO, (Hutson and Hoadley 1972b). Dichlorvos was detected (0.18 mg/L) in the blood of fetuses 5 minutes after oral administration of 6 mg/kg dichlorvos in sunflower oil to 5 pregnant rabbits on the day of delivery (Maslinska et al. 1979). A total of 38.2% of the [l-^{14}C-vinyl]dichlorvos (40 mg/kg in PVC pellets) was absorbed in 9 young male Yorkshire pigs (Potter et al. 1973a), the remainder was recovered in the pellets. In another experiment, where [l-^{14}C-vinylldichlorvos was mixed with feed, absorption of dichlorvos was demonstrated by the recovery of 4% of the radioactivity in the urine, 5% in the feces, and 6.6% in the expired air (Potter et al. 1973b). Small amounts (ppb) of organosoluble [^{32}P] (presumably dichlorvos or desmethyl dichlorvos) was detected in the milk of a lactating cow after the oral administration of 1 mg/kg/day [^{32}P]dichlorvos for 7 days followed by a dose of 20 mg/kg on day 8 in gelatin capsules (Casida et al. 1962). Dichlorvos was effectively absorbed in 6 male and 6 female rats following the oral administration of 3.6 mg/kg [methyl-^{14}C]dichlorvos in arachis oil as indicated by the recovery of 64.6% of the dose in urine (Hutson and Hoadley 1972a). Identical recovery was obtained in 6 male and 6 female mice given 22 mg/kg [methyl-^{14}C]dichlorvos (Hutson and Hoadley 1972a). When [vinyl-^{14}C]dichlorvos was administered orally to 2 male Syrian hamsters at a dose of 3.7 mg/kg and to a female at a dose of 1.5 mg/kg, it was rapidly absorbed and 11.9-21.8% of the dose was recovered in the urine.

Evidence for rapid absorption of dichlorvos by the oral route includes death of Swiss mice within 9 minutes after a single gavage dose of 150 mg/kg (Mohammad et al. 1989) and in crossbred swine within 15-30 minutes receiving 100-560 mg/kg in an LD_{50} study (Stanton et al. 1979). Signs of cholinergic toxicity (vomiting, diarrhea) were observed in greyhound dogs within 7-15 minutes of receiving 11 mg/kg dichlorvos by gelatin capsule (Snow and Watson 1973).

2.3.1.3 Dermal Exposure

Dichlorvos appears to be rapidly absorbed by dermal exposure, although absorption by this route has not been well characterized. Monkeys exposed dermally to dichlorvos in xylene solution at doses of

50, 75, or 100 mg/kg exhibited signs of neurotoxicity within 15-20 minutes of administration, indicating rapid absorption (Durham et al. 1957). Similar results were seen in Sherman rats during an LD_{50}, study (LD_{50}s were 107 mg/kg for males and 75 mg/kg for females). In this study, all rats that died did so within 20 minutes (Durham et al. 1957).

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

Inhalation exposure of rats and mice to a concentration of 90 mg/m^3 (10 ppm) dichlorvos in air for 4 hours produced mild signs of toxicity such as lethargy and pupil constriction (Blair et al. 1975). Dichlorvos concentrations were very low or undetectable in blood (<0.2 mg/kg), liver, testes, lung, and brain (<0.1 mg/kg) of rats. In contrast, kidneys and fat contained concentrations as high as 2.4 and 0.4 mg/kg tissue, respectively. In rats, dichlorvos seemed to have been trapped in the trachea as indicated by its higher concentration in the trachea compared to lungs. When rats were exposed for 4 hours to 10 mg/m^3 (1.1 ppm) in air, only the kidneys of the male animals contained detectable or measurable concentrations (0.08 mg/kg). The results in mice were different from rats, the mice having higher concentrations of dichlorvos in fat, lung, and testes, but much lower concentrations in the kidneys. No residues (<0.00l mg/kg) of dichlorvos were detected in blood, liver, kidney, renal fat, or lung tissues of rats exposed to 0.5 or 0.05 mg/m^3 (0.06 or 0.006 ppm) for 14 days. On the other hand, exposure of male rats to 50 mg/m^3 (6 ppm) resulted in detectable dichlorvos (1.7 mg/kg) in the kidneys after 2 and 4 hours exposure time. On removal of rats from exposure, the concentration of dichlorvos in the kidneys decreased rapidly with an estimated half-life of 13.5 minutes. Dichlorvos disappeared from the blood within 15 minutes after exposure (Blair et al. 1975).

Neither dichlorvos nor its metabolite desmethyl dichlorvos was detected in pigs exposed to 0.092 mg/m^3 (0.01 ppm) for 23 hours a day for 24 days. When young pigs were exposed for 24 days via inhalation to 0.15 mg/m^3 [l-^{14}C-vinyl]dichlorvos, radioactivity was detected in various tissues (blood, liver, lungs), but no intact dichlorvos was detected (Loeffler et al. 1976).

2.3.2.2 Oral Exposure

Little information is available on the distribution of dichlorvos after oral administration. It is probable that a large “first-pass effect” takes place in the liver when dichlorvos is absorbed from the gastrointestinal tract (Gaines et al. 1966). This may reduce intact dichlorvos concentrations below detection limits in tissues. Dichlorvos (0.18 mg/L) appeared in the blood of fetuses 5 minutes after oral administration of 6 mg/kg dichlorvos in sunflower oil to pregnant rabbits on the day of delivery (Maslinska et al. 1979). Organosoluble [^{32}P] (presumably dichlorvos or desmethyl dichlorvos) was detected in the milk of a lactating cow 30 minutes after oral administration of 1 mg/kg/day [^{32}P]dichlorvos for 7 days followed by a dose of 20 mg/kg on day 8 in gelatin capsules (Casida et al. 1962).

2.3.2.3 Dermal Exposure

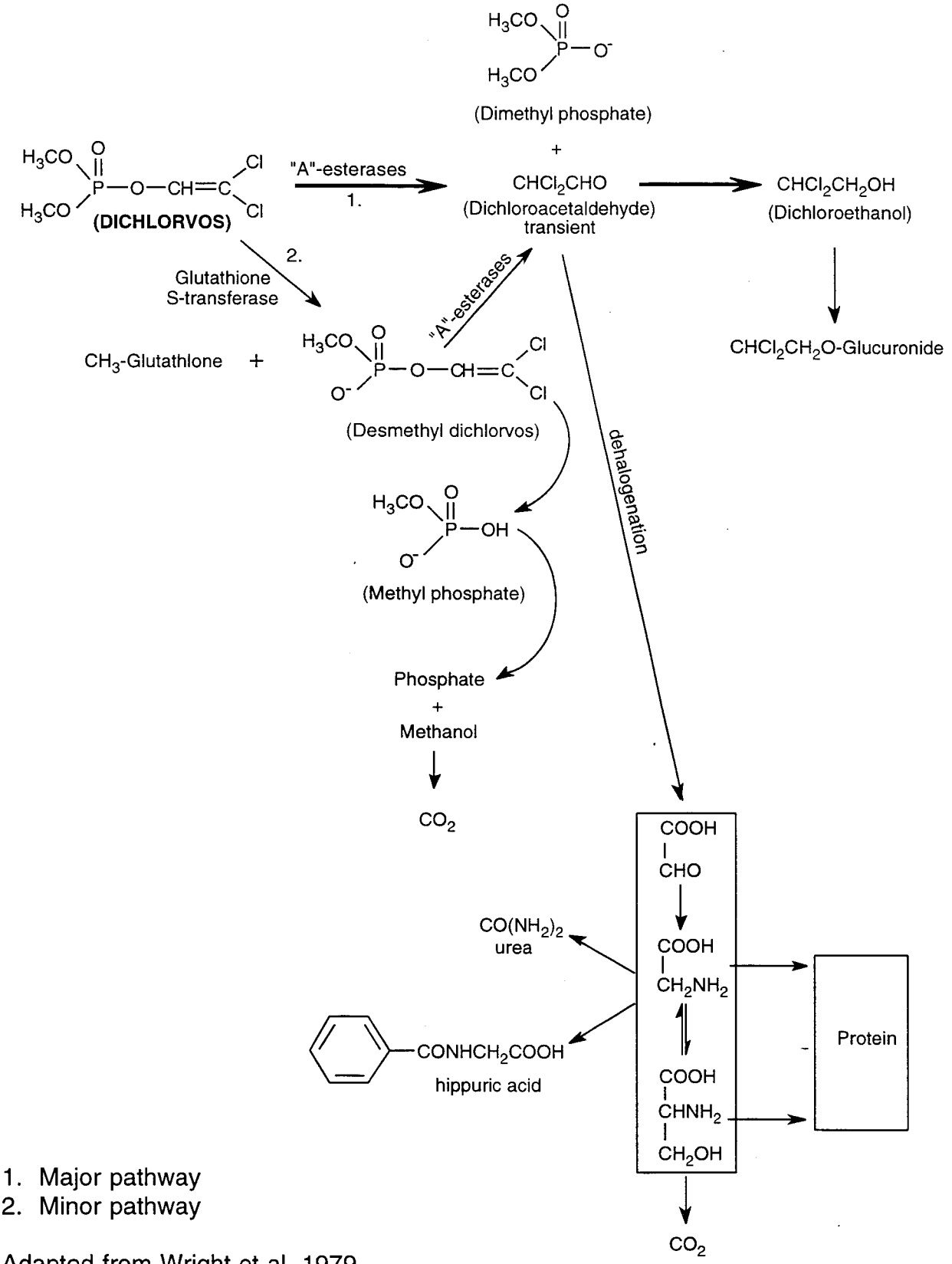
No information was located on tissue distribution after dermal exposure to dichlorvos.

2.3.3 Metabolism

In viva and in vitro studies have established the liver as the major site of dichlorvos detoxification (Casida et al. 1962; Gaines et al. 1966). Other tissues also metabolize dichlorvos *in vitro;* [^{32}P]dichlorvos was metabolized by blood, adrenal, kidney, lung and spleen tissue mostly to dimethyl phosphate. Other metabolites found were desmethyl dichlorvos, monomethyl phosphate, and inorganic phosphate (Loeffler et al. 1976). Pathways of metabolism for .dichlorvos are shown in Figure 2-3.

Metabolism studies of dichlorvos have been carried out in several animal species including human beings (Hutson and Hoadley 1972b), rats (Casida et al. 1962; Hutson and, Hoadley 1972a; Hutson et al. 1971), mice (Hutson and Hoadley 1972a, 1972b), Syrian hamsters (Hutson and Hoadley 1972b), pigs (Loeffler et al. 1976; Potter et al. 1973a, 1973b), goats (Casida et al. 1962), and cows (Casida et al. 1962) using radiolabeled dichlorvos and various routes of administration. These studies have shown that dichlorvos metabolism is generally similar in different species. Differences among species are mostly quantitative and related to the rate of metabolic pathways.

Figure 2-3. Mammalian Pathways of Metabolism of Dichlorvos



1. Major pathway
2. Minor pathway

Adapted from Wright et al. 1979

Dichlorvos breaks down via two enzymatic mechanisms. The first is glutathione-independent, catalyzed by "A"-type esterases, and produces dimethyl phosphate and dichloroacetaldehyde (Wright et al. 1979). The second is glutathione-dependent and results in formation of desmethyl dichlorvos and S-methyl glutathione (see Figure 2-3). Subsequent degradation of desmethyl dichlorvos to dichloroacetaldehyde and monomethyl phosphate is also catalyzed by "A"-type esterases (Wright et al. 1979). S-methyl-glutathione is broken down to methylmercapturic acid and excreted in the urine of animals treated with dichlorvos.

Several in vitro studies have examined the metabolism of dichlorvos in blood. An "A"-type esterase activity distinct from paraoxonase has been characterized in human serum (Traverso et al. 1989). A K_m of 7.1 mM was reported for the degradation of dichlorvos and a V_{max} of 143 nmol/min/mL. A K_m of 4 mM has also been reported in human serum for dichlorvos (Reiner et al. 1980). Half-times for degradation of dichlorvos in whole blood were 8.1 minutes for human males and 11.2 minutes for human females (Blair et al. 1975).

The vinyl moiety of the dichlorvos molecule undergoes two routes of biotransformation: conversion to dichloroethanol and subsequent formation of dichloroethanol glucuronide; or dehalogenation and incorporation of the carbon atoms into various metabolic pathways in the body. These pathways result in the production of hippuric acid, urea, carbon dioxide, and other endogenous compounds that result in a prolonged half-life of radioactivity in the tissues following the administration of [vinyl-^{14}C]dichlorvos. Both radiolabelled dichloroethanol glucuronide and urea have been identified in the urine of men treated with [vinyl-^{14}C]dichlorvos indicating that both pathways occur in humans (Hutson and Hoadley 1972b). In other animal species, most of the radioactivity in carcasses and tissues was identified as glycine, serine, and other body compounds resulting from the metabolism of the vinyl carbon atoms (Hutson and Hoadley 1972b; Loeffler et al. 1976). Neither dichlorvos nor its metabolites accumulated in any tissues of animals treated with dichlorvos.

The metabolism of some dichlorvos metabolites has been studied in experimental animals. Most of the single oral dose of 500 mg of [^{32}P]dimethylphosphate/kg body weight in rats was eliminated, with urine containing about 50% unmetabolized dimethylphosphate (Casida et al. 1962). On the other hand, when a single oral dose of 500 mg [^{32}P]desmethyl dichlorvos was administered to rats, 14% of the dose was recovered in the urine in 90 hours, with 86% of the radioactivity identified as phosphoric acid and 14% as unchanged desmethyl dichlorvos. The bone contained a high proportion of

radioactivity suggesting the incorporation of the phosphoric acid that was produced via rapid complete degradation of dichlorvos (Casida et al. 1962). Also, female rats given an intraperitoneal injection of [1-^{14}C]dichloroacetaldehyde excreted 32% of the radioactivity in expired air as carbon dioxide within 24 hours (Casida et al. 1962).

2.3.4 Elimination and Excretion

Following a single oral dose of 5 mg/kg body weight [^{14}C]dichlorvos in a human male, 27% of the radioactivity was eliminated as $^{14}CO_2$, while only 9% of the radioactivity was eliminated in the urine, Radioactivity excreted in urine decreased with time and none was detected by day nine (Hutson and Hoadley 1972b).

Rats given a single oral dose of 0.1-80 mg/kg body weight [^{32}P]dichlorvos excreted 66-70% of the dose in the urine and approximately 10% in the feces during a 6-day period after dosing (Casida et al. 1962). After a single oral dose of 1 mg (rats) or 0.5 mg (mice) [methyl-^{14}C]dichlorvos in 0.5 mL of arachis oil, approximately 65% of the radioactivity was eliminated in the urine, while only 15% was recovered in the expired air over the following 4 days (Hutson and Hoadley 1972a). Following the administration of a single oral dose of 20 mg [^{32}P]dichlorvos to a cow, 40% of the dose was eliminated in the urine and 50% in the feces (Casida et al. 1962). The milk contained organosoluble radioactivity that was significantly above background within two hours of dosing. The excretion of radioactivity via various routes 24 hours following the administration of a single oral dose of [vinyl-^{14}C] dichlorvos in human, rat, mouse, and hamster are listed in Table 2-5 (Hutson and Hoadley 1972b).

Several metabolism studies have shown the difficulty of determining the biological half-life of dichlorvos in animals. Orally administered dichlorvos undergoes rapid biotransformation, so that no intact dichlorvos could be detected in the blood or tissues of treated animals (Casida et al. 1962; Hutson and Hoadley 1972a, 1972b; Hutson et al. 1971; Potter et al. 1973a, 1973b). Intact dichlorvos has been detected in most tissues of the body only in rats and mice exposed by inhalation to the extremely high level of 90 mg dichlorvos/m3 (10 ppm) for 4 hours (Blair et al. 1975). The half-life of dichlorvos was 13.5 minutes in the kidney of rats exposed to 50 mg/m^3 for 2 or 4 hours.

Table 2-5. Percentage of Radioactivity Excreted by Males 24 Hours Following a Single Oral Dose of [^{14}C-vinyl]dichlorvos in Various Species

Species	Number of Animals	Carbon Dioxide	Urine	Feces
Human	1	27 (8 hours only)	7.6	No data
Rat	3	28.8	9.8	1.5
Mouse	1	23.1	27.4	3.2
Hamster	2	33.5	14.7	2.9

Source: Hutson and Hoadley 1972b

Neither dichlorvos nor any of its metabolites are stored in the tissues of exposed animals (Wright et al. 1979). Because small fractions of dichlorvos molecules, including carbon, phosphorus, and chlorine atoms, undergo reactions similar to natural components of the tissues, these dichlorvos-derived fractions are retained in the body for several days.

2.3.5 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 et al. 1987; Andersen and Krishnan 1994). 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 parametrization, (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) is 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 2-4 shows a conceptualized representation of a PBPK model.

If PBPK models for dichlorvos 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.

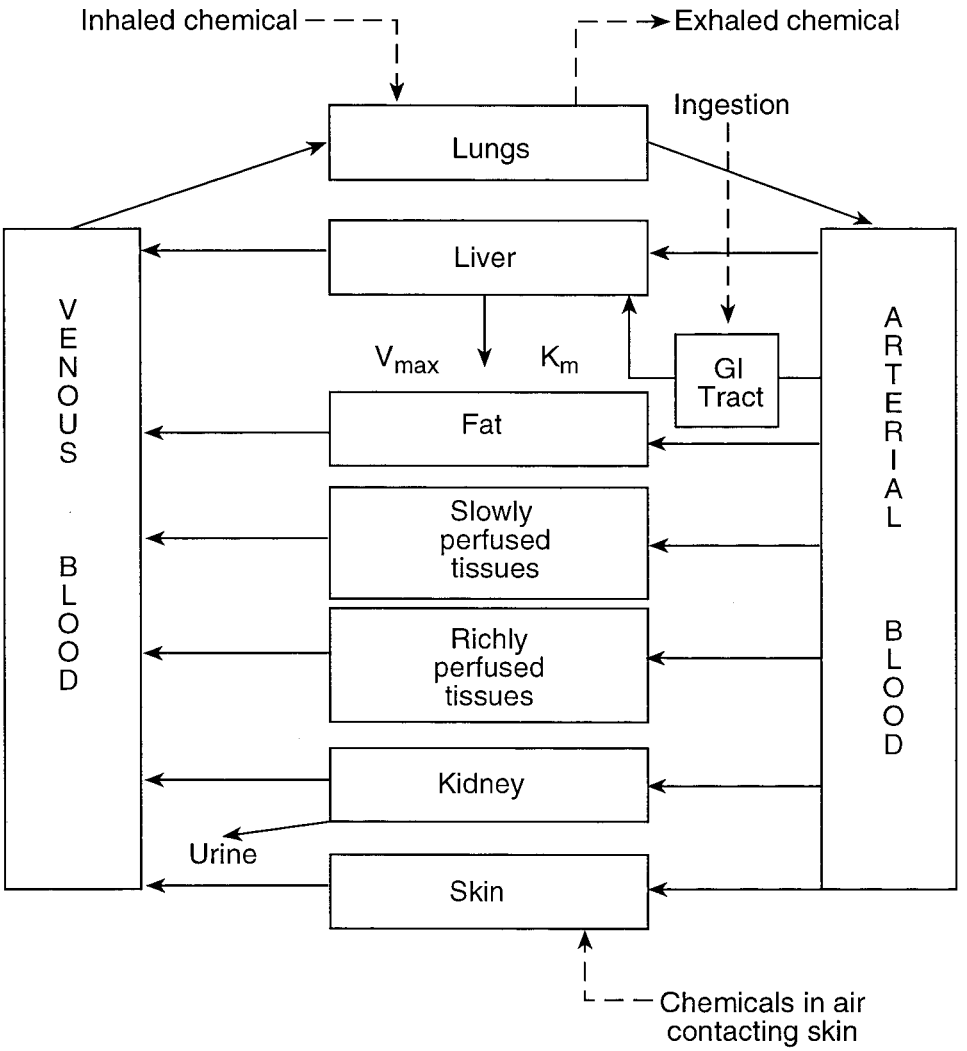
No PBPK models are available for dichlorvos.

2.4 MECHANISMS OF ACTION

2.4.1 Pharmacokinetic Mechanisms

The major characteristics of the pharmacokinetics of dichlorvos are its rapid absorption and rapid metabolism. This metabolism is so rapid in mammals that distribution is very difficult to study. Dichlorvos is a small (molecular weight = 221 daltons), lipophilic molecule and would be expected to be absorbed rapidly across cell membranes. Dichlorvos is absorbed by passive diffusion from the gut, lungs, and skin to the blood. Some dichlorvos can be inactivated by serum cholinesterase or

Figure 2-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1992

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.

erythrocyte and neural acetylcholinesterase, but based on total esterase activity in the body, the liver is probably the major site of metabolism. Glutathione-dependent demethylation can also take place, but this appears to be important only in high-dose situations (Wright et al. 1979). The toxicity of a given dose of dichlorvos depends on how rapidly neural acetylcholinesterase is inhibited; if this occurs before metabolic processes can reduce the blood level of dichlorvos, significant toxicity will take place.

Spontaneous reactivation of inhibited neural acetylcholinesterase has been studied *in vivo* (Pachecka et al. 1977; Reiner and Plestina 1979). After a single oral dose of 40 mg/kg dichlorvos in male rats (strain not stated), brain acetylcholinesterase was inhibited 47% after 5 minutes and 83% after 15 minutes (Pachecka et al. 1977). Brain acetylcholinesterase was 60% inhibited at 2 hours, 36% inhibited at 12 hours, and near control levels at 48 hours. Following intravenous administration of dichlorvos in male rats (Reiner and Plestina 1979), the highest degree of inhibition after a dose of 2.5 mg/kg was 85% at 30 minutes. Brain acetylcholinesterase inhibition returned to normal with a half-time of 2 hours. These relatively rapid times for reactivation indicate that recovery after a single dose is primarily by spontaneous reactivation.

2.4.2 Mechanisms of Toxicity

Dichlorvos exerts its toxicity by inhibiting neural acetylcholinesterase. The dichlorovinyloxy group of this molecule withdraws electrons from the phosphorus atom, leaving it susceptible to nucleophilic attack (Wright et al. 1979). One potential nucleophile is the serine hydroxyl group located at the active site of acetylcholinesterase. The products of this reaction are a dimethoxy-phosphorylated acetylcholinesterase molecule and dichloroacetaldehyde. The phosphorylated form of acetylcholinesterase is incapable of hydrolyzing acetylcholine. If this enzyme is inhibited, acetylcholine accumulates in the synapse and can interfere with neuron functioning.. The consequences of the disturbed cholinergic neurotransmission in the parasympathetic autonomic nervous system can include lacrimation, perspiration, miosis, nausea, vomiting, diarrhea, excessive bronchial secretions, bradycardia, increased salivation, and increased urinary frequency and incontinence. Effects on motor nerve fibers in the skeletal muscles can include muscle fasciculations, cramps, muscle weakness, and flaccidity. Effects on cholinergic synapses in the central nervous system result in drowsiness, fatigue, mental confusion, headache, convulsions and coma.

There is also evidence that repeated exposure to dichlorvos (5 mg/kg/day by intraperitoneal injection for 30 consecutive days) depletes both oxidized and reduced glutathione levels in rat brain (Julka et al. 1992). Lowered glutathione levels may decrease the rate of detoxification of dichlorvos by the glutathione-dependent metabolic pathways. The full significance of depleted brain glutathione is not known for dichlorvos metabolism.

The nervous system can accept a certain amount of acetylcholinesterase inhibition without overt toxic effects. In humans and animals, toxic signs are generally not seen until at least 20% of this enzyme (erythrocyte acetylcholinesterase used as a marker) has been inhibited (Ecobichon 1991). In an animal study, brain acetylcholinesterase after a 2-year inhalation exposure to dichlorvos was inhibited more than 90% compared to control animals (Blair et al. 1976), yet signs of cholinergic overstimulation were not observed. With dichlorvos and other organophosphate compounds, the best predictor of toxicity is not necessarily the actual percentage inhibition of acetylcholinesterase, but rather how rapidly this inhibition has occurred. Rapid inhibition does not give the nervous system time to adapt to acetylcholinesterase inhibition. This adaptation appears to involve desensitization and downregulation of muscarinic receptors (Fitzgerald and Costa 1993).

2.4.3 Animal-to-Human Extrapolations

Metabolic pathways for dichlorvos do not appear to be significantly different among animal species or between animal species and humans (Wright et al. 1979). Degradation by “A”-type esterases is the major pathway in all species examined. Metabolism also appears to be independent of both duration and route of exposure. Given the lack of information on what levels of dichlorvos exposure are necessary to produce toxicity in humans, selection of a suitable animal model for human toxicity is difficult. There do appear to be differences in sensitivity to dichlorvos among animal species. Orally exposed greyhound dogs (Snow and Watson 1973) and rabbits exposed by inhalation (Thorpe et al. 1972) appear to be more susceptible to dichlorvos toxicity than mice or rats similarly exposed.

2.5 RELEVANCE TO PUBLIC HEALTH

Overview

Dichlorvos is an organophosphorus insecticide that has been in use in the United States and elsewhere since the early 1960s. Like other insecticides in this class, dichlorvos is not only extremely toxic to insects, but also can be toxic to humans if high enough doses are received. The toxicity of dichlorvos results from its inhibition of neural acetylcholinesterase. This enzyme is necessary to hydrolyze acetylcholine and terminate its action at synapses and neuromuscular junctions. The clinical signs of dichlorvos toxicity are the result of overstimulation of the parasympathetic autonomic nervous system, somatic nerve fibers, and cholinergic pathways in the brain. After acute exposure to high concentrations of dichlorvos by any route, signs such as lacrimation, perspiration, miosis, nausea, vomiting, diarrhea, bronchial secretion, dyspnea, increased salivation, and urinary frequency and incontinence can result from overstimulation of the parasympathetic autonomic nervous system. The actions of dichlorvos at neuromuscular junctions can result in muscle fasciculations (especially in fine facial muscles), cramps, muscle weakness, and paralysis. Dichlorvos can also act in the central nervous system to produce drowsiness, fatigue, mental confusion, headache, convulsions, coma, and depression of respiratory centers in the brain.

There is limited information on the toxicity of dichlorvos to humans. The potential hazards of this chemical were well known before it came into use because of experience with other organophosphorus compounds. Exposure to levels of dichlorvos high enough to cause clinical symptoms of organophosphorus poisoning has been very rare in the United States. Limited studies of volunteers show that dichlorvos is rapidly metabolized by esterases in the liver and the blood; intact dichlorvos has never been detected in humans. Studies in laboratory animals show similar results. The metabolites of dichlorvos are polar compounds that are excreted into the urine; thus, no potential for bioaccumulation exists. Limited laboratory studies in animals have not shown adverse reproductive or developmental effects. However, sperm abnormalities occurred in rats treated with dichlorvos by intraperitoneal injection.

Dichlorvos is mutagenic in a number of in vitro test systems. There is also evidence in studies on rats and mice that dichlorvos is carcinogenic in these species. Based on this evidence, the EPA has classified dichlorvos as a probable human carcinogen. There is no direct evidence that dichlorvos is

carcinogenic in humans; however. the animal data were used to predict exposure levels that would increase the risk of cancer in humans. The EPA has calculated that a lifetime of drinking water containing 0.1 μg/L would cause one extra case of cancer in one million people.

Dichlorvos evaporates readily, so the most likely human route of exposure is by inhalation. This is most likely to occur during and/or after pesticide application. The U.S. Occupational Safety and Health Administration (OSHA) has set a Permissible Exposure Limit (PEL) of 1 mg/m^3 (0.11 ppm) for dichlorvos in workplaces. Health effects could occur in workplaces if proper industrial hygiene and safety precautions are not followed. The exposure of the general population to dichlorvos is negligible. Dichlorvos has not been detected in drinking water in the United States and rarely in outdoor air (only at manufacturing sites). Monitoring of the food supply by the U.S. Food and Drug Administration (FDA) and other government agencies has very rarely detected dichlorvos. Thus, the risk of adverse health effects in the general population from dichlorvos exposure appears to be negligible. Dichlorvos is degraded by water and does not persist in the atmosphere or in bodies of water for more than a few days.

For people living near hazardous waste sites, the potential for adverse health effects would depend on the amount of dichlorvos to which they were exposed. Dichlorvos has been detected in at least 3 of the 1,416 hazardous waste sites that have been proposed for inclusion on the EPA National Priority List (NPL) (HAZDAT 1995). However, the number of sites evaluated for dichlorvos is not known. The most likely routes of exposure for people living near hazardous waste sites would be by breathing dichlorvos-contaminated air, drinking dichlorvos-contaminated water, or skin contact with dichlorvos-contaminated soil. Monitoring of the air, drinking water, and soil levels of dichlorvos at these sites is necessary to predict the possibility of adverse health effects.

Minimal Risk Levels for Dichlorvos

Six minimal risk levels (MRLs) have been derived from the database compiled for this-profile; three for inhalation exposure (acute, intermediate, and chronic duration) and three for oral exposure (acute, intermediate, and chronic duration). In all cases, the toxic end point used for MRL derivation is inhibition of either erythrocyte or neural acetylcholinesterase activity. The neural form of this enzyme is the target for dichlorvos toxicity; the erythrocyte form is genetically identical (Taylor et al. 1993) and can be used to approximate neural activity (Hayes 1982). There is no evidence for toxic effects

from dichlorvos exposure by any route or for any duration unless activity of either the erythrocyte or neural form of this enzyme is reduced by at least 20%.

Inhalation MRL.s

- An MRL of 0.002 ppm has been derived for acute-duration inhalation exposure (14 days or less) to dichlorvos.

This MRL is based on a study in male Sprague-Dawley rats which were exposed continuously to atmospheres containing 8 different concentrations of dichlorvos ranging from 0.02 to 6.3 ppm over a 3-day period (Schmidt et al. 1979). A NOAEL of 0.20 ppm was observed for inhibition of erythrocyte acetyl-cholinesterase. This NOAEL was adjusted by dividing by uncertainty factors of 10 for human variability and 10 for interspecies extrapolation. A clear dose-response relationship existed between exposure level and inhibition of erythrocyte acetylcholinesterase.

Limited information on volunteers exposed to dichlorvos-containing atmospheres indicates that the 0.002 ppm dichlorvos level should be protective for health effects in populations potentially exposed near hazardous waste sites. A group of volunteers exposed to 0.23 ppm dichlorvos (l00-fold higher than the MRL level) for 2 hours a day for 4 consecutive days had no inhibition of erythrocyte acetyl-cholinesterase 24 hours after the exposure or clinical signs of dichlorvos toxicity such as miosis (Witter et al. 1961). Miosis and significant depression of erythrocyte acetylcholinesterase activity (50%) were seen in this same study in rhesus monkeys exposed to 1.43 ppm (7 times higher than the humans). One volunteer exposed to 0.08 ppm dichlorvos for 20 hours also showed no clinical signs of dichlorvos toxicity (Blair et al. 1975).

OSHA has established a PEL for dichlorvos of 0.1 ppm for a l0-hour workday. Practical use levels for insect control with dichlorvos are approximately 0.02 ppm. No human toxicity has been associated with this level of exposure (Hayes 1982).

- An MRL of 0.0003 ppm has been derived for intermediate-duration inhalation exposure (14 days to one year) to dichlorvos.

This MRL was derived from a NOAEL of 0.03 ppm dichlorvos for inhibition of brain acetylcholin-esterase activity in a rat developmental study (Thorpe et al. 1972). The MFU was derived by

adjusting the NOAEL by a factor of 10 for animal to human extrapolation and another factor of 10 for human variability.

Groups of 15 pregnant Carworth E rats were exposed to atmospheres containing 0, 0.25, 1.25. or 6.25 mg/m^3 (0, 0.03, 0.14, or 0.69 ppm) throughout their 20-day gestation period. Exposure of dams to all 3 concentrations of dichlorvos had no effect on developmental parameters (the number of fetal resorptions, late fetal deaths, litter size, or mean weight per fetus). Exposure at 0.03 ppm had no effect on erythrocyte or brain acetylcholinesterase. Exposure at 0.14 ppm resulted in a 29% inhibition of erythrocyte and a 28% inhibition of brain acetylcholinesterase, while exposure at 0.69 ppm resulted in 88% inhibition of erythrocyte acetylcholinesterase and an 83% inhibition of brain acetyl-cholinesterase. Brain and erythrocyte acetylcholinesterase activities were inhibited 83 and 88%, respectively, in dams in the high exposure (0.69 ppm) group. A NOAEL of 0.03 ppm was established for the neurological effect of brain acetylcholinesterase inhibition. A NOAEL of 0.03 ppm for brain acetylcholinesterase inhibition was also noted for rabbits exposed for a 28-day gestation period in this study (Thorpe et al. 1972). Significant maternal toxicity was observed in rabbits exposed to 0.69 and 0.44 ppm dichlorvos, although this appears to have been related to mechanical failures that allowed higher concentrations of dichlorvos to enter the chambers for brief periods. However, no adverse developmental effects on the offspring were observed.

No human studies were available for intermediate-duration inhalation exposure to dichlorvos. A NOAEL for developmental effects of 0.69 ppm was also observed in the MRL study (Thorpe et al. 1972), but the NOAEL for neurological effects of 0.03 ppm was used to derive the MRL since the target of dichlorvos toxicity is, neural acetylcholinesterase. The database for intermediate-duration inhalation exposure to dichlorvos in animals is limited to the MRL study and a study in which pigs were exposed to dichlorvos at concentrations up to 0.015 ppm dichlorvos for 24 days (Loeffler et al. 1976). A NOAEL of 0.015 ppm dichlorvos was identified for erythrocyte acetylcholinesterase inhibition.

The intermediate-duration inhalation exposure MRL of 0.0003 ppm dichlorvos should be protective against health effects in populations potentially exposed at hazardous waste sites. This level is approximately l00-fold lower than that used in insect control (0.02 ppm), a level that has not resulted in human toxicity (Hayes 1982).

- An MRL of 0.00006 ppm has been derived for chronic-duration inhalation exposure (365 days or longer) to dichlorvos.

This MRL is based on a 2-year inhalation study in Carworth E rats (Blair et al. 1976). A NOAEL of 0.006 ppm in the male rats in this study was observed for inhibition of erythrocyte acetylcholinesterase. This NOAEL was adjusted by dividing by uncertainty factors of 10 for human variability and 10 for interspecies extrapolation. A dose-response relationship existed between exposure level and inhibition of erythrocyte acetylcholinesterase.

Groups of 50 Carworth E strain rats of both sexes were exposed to atmospheres containing 0, 0.05, 0.5, or 5 mg dichlorvos/m3 (0, 0.006, 0.06, or 0.6 ppm) for 23 hours a day for 2 years as part of a carcinogenicity study (Blair et al. 1976). At the end of the study, the surviving rats were killed, blood was collected, and half the brain was used to determine brain acetylcholinesterase. Plasma cholinesterase and erythrocyte acetylcholinesterase were also measured. In males treated at 0.006 ppm, a NOAEL for brain and erythrocyte acetylcholinesterase was identified. Females at this dose had a 12% reduction in erythrocyte acetylcholinesterase; this is also a NOAEL, since erythrocyte acetylcholinesterase inhibition of 20% or less is not considered an adverse effect.

This is the only chronic inhalation study available for the derivation of an MRL. The EPA used this study to derive a reference concentration (RfC) of 0.0005 mg dichlorvos/m^3 (0.00006 ppm) for lifetime exposure to dichlorvos, the same value as derived for the MRL. The RfC "is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime" (IRIS 1995).

Oral MRLs

- . An MRL of 0.004 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to dichlorvos.

This MRL is based on a study in male Sprague-Dawley rats that were treated for 14 days with dichlorvos by corn oil gavage (Teichert et al. 1976). A LOAEL of 4 mg/kg/day was identified on the basis of a 44% inhibition of brain acetylcholinesterase. The MRL was derived by dividing the

LOAEL value by 10 for use of a LOAEL (no NOAEL was identified in this study), by 10 for interspecies extrapolation, and by 10 for human variability.

Male Sprague-Dawley rats were treated daily for 14 days with 4 mg/kg dichlorvos by gavage in corn oil (Teichert et al. 1976). Dichlorvos purity was 99%. Control animals received corn oil only. Ten animals were used in the control group and 11 in the treatment group. At the end of the 14-day dosing period, the rats were decapitated; the brains were removed, homogenized in 10 volumes of 0.3% Triton X-100, and centrifuged for 10 minutes. Aliquots of the supematant were assayed for acetylcholinesterase activity by the hydrolysis of radioactive acetylcholine. Dichlorvos treatment at 4 mg/kg/day over a 14-day period resulted in a 44% depression of brain acetylcholinesterase activity, which is considered a less serious LOAEL for neurological effects. In this same study, a single gavage dose of 40 mg/kg dichlorvos resulted in a 70% depression of brain acetylcholinesterase 1 hour later, demonstrating, a dose-severity relationship.

This is the only reliable study located for acute-duration oral exposure to dichlorvos where doses ranging from 5 to 10% of the LD_{50} were administered on a daily basis and brain acetylcholinesterase (one of the targets for dichlorvos) was measured rather than erythrocyte acetylcholinesterase. Most of the acute-duration oral studies for dichlorvos in rodent species were LD_{50} studies; representative LD_{50}, values for the rat range from 56 to 97.5 mg/kg (Durham et al. 1957; Gajewski and Katkiewicz 1981; Ikeda et al. 1990). The 44% inhibition of brain acetylcholinesterase reported in this study is considered a less serious LOAEL; clinical signs were not reported in this study, but in another oral dosing study in Fischer 344 rats (NTP 1989) over an 11-day period, no clinical signs of dichlorvos neurotoxicity were reported in animals receiving up to 16 mg/kg/day dichlorvos. In a developmental study where CF-1 mice and New Zealand rabbits were orally exposed to dichlorvos during pregnancy (60 mg/kg/day over gestation days 6-l 5 in mice; 5 mg/kg/day over gestation days 6-l 8 in rabbits), no treatment-related effects were observed in the offspring.

This MRL should be protective against health effects in individuals potentially exposed to dichlorvos at hazardous waste sites. Oral exposure at this level would not be expected to have any effect on neural acetylcholinesterase activity, the target for dichlorvos toxicity. The FAO/WHO Joint Meeting on Pesticide Residues has established an acceptable daily intake of dichlorvos for humans of 0.004 mg/kg/day (FAO/WHO 1967).

- An MRL of 0.003 mg/kg/day has been derived for intermediate-duration oral exposure (15-364 days) to dichlorvos.

This minimal risk level is based on a 21-day study in male volunteers who consumed 0.033 mg/kg/day in either a capsule form or in a 3-ounce container of gelatin at meals (Boyer et al. 1977). A NOAEL of 0.033 mg/kg/day was observed for inhibition of erythrocyte acetylcholinesterase. The MRL was derived by dividing the NOAEL by an uncertainty factor of 10 for human variability.

Boyer et al. (1977) reported on a study designed to determine if different formulations would change the effect of dichlorvos on serum cholinesterase and erythrocyte acetylcholinesterase. Plasma cholinesterase and erythrocyte acetylcholinesterase were determined twice a week for 3 weeks in 30 male volunteers. The 24 men with the most stable enzyme activities were used in the study. Two treatment groups of 6 men each received 0.9 mg dichlorvos 3 times daily either in a pre-meal capsule filled with cottonseed oil or in a 3-ounce container of gelatin. Two other groups of 6 men each received placebo capsules or gelatin. The treated volunteers received 0.9 mg dichlorvos 3 times a day or 2.7 mg/day. The average weight of the volunteers was 81 kg, resulting in an average dose of 0.033 mg/kg/day. Dosing was started and carried out for a 21-day period during which plasma cholinesterase and erythrocyte acetylcholinesterase were measured twice a week by a pH titration method. Following the termination of dosing, plasma and erythrocyte activities were monitored twice weekly for 7 weeks. The observation of each individual's cholinesterase activities was converted to a percentage of his pretrial average determinations. No clinical signs of neurotoxicity were noted in any of the subjects (tremor, pupillary response to light, and skin moisture were assessed). A NOAEL of 0.033 mg/kg/day dichlorvos was observed for inhibition of erythrocyte acetylcholinesterase.

The reduction of serum cholinesterase observed in the study confirms that dichlorvos was absorbed by the volunteers under these conditions. No signs of clinical neurotoxicity were observed at any time in the volunteers. The dose given appeared to have been chosen for the express purpose of not causing erythrocyte acetylcholinesterase inhibition. This study was chosen for MRL derivation because it was the only one located that defined a NOAEL for humans for the end point of erythrocyte acetylcholinesterase inhibition. Several animal studies on intermediate-duration oral exposure to dichlorvos are available. NOAEL values for erythrocyte acetylcholinesterase inhibition were 3.5 mg/kg/day in Sherman rats exposed for 90 days by feed (Durham et al. 1957), 8 mg/kg/day in Fischer 344 rats

exposed by gavage for 32 days (NTP 1989) and 40 mg/kg/day in B6C3F$_1$ mice exposed by gavage for 33 days (NTP 1989).

This MRL should be protective against health effects in individuals potentially exposed to dichlorvos at hazardous waste sites. Oral exposure at this level would not be expected to have any effect on neural acetylcholinesterase activity, the target for dichlorvos toxicity. The FAO/WHO Joint Meeting on Pesticide Residues has established an acceptable daily intake of dichlorvos for humans of 0.004 mg/kg/day (FAO/WHO 1967).

- An MRL risk level of 0.0005 mg/kg/day has been derived for chronic-duration oral exposure (364 days or more) to dichlorvos.

This MRL level is based on a chronic feeding study in dogs (AVMAC Chemical Co. 1990; IRIS 1995). Groups of Beagle dogs (4 per sex per dose, approximately 6-7 months old) were administered dichlorvos daily by gelatin capsule for 52 weeks at dose levels of 0, 0.1, 1.0, and 3.0 mg/kg/day. The 0.1 mg/kg/day dose level was lowered to 0.05 mg/kg/day on day 22 due to inhibition of serum cholinesterase noted after 12 days on dichlorvos (the authors were attempting to assure a no-effect level for serum ChE). Observations included clinical signs, body weight, food consumption, ophthalmology, blood chemistry, necropsy, and histopathology. Serum cholinesterase and erythrocyte acetylcholinesterase were measured throughout the study. At termination of the study, the brain was weighed and brain acetylcholinesterase was measured. Histopathology was performed on the brain (with brainstem), cervical spinal cord, lumbar spinal cord, and the sciatic nerve. Serum cholinesterase and erythrocyte acetylcholinesterase were unchanged in the 0.05 mg/kg/day groups, and decreased in a dose-dependent manner at 1.0 and 3.0 mg/kg/day. Levels of inhibition did not increase over time of measurement (2-52 weeks). At termination of the study, brain acetylcholiesterase was unchanged at 0.05 mg/kg/day. It was decreased 22% in males at 1.0 mg/kg/day, but not in females. At 3.0 mg/kg/day, brain acetylcholinesterase was decreased 47% in males and 29% in females. No treatment-related changes were seen on histopathology for any of the tissues examined.

The MRL was derived from the NOAEL level of 0.05 mg/kg/day for inhibition of brain acetylcholinesterase by adjusting by a factor of 10 for human variability and 10 for animal-to-human extrapolation.

The EPA has derived a reference dose (RfD) of 0.0005 mg/lcg/day based on a NOAEL for brain acetylcholinesterase inhibition of 0.05 mg/kg/day dichlorvos based on this study (IRIS 1995).

Death. There have been several reports of deaths in humans as a result of exposure to dichlorvos (Hayes 1982). In one case, a woman who drank a pesticide containing dichlorvos died the following day, but the actual amount ingested was not determined. A 19-month-old girl died after eating a cakelike bait that contained dichlorvos. In this case, the amount of dichlorvos consumed was not determined. The bait also contained malathion, raising the possibility that an interaction may have occurred and contributed to the death. Two pesticide workers in Costa Rica were also reported to have died after spilling a dichlorvos-containing pesticide on their skin and failing to promptly wash it off (Hayes 1982). The concentration of dichlorvos in the pesticide was not reported, nor was the quantity spilled on the skin.

Deaths have been reported in rats and rabbits after inhalation exposure to dichlorvos. In an early toxicity study (Durham et al. 1957), rats exposed to air saturated with dichlorvos died between 7 and 62 hours after exposure began. Dichlorvos measured in the incoming air was 306 mg/m^3 (34 ppm). The signs of poisoning preceding death included slow labored respiration, sialorrhea, and paleness of the extremities. Deaths were also reported among pregnant rabbits exposed by inhalation to dichlorvos for 28 days at 4 and 6.25 mg/m^3 (0.44 and 0.69 ppm) (Thorpe et al. 1972). Before death, the animals were anorexic, lethargic, showed tremors, and had nasal discharges and diarrhea-all signs of dichlorvos neurotoxicity. Rats exposed to dichlorvos at levels up to 56 mg/m^3 (6.2 ppm) for up to 14 days survived (Schmidt et al. 1979) as did pregnant rats exposed by inhalation at levels up to 6.25 mg/m^3 (0.69 ppm) (Thorpe et al. 1972). No studies on inhalation of dichlorvos in non-pregnant rabbits were located, so whether this greater sensitivity is a species difference or a consequence of pregnancy cannot be determined. Exposure of rats by inhalation for 2 years at 5 mg/m^3 (0.55 ppm) did not increase the death rate over controls in males or females (Blair et al. 1976).

Deaths have been reported after single oral doses in a number of LD_{50} studies. LD_{50}values in rats range from 56 mg/kg in female Sherman rats (Durham et al. 1957) to 97.5 mg/kg in male Fischer 344 rats (Ikeda et al. 1990). In mice, LD_{50} values of 133 mg/kg for female CD-l mice and 139 mg/kg for males have been reported (Haley et al. 1975). In this study a reliable LD_1 value of 84 mg/kg in male mice and 95 mg/kg in female mice was reported. Single acute oral doses of 150 mg/kg in male Swiss mice caused 100% lethality within 9 minutes (Mohammad et al. 1989). An oral LD_{50} of 157 mg/kg

has also been reported in crossbred pigs (Stanton et al. 1979). In intermediate-duration studies, all rats died after being orally exposed by feed at 180 mg/kg/day dichlorvos or more for 6 weeks (NCI 1977). All Fischer 344 rats exposed by gavage to dichlorvos at levels of 32 or 64 mg/dichlorvos died within 13 weeks. as did 6 of 10 female rats exposed to 16 mg/kg/day dichlorvos (NTP 1989). Mice appear to be more resistant to dichlorvos toxicity in intermediate-duration studies. In a 6-week study where mice were orally exposed through feed, deaths were not reported until levels of 720 mg/kg/day were reached (NCI 1977). A 13-week gavage study in the same strain of mice reported deaths at 80 mg/kg/day (NTP 1989). These studies suggest that dichlorvos is less toxic when consumed in feed than when administered by gavage, possibly due to slower absorption and the intermittent nature of exposure when consumed in feed. In a 2-year study on Fischer 344 rats and $B6C3F_1$ mice, oral exposure to dichlorvos at doses of 4 and 8 mg/kg/day dichlorvos in rats, and 10, 20, and 40 mg/kg/day in mice had no effect on survival. Deaths have also been reported after dermal exposure in monkeys and rats (Durham et al. 1957; Gajewski and Katkiewicz 1981). Monkeys receiving daily doses of 50 mg/kg/day dichlorvos and above died within 10 days; LD_{50}, values of 70-107 mg/kg have been reported in rats.

Parenteral doses causing death are considerably lower than those required by the inhalation, oral or dermal routes. An intravenous dose of 2.2 mg/kg undiluted dichlorvos was fatal in a greyhound dog (Snow and Watson 1973). The clinical signs of toxicity before death (hyperpnea, dyspnea, severe and progressive cyanosis) were the same as those seen in dogs that had been acutely poisoned orally. The importance of the liver in reducing dichlorvos toxicity was demonstrated in a study where rats were perfused with dichlorvos either through the femoral vein (dichlorvos entering the circulation directly) or the intestinal vein (dichlorvos passing through the liver before reaching the general circulation) (Gaines et al. 1966). Time to onset of symptoms was delayed about 3 times as long in rats infused through the intestinal vein compared to rats perfused through the femoral vein. All the rats perfused through the femoral vein died within 31 minutes (total dose 5 mg/kg), while none of the rats perfused through the intestinal vein died even though their total dose was 3 times as high (15.2 mg/kg).

Systemic Effects. The systemic effects seen after dichlorvos exposure can generally be explained as the result of dichlorvos neurotoxicity.

Respiratory Effects. Labored breathing has been reported in greyhound dogs (Snow and Watson 1973) and monkeys (Durham et al. 1957) after acute oral exposure to high doses of dichlorvos. This

effect appears to be secondary to acetylcholinesterase inhibition in the respiratory tract. Increased cholinergic activity would be expected to cause increased bronchial secretions and bronchoconstriction and partial depolarization block at neuromuscular junctions could lead to labored breathing.

Gastrointestinal Effects. Diarrhea, sometimes bloody, has been reported after acute oral exposure to dichlorvos in greyhound dogs (Snow and Watson 1973) and after intermediate-duration inhalation exposure in rabbits (Thorpe et al. 1972). Cholinergic stimulation in the gastrointestinal tract would be expected to cause increased intestinal motility and result in diarrhea.

Dermal Effects. One case of contact dermatitis as a result of dichlorvos exposure was located. A 52-year-old male truck driver who had been hauling pesticide containers presented with dermatitis of his neck, anterior chest, dorsal hands, and forearms (Mathias 1983). On the previous day, several containers spilled in his truck and he apparently had direct dermal contact with a pesticide containing 5% dichlorvos, 15% petroleum distillates, and 80% trichloroethane. A faint papular dermatitis was present over the dorsal arms, hands, and V of the neck. Vertical erythematous, slightly scaling streaks were present over the lateral and posterior neck, a pattern suggesting that liquid droplets had produced the dermatitis. The dermatitis was treated with 1% hydrocortisone ointment. Follow-up examination 6 weeks later demonstrated persistent vertical, mildly erythematous streaks over the posterior and lateral neck; the arms and anterior chest had cleared. Dermatitis resolved completely approximately 10 weeks after onset. The persistence of dermatitis 2 months after exposure is very unusual, and the author speculated that this may be related to some unique local toxic effect of dichlorvos.

Ocular Effects. Miosis was observed in monkeys after inhalation exposure to dichlorvos at 12.9 mg/m^3 for 2 hours a day (Witter et al. 1961). This condition was no longer present the next morning.

Body Weight Effects. Male Carworth E rats exposed to atmospheres containing 5 mg/m^3 (0.6 ppm) for 2 years (Blair et al. 1976) were consistently 20% or more below the body weight ef control rats from the tenth week of treatment. However, no significant changes in body weight were noted in rats or mice of either sex after a 2-year oral exposure at doses ranging from 4 to 40 mg/kg for 5 days a week (NIT 1989).

Immunological and Lymphoreticular Effects. Twelve of 18 subjects in an occupational study of flower growers who had positive patch test reactions to triforine (1,4-bis [2,2,2-trichlor-I-formamidoethyl] piperazine) also showed positive reactions to dichlorvos (Ueda et al. 1994). These subjects may have also been occupationally exposed to dichlorvos.

Immunosuppression after oral exposure to dichlorvos in rabbits has been reported in two studies (Desi et al. 1978, 1980). A dose-related suppression of the humoral immune response induced by *S. typhimurium* was observed in rabbits administered 0.3-2.5 mgikg dichlorvos 5 days a week for 6 weeks (Desi et al. 1978). A single oral dose of 120 mg/kg dichlorvos in male C57B1/6 mice that had been inoculated with sheep erythrocytes 2 days earlier suppressed the primary IgM response observed 48 hours later (Casale et al. 1983). Severe signs of dichlorvos neurotoxicity were noted and the authors stated that the immunosuppression observed in this study may have been mediated indirectly by toxic chemical stress.

In a guinea pig maximization test conducted in this study, induction with dichlorvos by intradermal injection and topical application and subsequent challenge with topical dichlorvos solutions showed sensitization (Ueda et al. 1994). Cross-reactivity with dichlorvos was demonstrated in animals that had been induced with triforine. It is unknown if the immunosuppression seen with dichlorvos in animals is a direct effect or mediated by a cholinergic pathway.

Neurological Effects. Dichlorvos exerts its toxic effects in humans and animals by inhibiting neural acetylcholinesterase. This enzyme is present at cholinergic synapses throughout the central and peripheral nervous systems and is responsible for hydrolyzing acetylcholine released from the presynaptic terminal. If this enzyme is inhibited, acetylcholine accumulates in the synapse, resulting in increased firing of the post-synaptic neuron or increased contractions in muscle. The consequences of this increased cholinergic activity in the parasympathetic autonomic nervous system can include lacrimation, perspiration, miosis, nausea, vomiting, diarrhea, excessive bronchial secretions, bradycardia, increased salivation, increased urinary frequency, and incontinence. Effects on motor nerve fibers in the skeletal muscles can include muscle fasciculations, cramps, muscle weakness, and paralysis. Effects on cholinergic synapses in the central nervous system result in drowsiness, fatigue, mental confusion, headache, convulsions, and coma.

This same enzyme is present in erythrocytes, where it is known as erythrocyte acetylcholinesterase. Both forms of acetylcholinesterase are produced by the same gene (Taylor et al. 1993). Erythrocyte and neural acetylcholinesterase are inhibited to roughly the same extent by exposure to dichlorvos and other organophosphorus compounds, and measurement of erythrocyte acetylcholinesterase is used as an indicator of the extent of inhibition of neural acetylcholinesterase (Hayes 1982). Another enzyme capable of hydrolyzing acetylcholine circulates in the blood and is called serum cholinesterase. The activity of this enzyme is inhibited by dichlorvos at lower levels of exposure than required for inhibition of erythrocyte acetylcholinesterase and is often used as a biomarker of exposure.

Male volunteers exposed to atmospheres containing 0.25 or 0.7 mg/m^3 showed no symptoms of neurological toxicity (Blair et al. 1975). One volunteer was exposed to 0.25 mg/m^3 for 10 hours, and another was exposed to 0.7 mg/m^3 for 20 hours.

Another group of seven male volunteers was exposed to dichlorvos-containing atmospheres in a simulated aircraft cabin to determine safe levels for aircraft insect control (Witter et al. 1961). In this study, the volunteers were exposed to dichlorvos on 4 consecutive days for either 1 or 2 hours. The average dichlorvos concentration in the first experiment was 0.49 mg/m^3 (0.05 ppm); in a second experiment with the same group, it was 2.1 mg/m^3 (0.23 ppm). In the first experiment at 0.49 mg/m^3, no changes were observed in serum cholinesterase or erythrocyte acetylcholinesterase in any of the men whether they had been exposed for 1 or 2 hours a day over the 4-day exposure period. There was a small inhibition of serum cholinesterase (about 20%) in 2 of 3 volunteers exposed for 2 hours a day for 4 consecutive days at 2.1 mg/m^3. No changes were seen in erythrocyte acetylcholinesterase in any of the men exposed to 2.1 mg/m^3 for either 1 or 2 hours a day over the 4-day period. In this study, there was a 24-hour interval between exposure and the time blood was sampled for cholinesterase activities.

In the same study, groups of 2 rhesus monkeys (one of each sex) were exposed to atmospheres containing 0.48, 2.3, 2.6, or 12.9 mg/m^3 (0.05, 0.25, 0.29, or 1.43 ppm) for 2 hours a day on 4 consecutive days (Witter et al. 1961). Exposure for 2 hours a day on 4 consecutive days at 0.48 mg/m^3 had no effect on serum cholinesterase or erythrocyte acetylcholinesterase. Similar exposures at 2.3 and 2.6 mg/m^3, and the same sampling interval, had no effect on erythrocyte acetylcholinesterase but caused about a 20% inhibition of serum cholinesterase. Exposure at 12.9 mg/m^3 had significant toxic effects. The monkeys exposed at this level showed substantial inhibition of both

cholinesterases from the first day of exposure. Activity fell throughout the 4-day exposure period; serum cholinesterase was inhibited about 40-50% in both monkeys while erythrocyte acetylcholinesterase fell about 40% in one monkey and 67% in the other. Miosis was also noted in both monkeys at the end of each 2-hour exposure period. but was no longer present the following morning. Cholinesterase determinations after exposure was terminated indicated that 40-50 days were required for a return to pre-exposure levels.

Rhesus monkeys housed in a chamber whose walls and ceiling had been sprayed with an emulsion of dichlorvos were observed for 2 weeks (Durham et al. 1957). The original concentration in the chamber was approximately 6 mg/m^3 (0.66 ppm); it fell to about 1 mg/m^3) (0.11 ppm) after 3 days and was about 0.1 mg/m^3 (0.01 ppm) for the remainder of the 2 weeks. No signs of neurological toxicity were observed. By the end of the first week, both serum cholinesterase and erythrocyte acetylcholinesterase had fallen from their pre-exposure levels. Inspection of a graph in this report indicates that levels of both blood cholinesterases fell about 50-60% during the first week of the study. Serum cholinesterase recovered partially in the second week, but erythrocyte acetylcholinesterase did not. After exposure was terminated, the activities of both enzymes were normal in about 5 weeks.

Ten Sherman rats of each sex housed in the same chamber as the monkeys were also monitored in this experiment (Durham et al. 1957). No clinical signs of neurological toxicity were observed in the rats over the 2-week exposure period. There was a slight decrease in serum cholinesterase and erythrocyte acetylcholinesterase at the end of the first week (about 10% for each enzyme). At the end of 2 weeks, there was no difference between exposed rats and controls. Bronchial and erythrocyte acetylcholinesterase were measured in male Sprague-Dawley rats exposed to atmospheres ranging from 0 to 56.64 mg/m^3 (0-6.3 ppm) over a 3-day period (Schmidt et al. 1979). A dose-dependent reduction in both bronchial and erythrocyte acetylcholinesterase was observed. Bronchial tissue acetylcholinesterase measured in homogenates from treated rats at 0.83 and 1.82 mg/m^3 (0.09 and 0.20 ppm) was lower than in control rats; 50% inhibition of bronchial tissue acetylcholinesterase took place at a dose (1.82 mg/m^3) that had no effect on erythrocyte acetylcholinesterase. Erythrocyte cholinesterase was inhibited more than 80% at 8.2 mg/m^3 (0.91 ppm) after 3 days exposure. Clinical signs were not reported in this study, so the toxicological significance of this level of inhibition in the male Sprague-Dawley rats cannot be assessed.

Several studies in animals have addressed neurological end points after intermediate-duration inhalation exposure to dichlorvos. In a study of pregnant Carworth E rats exposed over their gestation period (20 days), dams exposed to atmospheres containing 6.25 mg/m^3 (0.69 ppm) were less active than controls (Thorpe et al. 1972). Exposure at 0.25 mg/m^3 (0.03 ppm) had no effect on erythrocyte or brain acetylcholinesterase. Exposure at 1.25 mg/m^3 (0.14 ppm) resulted in a 30% inhibition of erythrocyte and brain acetylcholinesterase, while exposure at 6.25 mg/m^3 resulted in 88% inhibition of erythrocyte acetylcholinesterase and an 83% inhibition of brain acetylcholinesterase. A similar experiment with pregnant Dutch rabbits over 28 days (Thorpe et al. 1972) showed inhibition of 14% and l0%, respectively, in erythrocyte and brain acetylcholinesterase at exposure of 0.25 mg/m^3. At exposures of 1.25 mg/m^3, erythrocyte acetylcholinesterase was inhibited 68% and brain acetyl-cholinesterase was inhibited 56% compared to controls.

In a 2-year chronic inhalation study with dichlorvos (Blair et al. 1976), 50 Carworth E rats of each sex were exposed to atmospheres containing 0, 0.05, 0.5, or 5 mg/m^3 (0, 0.0055, 0.055, or 0.55 ppm). No clinical signs of neurological toxicity were seen in any of the groups, although 6 control rats and 9 treated rats were reported as showing involuntary convulsive movements when being weighed. Acetylcholinesterase activity was measured in brain and erythrocytes, as was serum cholinesterase at the termination of this study. In male animals exposed to 0.05 mg/m^3, no significant differences with control animals were seen for any of the cholinesterases. Female animals at this dose had a statistically significant decrease of 12% in erythrocyte acetylcholinesterase. At 0.5 mg/m^3, brain cholinesterase was 10% lower compared to controls in both male and female rats. Females at this dose also showed erythrocyte acetylcholinesterase inhibition of 31%, while the males were unaffected. At 5 mg/m^3, brain acetylcholinesterase was inhibited by 79 and 81% in male and female rats, respectively. Erythrocyte acetylcholinesterase inhibition at this dose was 96% in the male rats and 95% in the female rats.

Neurological effects have been seen in a number of studies in animals after acute oral exposure to dichlorvos. Male Fischer 344 rats exposed to dichlorvos by olive oil gavage during an LD_{50}, study had signs of excessive cholinergic stimulation including salivation, tremors, lacrimation, fasciculations, irregular respiration, and prostration (Ikeda et al. 1990). In greyhound dogs receiving 11 or 22 mg/kg in a single dose, signs of neurological toxicity appeared within 7-15 minutes of dosing (Snow and Watson 1973). Restlessness was seen initially, followed by increased salivation, muscle fasciculations, involuntary urination, and repeated diarrhea, sometimes bloody. There was no apparent difference in

severity of clinical signs between dogs given 11 or 22 mg/kg. In dogs where erythrocyte acetylcholinesterase was determined (7 of 9), activity was decreased by at least 75%. One dog with 97% inhibition of erythrocyte acetylcholinesterase survived although suffering severe symptoms. Crossbred pigs receiving single doses from 18 to 560 mg/kg had clinical signs of neurological toxicity including hypoactivity, vomiting, ataxia, muscle fasciculations, uncoordinated movements, frothy salivation, and defecation (Stanton et al. 1979).

In a 21-day study in volunteers given dichlorvos orally, no signs of neurological toxicity were seen at doses of 0.033 mg/kg/day. They were then given 0.9 mg dichlorvos 3 times a day for 21 days in either a pre-meal capsule or in a 3-ounce container of gelatin. Serum cholinesterase and erythrocyte acetylcholinesterase were measured twice a week during the exposure period. Once a week each volunteer had his vital signs measured and an examination made for tremor, pupillary response to light, and skin moisture. Following the termination of the study, serum cholinesterase and erythrocyte acetylcholinesterase were measured weekly for the next 7 weeks. No clinical signs of neurological toxicity were observed in any of the volunteers. Erythrocyte acetylcholinesterase was not inhibited at 0.033 mg/kg/day in either the gelatin or capsule formulation. Serum cholinesterase was inhibited on average 38% in the group given the pre-meal capsule and 28% in the gelatin group. Measurements after the dosing period indicated that the half-life for regeneration of serum cholinesterase was 13.7 days.

In a 90-day study in female Sherman rats, groups of 10 animals were exposed to doses ranging from 0 to 69.9 mg/kg/day in their feed. Two animals from each group were bled on days 3, 11, 60, and 90, and sernm cholinesterase and erythrocyte acetylcholinesterase were determined. No clinical signs of neurological toxicity were noted in any dosage group. Cholinesterase data were presented graphically so the percentage inhibition of the cholinesterases can only be estimated. For serum cholinesterase, doses of 0.4 and 1.5 mg/kg/day appeared to have no effect. Doses of 3.5 and 14.2 mg/kg/day appeared to have been inhibited by 25-40% of control by the third day of feeding, remained at this level up to 60 days, and rose to near control level by the termination of the experiment at 90 days. Serum cholinesterase in rats consuming 35.7 and 69.9 mg/kg/day fell by 50% after 3 days and remained at this level throughout the experiment. Erythrocyte acetylcholinesterase was unaffected at doses up to 3.5 mg/kg/day. Activity was inhibited by 30% after 3 days at 14.2 mg/kg/day and remained at this level until the end of the experiment. At 35.7 and 69.9 mg/kg/day, erythrocyte

acetylcholinesterase was inhibited by about 50% after 3 days and 80% after 10 days. There appeared to be some recovery to about 50% of control by the end of the experiment.

Inhibition of erythrocyte and brain acetylcholinesterase, but not signs of neurotoxicity, were reported in Beagles receiving 0.05, 1.0, or 3 mg/kg/day dichlorvos in capsules for 52 weeks (AMVAC Chemical Corp. 1990; IRIS 1995). No changes were noted at 0.05 mg/kg/day, but erythrocyte acetyl-cholinesterase was inhibited 43-53% in dogs receiving 1 mg/kg/day and 81-87% in dogs receiving 3 mg/kg/day. Brain acetylcholi-esterase was inhibited 22% in dogs receiving 1 mg/kg/day and 47% in dogs receiving 3 mg/kg/day.

In a study where 3 cynomolgus monkeys were exposed to dichlorvos in xylene by daily dermal doses on a shaved area between the shoulder blades (Durham et al. 1957), cholinergic signs appeared within 10-20 minutes of dosage. Signs of toxicity in general order of appearance were nervousness, incoordination, muscle fasciculations, excessive salivation, labored breathing, miosis, and inability to move. The authors stated that at any given dose, the cholinergic signs tended to become more severe with subsequent doses. Serum cholinesterase and erythrocyte acetylcholinesterase were measured in one of the monkeys which received 75 mg/kg/day. After 2 doses, erythrocyte acetylcholinesterase had declined by about 67%, while serum cholinesterase was unchanged. When these values were measured shortly after the next day's dosage, the serum cholinesterase had fallen by about 33%, while erythrocyte acetylcholinesterase remained inhibited by about 67%. The serum cholinesterase recovered after 2 days without dosing, but the erythrocyte acetylcholinesterase did not. After 5 doses, the erythrocyte acetylcholinesterase had fallen by 90% and stayed there until death occurred after 12 days, during which 10 doses were administered.

Symptoms of neurological toxicity were also observed in Sherman rats dermally exposed to dichlorvos during an LD_{50}, experiment (Durham et al. 1957). Rats which survived exhibited bulging eyes, excessive lacrimation, and generalized muscle fasciculation and tremors. Surviving rats appeared to be completely recovered after 24 hours.

In a study involving daily dermal dosage in white Leghorn chickens, 3 hens receiving 2.8-3.8 mg/kg/day exhibited a staggering gait after 14 days of treatment (Francis et al. 1985). Three hens receiving doses between 0.54 and 0.71 mg/kg/day showed no signs of neurotoxicity over 90 days of dosing.

Dichlorvos does not appear to cause organophosphate-induced delayed neurotoxicity (OPIDN. Certain organophosphate compounds can inhibit an enzyme called neuropathy target esterase (NIT), as well as neural acetylcholinesterase. Significant inhibition of NTE followed by irreversible binding to this enzyme (aging) results in a progressive, irreversible neuropathy in humans and experimental mammals and hens (Coppock et al. 1995; Johnson 1981). The in viva substrate for this enzyme is unknown, as is the biochemical mechanism underlying the subsequent development of neuropathy. In hens that had been pre-medicated with atropine to protect against the acute cholinergic effects of dichlorvos, subcutaneous injection of dichlorvos did not result in OPIDN (Durham et al. 19.56; Lotti and Johnson 1978). Mild signs of ataxia were noted in atropine-pretreated hens 2 weeks after a single subcutaneous dose of 100 mg/kg dichlorvos (Caroldi and Lotti 1981). Subcutaneous administration of 100 mg/kg dichlorvos to atropine pre-treated hens followed by the same dose within l-3 days produced ataxia in the hens (Johnson 1978). When dichlorvos was administered by a single intraperitoneal injection (5-60 mg/kg in hens and 5-30 mg/kg in rats), no significant pathological lesions of the OPIDN type were observed (Ehrich et al. 1995), or gait alterations characteristic of this neuropathy.

Hens protected from the parasympathomimetic effects of dichlorvos by atropine developed clinical signs of ataxia (Francis et al. 1985; Johnson 1978). However, the apparent clinical signs of axonalpathy in the hens were not confirmed by histopathology. A number of different animal species have been experimentally intoxicated with dichlorvos. It has also been used as a systemic parasiticide in a number of domestic animal species (Hayes 1982). Humans have been intoxicated with dichlorvos and, because of medical intervention, recovered. In none of these studies and human poisoning incidents has OPIDN been reported. It is unlikely that dichlorvos OPIDN will occur in humans even in victims who have high levels of exposure and survive because of timely medical intervention. However, the interactions between dichlorvos and other pesticides for inducing OPIDN is not known.

While signs of delayed neuropathy can be produced in animal models, this only occurs at doses far above the LD_{50}. It is unlikely that dichlorvos would produce OPIDN in humans at doses that were not lethal because of acute cholinergic effects.

Several effects on brain chemistry have been observed in studies where dichlorvos was administered intraperitoneally. Glutathione peroxidase activity was inhibited and the reduced and oxidized forms of

glutathione were depleted in female Wistar rats receiving 5 mg/kg/day (Julka et al. 1992). This suggests that glutathione-dependent metabolism of dichlorvos depletes glutathione levels and possibly leaves the brain vulnerable to oxidative damage. Dopamine, norepinephrine, and 5-hydroxy tryptamine levels were also reported depleted in the brain after 10 days of intraperitoneal administration at 3 mg/kg/day in male rats (Ali et al. 1979).

Reproductive Effects. No incident reports or epidemiological studies in humans on reproductive effects associated with dichlorvos exposure are available. In a reproductive toxicity study involving male CF-1 mice, groups of 16 mice were exposed to atmospheres containing 0, 30, or 55 mg/m^3 (0, 3.3, or 6.1 ppm, respectively) for 16 hours (Dean and Thorpe 1972). There were no differences between control and treated mice in the number of early fetal deaths, late fetal deaths, or live fetuses found in the pregnant females. The percentage of pregnancies for females mated to males exposed to 30 and 55 mg/m^3 (3.3 and 6.1 ppm) for 16 hours was similar to the controls (73-88%, mean 80.9%). Under these exposure conditions, dichlorvos does not appear to affect the fertility of male CF-1 mice.

Sperm abnormalities were seen in C57BL/C3H mice injected intraperitoneally with 10 mg/kg/day for 5 days (Wyrobek and Bruce 1975). About 6% of the sperm from dichlorvos-treated animals was abnormal compared to 1.8% of sperm from untreated animals.

Developmental Effects. No studies in humans on developmental effects associated with dichlorvos exposure are available. Several animal studies examining developmental toxicity during continuous inhalation exposure to dichlorvos are available. Groups of 15 pregnant Carworth E rats were exposed to atmospheres containing 0, 0.25, 1.25, or 6.25 mg/m^3 throughout their 20-day gestation period (Thorpe et al. 1972). At the end of 20 days, the rats were sacrificed and the uteri removed for examination. The number of live fetuses, stillbirths, and resorption sites were noted, and live fetuses were examined for external malformations. Exposure of dams to all three concentrations of dichlorvos had no effect on the number of fetal resorptions, late fetal deaths, litter size, or mean weight per fetus. One fetus in the litter of one dam in the 0.25 mg/m^3 group had skeletal defects and gastroschisis. No other fetuses from dams exposed to the same or higher concentrations had these defects, so the authors concluded that they were not exposure related. Brain and erythrocyte acetyl-cholinesterase activities were inhibited 83% and 88%, respectively, in dams in the high-dose (6.25 mg/m^3) group, suggesting that even very high levels of acetylcholinesterase inhibition are not associated with teratogenicity.

In a parallel experiment conducted on groups of 20 pregnant Dutch rabbits in this same study (Thorpe et al. 1972), similar results were seen. Dams exposed to 6.25 mg/m^3 in the previous study had high mortality (16 of 20 died) so the doses used in this experiment were 0, 0.25, 1.25, 2, and 4 mg/m^3 over the 28 day rabbit gestational period. Sizes of litters, fetal resorptions, and late fetal deaths were unaffected by inhalation exposure to dichlorvos. Mean weight per fetus was slightly depressed in dams exposed to 4 mg/m^3, but the authors ascribed this to maternal toxicity. (Clinical signs were not reported, but 6 dams out of 20 in this group died during the study.) Three fetuses from groups that had not been exposed to dichlorvos had gastroschisis. Two dead fetuses from one litter in the 4 mg/m^3 had cleft palates.

No adverse developmental effects were observed in CF-1 mice treated by gavage with 60 mg/kg/day dichlorvos during gestation days 6-15 (Schwetz et al. 1979). There was no significant effect on implantations, mean number of fetuses per litter, incidence or distribution of resorptions, or on fetal body measurements. Similar results were observed in New Zealand rabbits treated by gavage with 5 mg/kg/day over gestation days 6-18 (Schwetz et al. 1979).

Genotoxic Effects. Dichlorvos is an electrophile and possesses a structural alert for methylating activity. Dichlorvos has been tested for genotoxicity in a number of in viva and *in vitro* systems. In general, dichlorvos was not genotoxic in *in vivo* studies (see Table 2-6) but was generally genotoxic or mutagenic in *in vitro* tests when metabolizing enzymes (S9 fraction) were not present (see Table 2-7).

In the sex-linked lethal mutation test in *Drosophila melunogaster,* dichlorvos gave negative results when the flies were exposed by inhalation (Jayasuriya et al. 1973; Sobels and Todd 1979). Multiple generations of flies exposed to food containing dichlorvos for 18 months had increased mutations (Hanna and Dyer 1975). Salivary gland chromosome abnormalities were reported in larvae fed 1 ppm dichlorvos in food in one study (Gupta and Singh 1974), while another study at lower levels showed no effect (Kramers and Knaap 1978).

No dominant lethal mutations were reported in ICR mice given a single intraperitoneal dose or consecutive daily doses of 5 or 10 mg/kg orally (Epstein et al. 1972). In a similar study where dichlorvos was administered by inhalation (30 or 55 mg/m^3 (3.3 or 6.1 ppm) to CF-1 mice, no dominant lethal mutations were observed (Dean and Thorpe 1972).

Table 2-6. Genotoxicity of Dichorvos *In Vivo*

Species (test system)	Test	Results	Reference
Drosophila melanogaster	sex-linked lethal mutation	—	Jayasuriya et al. 1973
D. melanogaster	sex-linked lethal mutation	—	Sobels and Todd 1979
D. melanogaster	sex-linked lethal mutation	—	Kramers and Knapp 1978
D. melanogaster	chromosome abnormalities	+	Gupta and Singh 1974
Mouse (ICR/Ha)	dominant lethal	—	Epstein et al. 1972
Mouse (CF-1)	dominant lethal	—	Dean and Thorpe 1972
Mouse (Q)	dominant lethal	—	Degraeve et al. 1972
Mouse (CF1)	dominant lethal	—	Dean and Blair 1976
Mouse (Q)	dominant lethal	—	Degraeve et al. 1972
Mouse (Q)	chromosome damage	—	Moutschen-Dahmen et al. 1981
Mouse (Swiss)	chromosome aberrations	—	Paik and Lee 1977
Mouse (F-1)	chromosome aberrations	—	Dean and Thorpe 1972
Hamster (Chinese)	chromosome aberrations	—	Dean and Thorpe 1972
Hamster (Syrian)	chromosome aberrations	—	Dzwonkowska and Hubner 1986

+ = Positive result; – = negative result

Table 2-7. Dichlorvos Genotoxicity *In Vitro*

Species (test system)	Reverse test	Result: With activation	Result: Without activation	Reference
Salmonella typhimurium				
TA98	mutation	–		Braun et al. 1982
TA100		+		
TA1535		–		
TA1536		–		
TA1537		–	–	
TA1538		–	–	
S. typhimurium				
TA98	reverse mutation		–	Moriya et al. 1982
TA100			+	
TA1535		–	+	
TA1536			–	
TA1537			–	
TA1538			–	
S. typhimurium				
TA1530	reverse mutation		+	Hanna and Dyer 1975
TA1535			+	
LTZ his C117			–	
his G 46			–	

Table 2-7. Dichlorvos *In Vitro* (continued)

Species (test system)	Reverse test	Result: With activation	Result: Without activation	Reference
S. typhimurium				
TA1535	reverse mutation		+	Shirasu et al. 1976
TA1536			–	
TA1537			–	
TA1538			–	
Mammalian cells				
Hamster (Chinese U79)	mutation induction		–	Aquilina et al. 1984
Hamster (Chinese U79)	DNA strand breakage		+	Green et al. 1974
Hamster (Chinese ovary)	nuclease resistance		+	Nishio and Uyeki 1982
Hamster (Chinese ovary)	sister chromatid exchange		+	Nishio and Uyeki 1982
Hamster (Chinese U79)	sister chromatid exchange		+	Tezuka et al. 1980
Hamster (Syrian embryo)	adenovirus transformation		+	Hatch et al. 1986
Mouse (Peripheral blood lymphocyte)	sister chromatid exchange		–	Kligerman et al. 1985
Human (epithelial line EUE)	unscheduled DNA synthesis		+	Aquilina et al. 1984
Human (kidney T-cells)	DNA single-strand breakage		–	Bootsman et al. 1991
Human (lympocytes, fetal lung fibroblasts)	exchange		–	Nicholas et al. 1978

NA = not applicable; ND = no data; – = negative results; + = positive results

Male Q strain mice which received drinking water containing 2 mg/L dichlorvos for 7 weeks did not show chromosome damage in bone marrow cells, spermatogonia, or primary spermatocytes (Moutschen-Dahmen et al. 1981). In a micronucleus test, Swiss mice given daily intraperitoneal injections of dichlorvos (0.0075 or 0.015 mg/kg) for 2 days showed no aberrations in structure or number of chromosomes in bone marrow cells. CF-1 mice exposed to 64-72 mg/m^3 for 16 hours or to 5 mg/m^3 for 21 days did not show chromosome abnormalities (Dean and Thorpe 1972). In Syrian hamsters, however, intraperitoneal injections at 3, 6, 15, and 30 mg/kg did cause increases in the number of cells with aberrant chromosomes (Dzwonkowska and Hubner 1986).

Dichlorvos was positive for binding to calf thymus DNA in vitro (Lofroth 1970; Segerbeck 1981). Dichlorvos was negative for DNA binding in viva in rats (Wooder et al. 1977) and in mice (Segerbeck 1981).

In mutagenicity tests with *S. typhimurium* tester strains, dichlorvos has generally been positive without metabolic activation and negative in the presence of S9. Dichlorvos in the presence of metabolic activation was negative in strains TA 98 (Braun et al. 1982), TA 1535 (Braun et al. 1982; Carere et al. 1976; Moriya et al. 1978) and TA 1536, 1537, and 1538 (Braun et al. 1982; Carere et al. 1976). Without metabolic activation, dichlorvos was positive in strain TA 100 (Moriya et al. 1978), strain 1530 (Hanna and Dyer 1975), strain 1535 (Carere et al. 1978; Hanna and Dyer 1975; Moriya et al. 1978; Shirasu et al. 1976), but negative in strains 1536, 1537, and 1538 (Moriya et al. 1983; Shirasu et al. 1976).

The lack of dichlorvos genotoxicity in *in vivo* studies is most likely due to the fact that while dichlorvos possesses methylating activity, the phosphorous atom of the molecule is a stronger electrophile than the methyl carbon atoms (Wright et al. 1979). In tissues and blood, dichlorvos is much more likely to react with “A”-type esterases, serum cholinesterase, or acetylcholinesterase than with DNA (WHO 1989).

Cancer. In a 2-year carcinogenicity study of inhalation exposure to dichlorvos, groups of 50 Carworth rats of each sex were exposed at levels of 0, 0.05, 0.5, or 5 mg/m^3 (Blair et al. 1976). Only 11 of the unexposed male controls and 25 of the unexposed female controls survived to the end of the study. Survival was actually highest in the rats receiving the highest dose of dichlorvos (32 of 50 males and 34 of 50 females). Microscopic examination revealed a wide range of lesions in all

groups; the authors stated that these are commonly seen in old rats of this strain. There was a high incidence in all groups of chronic nephrosis, focal myocardial fibrosis, degenerative artery disease, lymphoid hyperplasia of the spleen. and testicular atrophy. Common tumors in all groups were adenomas of the anterior pituitary gland, parafollicular cell adenomas, and carcinomas of the thyroid gland, adrenal pheochromocytomas, and mammary fibroadenomas in the females. Examination of the lungs (presumably the tissue receiving the highest dose) revealed minor changes in all groups. Peribronchial and perivascular lymphoid aggregates, mild degrees of bronchiolitis and focal alveolar thickening were noted. Electron microscopic examination of bronchi, bronchioli, and alveoli of a small number of control and high-dose group animals showed no differences between the groups. None of the lesions in the study was associated with dichlorvos exposure.

The high mortality of the control animals in this study makes interpretation of the carcinogenicity data problematic. The possibility also exists that exposure by the oral and dermal routes may have occurred. However, no significant increase in neoplastic or non-neoplastic lesions was found in the nasal and respiratory tract tissues that received the highest dose of dichlorvos.

In a carcinogenicity study in Osborne-Mendel rats, groups of 50 animals of each sex were originally dosed through feed at levels of 45 and 90 mg/kg/day (NCI 1977). During the initial 3 weeks of dosage, acute signs of toxicity were observed including tremor and diarrhea in the 90 mg/kg/day group. For this reason, the dosages were then lowered to 30% of the original. The TWA doses over the go-week period of dosing were 13.5 and 29.3 mg/kg/day. After the go-week dosing period, the animals were observed for a further 30 weeks until sacrifice. Adverse clinical signs (hematuria, rough coats, epistaxis) were noted in control and dosed animals, gradually increasing during the second year of the study. The authors stated that at the end of the study the rats were in generally poor condition. The matched control groups had significantly lower survival than the treated groups at the end of the study, mainly due to deaths during the 30-week observation period after treatment. At the termination of the study, only 2 of 10 male rats and 5 of 10 female rats survived in the matched control groups. For this reason, these control rats were pooled with control rats from concurrent studies for comparison with the treated groups. Of the male rats, 76% of the high-dose and 64% of the low-dose group survived to the end of the study as did 84% of the high-dose and 80% of the low-dose females.

Numerous inflammatory, degenerative, and proliferative lesions commonly seen in aged rats occurred with approximately equal frequency in the treated and the pooled control rats. Several non-neoplastic

lesions occurred more frequently in the treated rats than in the controls. These included aggregates of alveolar macrophages in the lungs, interstitial fibrosis of the myocardium, and focal follicular cell hyperplasia of the thyroid gland in the male rats. Benign endocrine neoplasms occurred frequently in both test and control rats. There was a high incidence of benign mammary neoplasms in both control and treated rats. Because of the low survival of the matched control rats, control animals from other concurrent studies were pooled for statistical analysis. The authors stated that on the basis of variability of both the incidence and type of spontaneous lesions and the lack of significant proportions of tumors in the dosed groups compared to the controls, no statistical significance could be attached to the incidence of the tumors seen in the dichlorvos-treated rats in this study. Because of the poor survival of control animals in this study, the results are difficult to interpret.

In another carcinogenicity study in rats, groups of 50 Fischer 344 rats of each sex were dosed with dichlorvos by oral gavage in corn oil at levels of 0, 4, or 8 mg for 5 days a week for 103 weeks (NTP 1989). No significant differences in survival were noted between any groups. Survival rates were: 31 of 50 for male and female controls, 25 of 50 males and 26 of 50 females in the low-dose group, and 24 of 50 males and 26 of 50 females in the high-dose group.

Statistically significant increases in neoplasms were observed in the pancreas and hematopoietic system in male rats and in the mammary gland in female rats. Adenoma of the exocrine pancreas exhibited a significant positive trend and the incidences were greater in treated than control groups (15 of 50 in vehicle control, 23 of 49 in the low-dose group and 30 of 50 in the high-dose group). When horizontal sections of the pancreas were examined, additional adenomas were observed. When the data from both methods were combined, the incidences of pancreatic adenoma were 25 of 50 in the controls, 30 of 50 in the low-dose, and 33 of 50 in the high-dose group. The incidence for the treated groups was statistically significant compared to the vehicle controls.

A significant positive trend for mononuclear cell leukemia was also observed in the male rats. This neoplasm was found in 11 of 50 controls, 20 of 50 in the low-dose group, and 21 of 50 in the high-dose group. A significant positive trend also occurred for mammary gland tumors in female rats. Fibroadenoma, adenoma, or carcinoma occurred in 11 of 50 control rats, 20 of 50 in the low-dose group, and 17 of 50 in the high-dose group. Peer review panels characterized these results as “some evidence” of carcinogenic activity in male rats and “equivocal evidence” in female rats. The control

animals in these studies had substantially higher incidences of neoplasms at these sites than the historical incidence compiled from other studies.

Dichlorvos was also tested for carcinogenicity in male and female $B6C3F_1$ mice by a similar 2-year protocol (NTP 1989). Because of higher toxicity in male mice during the dose-finding study, groups of 50 male mice were dosed by corn oil gavage at 0, 10, or 20 mg/kg for 5 days a week, and the females dosed at 0, 20, or 40 mg/kg for 5 days a week.

The only neoplasm that occurred with a significant positive trend in treated compared to control mice was squamous cell papilloma and carcinoma of the forestomach. The overall incidence of this lesion in male mice was 1 of 50 in the controls, 1 of 50 in the low-dose group, and 5 of 50 in the high-dose group. In the females, overall incidences were 5 of 49 in the control group, 6 of 49 in the low-dose group and 18 of 50 in the high-dose group. Incidence in male controls was near the historical incidence of l%, but was higher in the female controls (10% compared to 1%). Peer review panels characterized the level of carcinogenic activity as “some evidence” in male mice and “clear evidence” in female mice.

The mechanism of dichlorvos-induced carcinogenicity is not known. A study of $B6C3F_1$ mouse forestomach from mice treated with dichlorvos by gavage in corn oil (Benford et al. 1994) showed increases in replicative DNA synthesis (associated with increased cell proliferation). Unscheduled DNA synthesis (associated with DNA repair) was not increased by dichlorvos treatment, but was increased by 1 -methyl-3-nitro-1 -nitrosoguanidine, a known genotoxic forestomach carcinogen. The authors concluded that the forestomach tumors seen in the 2-year carcinogenicity study (NTP 1989) may have been mediated by enforced cellular proliferation rather than by a genotoxic mechanism.

Two organizations have reviewed the evidence for dichlorvos carcinogenicity in humans from the results obtained in test systems. The EPA has classified dichlorvos as a probable human carcinogen (Category B2) on the basis of significant increases of forestomach tumors in mice and-leukemias and pancreatic acinar adenomas in rats. Supporting evidence included observation of tumors at other sites in the rat and the observation that dichlorvos and a major metabolite, dichloroacetaldehyde, are mutagenic in in vitro test systems. A structurally related compound, dichloropropene, also causes forestomach tumors in rodents (IRIS 1995). The International Agency for Research on Cancer (IARC) has classified dichlorvos as possibly carcinogenic to humans (Group 2B) based on inadequate evidence

in humans for the carcinogenicity of dichlorvos and sufficient evidence in experimental animals for the carcinogenicity of dichlorvos (IARC 1991).

2.6 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 (NRC 1989). The preferred biomarkers of exposure are generally the substance itself or 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 dichlorvos are discussed in Section 2.6.1.

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 (NAWNRC 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 dichlorvos are discussed in Section 2.6.2.

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 2.8, Populations That Are Unusually Susceptible.

2.6.1 Biomarkers Used to Identify or Quantify Exposure to Dichlorvos

Dichlorvos at high doses will cause classical symptoms of organophosphate toxicity, such as miosis, tremor, increased salivation, lacrimation, pulmonary secretions, and perspiration. If these symptoms occur together and the individual has recently been in contact with pesticides containing dichlorvos, it is highly likely that exposure to dichlorvos has occurred. Exposure to toxic concentrations of dichlorvos or other organophosphates can be confirmed by blood tests. Dichlorvos can reduce the activity of two enzymes in the blood, serum cholinesterase and erythrocyte acetylcholinesterase. Serum cholinesterase appears to be more sensitive to inhibition by dichlorvos and other organophosphates than erythrocyte acetylcholinesterase. However, serum cholinesterase activity recovers more rapidly than erythrocyte acetylcholinesterase because of the higher turnover rate of serum proteins compared to erythrocytes. Exposures that occurred two weeks or more before testing probably would not be reflected in an inhibition of serum cholinesterase. Because of the human variability in activity of these enzymes, follow-up determinations showing a rise back to a constant activity are more reliable evidence that an exposure has taken place than a single determination.

Confirmation that specific exposure to dichlorvos has taken place is difficult and requires sophisticated analytical chemistry. Intact dichlorvos has not been detected in humans and only rarely in animals. This is because of the rapid metabolism of dichlorvos by esterases in the liver and blood (see Section 2.3). The major metabolic products of dichlorvos are dimethyl phosphate and the glucuronide conjugate of dichloroethanol. These compounds are rapidly excreted into the urine and will have left the body within a day or two of cessation of exposure. Dimethyl phosphate has been measured in the urine of pesticide applicators by extraction with an ion exchange resin, derivitization, and gas chromatography (Das et al. 1983). Dichloroethanol has been detected in the urine of a human volunteer after glucuronidase treatment and gas-liquid chromatography (Hutson and Hoadley 1972b). However, because of interference by endogenous urine components, the measurement had a relatively high error. Exposure to naled and trichlorphon, two organophosphates that are converted in the body

to dichlorvos, would also have to be ruled out before a definitive determination of dichlorvos exposure could be made (Hayes 1982).

2.6.2 Biomarkers Used to Characterize Effects Caused by Dichlorvos

The toxic effects of dichlorvos are caused by its inhibition of neural acetylcholinesterase in the peripheral and central nervous systems. This inhibition is reflected by the level of depression of erythrocyte acetylcholinesterase activity in the blood.

The nervous system can accept a certain amount of acetylcholinesterase inhibition without overt toxic effects. In humans and animals, toxic signs are generally not seen until at least 20% of this enzyme (measured as erythrocyte acetylcholinesterase) has been inhibited (Ecobichon 1991). In an animal study, brain acetylcholinesterase after a 2-year inhalation exposure to dichlorvos was inhibited more than 90% compared to control animals (Blair et al. 1976), yet no symptoms of cholinergic overstimulation were observed. With dichlorvos and other organophosphate compounds, the best predictor of toxicity is not necessarily the actual percent inhibition of acetylcholinesterase, but rather how rapidly this inhibition has occurred. Rapid inhibition does not give the nervous system time to physiologically adapt to acetylcholinesterase inhibition. This adaptation appears to involve desensitization and down-regulation of muscarinic receptors (Fitzgerald and Costa 1993).

For more information on biomarkers for renal and hepatic effects of chemicals see ATSDR/CDC *Subcommittee Report on Biological Indicators of Organ Damage* (1990) and for information on biomarkers for neurological effects see OTA (1990).

2.7 INTERACTIONS WITH OTHER CHEMICALS

The major interaction of dichlorvos with other chemicals would be with chemicals that have the same mechanism of action (i.e., organophosphate and carbamate pesticides). Simultaneous exposure to dichlorvos and one of these chemicals could possibly have an additive effect on inhibition of neural acetylcholinesterase. There has been one case of serious human toxicity caused by ingestion of a bait cake that contained both dichlorvos and malathion (Hayes 1982). Chemicals which can react with the serine residue at the active site of the “A”-type esterases (e.g., diisopropylfluorophosphate [DEP]) could also increase the toxicity of dichlorvos by interfering with metabolism.

2.8 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to dichlorvos than will most persons exposed to the same level of dichlorvos 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 may result in reduced detoxification or excretion of dichlorvos, or compromised function of target organs affected by dichlorvos. Populations who are at greater risk due to their unusually high exposure to dichlorvos are discussed in Section 5.6, Populations With Potentially High Exposure.

People with impaired “A”-type esterase function would be unusually susceptible to dichlorvos exposure , because of an impaired ability to metabolize dichlorvos absorbed by the body. This population would primarily be composed of people suffering from liver diseases. Pregnant women have lower levels of serum cholinesterase and are more susceptible to agents such as succinylcholine which is metabolized by this enzyme. Dichlorvos can bind stoichiometrically to this enzyme and inhibit its activity, so pregnant women are at least hypothetically more susceptible to dichlorvos exposure than other populations. A similar effect could be expected in individuals with inherited abnormally low serum cholinesterase levels.

2.9 METHODS FOR REDUCING TOXIC EFFECTS

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

> “Cholinesterase inhibitor pesticides” in *Handbook* of Poisonilzg, 1987, Appleton and Lange, Norwalk, CT; R.H. Dreisbach and W.O. Robertson. pp 11 l-l 18.
>
> “Organophosphates and other insecticides” in *Clinical Management of Poisoning and Drug Overdose,* 2nd. edition, 1990, W.B. Saunders, Philadelphia; L.M. Haddad and J.F. Winchester, eds. pp 10761087.

"Insecticides: organophosphates and carbamates" in *Goldfrank's Toxicologic Emergencies,* 5th edition, 1994, Norwalk, CT; L.R. Goldfrank, N.E. Flomenbaum, N.A. Lewis, R.S. Weisman, M.A. Howland, and R. S. Hoffman. pp 1105-l 119.

2.9.1 Reducing Peak Absorption Following Exposure

If exposure has occurred by the oral route, gastric lavage would reduce peak absorption following exposure. Dichlorvos generally does not absorb to other materials. so treatment with activated charcoal, for example would probably be ineffective. If exposure has occurred by the dermal route, rinsing the exposed skin with large amounts of flowing water and soap would greatly reduce exposure.

2.9.2 Reducing Body Burden

Because dichlorvos does not accumulate in the body and is rapidly metabolized to less toxic metabolites which are rapidly excreted into the urine, specific efforts to reduce the body burden would not appear to be necessary.

2.9.3 Interfering with the Mechanism of Action for Toxic Effects

Dichlorvos has the same mechanism of action as other organophosphorus insecticides. Poisonings with these types of chemicals are common enough that specific and effective medical interventions have been developed. The life-threatening effects of dichlorvos poisoning are related to its effects on the respiratory system (respiratory depression, bronchospasm, increased bronchial secretions, pulmonary edema. muscular weakness). If these symptoms are present, artificial respiration and suctioning are performed via an endotracheal tube. Atropine is used to counteract the muscarinic effects of dichlorvos with care being taken that symptoms of atropine overdose do not occur (dry mouth, dilatation of the pupils). The inhibited neural acetylcholinesterase can be reactivated by intravenous administration of specific antidotes such as pralidoxime.

2.10 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 dichlorvos 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 dichlorvos.

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.

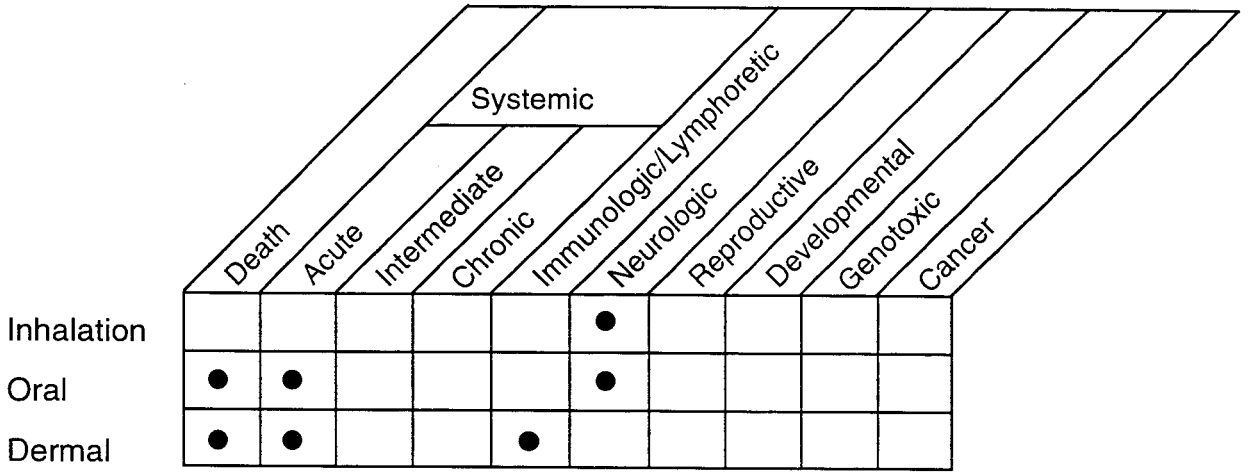
2.10.1 Existing information on Health Effects of Dichlorvos

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to dichlorvos are summarized in Figure 2-5. The purpose of this figure is to illustrate the existing information concerning the health effects of dichlorvos. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 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.

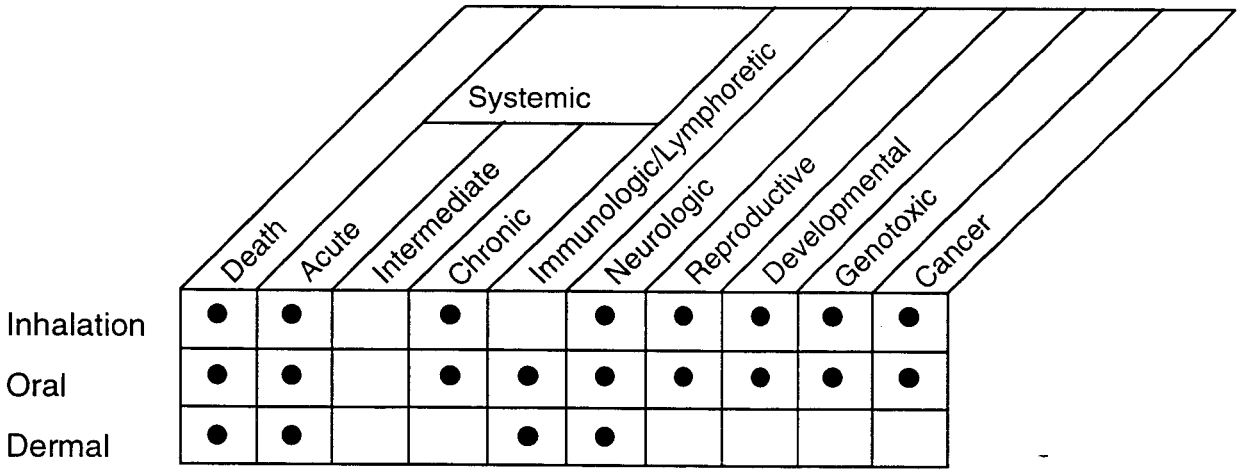
As shown in Figure 2-5, information is available for humans on death after oral and dermal exposure, and on systemic and neurological effects after inhalation and oral exposure. This information is generally for acute-duration exposure only, except for one 21-day study of oral exposure in volunteers that examined the neurological end point of erythrocyte acetylcholinesterase inhibition. Nonneurological end points generally have not been observed in humans, except those that result secondarily from the neurological toxicity of dichlorvos.

Animal data exist for death by inhalation, oral, and dermal routes for acute durations, and inhalation and oral routes for intermediate and chronic durations. Data also exist for systemic effects that are secondary to the neurotoxicity of dichlorvos. Limited immunological data are available only for the oral route for the acute and intermediate duration. Data are available for neurological effects for all

Figure 2-5. Existing Information on Health Effects of Dichlorvos



Human



Animal

● Existing Studies

routes and all durations except dermal intermediate and chronic. Reproductive and developmental studies are available for the inhalation and oral route. Genotoxic and cancer studies are available for the inhalation and oral route.

2.10.2 Identification of Data Needs

Acute-Duration Exposure. Populations in the vicinity of hazardous waste sites may be exposed to dichlorvos for brief periods. Exposure would most likely occur by the inhalation route, but dermal exposure by contact with contaminated soil is also possible. Cases of accidental and intentional poisonings in humans (Hayes 1982) indicate that the central and peripheral nervous systems are the major target organs for dichlorvos toxicity by the oral and dermal routes. It can be inferred from animal studies that this is true for inhalation exposure as well (Durham et al. 1957). The specific target of dichlorvos is the enzyme which catalyzes the hydrolysis of the neurotransmitter acetylcholine, neural acetylcholinesterase. Minimal risk levels (MRLs) have been derived for the acute duration for both the inhalation route (0.002 ppm) (Schmidt et al. 1979) and the oral route (0.004 mg/kg/day) (Teichert et al. 1976) based on inhibition of erythrocyte acetylcholinesterase and neural acetyl-cholinesterase activity. The study used for the acute-duration oral MRL (Teichert et al. 1976) did not include a dose at which no adverse effects occurred (NOAEL); an acute-duration oral study in animals which included doses of dichlorvos that produced both a NOAEL and a LOAEL would be valuable. Little information on acute-duration dermal exposure is available except for lethality studies. Since this is a possible route of exposure from contaminated soil at hazardous waste sites, studies which establish a threshold value for acetylcholinesterase inhibition in a sensitive species would be useful.

Intermediate-Duration Exposure. A well-conducted study is available for human intermediateduration oral exposure to dichlorvos (Boyer et al. 1977). This study was used to derive an oral MRL of 0.003 mg/kg/day dichlorvos based on a NOAEL of 0.033 mg/kg/day for erythrocyte acetyl-cholinesterase inhibition. An intermediate-duration inhalation study in rats (Thorpe et al. 1972) was used to derive an inhalation MRL of 0.0003 ppm dichlorvos based on a NOAEL of 0.03 ppm for brain acetylcholinesterase inhibition. Results in animal studies indicate that the toxic effects of intermediate-duration exposure dichlorvos are similar to those for the acute duration. Several studies have identified immunosuppression in rats treated orally with dichlorvos over the intermediate duration (Desi et al. 1978, 1980). This effect may be secondary to central cholinergic stimulation; further studies are needed to clarify this point. No studies on intermediate-duration dermal exposure to

dichlorvos were located: studies on this topic would be helpful to establish threshold values of acetylcholinesterase inhibition.

Chronic-Duration Exposure and Cancer. Chronic-duration exposure is the most likely type of exposure that would be experienced by people living near hazardous waste sites. Because of the physical properties of dichlorvos, this exposure is most likely to be by the inhalation route. The only chronic-duration study by the inhalation route was done on rats in 1976 (Blair et al. 1976). A chronicduration inhalation MRL of 0.00006 ppm was derived, based on brain acetylcholinesterase inhibition in this study. While this study found no evidence of an increased incidence of cancer in these rats, it does not meet present-day standards for carcinogenicity studies of this type. A chronic-duration oral study has been done in rats and mice, and produced evidence of carcinogenicity by this route (NTP 1989). Since inhalation is the most likely route of exposure in humans for dichlorvos, a chronic duration inhalation study in rats and mice could help public health assessment of potential risks of dichlorvos.

Genotoxicity. Dichlorvos has been tested in virtually every available genotoxicity test. In bacteria, dichlorvos bound covalently to DNA and caused DNA damage and point mutations. Dichlorvos induced gene conversion, mutation and aneuploidy in yeast and fungi. In *D. melunogasrer*, chromosomal but not sex-linked recessive lethal mutations were produced. In mammalian cells *in vitro,* dichlorvos caused DNA strand breaks, mutation, sister chromatid exchange, chromosomal aberrations and cell transformation. The effects on *in vitro* systems were in general greatly reduced when metabolic activation was present. Dichlorvos was negative for genotoxicity in *in vivo* tests for the induction of unscheduled DNA synthesis, sister chromatid exchange, micronucleus formation, chromosomal aberrations, or dominant lethal mutation by the inhalation route. The database for dichlorvos genotoxicity is extensive and no data needs have been identified.

Reproductive Toxicity. No information on reproductive toxicity in humans after dichlorvos exposure is available. Dichlorvos did not cause reproductive toxicity by the inhalation route over the acute duration in male mice (Dean and Thorpe 1972). No gross or histological evidence of treatmentrelated damage to reproductive tissues (prostate, testes, epididymis, ovaries or uterus) was seen in rats (4 or 8 mg/kg/day) or mice (10, 20, or 40 mg/kg/day) orally exposed to dichlorvos by gavage for 2 years (NTP 1989). No reproductive studies on dichlorvos by the oral or dermal routes are available;

animal reproductive toxicity studies by these routes would be useful. No multi-generational reproductive studies on dichlorvos are available.

Developmental Toxicity. No information on developmental toxicity in humans after dichlorvos exposure is available. Dichlorvos did not cause developmental toxicity in rats exposed throughout pregnancy by the inhalation route to up to 0.69 ppm or up to 0.44 ppm in rabbits (Thorpe et al. 1972). Similar results were obtained with mice and rabbits exposed to 0.44 ppm dichlorvos for 7 hours a day during the organogenesis period of gestation (Schwetz et al. 1979). Developmental toxicity was not observed in mice exposed orally by gavage to 60 mg/kg/day dichlorvos over gestation days 6-15, or in rabbits exposed similarly to 5 mg/kg/day dichlorvos over gestation days 6-18 (Schwetz et al. 1979). The database for dichlorvos developmental toxicity is adequate, except that a multi-generation developmental study in rabbits would be useful.

Immunotoxicity. No information was located on immunotoxicity in humans after dichlorvos exposure. Only a few studies were located that addressed immunotoxicity in animals after dichlorvos exposure. Immunosuppression after oral exposure to dichlorvos in rabbits has been reported in three studies (Casale et al. 1983; Desi et al. 1978, 1980). A dose-related suppression of the humoral immune response induced by *S. typhimurium* was observed in rabbits (Desi et al. 1978). A single oral dose of 120 mg/kg dichlorvos in male C57B l/6 mice that had been inoculated with sheep erythrocytes 2 days earlier suppressed the primary IgM response observed 48 hours later (Casale et al. 1983). Severe signs of dichlorvos neurotoxicity were noted, and the authors stated that the immuno-suppression observed in this study may have been mediated indirectly by toxic chemical stress. It is unknown if the immune suppression noted after dichlorvos exposure in these studies is secondary to cholinergic stimulation. Immunotoxicity studies employing atropine prophylaxis to counteract the anticholinesterase effect of dichlorvos are necessary to resolve this question. Additional studies examining potential longer-term effects of dichlorvos on the immune system by all three routes as well as short-term effects by the inhalation and dermal routes would be important for estimating human susceptibility for populations exposed for varying lengths of time at hazardous waste sites.

Neurotoxicity. A few case reports in humans indicate that the central and peripheral nervous systems are the targets of dichlorvos toxicity after oral and dermal exposure (Hayes 1982). Numerous animal studies by the inhalation, oral and dermal routes corroborate these findings (Durham et al. 1957; Snow and Watson 1973; Stanton et al. 1979) and have identified the molecular target for

dichlorvos neurotoxicity, neural acetylcholinesterase. The database for neurotoxicity was sufficient to derive MRLs for acute-, intermediate-, and chronic-duration inhalation exposure and for acute- and intermediate-duration oral exposure based on observation of NOAELs and one less serious LOAEL for inhibition of neural and/or erythrocyte acetylcholinesterase. Further studies are needed to elucidate the mechanism of adaptation to dichlorvos neurotoxicity. In longer term experiments (Blair et al. 1976), levels of inhibition that would cause serious toxicity if caused by an acute dose (up to 90% for neural acetylcholinesterase) are tolerated without clinical signs of dichlorvos neurotoxicity. Studies that determine a threshold dose for dichlorvos neurotoxicity by the dermal route are also needed since this is a potential route for exposure to populations near hazardous waste sites.

The adverse neurological effects of dichlorvos can be explained largely by its inhibition of neural acetylcholinesterase, but the possibility exists that other potential targets in the nervous system exist. For example, there is evidence that chlorpyrifos oxon (the active metabolite of the organophosporus insecticide chlorpyrifos) can inhibit muscarinic receptor binding *in vitro.* This inhibition occurs at concentrations lower than those necessary to inhibit neural acetylcholinesterase (Huff et al. 1994). *In vitro* receptor binding in brain membrane preparations or in cultured cells would determine if dichlorvos has a similar effect on muscarinic receptor function. Little is known about the potential for interaction between dichlorvos and other neurotoxic agents; further studies on this topic would be useful.

Epidemiological and Human Dosimetry Studies. At the present time, very few people are exposed to dichlorvos outside occupational groups. The major group potentially exposed, pest control workers, generally use several different pesticides, and it would be virtually impossible to identify a group exposed primarily to dichlorvos. The lack of adequate analytical methods that would specifically quantify dichlorvos exposure (as opposed to other organophosphorus or carbamate pesticides) precludes human dosimetry studies. Thus, no data needs for epidemiological or human dosimetry studies were identified.

Biomarkers of Exposure and Effect.

Exposure. Reliable biomarkers for exposure to dichlorvos already exist (serum cholinesterase, erythrocyte acetylcholinesterase, and clinical symptoms of neurotoxicity). However, reliable methods

to distinguish dichlorvos intoxication from that caused by other organophosphorus compounds do not exist.

Effect. Reliable biomarkers for the effect of dichlorvos exist (cholinergic symptoms of neurotoxicity and erythrocyte acetylcholinesterase). There is no evidence that toxic effects occur in humans at levels of dichlorvos that do not significantly inhibit erythrocyte acetylcholinesterase.

Absorption, Distribution, Metabolism, and Excretion. Very little information is available on the toxicokinetics of dichlorvos in humans. Dichlorvos appears to be rapidly absorbed by all routes of exposure. This rapid rate of absorption is inferred from the time to onset of clinical signs and/or cholinesterase inhibition because of the difficulty in assaying dichlorvos in biological tissues. This is due to the rapid hydrolysis of dichlorvos by tissue esterases, particularly in the liver and the serum. The half-life of dichlorvos in human blood *in vitro* is about 10 minutes (Blair et al. 1975). Distribution is also inferred from cholinesterase inhibition, but there does not appear to be any preferential distribution to particular tissues. Dichlorvos does not appear to be stored or concentrated in any tissue (Casida et al. 1962). The major metabolites of the esterase-catalyzed degradation of dichlorvos are dimethyl phosphate and dichloroacetaldehyde (Wright et al. 1979). Dimethyl phosphate is excreted in the urine, while dichloroacetaldehyde can be reduced to dichlorethanol or dehalogenated to glyoxal, which enters 2-carbon metabolism (Hutson et al. 1971). Dichloroethanol is either conjugated to glucuronic acid and excreted or dehalogenated and further metabolized. There is also some evidence that dichlorvos can be demethylated in a glutathione-dependent process (Blair et al. 1975).

Further characterization of the esterases involved in dichlorvos degradation is necessary to determine if any possibility exists that human polymorphism may make some groups more susceptible to dichlorvos toxicity than others. Since inhalation exposure is the most likely route of exposure at hazardous waste sites, determination of the blood-gas partition coefficient for dichlorvos in human blood is needed to more accurately quantify the potential health risks for a given atmospheric level of dichlorvos.

Comparative Toxicokinetics. Differences in sensitivity to dichlorvos toxicity appear to exist in mammalian test animal species. Generally, for a given dose, rabbits are the most sensitive, followed in order by dogs, rats, and mice (NTP 1989; Snow and Watson 1973; Thorpe et al. 1972). The comparative toxicokinetic parameters that might explain these differences are unknown. Further

studies via all three exposure routes in test species would be useful in determining similarities and differences between humans and animals and the effects of pregnancy on dichlorvos metabolism.

Methods for Reducing Toxic Effects. Further studies on retarding gastrointestinal absorption of dichlorvos would be useful in the treatment of poisoning. No methods exist for reducing the body burden of dichlorvos. The medical management of the toxic effects of dichlorvos (respiratory support, atropine treatment, reactivation of neural acetylcholinesterase with oximes) is similar to that for poisoning by other organophosphorus pesticides. Any improvements in the management of organophosphorus poisoning would apply to dichlorvos.

2.10.3 Ongoing Studies

M. Cunningham of the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, is conducting feeding studies in mice with dichlorvos and several other chemicals to investigate the relationship between cell proliferation and carcinogenesis.