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 chlorfenvinphos. 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 lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELS have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in

determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (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.

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

2.2.1.1 Death

There are no reports of deaths in humans exposed by acute-, intermediate-, or chronic-duration inhalation to chlorfenvinphos.

No studies were located regarding lethality in animals after intermediate- or chronic-duration inhalation exposure to chlorfenvinphos. A study investigated whether the difference in the route of absorption or the mode of lethality is responsible for the higher lethality of the micron-sized (>1 µm) aerosols in male rats. The study reported 0, 60, and 100% mortality in rats at the 83, 144, and 236 mg/m^3 ambient air concentrations, respectively. The deaths occurred 3 and 4 hours after initiation of the exposure to micron-sized chlorfenvinphos aerosols. There was 0, 80, and 100% mortality in rats at the 254, 471, and 1,019 mg/m^3 ambient air concentrations, respectively. The LC_{50} value calculated from the mortality of the rats was markedly increased by cannulation (from 133 mg/m^3 for non-cannulated to 489 mg/m^3 for cannulated rats) for the micron-sized aerosol ambient air concentrations. However, there was essentially no difference in the LC_{50} values (509 mg/m^3 for non-cannulated and 475 mg/m^3 for cannulated rats) calculated from the mortality of the rats that were administered the more inhalable submicron-sized aerosols ambient air concentrations. These data indicate that the micron-sized aerosols were about 4 times more potent in producing lethality than the submicron-sized aerosols. This is also an indication that swallowed chlorfenvinphos contributed to the lethality; this was more evident for the less inhalable micron-sized aerosol ambient air concentrations. Elapsed time from the start of exposure to death was not changed by the cannulation in both the micron-sized and the submicron-sized aerosols. The authors surmised that death from acute exposure to chlorfenvinphos aerosols probably derives from inhibition of acetylcholinesterase(AChE) activity (Takahashi et al. 1994). In another study with rats, the acute lethality of chlorfenvinphos was unaffected in male rats in snout-only or whole body exposures (Tsuda et al. 1986). The LC_{50} for death in rats is shown in Table 2-1 and plotted in Figure 2-1.

2.2.1.2 Systemic Effects

No studies were located regarding the gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, or body weight effects in humans or animals following acute-, intermediate-, or chronic-duration inhalation exposure to chlorfenvinphos. Existing human data on the metabolic effects of the

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

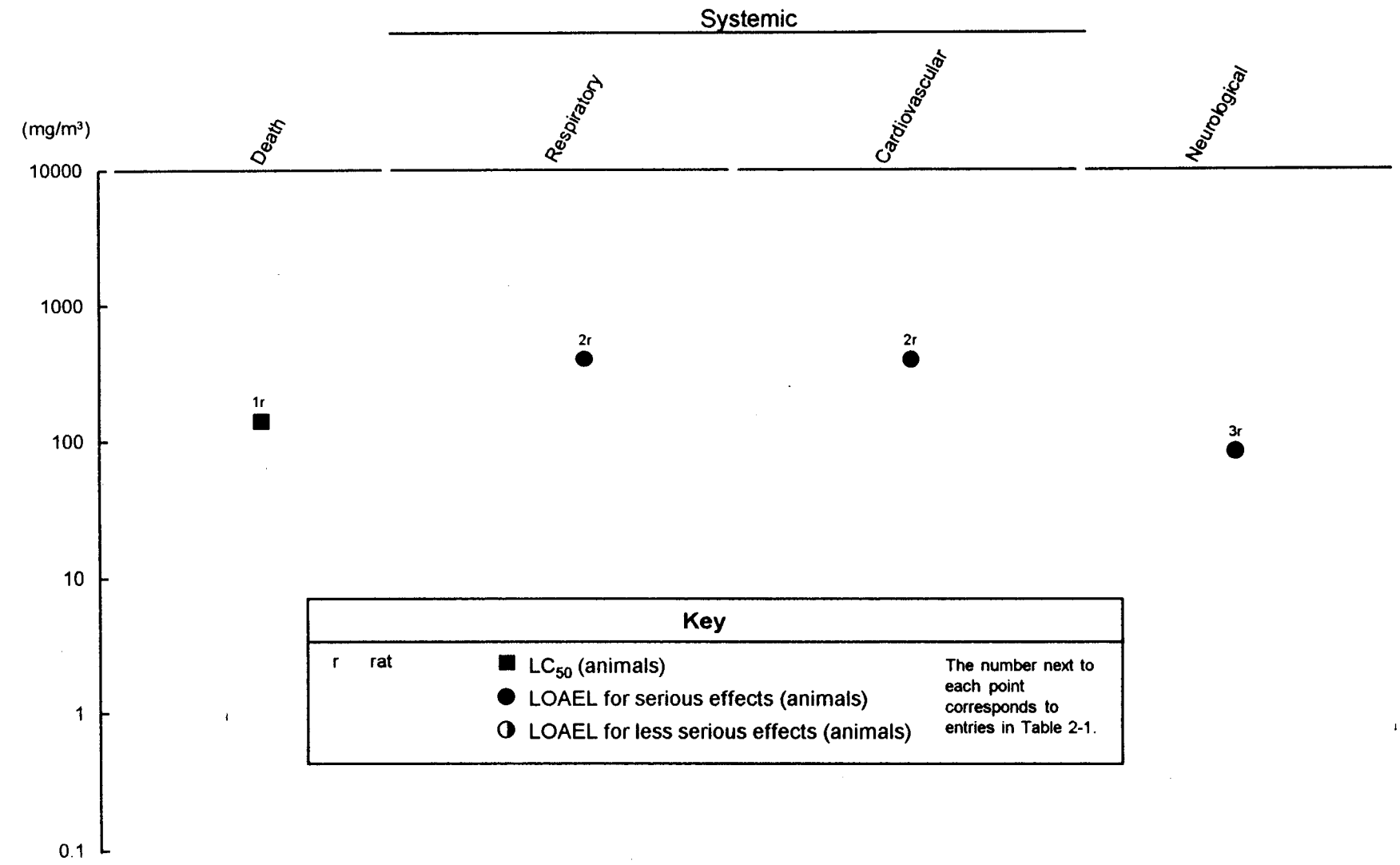
Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference
ACUTE EXPOSURE							
Death							
1	Rat (Fischer- 344)	once				133 M (LC_{50})	Takahashi et al. 1994
Systemic							
2	Rat (Fischer- 344)	4 hr	Resp			390 M (apnea)	Takahashi et al. 1994
			Cardio			390 M (progressive increase in blood pressure, bradycardia)	
Neurological							
3	Rat (Fischer- 344)	4 hr				83 M (salivation, urination, exophthalmos, twitches, and tremors)	Takahashi et al. 1994

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

Cardio = cardiovascular; hr = hour; LC_{50} = lethal concentration, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed- adverse-effect level; Resp = respiratory

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

Acute (≤14 days)



substance are limited to chronic-duration exposure. Existing animal data on the respiratory and cardiovascular effects are limited to acute-duration exposure.

The highest NOAEL value and all LOAEL values for adverse systemic effects in each reliable study for each species and duration category are shown in Table 2-1 and plotted in Figure 2-1.

Respiratory Effects. No studies were located regarding respiratory effects in humans after exposure to chlorfenvinphos for any duration category.

Acute-duration exposure of rats to inhalation aerosol chlorfenvinphos ambient air concentrations produced cardiorespiratory changes in the treated rats at high ambient air concentrations. Male Fischer 344 rats exposed to the micron-sized (>1 µm) or submicron-sized (<1 µm) chlorfenvinphos aerosols for 4 hours suffered cardiorespiratory effects. Rats exposed to the lethal concentration of 1,220 mg/m^3 of the submicron-sized aerosols showed a progressive increase in blood pressure followed by an apnea during which the blood pressure was maximally increased. Rats exposed to 390 mg/m^3 of the micron-sized aerosols also exhibited cardiorespiratory changes similar to changes caused by the submicron-sized aerosols (data were not shown in the report). No significant qualitative difference was observed in cardiorespiratory changes between the micron-sized and the submicron-sized aerosols, suggesting that the mode of lethality is not different between the two types of aerosols. A LOAEL of 390 mg/m^3 for apnea was established in this study (Takahashi et al.
1994). In a previous study (Takahashi et al. 1991), Sprague-Dawley rats exhibited similar signs at an intravenous dose of 16 mg/kg of chlorfenvinphos.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after exposure to chlorfenvinphos for any duration category.

Acute exposure of rats to inhalation aerosol chlorfenvinphos ambient air concentrations produced cardiorespiratory changes in the treated rats at high ambient air concentrations. Adult male Fischer 344 rats exposed to the micron-sized (>1 µm) or submicron-sized (<1 µm) chlorfenvinphos aerosols for 4 hours suffered cardiorespiratory effects. Rats exposed to the lethal concentration of 1,220 mg/m^3 of the submicron-sized aerosols showed a progressive increase in blood pressure followed by apnea during which the blood pressure was maximally increased. Bradycardia was observed in the electrocardiograph (ECG) at the pressor period, which was characterized by a prolonged TP time without a change in PQ, QRS, and ST time. Rats exposed to 390 mg/m^3 of the micron-sized aerosols also exhibited cardiorespiratory changes similar to those

caused by the submicron-sized aerosols (data were not shown in the report). No significant qualitative difference was observed in cardiorespiratory changes between the micron-sized and the submicron-sized aerosols, suggesting that the mode of lethality is not different between the two types of aerosols. A LOAEL of 390 mg/m^3 for progressive increased blood pressure and bradycardia was established in this study (Takahashi et al. 1994).

Metabolic Effects. The information on the metabolic effects of chronic-duration inhalation exposure to chlorfenvinphos provides only inconclusive evidence. Examinations of 31 manufacturing workers who directly handled the chlorfenvinphos (and other similar compounds) for 19–53 years revealed significantly lowered NBT (nitroblue tetrazolium)-dye reduction in both stimulated and non-stimulated cells, as well as a significant decrease of the spontaneous E rosette formation (not influenced by exposure time) in the blood (early E rosettes, 52%; late E rosettes, 57%) as compared to controls (early E rosettes, 57%; late E rosettes, 63%). No correlation was found between the spontaneous E rosette formation and acetylcholinesterase activity. The authors of this study concluded that depressed NBT-dye reduction and diminished spontaneous E rosette formation may be regarded as a probable mode of the effect of organophosphoric chemicals on metabolic and membrane damage to human cells. About half of the subjects in the study were smokers (Wysocki et al. 1987). However, the data from this study are not reliable for evaluating the inhalation toxicity of chlorfenvinphos because the workers were also concurrently exposed to greater concentrations of other known toxic substances.

No studies were located regarding metabolic effects in animals after exposure to chlorfenvinphos for any duration category.

2.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding the immunological and lymphoreticular effects in humans following acute- or intermediate-duration inhalation exposure to chlorfenvinphos. However, chronic-duration inhalation exposure to organophosphoric pesticides caused a depression of immune responses and, consequently, produced damage to humoral mechanisms in humans. Examination of 31 manufacturing workers who directly handled the organophosphoric pesticide chlorfenvinphos (and other compounds) for 19–53 years revealed significantly lowered NBT-dye reduction in both stimulated and non-stimulated cells, and a decreased percentage of phagocytic cells ($P<0.001$, 0.05, and 0.002, respectively) in pesticide workers occupationally exposed to an estimated average ambient chlorfenvinphos concentration of 0.21 mg/m^3. These analyses parameters of the NBT test showed a positive linear correlation with the degree of acetylcholinesterase activity reduction. The exposure time had no effect on NBT reduction test

parameters, but there was a negative linear correlation with the phagocytic index of the NBT test ($r = -0.4879$, $P<0.01$). A significant decrease of the spontaneous E rosette formation (not influenced by exposure time) was found in the blood of the exposed workers (early E rosettes, 52%; late E rosettes, 57%), as compared to controls (early E rosettes, 57%; late E rosettes, 63%). No correlation was found between the spontaneous E rosette formation and acetylcholinesterase activity. No significant differences were found in number of white blood cells, mainly neutrophils, in the two examined subgroups, but the absolute lymphocyte count in the peripheral blood of the exposed subjects was lower when compared to controls (1,941.9 versus 2,380 cells/mm^3, $P<0.05$). The authors of this study concluded that depressed NBT-dye reduction and diminished spontaneous E rosette formation may be regarded as a probable mode of action of organophosphoric chemicals on metabolic and membrane damage to human cells. Acetylcholinesterase activity, which showed a time-independent positive linear relationship to lowered NBT-dye reduction in the subjects, was above 2 µmol in 17 of the subjects (with an average age of 38.5 years). Eighteen (58.1%) of the subjects examined showed symptoms of chronic bronchitis; seven (23.3%) subjects in the control group also had signs indicating previous (anamestic) chronic bronchitis. It is generally understood that chronic bronchitis stems from changes in humoral rather than cellular immune response. About half of the subjects were smokers. The maximal estimated airborne concentrations of substances in the workplace were: 0.654 mg/m^3 (formothion), 0.483 mg/m^3 (sumithion), trace (DDVP), 0.21 mg/m^3 (chlorfenvinphos), and 1.09 mg/m^3 (malathion) (Wysocki et al. 1987). The data from this study are not reliable for evaluating the inhalation toxicity of chlorfenvinphos because the workers were also concurrently exposed to greater concentrations of other known immunotoxic substances.

No studies were located regarding immunological and lymphoreticular effects in animals after acute-, intermediate-, or chronic-duration inhalation exposure to chlorfenvinphos.

2.2.1.4 Neurological Effects

No studies were located regarding neurological effects in humans after acute- or intermediate-duration inhalation exposure to chlorfenvinphos. The single study that reported neurological effects in humans from inhalation exposure to chlorfenvinphos involved occupational exposure. A group of nine gardeners (pesticide mixers) who worked with the organo-phosphates (dimethoate, formothion, isofenphos and occasionally chlorfenvinphos) for an unspecified duration complained of headaches. The gardeners had a mean difference (before and after exposure) of 0.56 nmol/mL for acetylcholinesterase and 2.67 nmol/mL for butyryl

cholinesterase. Since the symptoms could also result from exposure to the other organophosphates (dimethoate, formothion, isofenphos) to which the workers were also exposed, the role of chlorfenvinphos exposure in this incident is not certain. In addition, no data were given on air concentrations of the organophosphate pesticides (Kolmodin-Hedman and Eriksson 1987).

No studies were located regarding neurological effects in animals after intermediate- or chronic-duration inhalation exposure to chlorfenvinphos. Acute exposure of rats to aerosols of chlorfenvinphos produced neurological signs indicative of cholinergic response stemming from inhibition of acetylcholinesterase activity. Surviving adult male Fischer 344 rats (27 of 60) exposed to the micron-sized (>1 µm) or submicron-sized (<1 µm) chlorfenvinphos aerosols for 4 hours (83, 130, 144, 236, 254, 322, 471, 623, 1,019, or 1,065 mg/m^3) exhibited cholinergic signs: salivation, urination, exophthalmos, twitches, and tremors, at $83 mg/$m^3$. The inhalation experiments were conducted using a nose-only inhalation chamber. (To examine the toxicological significance of swallowed chlorfenvinphos, a drain cannula was placed in the esophagus under pentobarbital sodium anesthesia.) Toxic signs were not assessed in detail during the exposure because the rats were in the animal holder. There were no differences in toxic signs between the micron-sized and the submicron-sized aerosols (Takahashi et al. 1994).

All LOAEL values for neurological effects in each reliable study for each species and duration category are shown in Table 2-1 and plotted in Figure 2-1.

No studies were located regarding the following health effects in humans or animals after acute-, intermediate-, or chronic-duration inhalation exposure to chlorfenvinphos:

2.2.1.5 Reproductive Effects

2.2.1.6 Developmental Effects

2.2.1.7 Genotoxic Effects

Other genotoxicity studies for chlorfenvinphos are described in Section 2.5.

2.2.1.8 Cancer

No studies were located regarding carcinogenic effects in humans or animals following inhalation exposure to chlorfenvinphos.

2.2.2 Oral Exposure

2.2.2.1 Death

There are no reports of deaths in humans exposed by intermediate- or chronic-duration ingestion of chlorfenvinphos. A 16-month-old child who accidentally drank an unspecified amount of chlorfenvinphos, used for flea treatment in the home, died despite treatment in a hospital (Felthous 1978).

Chlorfenvinphos is extremely toxic to rodents and dogs by the oral route in acute doses. The oral LD_{50} values for rats, rabbits, and dogs have been estimated to be 9.7, 300, 50.5 mg/kg, respectively (Ambrose et al. 1970). An acute oral LD_{50} of 23 mg/kg was calculated from the mortality data of an unspecified number of male Wistar rats that were given varying doses of chlorfenvinphos in olive oil (Hutson and Logan 1986). Similarly, the acute oral LD_{50} of chlorfenvinphos in male rats as a single dose in olive oil was estimated to be 15.4 mg/kg. Animals that died from the effects of chlorfenvinphos did so within 12 hours of dosing; no further deaths were noted up to 72 hours after dosing. The chlorfenvinphos LD_{50} value for rats pretreated with dieldrin (0.2 mg/kg) was estimated to be 157 mg/kg; thus, dieldrin pretreatment induced a 10-fold protective effect against the acute toxicity of the organophosphate (Hutson and Wright 1980). After prolonged (105 minutes) exhibition of cholinergic signs, death occurred at the 20 mg/kg dose when Sprague-Dawley rats were orally administered single doses of 1.25, 5, or 20 mg/kg chlorfenvinphos (Takahashi et al. 1991). In another study with Wistar rats, the acute oral LD_{50} was significantly decreased in rats fed a protein-deficient (4.5%) diet during the 60 days of administration. The 60-day rat LD_{50} value was reduced from a control value of 23.0 mg/kg to 7.4 mg/kg in males and 25.5 mg/kg to 10.2 mg/kg in females (Puzynska 1984). Pretreatment of male Fischer 344 rats with 15 mg/kg chlorfenvinphos followed by a further 15 mg/kg dose significantly reduced the male oral LD_{50} for chlorfenvinphos from a positive control value of 34.3 mg/kg to 105.6 mg/kg; about 3.08-fold ($P<0.05$). Deaths occurred between 2 hours and 1 day after oral administration of chlorfenvinphos (Ikeda et al. 1992).

Chlorfenvinphos appears to be less toxic to mice than to rats. In a lethality study with BDF1 mice of both sexes, the oral LD_{50} and maximum tolerated dose (MTD) for chlorfenvinphos (suspended in methylcellulose) were estimated to be 148 mg/kg and 109 mg/kg, respectively. The mice were evaluated 5 days after intragastric administration of the chlorfenvinphos doses (Kowalczyk-Bronisz et al. 1992). A mouse acute oral LD_{50} of 100–200 mg/kg for chlorfenvinphos was cited from an earlier study in which male CFI mice were used (Hutson and Logan 1986).

A rabbit acute oral LD_{50} of >500–1,000 mg/kg for chlorfenvinphos was cited from an earlier study in which male New Zealand White rabbits were used (Hutson and Logan 1986).

A dog acute oral LD_{50} of >5,000 mg/kg for chlorfenvinphos was cited from an earlier study in which female Beagle dogs were used (Hutson and Logan 1986).

In prolonged exposures, no significant effect on mortality and survival was reported for rats and dogs given chlorfenvinphos in the diet at doses as high as 90 mg/kg/day for 12 weeks or 24 mg/kg/day for 104 weeks (Ambrose et al. 1970).

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

2.2.2.2 Systemic Effects

No studies were located regarding the hematological, cardiovascular, gastrointestinal, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, body weight, or other systemic effects in humans following acute-, intermediate-, or chronic-duration oral exposure to chlorfenvinphos. Existing human data on the respiratory and neurological effects are limited to acute-duration exposures. No studies were located regarding ocular effects in animals following acute-, intermediate-, or chronic-duration oral exposure to chlorfenvinphos.

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

Respiratory Effects. No human studies were located that reported direct effects on the respiratory system following oral exposure to chlorfenvinphos. Respiratory effects in humans following accidental

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

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
	ACUTE EXPOSURE						
	Death						
1	Rat (Wistar)	once (GO)				9.7 M (LD_{50})	Ambrose et al. 1970
2	Rat (Wistar)	once (GO)				23 M (LD_{50})	Hutson and Logan 1986
3	Rat (Carworth Farm E)	once (GO)				15.4 M (LD_{50})	Hutson and Wright 1980
4	Rat (Fischer- 344)	once (GO)				34.3 M (LD_{50})	Ikeda et al. 1992
5	Rat (Sprague-Dawley)	once (NS)				20 (100% mortality)	Takahashi et al. 1991
6	Mouse (BDF1)	once (GO)				148 (LD_{50}) 109 (MTD)	Kowalczyk-Bronisz et al. 1992
7	Dog (Mongrel)	once (GO)				50.5 (LD_{50})	Ambrose et al. 1970
8	Rabbit (NS)	once (GO)				300 M (LD_{50})	Ambrose et al. 1970

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

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
	Systemic						
9	Rat (Wistar)	10 d *ad libitum* (F)	Bd Wt	2.4 F			Barna and Simon 1973
			Metabolic		2.4 F (30% increased gastrointestinal absorption of glucose; 32% decreased gastrointestinal absorption of Na^+)		
10	Rat (Fischer- 344)	once (GO)	Hepatic		15 M (30% increased P-450 activity; 40% increased aminopyrine-*N*-demethylase activity; 27% increased aniline hydroxylase activity)		Ikeda et al. 1991
11	Rat (Wistar)	once (GO)	Endocr		6.15 M (>300% elevation of plasma corticosteroids)		Osicka-Koprowska et al. 1984
12	Rat (Wistar)	once (GO)	Hepatic		30 M (51% increase in serum sorbitol dehydrogenase; 37% to 109% increase in liver aromatic aminotransferase activity)		Puzynska 1984
					30 F (105% increase in serum sorbitol dehydrogenase; 32% decrease in serum glucose-6-phosphate isomerase)		

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

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
	Neurological						
13	Rat (Wistar)	10 d *ad libitum* (F)			2.4 [b] F (52% inhibition of plasma cholinesterase activity; 30% inhibition of erythrocyte activity)		Barna and Simon 1973
14	Rat (Wistar)	once (GO)			13 M (20% decrease in the noradrenaline level)		Brzezinski 1978
15	Rat (Fischer- 344)	once (GO)				30 M (salivation, fasciculation, lacrimation, tremors, irregular respiration, and prostration)	Ikeda et al. 1992
16	Rat (Wistar)	once (GO)			6.2 M (10-30% decrease in brain and blood cholinesterase activity)		Osicka-Koprowska et al. 1984
17	Rat (Wistar)	once (GO)		1 M	2 M (38% decrease in brain cholinesterase activity)		Osumi et al. 1975

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

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less Serious (mg/kg/day)		Serious (mg/kg/day)	
18	Rat (Wistar)	once (GO)			30 M	(87% inhibition of serum acetylcholinesterase activity; 58% inhibition of brain acetylcholin-esterase activity; 20% increase in brain aromatic amino-transferase activity)		Puzynska 1984
					30 F	(93% inhibition of serum acetylcholinesterase activity; 39% inhibition of brain acetylcholinesterase activity; 30% to 42% increase in brain aromatic amino-transferase activity; 32% elevation of brain glucosephosphate isomerase)		
19	Rat (Sprague-Dawley)	once (NS)		1.25			5 M (>90% reduction of erythrocyte cholinesterase activity; fasciculations, twitches, convulsions, chromodacryorrhea, exophthalmos, gasping, lacrimation, prostration, salivation, Straub tail reflex, urination)	Takahashi et al. 1991

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

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
	INTERMEDIATE EXPOSURE						
	Systemic						
20	Rat (Wistar)	12 wk *ad libitum* (F)	Resp	90 M			Ambrose et al. 1970
			Cardio	90 M			
			Gastro	90 M			
			Hepatic	90 M			
			Renal	0.9 M	2.7 M (significant reduction of relative kidney weight)		
				1 F	3 F (significant reduction of relative kidney weight)		
			Endocr	90 M			
			Dermal	90 M			
21	Rat (Wistar)	30 d *ad libitum* (F)	Bd Wt	0.8 F			Barna and Simon 1973
			Metabolic		0.8 F (12% increased gastrointestinal absorption of glucose; 23% decreased gastrointestinal absorption of Na^+)		
22	Dog (Mongrel)	12 wk *ad libitum* (F)	Resp	10 M			Ambrose et al. 1970
			Cardio	10 M			
			Gastro	10 M			
			Musc/skel	10 M			
			Hepatic	10 M			
			Renal	10 M			
			Endocr	10 M			
			Bd Wt	10 M			
			Other	10 M			

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

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference
	Immunological/Lymphoreticular						
23	Mouse (C57BL/ 6N)	90 d 1 x/d (GO)			1.5 [c] (190% increase of spleen endogenous colonies; 162% increase of spleen exogenous colonies; 50% reduction in thymus weight)		Kowalczyk-Bronisz et al. 1992
				1.5	3 (15% decrease in plaque-forming cells; 25% reduction in EA rosettes-forming cells)		
24	Rabbit (NS)	90 d 1 x/d (GO)			10 (16% elevation of serum hemagglutinin; 66% elevation of hemolysin activity; increased antibody production)		Roszkowski 1978
	Neurological						
25	Rat (Wistar)	12 wk *ad libitum* (F)		2.7	9 M (significant depression of plasma and erythrocyte cholinesterase activity)		Ambrose et al. 1970
				3	10 F (significant depression of plasma and erythrocyte cholinesterase activity)		
26	Rat (Sprague-Dawley)	3-6 mo (F)			10.5 (38% inhibition of whole blood acetylcholinesterase)		Maxwell and LeQuesne 1982
27	Rat (Sprague-Dawley)	1 yr (F)			10.5 (abnormal response to muscle stimuli)		Maxwell and LeQuesne 1982

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

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
28	Rat (Sprague-Dawley)	3 mo (F)			10.5 (abnormal response to muscle stimuli)		Maxwell and LeQuesne 1982
	Reproductive						
29	Rat (Wistar)	3 gen *ad libitum* (F)				3 F (50% decrease in fertility in F/2 generation)	Ambrose et al. 1970
	Developmental						
30	Rat (Wistar)	12 wk *ad libitum* (F)		2.7 3	9M (significant depression of growth) 10 F (significant depression of growth)		Ambrose et al. 1970
31	Rat (Wistar)	3 gen *ad libitum* (F)				10 (66% decrease in pup viability; 46% decrease in lactation index)	Ambrose et al. 1970

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

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
	CHRONIC EXPOSURE						
	Systemic						
32	Rat (Wistar)	104 wk *ad libitum* (F)	Resp	21 M			Ambrose et al. 1970
			Cardio	21 M			
			Gastro	21 M			
			Hemato	21 M			
			Musc/skel	21 M			
			Hepatic	2.1	7M (significant increase in relative liver weight)		
			Renal	21 M			
			Endocr	21 M			
			Dermal	21 M			
			Bd Wt	2.4	8 F (significant decrease in body weight gain)		
33	Dog (Beagle)	104 wk *ad libitum* (F)	Cardio	10 M 50 F			Ambrose et al. 1970
			Hemato	10 M 50 F			
			Hepatic	10 M 50 F			
			Renal	10 M 50 F			
			Endocr	10 M 50 F			
			Bd Wt	10 M 50 F			

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

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
	Neurological						
34	Rat (Wistar)	104 wk *ad libitum* (F)			0.7[d] M (45% inhibition of plasma cholinesterase activity; 33% inhibition of erythrocyte cholinesterase activity) 0.8 F (48% inhibition of plasma cholinesterase activity; 20% inhibition of erythrocyte cholinesterase activity)		Ambrose et al. 1970
35	Dog (Beagle)	104 wk *ad libitum* (F)		2 10	10 M (36% inhibition of erythrocyte cholinesterase) 50 F (36% inhibition of erythrocyte cholinesterase)		Ambrose et al. 1970

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

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
Reproductive							
36	Rat (Wistar)	104 wk *ad libitum* (F)		21 M			Ambrose et al. 1970

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

[b]Used to derive an acute oral minimal risk level (MRL) of 0.002 mg/kg/day. Dose divided by an uncertainty factor of 1000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

[c]Used to derive an intermediate oral MRL of 0.002 mg/kg/day. Dose divided by an uncertainty factor of 1000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

[d]Used to derive a chronic oral MRL of 0.0007 mg/kg/day. Dose divided by an uncertainty factor of 1000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

Bd Wt = body weight; d = day(s); EA = erythrocyte acetylcholinesterase; Endocr = endocrine; F = female; (GO) = gavage, oil; gen = generation; Hemato = hematological; Ld = lactation day(s); LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; x = time(s); yr = year(s)

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

Acute (≤14 days)

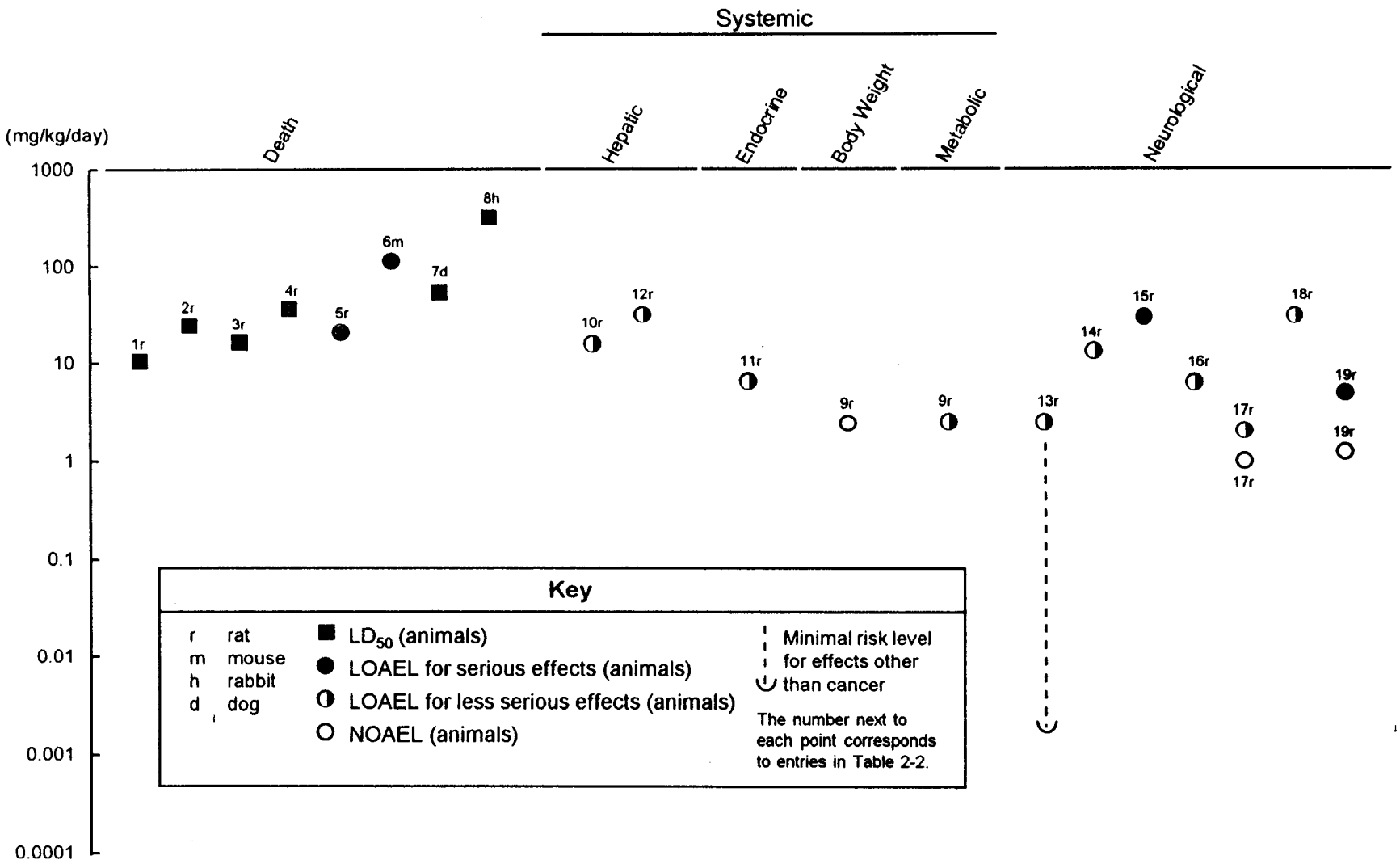


Figure 2-2. Levels of Significant Exposure to Chlorfenvinphos - Oral (cont.)

Intermediate (15-364 days)

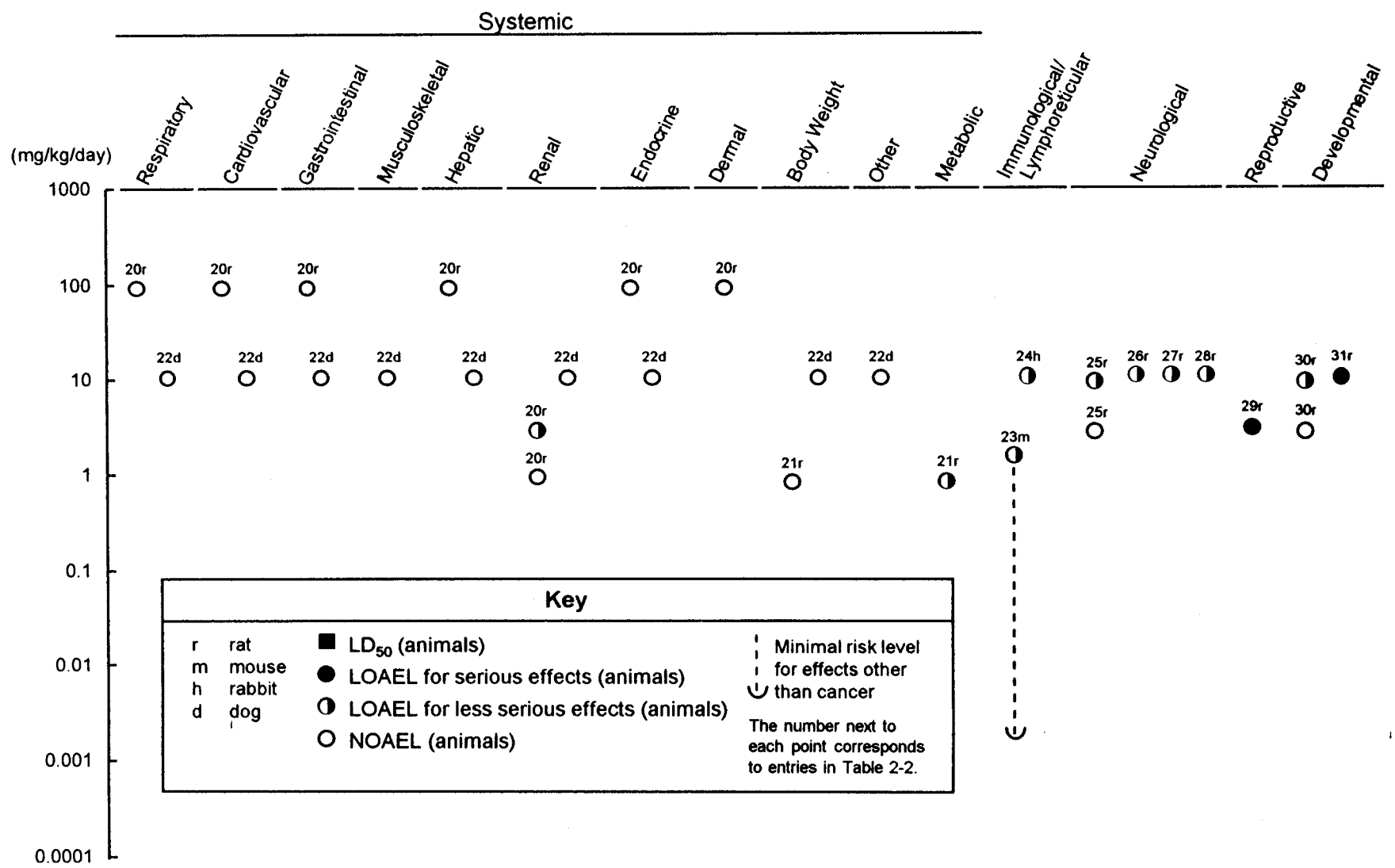
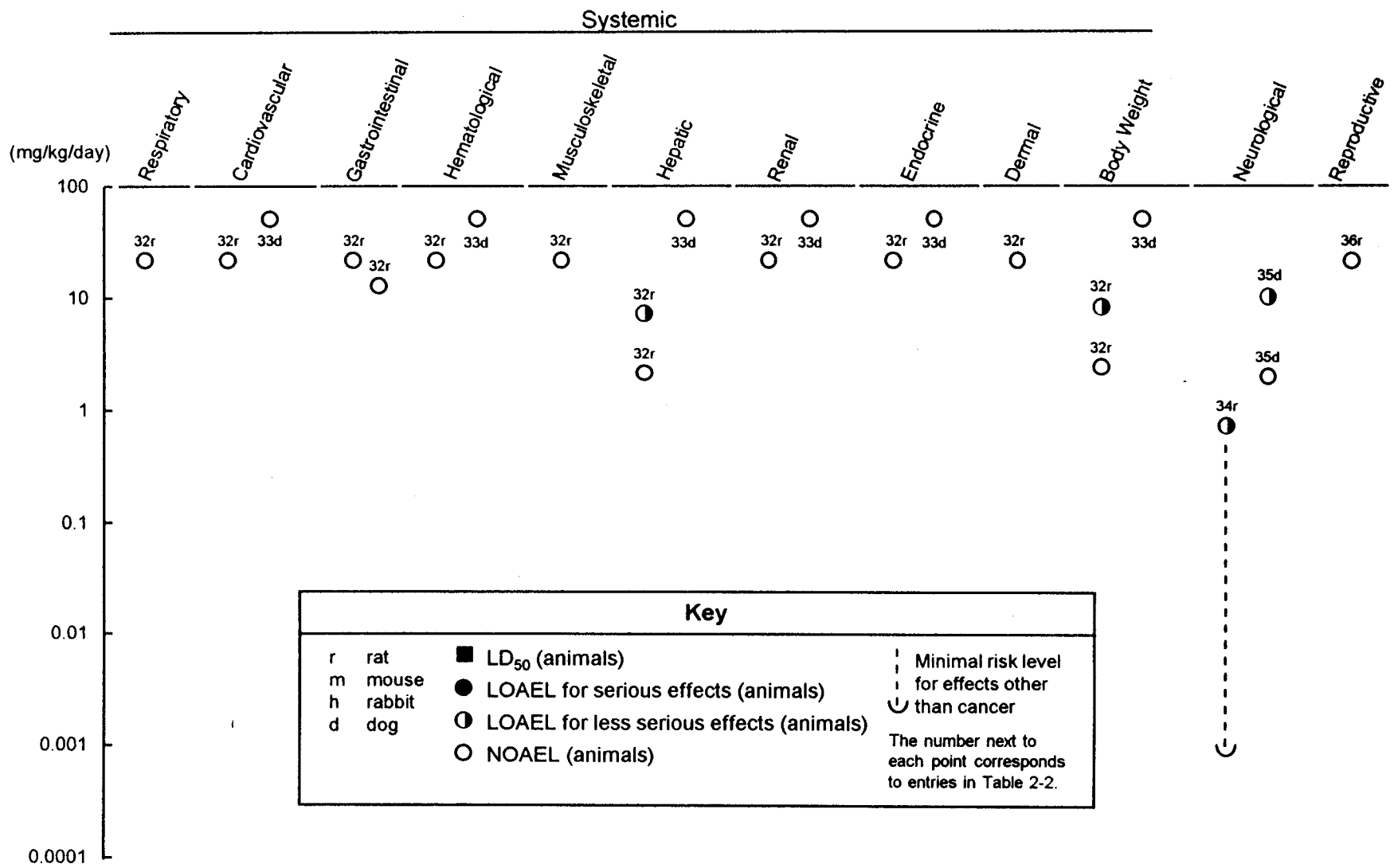


Figure 2-2. Levels of Significant Exposure to Chlorfenvinphos - Oral (cont.)

Chronic (≥365 days)



ingestion of a mange-mite medication containing organic phosphate or intentional ingestion of the preparation Enolofos®, which contains 50% chlorfenvinphos, in suicide attempts reported in two clinical reports stemmed from central cholinergic disturbances (Cupp et al. 1975; Pach et al. 1987).

In animal studies, no effects on the respiratory system were noted in rats and dogs orally administered chlorfenvinphos at doses as high as 100 mg/kg/day (rats) or 50 mg/kg/day (dogs) for 12 weeks; or in rats at doses as high as 24 mg/kg/day for 104 weeks (Ambrose et al. 1970).

Cardiovascular Effects. Evidence from animal studies indicates that chlorfenvinphos is not directly toxic to the cardiovascular system but may modulate the function of the cardiovascular system via its effect on the central nervous system. Relative heart-to-body weight ratios of weanling albino (Wistar) rats were not significantly altered when administered daily chlorfenvinphos doses of 0.27, 0.9, 2.7, 9, or 90 mg/kg/day (males): 0.3, 1, 3, 10, or 100 mg/kg/day (females) in the diet for 12 weeks. Similarly, no effects on relative heart-to-body weight ratios were reported for Wistar rats given chlorfenvinphos at doses of 0.7, 2.1, 7, or 21 mg/kg/day (males) or 0.8, 2.4, 8, or 24 mg/kg/day (females) in the diet for 104 weeks in another part of the same study (Ambrose et al. 1970). However, cardiovascular function was not assessed in these studies. No gross or microscopic histopathology in heart tissues or changes in relative heart weights were evident in mongrel dogs administered daily chlorfenvinphos doses of 0.01, 0.1, 1, or 10 mg/kg/day (males) or 0.05, 0.5, 5, or 50 mg/kg/day (females) for 12 weeks (Ambrose et al. 1970). However, cardiovascular function was not assessed in this study. Similarly, no effects on heart-to-body weight ratios were reported for Beagle dogs given chlorfenvinphos at doses of 0.3, 2, or 10 mg/kg/day (males), or 1.5, 10, 50 mg/kg/day (females) in the diet for 104 weeks in another part of the same study (Ambrose et al. 1970).

A study concluded that the alteration of brain and liver activities of the aromatic amino acid transferases may be due to the inhibitory effect of chlorfenvinphos on noradrenaline (norepinephrine) activity (Puzynska 1984). In other studies, chlorfenvinphos was shown to independently inhibit noradrenaline (norepinephrine) activity *in vivo* in rats rapidly (3 hours) at doses as low as 4 mg/kg (Brzezinski 1978; Osumi et al. 1975). On this basis, it has been postulated that chlorfenvinphos may also act via central noradrenergic mechanisms, disturbing the dynamic equilibrium between the rate of formation and utilization of noradrenaline (norepinephrine). It was postulated that this action via central noradrenergic mechanisms by chlorfenvinphos may be responsible for the changes in blood pressure observed in other studies after chlorfenvinphos intoxication (Brzezinski 1978).

Gastrointestinal Effects. No gross or microscopic histopathology was evident in the gastrointestinal tract of rats and dogs given oral doses of up to 100 mg/kg/day (female rats) or 50 mg/kg/day (female Beagle dogs) chlorfenvinphos for 12 weeks (Ambrose et al. 1970). Similarly, no gross or microscopic histopathology was evident in the stomach, or small and large intestine of weanling albino (Wistar) rats of both sexes chronically (104 weeks) given daily dietary chlorfenvinphos doses of 0.7, 2.1, 7, or 21 mg/kg/day (males) or 0.8, 2.4, 8, or 24 mg/kg/day (females) (Ambrose et al. 1970).

Hematological Effects. Information on the hematological effects from oral exposure to chlorfenvinphos is limited. Investigations conducted to evaluate the serological effects of chlorfenvinphos on 4 groups of rabbits of both sexes at a doses of 10 mg/kg for 90 days found significant increases of hemolysin and hemagglutinin serum titer, as compared to controls. Hemagglutinin and hemagglutinin IgG titer were increased by 16 and 18%, respectively, while hemolysin and hemolysin IgG titer were elevated by 66 and 102%, respectively (Roszkowski 1978). In a 104-week oral study in Beagle dogs, no effects were reported on monitored hematological parameters (hemoglobin, hematocrit, total/differential leucocyte counts) at doses of 10 mg/kg/day for male dogs and 50 mg/kg/day for female dogs (Ambrose et al. 1970). Similarly, no effects were seen on monitored hematological parameters (hemoglobin, hematocrit, total/differential leucocyte counts) in rats orally administered chlorfenvinphos (21 mg/kg/day, males; 24 mg/kg/day, females) for 104 weeks (Ambrose et al. 1970).

Musculoskeletal. In animal studies, no gross or microscopic histopathology changes in the musculoskeletal system were observed in dogs orally administered chlorfenvinphos at doses as high as 50 mg/kg/day (females) for 12 weeks, or in rats at doses as high as 24 mg/kg/day (females) for 104 weeks (Ambrose et al. 1970).

Hepatic Effects. The limited information on the hepatic effects of chlorfenvinphos indicates that the substance is not significantly hepatotoxic by the oral route in acute- or intermediate-duration exposure. No changes in liver weight (relative to body weight) were reported in Fischer 344 rats given a single oral chlorfenvinphos dose of 15 mg/kg. However, P-450 activity was increased by 30%. Aminopyrine-*N*-demethylase and aniline hydroxylase activities were also increased by 40 and 27%, respectively (Ikeda et al. 1991). Mature Wistar rats of both sexes were kept on diets containing 4.5% of casein (low-protein diet), 26% of casein (optimal-protein diet), or standard (Murigran) diet and 30 mg/kg/day of chlorfenvinphos for 30 days to evaluate the effects of oral chlorfenvinphos exposure on serum activity of sorbitol dehydrogenase (SDH), and on brain and liver activities of the aromatic amino acids transferases L-phenylalanine

aminotransferase (Phen AT), L-tyrosine aminotransferase (Tyr AT), and L-tryptophan aminotransferase (Try AT). Both male and female rats exhibited disturbances in the activities of these enzymes. Chlorfenvinphos significantly decreased the activities of Phen AT (females = 41%, P<0.01), Tyr AT (females = 30, P<0.01), and Try AT (males = 20%, females = 42%, P<0.01) in the brain of the rats. Concomitantly, chlorfenvinphos significantly increased the activities of Phen AT (males = 37%, females = 54%, P<0.01), Tyr AT (males = 75, female = 150%, P<0.001)), and Try AT (males = 109%, females = 62%, P<0.001) in the liver of the rats, and developed a rise in the activity of sorbitol dehydrogenase (SDH) in serum (4.5% protein diet, males = NS, females = 94%; standard diet, males = 51%, females = 105%). There was also a 32% decrease in serum glucose-6-phosphate isomerase in females. In general, these changes were more pronounced in female rats fed standard Murigran diet. However, no changes in protein levels were reported in the study (Puzynska 1984).

In other animal studies, no gross or microscopic histopathology in liver tissues was evident in weanling albino (Wistar) rats administered daily chlorfenvinphos doses of 0.27, 0.9, 2.7, 9, or 90 mg/kg/day (males) or 0.3, 1, 3, 10, or 100 mg/kg/day (females) in the diet for 12 weeks (Ambrose et al. 1970). In a chronic study (104 weeks) in which this strain of rats (both sexes) was given daily dietary chlorfenvinphos doses of 0.7, 2.1, 7, or 21 mg/kg/day (males) or 0.8, 2.4, 8, or 24 mg/kg/day (females), increased relative liver weights were observed in males at the 7 mg/kg/day dose level. No gross or microscopic histopathology in the liver tissues examined or changes in relative liver weights were reported at any dose level (Ambrose et al. 1970). Hepatic function was not assessed in this study.

No gross or microscopic liver histopathology or changes in relative liver weights were evident in mongrel dogs administered daily chlorfenvinphos doses of 0.01, 0.1, 1, or 10 mg/kg/day (males) or 0.05, 0.5, 5, or 50 mg/kg/day (females) for 12 weeks (Ambrose et al. 1970). Similarly, no gross or microscopic liver histopathology or significant changes in relative kidney weights were evident in Beagle dogs (2/sex) fed daily dietary chlorfenvinphos doses of 0.3, 2, or 10 mg/kg/day (males), or 1.5, 10, or 50 mg/kg/day (females) for 104 weeks. No adverse effects on liver function as indicated by alterations in serum bromosulfalein (BSP), serum glutamic oxaloacetic transaminase (SGOT), and serum alkaline phosphatase (SAP) or in blood urea nitrogen (BUN) levels were reported for the test animals (Ambrose et al. 1970).

Renal Effects. The relative kidney weight ratios of weanling albino (Wistar) rats administered daily chlorfenvinphos doses of 0.27, 0.9, 2.7, 9, or 90 mg/kg/day (males), or 0.3, 1, 3, 10, or 100 mg/kg/day (females) in the diet for 12 weeks were significantly and irreversibly decreased at

the 2.7 mg/kg/day (males) and 3 mg/kg/day (females) dose levels (Ambrose et al. 1970). However, no quantitative data on the reduction of relative kidney weight were provided in this study. No gross or microscopic histopathology in the kidney and urinary bladder tissues examined or changes in relative kidney weights were evident in Wistar rats of both sexes given daily dietary chlorfenvinphos doses of 0.7, 2.1, 7, or 21 mg/kg/day (males) or 0.8, 2.4, 8, or 24 mg/kg/day (females) in a chronic study (104 weeks) (Ambrose et al. 1970). Renal function was not assessed in either study.

No gross or microscopic histopathology in kidney tissues or changes in relative kidney weights were evident in mongrel dogs administered daily chlorfenvinphos doses of 0.01, 0.1, 1, or 10 mg/kg/day (males) or 0.05, 0.5, 5, or 50 mg/kg/day (females) for 12 weeks (Ambrose et al. 1970). Similarly, no gross or microscopic renal histopathology or significant changes in relative kidney weights were evident in Beagle dogs fed daily dietary chlorfenvinphos doses of 0.3, 2, or 10 mg/kg/day (males), or 1.5, 10, or 50 mg/kg/day (females) for 104 weeks (Ambrose et al. 1970). However, renal function was not assessed in these studies.

Endocrine Effects. A significant increase (>300%) in plasma corticosterone was observed at 1 and 3 hours and in plasma aldosterone from 1 to 6 hours after treatment of male Wistar rats with a single chlorfenvinphos dose of 6.15 mg/kg (50% LD_{50}) by stomach tube. Maximal increase in plasma corticosteroid levels occurred within 1 hour, while the brain cholinesterase activity was only slightly inhibited at that time. The authors surmised that changes in plasma corticosteroids are not related to the decrease of cholinesterase activity in the brain (Osicka-Koprowska et al. 1984). The toxicological significance of these findings is unknown.

No gross or microscopic histopathology was evident in endocrine organs (pancreas, thyroid, adrenal, pituitary) of weanling albino (Wistar) rats or Beagle dogs given oral doses of up to 100 mg/kg/day (female rats) or 50 mg/kg/day (female Beagle dogs) chlorfenvinphos for 12 weeks (Ambrose et al. 1970). Similarly, no gross or microscopic histopathology was evident in endocrine organs (pancreas, thyroid, adrenal, pituitary) of weanling albino (Wistar) rats of both sexes chronically (104 weeks) given daily dietary chlorfenvinphos doses of 0.7, 2.1, 7, or 21 mg/kg/day (males) or 0.8, 2.4, 8, or 24 mg/kg/day (females) (Ambrose et al. 1970).

Dermal Effects. No adverse changes were observed in the skin of weanling albino (Wistar) rats of both sexes given oral doses of up to 100 mg/kg/day (female rats) chlorfenvinphos for 12 weeks (Ambrose et al. 1970). Similarly, no adverse changes were observed in the skin of

weanling albino (Wistar) rats of both sexes chronically (104 weeks) given daily dietary chlorfenvinphos doses of 0.7, 2.1, 7, or 21 mg/kg/day (males) or 0.8, 2.4, 8, or 24 mg/kg/day (females) (Ambrose et al. 1970).

Body Weight Effects. A significant but slightly reversible depression on growth was observed at 9 mg/kg/day (males) or 10 mg/kg/day (females) in weanling albino (Wistar) rats. The rats were administered daily chlorfenvinphos doses of 0.27, 0.9, 2.7, 9, or 90 mg/kg/day (males) or 0.3, 1, 3, 10, or 100 mg/kg/day (females) in the diet for 12 weeks (Ambrose et al. 1970). In an accompanying chronic study (104 weeks) in which this strain of rats (both sexes) was given daily dietary chlorfenvinphos doses of 0.7, 2.1, 7, or 21 mg/kg/day (males) or 0.8, 2.4, 8, or 24 mg/kg/day (females), no consistent difference in body weight gains in males was evident at any dose level tested, as compared to undosed controls. However, chlorfenvinphos exposure caused a significant decrease in body weight gain in females in the 8 and 24 mg/kg/day dose groups from the 26th week until near the end of the study. The decreased body weight gain became statistically insignificant at the end of the study (Ambrose et al. 1970). In another rat study, body weight gains in adult female albino (Wistar) rats were unaffected following orally administered Birlane® (chlorfenvinphos) at a dose of 0 or 2.4 mg/kg/day in the diet for 10 days or 0 or 0.8 mg/kg/day in the diet for 30 days (Barna and Simon 1973).

Likewise, no significant effect on body weight was observed in mongrel dogs following dietary chlorfenvinphos doses of 0.01, 0.1, 1, or 10 mg/kg/day (males), or 0.05, 0.5, 5, or 50 mg/kg/day (females) for 12 weeks (Ambrose et al. 1970). In a chronic study (104 weeks) in which Beagle dogs were given daily dietary chlorfenvinphos doses of 0.3, 2, or 10 mg/kg/day (males), or 1.5, 10, or 50 mg/kg/day (females), no significant changes in body weight were evident at any dose level tested, as compared to undosed controls (Ambrose et al. 1970).

Metabolic Effects. In animal studies, pairs of Carworth Farm E strain male rats orally administered a single [^{14}C]chlorfenvinphos dose of 2.5 or 13.3 mg/kg in olive oil (with or without prior monooxygenase induction with dieldrin) exhibited minimal changes in the metabolic profiles (Hutson and Wright 1980).

Other Systemic Effects. No significant effect on food consumption was observed in weanling albino (Wistar) rats administered daily chlorfenvinphos doses of 0.27, 0.9, 2.7, 9, or 90 mg/kg/day (males) or 0.3, 1, 3, 10, or 100 mg/kg/day (females) in the diet for 12 weeks (Ambrose et al. 1970). In a chronic study (104 weeks) in which weanling albino (Wistar) rats of both sexes were given daily dietary chlorfenvinphos doses of 0.7, 2.1, 7, or 21 mg/kg/day (males) or 0.8, 2.4, 8, or 24 mg/kg/day (females), no consistent

difference in food consumption was evident at any dose level tested, as compared to undosed controls (Ambrose et al. 1970). In dog studies, no significant effect on food consumption was observed in mongrel dogs given dietary chlorfenvinphos doses of 0.01, 0.1, 1, or 10 mg/kg/day (males) or 0.05, 0.5, 5, or 50 mg/kg/day (females) for 12 weeks (Ambrose et al. 1970). Similarly, no significant changes in food consumption were evident at any dose level tested in a chronic study (104 weeks) in which Beagle dogs were given daily dietary chlorfenvinphos doses of 0.3, 2, or 10 mg/kg/day (males) or 1.5, 10, or 50 mg/kg/day (females) (Ambrose et al. 1970).

2.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding the immunological and lymphoreticular effects in humans following acute-, intermediate-, or chronic-duration oral exposure to chlorfenvinphos.

The limited information on the immunological and lymphoreticular effects of chlorfenvinphos indicates that the substance is moderately immunotrophic to the rodent immune system in oral exposures. The gastrointestinal absorption of glucose was increased by 30% over control values in adult female albino (Wistar) rats orally administered Birlane® (chlorfenvinphos) at a dose of 0 or 2.4 mg/kg/day in the diet for 10 days. Similarly, gastrointestinal absorption of glucose was increased by 12% over control values following orally administered Birlane® (chlorfenvinphos) at a dose of 0 or 0.8 mg/kg/day in the diet of Wistar rats for 30 days. The changes in glucose and Na^+ absorption were not considered statistically significant ($P>0.05$) by the investigators (Barna and Simon 1973). It has been observed in other studies that an increased metabolic activity of neutrophils and monocytes during phagocytosis is accompanied by higher consumption of glucose and oxygen. Hydrogen peroxide is then derived from the pentose cycle, NAD-, and NADP-oxidase action (Kolanoski 1977 as cited in Wysocki et al. 1977). The relationship between increased gastrointestinal absorption of glucose and increased glucose utilization is not clear.

In an intermediate-duration dietary study with albino (Wistar) rats, there was a significant and irreversible reduction in relative spleen weight of female rats given $3 mg/kg/day chlorfenvinphos for 12 weeks. However, no gross or microscopic histopathology was e vident in the spleen and bone marrow tissues of the rats upon examination (Ambrose et al. 1970). In dogs, relative spleen weights were unaffected following dietary doses of 10 mg/kg/ day (males) or 50 mg/kg/day (females) to mongrel dogs for 12 weeks. In addition, no gross or microscopic histopathology was evident in the spleen and bone marrow tissues of the

dogs upon examination (Ambrose et al. 1970). No quantitative data on the reduction of relative spleen weights were provided in these studies.

A study was undertaken to evaluate selected serological and cytoimmunological reactions in rabbits subjected to a long-term poisoning with subtoxic oral doses (10 mg/kg in a soya oil solution with a small amount of food) of chlorfenvinphos for 90 days (shortened chronic poisoning). Both control group (soya oil) and treatment group rabbits were immunized with sheep red blood cells 6 days prior to ending the experiment. Chlorfenvinphos treatment significantly elevated serum hemagglutinin level (16%) and hemolysin activity (66%, $P<0.05$) and increased the number of nucleated lymphoid cells producing hemolytic antibody to sheep erythrocytes as compared to controls (treated 906, $P<0.05$ and controls 618). Spleen cytomorphology changes, manifested mainly as transformation of primary follicles into secondary ones with well developed germinal centers, were also observed (Roszkowski 1978). After 90 days of oral intoxication of C57BL/6 mice and (C57BL/6 x DBA/2)F1 (BDF1/Iiw) hybrid mice (6–8-weeks-old) with chlorfenvinphos (suspended in 1% methylcellulose solution), a dose-related decrease in number of hemolysin-producing cells was observed. Plaque-forming cells (PFC) were 58% at the 6 mg/kg dose group and 85% at the 3 mg/kg dose level, as compared to control values. Chlorfenvinphos treatment also caused reduction in E rosettes-forming cell numbers by 30% at the 6 mg/kg dose level, 25% at the 3 mg/kg dose level, and 45% at the 6 mg/kg dose level. Spleen colonies were stimulated as evidenced by the increase of endogenous spleen colonies; and exogenous spleen colonies (CFU-S) increased 190% at the 1.5 mg/kg dose level, 137% at the 6 mg/kg dose level, 162% at 1.5 mg/kg dose level, and 70% at the 6 mg/kg dose level, respectively. When the IgM PFC number was tested 3 weeks later, after the exposure to chlorfenvinphos in the small dose (1.5 mg/kg), an increase (about 40%) in plaque number was observed. There was a 50% reduction in thymus weight at the 1.5 mg/kg dose level, as compared to controls, as well as significant involution of the thymus. IgM levels returned to normal values, indicating the reversible nature of the immunotrophic effect of chlorfenvinphos (Kowalczyk-Bronisz et al. 1992). The LOAEL of 1.5 mg/kg/day, based on adverse immunologic/lymphoreticular effects in this study, was used to derive an intermediate oral MRL of 0.002 mg/kg/day for chlorfenvinphos.

In a chronic-duration (104 weeks) dietary study in which weanling albino (Wistar) rats of both sexes were given daily dietary chlorfenvinphos doses of 0.7, 2.1, 7, or 21 mg/kg/day (males) or 0.8, 2.4, 8, or 24 mg/kg/day (females), no histopathological changes in the spleen or bone marrow were evident at any dose level tested, as compared to undosed controls. In addition, no changes in absolute or relative spleen weights were reported (Ambrose et al. 1970). Likewise, no

histopathological changes in the spleen or bone marrow were evident in Beagle dogs given daily dietary chlorfenvinphos doses of 0.3, 2, or 10 mg/kg/day (males) or 1.5, 10, or 50 mg/kg/day (females) for 104 weeks. In addition, no changes in absolute or relative spleen weights were reported (Ambrose et al. 1970).

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

2.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans following intermediate- or chronic-duration oral exposure to chlorfenvinphos. In humans, chlorfenvinphos inhibits cholinesterase activity in the central and peripheral nervous system when ingested in acute-duration exposures (Cupp et al. 1975; Pach et al. 1987). A 16-year-old white male mistakenly took a full swallow of a mange-mite medication (identified as Dermaton® which contains 25% organophosphate, like chlorfenvinphos, as an active ingredient) prescribed by a veterinarian for his dog. Approximately 90 minutes later, he was hospitalized with symptoms of abdominal cramps, nausea, vomiting, generalized weakness, cold dry skin, constricted pupils, fine generalized muscular twitching, and apprehension. On physical examination, his blood pressure was 152/102, pulse was 96 and irregular, respiration was 24, and rectal temperature was 94.2 EF. While in the emergency room, gastric lavage of 4 L was done and 1 mg of atropine given intravenously. On transfer to the intensive care unit, respiration had increased in rate and depth, skin was warm and slightly diaphoretic, muscle twitching had increased, and he complained of double vision. Emesis persisted. The patient developed hypothermia which lasted for 2 hours. Four and one-half hours after admission, the patient was listless and apathetic, but oriented. Blood analysis showed plasma and erythrocyte cholinesterase levels of 0.3 and 1.1 µmol/minute, respectively. Twenty-four hours later, plasma and erythrocyte cholinesterase levels were 0.8 and 12.7 µmol/minute, respectively. He was given 1 g of pralidoxime intravenously over a 15-minute period, repeated in 1 hour. Forty-eight hours after admission, signs of improvement were evident. The patient was discharged 5 days after admission without residual effects (Cupp et al. 1975). Another clinical report described anticholinesterase symptoms (unconsciousness, absence of tendon reflexes, 80% reduction of erythrocyte acetylcholinesterase activity, 100% reduction in plasma pseudocholinesterase activity, respiratory failure, bronchial tree hypersecretion) in a 29-year-old male patient hospitalized 3 hours after a suicide attempt during which he drank about 50 mL of the preparation ‘Enolofos®, which contains 50% chlorfenvinphos (Pach et al. 1987).

The existing information on neurological effects in animals following acute-, intermediate-, and chronic-duration oral exposures to chlorfenvinphos indicates that the substance causes disruptions in the central and peripheral nervous system in rats manifested as cholinesterase inhibition (Barna and Simon 1973; Osicka-Koprowska et al. 1984; Takahashi et al. 1991) and interference with noradrenaline (norepinephrine) activity in central adrenergic mechanisms (Brzezinski 1978; Osumi et al. 1975). Inhibition of acetylcholinesterase activity results in accumulation of acetylcholine at muscarinic and nicotinic receptors leading to peripheral and central nervous system effects. These effects usually appear within a few minutes to a few hours after exposure depending on the extent of exposure. When chlorfenvinphos was evaluated for acute lethality in animals, death occurred within 12 hours in tested rats, rabbits, and dogs and was usually preceded by the characteristic signs of cholinergic response—salivation, lacrimation, muscle fasciculation, diarrhea, emesis, tremors, irregular respiration, and prostration (Ambrose et al. 1970, Ikeda et al. 1992).

In acute-duration studies, brain cholinesterase activity was strongly inhibited in male Fischer 344 rats (8 weeks old) that were orally treated with 30 mg/kg chlorfenvinphos or pretreated with 15 mg/kg for 24 hours before treatment with 30 mg/kg chlorfenvinphos. This was accompanied by clinical anticholinesterase symptoms which included salivation, fasciculation, lacrimation, tremors, irregular respiration, and prostration which resulted in deaths 1–24 hours after administration of the chlorfenvinphos doses. Although chlorfenvinphos pretreatment did not change the nature of these symptoms, it aggravated the inhibition of brain cholinesterase activity (Ikeda et al. 1992). Plasma and erythrocyte cholinesterase activities were inhibited by 52 and 30%, respectively, at the only tested Birlane® (chlorfenvinphos) dietary dose of 2.4 mg/kg/day administered to adult female albino (Wistar) rats for 10 days (Barna and Simon 1973). The LOAEL of 2.4 mg/kg/day from the 10-day dosing protocol of this study, based on adverse neurological effects in rats was used to derive an acute oral MRL of 0.002 mg/kg/day for chlorfenvinphos.

Brain and erythrocyte acetylcholinesterase, and plasma pseudocholinesterase activities were reduced by $90% and 50%, respectively, following acute oral treatment of male Sprague-Dawley rats with chlorfenvinphos. Rats administered chlorfenvinphos orally attained maximum inhibition of cholinesterase activity in less than two hours. The rats also exhibited cholinergic signs that included fasciculations, twitches, convulsions, chromodacryorrhea, exophthalmos, gasping, lacrimation, prostration, salivation, Straub tail reflex, and urination. The clinical signs lasted for 8 hours. No effects were observed at the 1.25 mg/kg dose level (Takahashi et al. 1994). Cholinesterase activity in the brain of male Wistar rats was unaffected 3 hours after oral administration of 1 mg/kg of chlorfenvinphos. However, at doses of 2 and 4 mg/kg, oral chlorfenvinphos produced marked decreases in the brain cholinesterase activity to 38 and 18% of

control (P<0.001), respectively. The maximum inhibition occurred 3 hours after administration, after which the cholinesterase activity elevated gradually. Activity in the brain decreased steadily with time and, at 72 hours, still remained 67% of control. Erythrocyte acetylcholinesterase activity also decreased after 4 mg/kg of chlorfenvinphos; the lowest level (20%, P<0.001) was attained 3 hours after treatment (Osumi et al. 1975).

Acute oral exposure to chlorfenvinphos was also associated with reversible sleep disturbance in male Wistar rats. Single oral chlorfenvinphos doses up to 1 mg/kg did not affect the awake-sleep cycle in the rats, but spontaneous electroencephalogram (EEG) showed a prominent arousal pattern and appearance of slow wave sleep and parasleep was markedly depressed in doses over 2 mg/kg. The duration of arousal pattern was proportional to the doses but, nonetheless, the awake-sleep cycle returned to control values on the second day; a rebound increase in parasleep occurred on the third day at doses over 4 mg/kg. Atropine at a dose of 2 mg/kg (administered to one rat 2 hours after chlorfenvinphos administration) was antidotal to the chlorfenvinphos-induced disturbance of EEG arousal pattern, depressing the EEG arousal pattern without affecting cholinesterase activity in the brain. As a positive control, physostigmine, a reversible cholinesterase inhibitor, in doses of 0.05 and 0.1 mg/kg, produced an increase in wakefulness during the first hour. Thereafter, the arousal pattern was reduced, and slow wave sleep and parasleep patterns increased, as compared with the control, 3–5 hours after the administration of chlorfenvinphos. According to these investigators, appearance of EEG arousal pattern after treatment with chlorfenvinphos is indicative of central cholinergic activation. A LOAEL of 2 mg/kg with a NOAEL of 1 mg/kg for 38% decrease in brain cholinesterase activity and reversible sleep disturbances was determined in this study (Osumi et al. 1975). This study was not used to calculate an acute oral MRL because it was deemed less appropriate because of the gavage (oral) route of administration. An oral feeding study is preferred for this purpose by ATSDR.

In intermediate-duration studies, plasma and erythrocyte cholinesterase activities were inhibited by 36 and 3%, respectively, at a chlorfenvinphos dose of 0.8 mg/kg/day following dietary administration of 0 or 0.8 mg/kg/day Birlane® (chlorfenvinphos) to adult female albino (Wistar) rats for 30 days (Barna and Simon 1973). However, the change in erythrocyte acetylcholinesterase activity was not considered significant and therefore, this study was not used to derive an intermediate MRL. Similarly, the change in plasma pseudocholinesterase was not used to derive an intermediate MRL because while the inhibition of plasma pseudocholinesterase may have some physiological significance, alteration in the levels of this enzyme is generally regarded more as a biomarker of exposure to organophosphate compounds than as an adverse neurological effect. In other intermediate rat studies, mature rats of both sexes kept on diets containing 4.5%

of casein (low-protein diet), 26% of casein (optimal-protein diet), or standard (Murigran) diet for 30 days exhibited depressed cholinesterase activities following oral exposure to chlorfenvinphos. These alterations consisted of significant inhibition in pseudocholinesterase activity in the serum (4.5% protein diet: males = 97%, females = 96%; standard diet: males = 87%, females = 93%) and brain (4.5% protein diet: males = 83%, females = 87%; standard diet: males = 58%, females = 39%), with more pronounced effects in the brain of female rats fed low-protein diets (87%). The activity of this enzyme returned to normal values 14 days after dosing (Puzynska 1984). Blood and plasma cholinesterase activity was depressed at $9 mg/kg/day (males) or $10 mg/kg/day (females), with a NOAEL of 2.7 mg/kg/day (males) or 3 mg/kg/day (females) following dietary administration of chlorfenvinphos doses of 0.27, 0.9, 2.7, 9, or 90 mg/kg/day (males), or 0.3, 1, 3, 10, or 100 mg/kg/day (females) in the diet to weanling albino (Wistar) rats for 12 weeks. No gross or microscopic histopathology was evident in the brain tissues examined (Ambrose et al. 1970). Similarly, plasma cholinesterase activity was consistently depressed, while erythrocyte cholinesterase activity was sporadically depressed in all dose groups in mongrel dogs administered daily chlorfenvinphos doses of 0.01, 0.1, 1, or 10 mg/kg/day (males) or 0.05, 0.5, 5, or 50 mg/kg/day (females) in the diet for 12 weeks. No gross or microscopic histopathology was evident in the brain and spinal cord tissues examined (Ambrose et al. 1970). Due to incomplete reporting of this study, it was not clear if there was a NOAEL for the inhibition of erythrocyte cholinesterase activity. No quantitative data on the depression of plasma or erythrocyte cholinesterase activity were provided in this study.

In another intermediate-duration study, whole blood cholinesterase activity in Sprague-Dawley rats was markedly inhibited at 3 and 6 months of exposure following exposure to 10.5 mg/kg/day chlorfenvinphos (0.9 and 0.6×10^{-6} mol/minute; control, 2.4 and 1.9×10^{-6} mol/minute, respectively, $P<0.001$). After 3 and 6 months of exposure to chlorfenvinphos, plasma cholinesterase activity was also markedly inhibited (0.4 and 0.4×10^{-6} mol/minute; control, 1.9 and 1.6×10^{-6} mol/minute, respectively; $P<0.001$). After 3–6 months, all 36 Sprague-Dawley rats in this study had repetitive and increasingly diminishing muscle fiber depolarization when given double stimuli. The greatest reduction in peak depolarization occurred with an interval of 4 muscle action potential amplitude (ms) and a large but slightly smaller reduction at 7 ms. These phases probably coincide with refractoriness of some muscle fibers due either to repetitive activity (at 4 ms) or reflex activity (at 7 ms). Double and repetitive stimulation at rates even as low as 0.5 Hz reduced or abolished the prolonged negative potential and repetitive activity. These abnormalities became more marked with time, even on constant dosing. Spike potentials were recorded between the direct response and reflex responses with latency similar to the repetitive activity potential. These electrophysiological abnormalities may be attributable to acetylcholinesterase inhibition at neuromuscular junctions (Maxwell and LeQuesne 1982).

In chronic-duration studies, chlorfenvinphos significantly inhibited both plasma and erythrocyte cholinesterase activities in a dose-dependent manner in weanling albino (Wistar) rats fed daily chlorfenvinphos doses of 0.7, 2.1, 7, or 21 mg/kg/day (males) or 0.8, 2.4, 8, or 24 mg/kg/day (females) mg/kg/day in the diet for 104 weeks. Plasma and erythrocyte cholinesterase activities were inhibited by 48% in females and 45% in males in the first week of treatment and by 20% in females and 33% in males in the fourth week of treatment, respectively, at the lowest dose tested (0.7 mg/kg/day for males and 0.8 mg/kg/day for females). No gross or microscopic histopathology was evident in the brain tissue examined (Ambrose et al. 1970). The LOAEL of 0.7 mg/kg/day, based on adverse neurological effects in rats in this study, was used to derive a chronic oral MRL of 0.0007 mg/kg/day for chlorfenvinphos.

Similarly, chlorfenvinphos significantly inhibited both plasma and erythrocyte cholinesterase activities in Beagle dogs fed daily chlorfenvinphos doses of 0.3, 2, or 10 mg/kg/day (males), or 1.5, 10, or 50 mg/kg/day (females) in the diet (moist) for 104 weeks. Plasma cholinesterase activities were significantly inhibited at all dietary levels through week 39 of the study; 49% inhibition at ambient air concentrations of 0.3 mg/kg/day (males) and 1.5 mg/kg/day (females) groups. During the first 12 weeks, erythrocyte cholinesterase activity was significantly and consistently inhibited (36%) only in the 10 mg/kg/day (males) and 50 mg/kg/day (females) dose groups. No gross or microscopic histopathology was evident in the brain and spinal cord tissues examined (Ambrose et al. 1970).

Besides its cholinergic action, chlorfenvinphos may also act via central noradrenergic mechanisms in rats by accelerating the noradrenaline (norepinephrine) turnover in the brain *in vivo*. Three hours after oral administration of 4 mg/kg of chlorfenvinphos, the brain noradrenaline (norepinephrine) level of male Wistar rats was reversibly decreased by 16% (Osumi et al. 1975). In another study with male Wistar rats, 13 mg/kg of oral chlorfenvinphos decreased cerebral noradrenaline (norepinephrine) level by 20% as compared to the control rats. Other rats that received oral chlorfenvinphos 30 minutes after pretreatment with disulfiram injection (400 mg/kg intraperitoneally) as positive controls exhibited a 50% decrease of cerebral noradrenaline (norepinephrine); the level was observed in time intervals of 1–3 hours, peaking at 89% decrease after 6 hours. Based on these observations, it was suggested that chlorfenvinphos accelerates the rate of noradrenaline (norepinephrine) disappearance from the rat brain *in vivo*. Thus, besides being a cholinergic agent, chlorfenvinphos may also act via central noradrenergic mechanisms, disturbing the dynamic equilibrium between the rate of formation and utilization of noradrenaline (norepinephrine). It was postulated that this action via central noradrenergic mechanisms by chlorfenvinphos may be responsible for the changes in blood pressure observed in other studies after chlorfenvinphos intoxication (Brzezinski 1978).

Sprague-Dawley rats fed chlorfenvinphos doses of 10.5 mg/kg/day for 1 year had repetitive and increasingly diminishing muscle fiber depolarization when given double stimuli. The greatest reduction in peak depolarization occurred with an interval of 4 muscle action potential amplitude (ms) and a large but slightly smaller reduction at 7 ms. These phases probably coincide with refractoriness of some muscle fibers due either to repetitive activity (at 4 ms) or reflex activity (at 7 ms). These electrophysiological abnormalities may be attributable to acetylcholinesterase inhibition at neuromuscular junctions (Maxwell and LeQuesne 1982).

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

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans following acute-, intermediate-, or chronic-duration oral exposure to chlorfenvinphos.

No studies were located regarding reproductive effects in animals following acute-duration oral exposure to chlorfenvinphos. The limited information on the reproductive toxicity of chlorfenvinphos indicates that chlorfenvinphos may interfere with the reproductive competence of rats. In a 3-generation reproductive study, chlorfenvinphos induced adverse reproductive effects (decreased fertility and maternal body weight gain) at a LOAEL of 2.7 mg/kg/day in albino (Wistar) rats given chlorfenvinphos at a doses of 2.7, 9, or 27 mg/kg/day (males) or 3, 10, or 30 mg/kg/day (females) in the diet for 11 weeks. The chlorfenvinphos dosed rats were mated for 20 days to produce an F/1a generation and remated, 10 days after weaning of the F/1a pups, to produce an F/1b generation. Each generation was fed the chlorfenvinphos doses for 11 weeks before mating. The study reported decreased maternal body weights of 3, 5, and 11%, respectively, for F/0 parental generation animals fed 0, 3, or 10 mg/kg/day; 9, 2, and 19%, respectively, for F/1 parental generation animals fed 0, 3, or 10 mg/kg/day; and 14 and 10%, respectively, for F/2 parental generation animals fed 0 or 3 mg/kg/day chlorfenvinphos. The changes in maternal body weight gain were not considered significant. However, fertility (pregnancy/mating x 100) was decreased by 49% in the F/1b parents at the 10 mg/kg/day dose level. In the F/2b parents, fertility was decreased by 50% at the 3 mg/kg/day dose level and by 84% in the 10 mg/kg/day dose level. No gross or microscopic histopathology was evident in male and female gonads examined. No adverse effects on gestation were noted at any exposure levels (Ambrose et al. 1970).

In intermediate-duration studies, chlorfenvinphos had no effect on relative testes weight at any of the doses tested in weanling albino (Wistar) rats administered daily chlorfenvinphos doses of 90 mg/kg/day (males) or 100 mg/kg/day (females) in diet for 12 weeks. No gross or microscopic histopathology was evident in any of the gonads (Ambrose et al. 1970).

In chronic-duration studies, no gross or microscopic gonad histopathology in either sex or changes in the relative weights of the testes in males were reported in albino (Wistar) rats administered daily chlorfenvinphos doses of 21 mg/kg/day (males) or 24 mg/kg/day (females) in the diet for 104 weeks (Ambrose et al. 1970). Similarly, no gross or microscopic gonad histopathology in either sex or changes in the relative weights of the testes in males were reported in mongrel dogs administered daily chlorfenvinphos doses of 0.01, 0.1, 1, or 10 mg/kg/day (males) or 0.05, 0.5, 5, or 50 mg/kg/day (females) in the diet for 12 weeks (Ambrose et al. 1970). Reproductive function was not evaluated in these studies.

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

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans following acute-, intermediate-, or chronic-duration oral exposure to chlorfenvinphos.

No studies were located regarding developmental effects in animals following acute-duration oral exposures in animals. Oral chlorfenvinphos interfered with development in intermediate- and chronic-duration exposures as well as multigenerational studies in animals. Weanling albino (Wistar) rats administered daily dietary doses of chlorfenvinphos (0.27, 0.9, 2.7, 9, or 90 mg/kg/day for males; 0.3, 1, 3, 10, or 100 mg/kg/day for females) for 12 weeks exhibited slightly reversible but significant depression of growth at doses of 9 mg/kg/day (males) or 10 mg/kg/day (females). No quantitative data on the depression of growth were provided in the study (Ambrose et al.1970). In a chronic feeding study (104 weeks) in which weanling albino (Wistar) rats were given chlorfenvinphos in the diet at doses of 0.7, 2.1, 7, or 21 mg/kg/day (males) or 0.8, 2.4, 8, or 24 mg/kg/day (females) for 104 weeks, chlorfenvinphos significantly decreased body weight gain of females at the 8 and 24 mg/kg/day ambient air concentrations from the 26th week till towards the end of the study. The decrease in body weight gain was not statistically significant at the end of the study. An increased relative liver weight was observed in males at the 7 mg/kg/day dose level, but no other signs of hepatopathology were

reported. No consistent differences in body weight gains in males, survival of the test animals, food consumption, or mortality were evident at any dose level tested, as compared to undosed controls. No gross or microscopic histopathology was evident in any of the tissues (heart, lungs, liver, kidney, urinary bladder, spleen, stomach, small and large intestine, skeletal muscle, skin, bone marrow, pancreas, thyroid, adrenal, pituitary) examined to indicate teratogenicity. No changes in organ-to-body weight were observed in the heart and kidney (Ambrose et al. 1970). Dietary exposure to chlorfenvinphos exposure caused decreases in viability and lactational indices in a 3-generation reproductive study with albino (Wistar) rats. In this study, the rats were given chlorfenvinphos in the diet at doses of 2.7, 9, or 27 mg/kg/day (males) or 3, 10, or 30 mg/kg/day (females) for 11 weeks. The chlorfenvinphos-dosed rats were mated for 20 days to produce an F/1a generation and remated 10 days after weaning of the F/1a pups to produce an F/1b generation. Each generation was fed the chlorfenvinphos doses for 11 weeks before mating. The pup viability index (pups surviving 5 days/pups born alive x 100) decreased by 66% for F/1b pups at a maternal dose of 10 mg/kg/day. No offspring in the 27 mg/kg/day (males) or 30 mg/kg/day (females) dose group survived beyond the F1 generation. The lactation index was also decreased by 46% in the F/1b offsprings at the 10 mg/kg/day dose level. No gross or microscopic histopathology was evident in any of the tissues examined. There were no gross signs of teratogenicity (Ambrose et al. 1970).

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

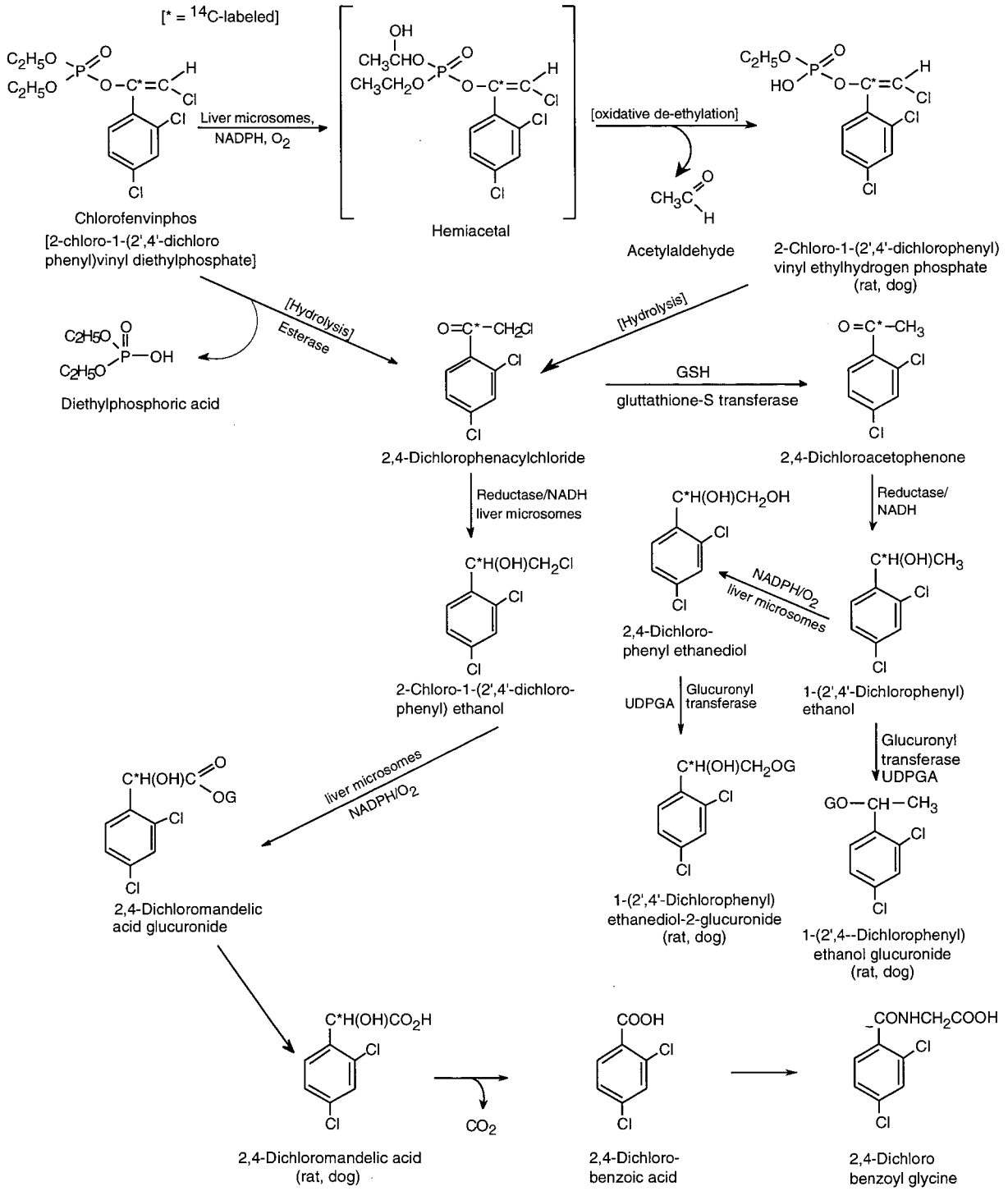
2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in animals after oral exposure to chlorfenvinphos. Other genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

No studies were located regarding carcinogenic effects in humans or animals following oral exposure to chlorfenvinphos.

Figure 2-3. Proposed Mammalian Metabolic Pathway for Chlorfenvinphos



2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death in humans after acute-, intermediate-, or chronic-duration dermal exposure to chlorfenvinphos.

The dermal LD_{50} values in rabbits for undiluted chlorfenvinphos and emulsifiable concentrate have been estimated to be 400 and 1,087 mg/kg, respectively (Ambrose et al. 1970).

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, hepatic, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, body weight, metabolic, or other systemic effects in humans or animals following acute-, intermediate-, or chronic-duration dermal exposure to chlorfenvinphos.

2.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans or animals after acute-, intermediate-, or chronic-duration dermal exposure to chlorfenvinphos.

2.2.3.4 Neurological Effects

No studies were located regarding neurological effects in humans after intermediate-, or chronic-duration dermal exposure to chlorfenvinphos. Dermally-applied chlorfenvinphos formulations significantly inhibited plasma cholinesterase activity in healthy human male volunteers without prior occupational exposure to the substance. Three different chlorfenvinphos formulations were used in this study: 80% weight per volume (w/v) emulsifiable concentrate (EC), mainly chlorfenvinphos and emulsifiers; 24% w/v EC, mainly isometric trimethyl benzenes with 4½% w/v emulsifiers; 25% w/w wettable powder (WP), mainly colloidal silica, florisil, triphenylphosphate, sodium triphosphates, empicol LZ and tamal. The formulations were administered separately in single applications to the forearm skin of 9 adult human males for periods up to 4 hours in doses of 4–10 mg chlorfenvinphos/kg body weight. The 80% EC and 25% EC formulations had no effect on cholinesterase activity levels in the volunteers. Only plasma and erythrocyte cholinesterase activities

of volunteers who received a single application of the 24% emulsifiable concentrate (5 and 10 mg chlorfenvinphos/kg body weight equivalent to a dermal dose of 5 mg/cm^2) were inhibited. Plasma and erythrocyte cholinesterase were inhibited by 53–76% and 9%, respectively (Hunter 1969).

No studies were located regarding neurological effects in animals after intermediate- or chronic-duration dermal exposure to chlorfenvinphos. Acute-duration dermal exposure of laboratory animals to chlorfenvinphos resulted in the depression of plasma cholinesterase without clinical symptoms. Two dogs of unspecified sex treated with a daily dose of 0.3% chlorfenvinphos applied topically to the spinal area from head to tail for 7 days (3 consecutive days, 1 day skipped, then 4 consecutive days) suffered 28% depression in plasma cholinesterase activity by day 8. No clinical signs resulting from cholinesterase depression were observed in any of the dogs (Vestweber and Kruckenberg 1972).

2.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals after acute-, intermediate-, or chronic-duration dermal exposure to chlorfenvinphos.

2.2.3.6 Developmental Effects

No studies were located regarding developmental effects in humans after acute-, intermediate-, or chronic-duration dermal exposure to chlorfenvinphos.

2.2.3.7 Genotoxic Effects

No studies were located regarding genotoxic effects of chlorfenvinphos in humans or animals following dermal exposure.

Other genotoxicity studies for chlorfenvinphos are described in Section 2.5.

2.2.3.8 Cancer

No studies were located regarding carcinogenic effects in humans or animals after dermal exposure to chlorfenvinphos.

2.3 TOXICOKINETICS

The absorption of chlorfenvinphos after inhalation exposure in humans or animals is unknown due to lack of data. Ingested chlorfenvinphos is rapidly absorbed in humans. In animals, oral chlorfenvinphos is also well absorbed to an extent of $67.1–72.5% of the administered dose. Although no information on the dermal absorption of chlorfenvinphos in animals is available, dermally applied chlorfenvinphos formulations were rapidly and extensively absorbed in humans; the rate and extent of absorption was dependent on solvents used in the preparation.

In humans, absorbed chlorfenvinphos is widely distributed and has been detected in compartments that include serum, cervical mucus, follicular and sperm fluids, and milk at levels of up to 0.42 µg/kg for environmental exposures and 15 ng/mL in an acute poisoning case. The distribution of absorbed chlorfenvinphos in animals is unknown because of lack of data.

In humans, absorbed chlorfenvinphos is extensively metabolized via oxidative dealkylation by liver microsomal fractions. Based on human and animal data, the mechanism for oxidative dealkylation of chlorfenvinphos proceeds initially via monooxygenation of the *alpha*-carbon atom of the alkoxy group to produce an unstable hemiacetal which breaks down by oxidative *O*- and *N*-alkylation mechanisms to acetaldehyde and 2-chloro-1-(2,4-dichlorophenyl) vinylethylhydrogen phosphate. Acetophenone produced in the succeeding step is reduced to the alcohol and conjugated by glutathione transferases. Data from animal studies suggest that electrophilic metabolic intermediates or epoxides may be produced in the metabolism of chlorfenvinphos.

The elimination or excretion of chlorfenvinphos in humans and animals after inhalation or dermal exposures is unknown due to lack of data. In humans, ingested chlorfenvinphos is rapidly removed from the blood and passed into the tissues. Chlorfenvinphos has been detected in human sperm fluid and milk samples. In animals, ingested chlorfenvinphos is rapidly eliminated via the urine (70–90%), feces (about 16%), and expired air (0.5%).

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

No studies were located regarding absorption of chlorfenvinphos after inhalation exposure in humans or animals.

2.3.1.2 Oral Exposure

A 29-year-old male was hospitalized with severe respiratory distress and bronchial tree hypersecretion. The patient had ingested about 50 mL of the preparation Enolofos®, which contains 50% chlorfenvinphos, in a suicide attempt. The concentration of chlorfenvinphos in the serum was 300 ng/mL upon admission. This is the only known human chlorfenvinphos poisoning case in which hemoperfusion intervention was employed. The mean clearance of chlorfenvinphos during hemoperfusion was low (68 mL/min) and only 0.42 mg of the poison was recovered. The poison level in the serum was low (15 ng/mL) immediately before the procedure, and gradually rose in successive blood samples indicating that chlorfenvinphos passes fairly easily from the tissues into blood. This may be related to secondary resorption from the digestive tract. The highest value of clearance was observed in the fourth hour of hemoperfusion, in contrast to the observations during hemoperfusion performed for other drug poisoning, when the value of the clearance was lowest in the last hour. The serum chlorfenvinphos levels decreased temporarily within a few hours even prior to the beginning of hemoperfusion, either due to rapid inactivation or to rapid passage into the tissues where the organophosphates accumulate (Pach et al. 1987).

In animal studies, a total of 67.1–72.5% of the administered radioactivity was recovered from the urine of pairs of Carworth Farm E strain male rats administered [^{14}C]chlorfenvinphos orally at doses of 2.5 or 13.3 mg/kg in olive oil (with or without prior monooxygenase induction with dieldrin). This is suggestive of gastrointestinal absorption of $67.1–72.5% (Hutson and Wright 1980).

2.3.1.3 Dermal Exposure

A study conducted to assess the potential dermal absorption of chlorfenvinphos for humans (since the most likely route of entry is through the skin in occupational exposures) applied the substance to the forearm skin of nine healthy human male volunteers who had no prior occupational exposure to the

substance. Three different chlorfenvinphos formulations were used in this study: 80% w/v EC, mainly chlorfenvinphos and emulsifiers; 24% w/v EC, mainly isometric trimethyl benzenes with 4.5% w/v emulsifiers; 25% weight per weight (w/w) wettable powder, mainly colloidal silica, florisil, triphenylphosphate, sodium triphosphates, empicol LZ and tamal. The formulations were administered separately in single applications to the forearm skin of nine adult human males for periods up to 4 hours in doses of 4–10 mg chlorfenvinphos/kg body weight. The 80% EC formulation was applied at doses of 4, 5, 10, or 10 mg/kg body weight for periods of 4, 3.7, 3.8, or 4 hours on approximate skin areas of 36, 38, 320, or 336 cm^2, respectively. The 24% EC formulation was applied at doses of 5, 5, 10, 10, or 10 mg/kg body weight for periods of 4, 3.8, 4.1, 4, or 4 hours on approximate skin areas of 272, 420, 800, 600, or 880 cm^2, respectively. The 25% WP formulation was applied at doses of 5 or 5 mg/kg body weight for periods of 4.2 or 3.8 hours on approximate skin areas of 80 or 70 cm^2, respectively. The extent and ease of absorption or permeability factor of the applied doses depended on the formulation, relating to solvents in the preparation. The dermal absorption of the 80% EC formulation was 1.43, 1.81, 0.06, or 0.32 mg/cm^2/hour, corresponding to applied doses of 4, 5, 10, or 10 mg/kg body weight for periods of 4, 3.7, 3.8, or 4 hours on approximate skin areas of 36, 38, 320, or 336 cm^2, respectively. The dermal absorption of the 24% EC formulation was 0.18, 0.12, 0.14, 0.20, or 0.08 mg/cm^2/hour corresponding to applied doses of 5, 5, 10, 10, or 10 mg/kg body weight for periods of 4, 3.8, 4.1, 4, or 4 hours on approximate skin areas of 272, 420, 800, 600, or 880 cm^2, respectively. The dermal absorption of the 25% wettable powder formulation was 0.35 mg/cm^2/hour corresponding to applied doses of 5 mg/kg body weight for periods of 4.2 hours on approximate skin areas of 80 cm^2. Concentrations of intact chlorfenvinphos of 14.4, 12.0, or 0.5 µg/L were found 24 hours post-exposure in the blood of volunteers for whom chlorfenvinphos absorption rates of 0.20, 0.14, or 0.08 mg/cm^2/hour, respectively, had been estimated. Concentrations of intact chlorfenvinphos of 22, 3.8, <2.8, 2.9, 2.6, 0.2, or <0.7 µg/L were found 8 hours later in the blood of volunteers for whom chlorfenvinphos absorption rates of 1.81, 1.43, 0.32, 0.18, 0.12, and 0.06 mg/cm^2/hour, respectively, had been estimated (Hunter 1969).

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

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

As an organophosphorus compound, chlorfenvinphos is not expected to accumulate in the body tissues because of its expected short half-life. However, chlorfenvinphos was found in some of the

41 specimens of cervical mucus, follicular and sperm fluids, and human milk that were examined in Germany. Chlorfenvinphos levels of 13.66, 1.69, 2.02, and 1.89 µg/kg were detected in 4 of the 11 samples of cervical mucus. Chlorfenvinphos levels of 0.42 µg/kg were detected in 1 of the 10 sperm fluid samples and 1 of the 10 human milk samples, respectively. The detection of chlorfenvinphos in the cervical mucus, which showed the highest levels, was unexpected because it was believed to be the most unlikely site for accumulation in the body. It was suggested that a connection exists between the activities of the cervical glands of the endocervix and the appearance of some pesticides in the cervical mucus which might show a new way for accumulation. Accordingly, the data indicate that organophosphorus environmental pollutants, like chlorfenvinphos, can appear in the human reproductive organs, exposing even germ-cells and, thus, present a risk of interference with the process of reproduction (Wagner et al. 1990). Since the data were generated from environmental exposure, the combined route of exposure may include inhalation.

No studies were located regarding the distribution of chlorfenvinphos after inhalation exposure in animals.

2.3.2.2 Oral Exposure

Although chlorfenvinphos is a hydrophilic substance, it has hitherto not been widely found in human tissues because it is not expected to persist in these tissues. As an organophosphorus compound, chlorfenvinphos is not expected to accumulate in the body tissues because of its expected short half-life. However, chlorfenvinphos was found in some of the 41 specimens of cervical mucus, follicular and sperm fluids, and human milk that were examined. Chlorfenvinphos levels of 13.66, 1.69, 2.02, and 1.89 µg/kg were detected in 4 of the 11 samples of cervical mucus. Chlorfenvinphos levels of 0.42 µg/kg were detected in 1 of the 10 sperm fluid samples and 1 of the 10 human milk samples, respectively. The detection of chlorfenvinphos in the cervical mucus, which showed the highest levels, was unexpected because it is the most unlikely compartment for accumulation in the body. It was suggested that a connection exists between the gland's activities and the appearance of some pesticides in the cervical mucus, which might show a new way for accumulation. Accordingly, these data indicate that new environmental pollutants, like chlorfenvinphos, can appear in the human organs, exposing even germ-cells and, thus, present a risk of adverse effects on reproduction (Wagner et al. 1990). Since the data were generated from environmental exposure, the combined route of exposure possibly include oral.

A 29-year-old male was hospitalized with severe respiratory distress and bronchial tree hypersecretion. The patient had ingested about 50 mL of the preparation Enolofos®, which contains

50% chlorfenvinphos, in a suicide attempt. The concentration of chlorfenvinphos in the serum was 300 ng/mL upon admission. This is the only human chlorfenvinphos poisoning case in which hemoperfusion intervention was employed. The mean clearance of chlorfenvinphos during hemoperfusion was low (68 mL/minute) and only 0.42 mg of the poison was recovered. The poison level in the serum was low (15 ng/mL) immediately before the procedure, and gradually rose in successive blood sampling indicating that chlorfenvinphos passes fairly easily from the tissues into blood. This may be related to secondary resorption from the digestive tract. The highest value of clearance was observed in the fourth hour of hemoperfusion, in contrast to the observations during hemoperfusion performed for other drug poisoning, when the value of the clearance was lowest in the last hour. The serum chlorfenvinphos levels decreased temporarily within a few hours even prior to the beginning of hemoperfusion, either due to rapid inactivation or to rapid passage into the tissues where the organophosphates accumulate (Pach et al. 1987).

No studies were located regarding the distribution of chlorfenvinphos in animals after oral exposure.

2.3.2.3 Dermal Exposure

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

Although chlorfenvinphos is a hydrophilic substance, it has hitherto not been widely found in human tissues because it is not expected to persist in these tissues. As an organophosphorus compound, chlorfenvinphos is not expected to accumulate in the body tissues because of its expected short half-life. However, chlorfenvinphos was found in some of the 41 specimens of cervical mucus, follicular and sperm fluids, and human milk that were examined. Chlorfenvinphos levels of 13.66, 1.69, 2.02, and 1.89 µg/kg were detected in 4 of the 11 samples of cervical mucus. Chlorfenvinphos levels of 0.42 µg/kg were detected in 1 of the 10 sperm fluid samples and 1 of the 10 human milk samples, respectively. The detection of chlorfenvinphos in the cervical mucus, which showed the highest levels, was unexpected because it is the most unlikely compartment for an accumulation in the body. It was suggested that a connection exists between the gland activities and the appearance of some pesticides in the cervical mucus, which might show a new way for accumulation. Accordingly, this data indicate that new environmental pollutants, like chlorfenvinphos, can appear in the human organs, contacting even germ-cells and, thus, present risk of adverse effects on reproduction (Wagner et al. 1990). Since the data were generated from environmental exposure, the combined route of exposure possibly includes dermal.

2.3.3 Metabolism

An adapted scheme for the mammalian metabolic pathway of chlorfenvinphos (Akintonwa 1984; Akintonwa 1985; Akintonwa and Itam 1988; Hunter et al. 1972; Hutson and Millburn 1991; Hutson and Wright 1980) is presented in Figure 2-3.

2.3.3.1 Inhalation Exposure

No studies were located regarding the metabolism of chlorfenvinphos after inhalation exposure in humans or animals.

2.3.3.2 Oral Exposure

In humans, the rates of chlorfenvinphos de-ethylation by liver microsomal fractions are 0.36 nmol/minute per mg protein (range 0.11–0.82) without induction and 1.03 nmol/minute per nmol of cytochrome P-450 (range 0.42–1.78) with induction (Hutson and Logan 1986).

In animal studies, pairs of Carworth Farm E strain male rats administered [^{14}C]chlorfenvinphos orally at doses of 2.5 or 13.3 mg/kg in olive oil (with or without prior monooxygenase induction with dieldrin) exhibited minimal changes in the metabolic profiles. Urine samples were collected at 12 and 32 hours and analyzed by chromatography for metabolites of chlorfenvinphos using authentic standards: de-ethylchlorfenvinphos or 2-chloro-1-(2',4'-dichlorophenyl) vinylethylhydrogen phosphate, 1-(2',4'-dichlorophenyl) ethanol, 1-(2',4'-dichlorophenyl) ethanediol, 2,4-dichloromandelic acid, and 2,4-dichlorobenzoyl glycine. The metabolites 2-chloro-1-(2,4-dichlorophenyl) vinylethylhydrogen phosphate, 1-(2,4-dichlorophenyl) ethanol, 1-(2,4-dichlorophenyl) ethanediol, 2,4-dichloromandelic acid, and 2,4-dichlorobenzoyl glycine were identified in the urine by this method. Increased monooxygenase induction (dieldrin pretreatment) favored the production of the glucuronide of 1-(2,4-dichlorophenyl) ethanol and decreased yield of 2,4-dichloromandelic acid and 2,4-dichloro-benzoyl glycine at a low dose level. At the high dose level, an increased yield of 1-(2,4-dichlorophenyl) ethanol, an increase in the relative yield of 2,4-dichloromandelic acid and 2,4-dichlorobenzoyl glycine, and a doubling in the relative yield of de-ethylchlorfenvinphos occurred with a concomitant reduction in the relative yields of the glucuronides. The authors suggested that the relatively low amount of radioactivity eliminated within 0–32 hours via the urine of high-dose rats was probably due to limited absorption/metabolism. The results support the conclusion that the effect of enzyme induction on the

metabolism of substrates of that enzyme are dose-dependent with respect to enzyme saturation. Therefore, alterations in metabolism are not necessarily a consequence of enzyme induction alone (Hutson and Wright 1980). The tissue distribution of organophosphoric ester metabolizing enzymes in the livers of mammalian species has been suggested to be an important factor in accounting for species specificity of the toxicity of some phosphate triester anticholinesterase agents, including chlorfenvinphos. These enzymes were identified as microsomal monooxygenases, esterases, and glutathione-s-transferases. The acute oral LD_{50} values of chlorfenvinphos for the rat, mouse, rabbit, and dog which have been estimated as 10, 100, 500, and 12,000 mg/kg, respectively, correlated fairly well with the relative rates of chlorfenvinphos *O*-dealkylation and relative *in vivo* chlorfenvinphos-induced decreases in the rates of hexobarbital metabolism in these species. The relative rates of dealkylation in the rat, mouse, rabbit, and dog have been estimated as 1, 8, 24, and 80, respectively. The relative rates of chlorfenvinphos-induced decreases in hexobarbital metabolism in the rat, mouse, rabbit, and dog have been estimated as 4, 17, 5, and 1, respectively (Hansen 1983).

An investigator in an earlier study had suggested that the enzyme system responsible for this reaction was found to be microsomal and required molecular oxygen and $NADPH_2$ for activity. The activity of this enzyme system in isolated washed rat, mouse, and dog liver microsomes had rates of product formation of 0.02, 0.65, and 2.00 nmol (per mg of microsomal protein per minute), respectively. The activity of this enzyme system in isolated washed rabbit liver microsomes had a rate of product formation similar to the other species used in this study. The maximum specific activity of liver microsomes with respect to the dealkylation of chlorfenvinphos achieved in dieldrin-pretreated and phenobarbital-pretreated mice was 3.6 nmol at 1.6 mg/kg daily for 62 weeks and 6.0 nmol at 12 mg/kg daily for 40 weeks, respectively. The maximum specific activity of liver microsomes with respect to the dealkylation of chlorfenvinphos achieved in dieldrin-pretreated and phenobarbital-pretreated dogs was 4.1 nmol (per mg of microsomal protein per minute) at 2.0 mg/kg daily for 4 weeks and 9.2 nmol (per mg of microsomal protein per minute) at 20 mg/kg daily for 4 weeks, respectively. The activity of the enzyme system oxygen: $NADPH_2$ oxidoreductase (with chlorfenvinphos as substrate) in isolated washed monkey liver microsomes had a rate of product formation of 1.00 nmol (per mg of microsomal protein per minute). The maximum specific activity of liver microsomes with respect to the dealkylation of chlorfenvinphos achieved in dieldrin-pretreated monkeys given oral chlorfenvinphos doses of 0.03 mg/kg/day for 6.15 years was 1.8 nmol (per mg of microsomal protein per minute). The mechanism of this reaction has been proposed to be mediated by oxidative dealkylation of chlorfenvinphos to the relatively nontoxic metabolite, 2-chloro-1-(2,4-dichlorophenyl) vinyl ethylhydrogen phosphate and acetaldehyde. The enzyme system was

readily inducible, especially in the rat, by the administration of phenobarbital or dieldrin. The maximum specific activity of liver microsomes with respect to the dealkylation of chlorfenvinphos achieved in dieldrin-pretreated and phenobarbital-pretreated rats was 12.4 nmol at 8 mg/kg/day for 4 weeks and 5.7 nmol at 14 mg/kg/day for 4 weeks, respectively. A 600-fold increase in specific activity was observed in the liver of dieldrin-pretreated rats. In rats pretreated with dieldrin at 200 ppm in the diet for 12 days, the acute LD_{50} for chlorfenvinphos was increased by a factor of 6–8 (Donninger 1971).

A quantitative study of the species distribution of phosphate esterases and glutathione *S*-alkyl transferase found that these enzymes are significantly less in quantity in the pig than in all the other species studied, suggesting a significantly varied distribution among some mammalian species. The author of the study concluded that the distribution of these enzymes is an important factor in accounting for the species specificity of at least some anticholinesterase agents and that the glutathione-dependent alkyl transferase is predominantly a methyl transferase. The contribution of these two enzyme systems to the detoxification of any particular phosphate triester is dependent on the structure and solubility of the molecule (Donninger 1971).

These findings were confirmed by three other reports. In the report by Hutson and Millburn (1991), the oral LD_{50} values for rat, mouse, rabbit, and dog are 10–30 mg/kg, 150, 500, and >5,000 mg/kg, respectively. The oral LD_{50} in the rabbit has been reported to be as high as >12,000 mg/kg in some studies. The difference in toxicity in rats and dogs was found to be due to differences in several pharmacokinetic and pharmacodynamic factors. These factors include differences in the rates of absorption and metabolism, bioavailability in blood, and rates of uptake by the brain, as well as the sensitivity of brain cholinesterase to the phosphorylating action of the compound. In studies using rats and dogs, the most important reaction in detoxification is the conversion of chlorfenvinphos to de-ethylchlorfenvinphos by oxidative de-ethylation. The reaction is catalyzed by a hepatic microsomal monooxygenase, probably cytochrome P-450. The relative rates of de-ethylation in liver slices were 1, 8, 24, and 88 for the rat, mouse, rabbit, and dog, respectively. Thus, an excellent inverse correlation between oral LD_{50} and rate of de-ethylation was established. Also evidence for the significance of the reaction *in vivo* was provided from experiments in which rats were protected 7-fold from the action of chlorfenvinphos by pre-treatment with dieldrin in the diet for 12 days. This treatment induces cytochrome P-450 microsomal monooxygenase and, thus, chlorfenvinphos de-ethylation. However, the author cautioned that species differences in toxicity response are often multi-factorial, and metabolism can be a minor component (Hutson and Millburn 1991).

In the second report, Ikeda et al. (1991) found the metabolism of chlorfenvinphos in kidney subcellular fractions and in the serum of Fischer 344 rats (orally pretreated with chlorfenvinphos followed by oral treatment with a similar chlorfenvinphos dose or 50 mg/kg phenobarbital in 24 hours), with or without the NADPH-generating system, to be negligible. Metabolism of chlorfenvinphos in the liver subcellular fraction without the NADPH-generating system was also practically negligible. However, when the NADPH-generating system was added to the liver subcellular fraction, chlorfenvinphos metabolism increased significantly (203% in the 9,000 g fraction and 178% in the microsomes) in the chlorfenvinphos-pretreated animals and in the phenobarbital-pretreated animals (565%). Additionally, chlorfenvinphos pretreatment increased cytochrome P-450 content (30%) in the hepatic microsomal fraction; phenobarbital pretreatment caused a 180% increase. Hepatic microsomal cytochrome b5 content and cytochrome P-450 reductase activity were also increased by chlorfenvinphos and phenobarbital pretreatment (121 and 130%, respectively, for chlorfenvinphos, and 126 and 139%, respectively, for phenobarbital). Both chlorfenvinphos and phenobarbital pretreatment significantly increased protein content ($P<0.001$) in the microsomal fraction. Chlorfenvinphos treatment did not increase liver weight (relative to body weight). Increases were also noted in cytochrome P-450-linked activities such as aminopyrine *N*-demethylase (40%) and aniline hydroxylase (27%) content in the hepatic microsomal fraction and hexobarbital sleeping time and zoxazolamine paralysis time. Both chlorfenvinphos and phenobarbital are potent inducers of cytochrome P-450, which is involved in the metabolic detoxication of chlorfenvinphos. Thus, the authors concluded that the increase in hepatic chlorfenvinphos metabolism may be due to the induction of the hepatic cytochrome P-450 system caused by the single oral short-term treatment with chlorfenvinphos. Also this induction may be one of the reasons for the decrease in plasma chlorfenvinphos concentration which may be responsible for the reduction in toxicity of subsequent exposure to chlorfenvinphos (Ikeda et al. 1991).

The third report was a theoretical analysis that predicted the mammalian biotransformation products based on the recognition of the structure of chlorfenvinphos, understanding of Types I and II metabolism of foreign compounds, and mechanistic biochemistry (Akintonwa 1984). This analysis also acknowledged that cytochrome P-450 monooxygenase (an inducible enzyme) is the relevant enzyme which mediates the biotransformation of chlorfenvinphos via oxidative dealkylation of to the relatively nontoxic metabolite, 2-chloro-1-(2,4-dichlorophenyl) vinyl ethylhydrogen phosphate or de-ethylchlorfenvinphos. Thirteen metabolites of chlorfenvinphos were predicted for mammals from theoretical biotransformation as justified by the known structure of chlorfenvinphos and understanding of biochemical reactions of monooxygenation, reduction, hydrolysis, glucuronidation, glutathione-S-transferase conjugation, and amino acid conjugation. The 13 metabolites predicted are: 2-chloro-

1-(2',4'-dichlorophenyl) vinyldiethyl phosphate; acetaldehyde; 2-chloro-1-(2',4'-dichlorophenyl) vinyl-ethylhydrogen phosphate; 2,4-dichlorophenacylchloride; 2-chloro-1-(2',4'-dichlorophenyl) ethanol; 2,4-dichloromandelic acid; 2,4-dichloromandelic acid ester glucuronide; 2,4-dichloroacetophenone; 1-(2',4'-dichlorophenyl) ethanol; 1-(2',4'-dichlorophenyl) ethanediol; 1-(2',4'-dichlorophenyl) ethanediol-2-glucuronide; 1-hydroxy-1-(2',4'-dichlorophenyl) acetyl glycine; and 1-(2',4'-dichlorophenyl) ethanediol. 2-Chloro-1-(2',4'-dichlorophenyl) vinylethylhydrogen phosphate, 2,4-dichloromandelic acid, 1-(2',4'-dichlorophenyl) ethanediol-2-glucuronide, and 1-(2',4'-dichlorophenyl) ethanediol are predicted specifically for the dog and rat. 1-Hydroxy-1-(2',4'-dichlorophenyl) acetyl glycine (the glycine conjugate of 2,4-dichloromandelic acid) was not confirmed in the rat while 2,4-dichlorohippuric acid was present in the dog, but not in the rat. While the theoretical or predictive approach to metabolite identification elucidates all the possible mechanistic pathways in the derivation of each metabolite and identifies all toxic or hazardous intermediates, only actual experimentation, which begins with theoretical prediction, can provide species differences in the proportion of metabolites and the toxicity of these metabolites. Thus, the theoretical approach to chlorfenvinphos metabolism in mammals effectively reveals that the monooxygenation of the vinyl group would produce an unstable epoxide (2-hydroxyl groups attached to a carbon) to yield 2,4-dichlorophenyl glyoxylate and 2,4-dichlorobenzoic acid through decarboxylation and oxygenation. The author of this study postulated that the 2,4-dichlorobenzoyl glycine (2,4-dichlorohippuric acid) was produced in the rat by this mechanism. The production of electrophilic metabolic intermediates or epoxides in the metabolism of chlorfenvinphos, which could react with nucleophilic cellular components (DNA, RNA, and proteins) leading to carcinogenesis, was considered unlikely by this theoretical approach (Akintonwa 1984).

2,4-Dichlorophenacyl chloride, an intermediary metabolite of chlorfenvinphos and dimethylvinphos, is excreted from mammals mainly as 1-(2,4-dichlorophenyl)ethyl glucuronide (Hutson et al. 1977). The authors assumed that this arose via the reductive dechlorination of the phenacyl halide to the acetophenone, which was then reduced to the alcohol and conjugated. A further investigation of the proposed reductive dechlorination step (using subcellular fractions of rat liver) led the authors to conclude that it is likely that the mechanism of reaction is a nucleophilic attack by sulphur (of the second GSH) on sulphur (of the chlorfenvinphos/GSH conjugate), with the expulsion of the phenacyl anion as the leaving group. The enzyme may be regarded as one of the glutathione transferases (Hutson et al. 1977).

An assay developed for determining monooxygenase activity in human fetal livers, as a measure of the rate of decrease in substrate (Supona®, chlorfenvinphos) concentration, was found reliable for incubations at 37 EC for periods up to 10 minutes. Incubations in excess of 10 minutes were

unreliable due to an unexpected increase in $E_{246\,nm}$ readings. The investigators concluded that hydrolysis, rather than monooxygenation, of chlorfenvinphos, was probably responsible for the observed increase in readings. Using 5- and 10-minute incubations, specific activity values for monooxygenase in whole liver homogenates of 13- and 16-week-old human fetuses were determined to be 6.47±0.84 and 5.26±0.46 µg/mg, respectively; no monooxygenase activity could be detected in whole liver homogenates of 24-week-old fetuses. The authors concluded that, similar to *in vivo* observations, the mechanism for oxidative dealkylation of chlorfenvinphos proceeds initially via monooxygenation of the *alpha*-carbon atom of the alkoxy group to produce an unstable hemiacetal which breaks down by oxidative *O*- and *N*-alkylation mechanisms to acetaldehyde and 2-chloro-1-(2,4-dichlorophenyl) vinylethylhydrogen phosphate (Akintonwa and Itam 1988).

2.3.3.3 Dermal Exposure

No studies were located regarding metabolism of chlorfenvinphos after dermal exposure in humans or animals.

2.3.4 Elimination and Excretion

2.3.4.1 Inhalation Exposure

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

2.3.4.2 Oral Exposure

A male patient, aged 29, was admitted to the hospital 3 hours after a suicide attempt during which he drank about 50 mL of the preparation Enolofos® which contains 50% chlorfenvinphos. The concentration of chlorfenvinphos in the serum was 300 ng/mL upon admission. In this, the only human chlorfenvinphos poisoning in which hemoperfusion intervention was employed, the mean clearance of chlorfenvinphos during hemoperfusion was low, 68 mL/minute; and only 0.42 mg of the poison was recovered. The level of the poison in the serum was low (15 ng/mL) immediately before the procedure and gradually rose in successive blood sampling. At all times in successive blood samples during the procedure, there was an increase of chlorfenvinphos level in the serum, indicating that chlorfenvinphos passes fairly easily from the tissues into blood. This may be related to secondary

resorption from the digestive tract. The highest value of clearance was observed in the fourth hour of hemoperfusion, in contrast to the observations during hemoperfusion performed for other drug poisoning, when the value of the clearance was lowest in the last hour. The serum chlorfenvinphos levels decreased temporarily within a few hours even prior to the beginning of hemoperfusion, either due to rapid inactivation or to rapid passage into the tissues where the organophosphates accumulate (Pach et al. 1987).

In animal studies, pairs of Carworth Farm E strain male rats administered [^{14}C]chlorfenvinphos orally at doses of 2.5 or 13.3 mg/kg in olive oil (with or without prior monooxygenase induction with dieldrin) eliminated about 50% of the administered radioactivity in the urine in the first 12 hours, 9–13% in the next 12 hours, 3.5–6% in the subsequent 16 hours, and 4.2–5% in the final 42 hours of monitoring. A total of 67.1–72.5% of the administered radioactivity was recovered from the urine. Only 15.2–16.9% of the administered radioactivity was eliminated in the urine in the first 12 hours, 2.5–8.2% in the next 12 hours, 6.1–6.6% in the subsequent 18 hours, and 0.7–1.4% in the final 42 hours of monitoring. Only 26.2–31.7% of the total dose was recovered in the urine in the high-dose animals. Dieldrin pretreatment resulted in a more rapid elimination as well as a greater percentage elimination of the administered [^{14}C]chlorfenvinphos doses (Hutson and Wright 1980). All of the Birlane® (chlorfenvinphos) dose of 0.8 mg/kg/day administered in the diet for 30 days to 2 groups of adult female albino (Wistar) rats was removed from the body (excreted or metabolized) in 4 days. The dose was excreted mostly in the urine (70–90%) and feces (about 16%) with minor amounts (0.5%) in expired air (Barna and Simon 1973).

2.3.4.3 Dermal Exposure

No studies were located regarding excretion of chlorfenvinphos after dermal exposure in humans or animals.

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.

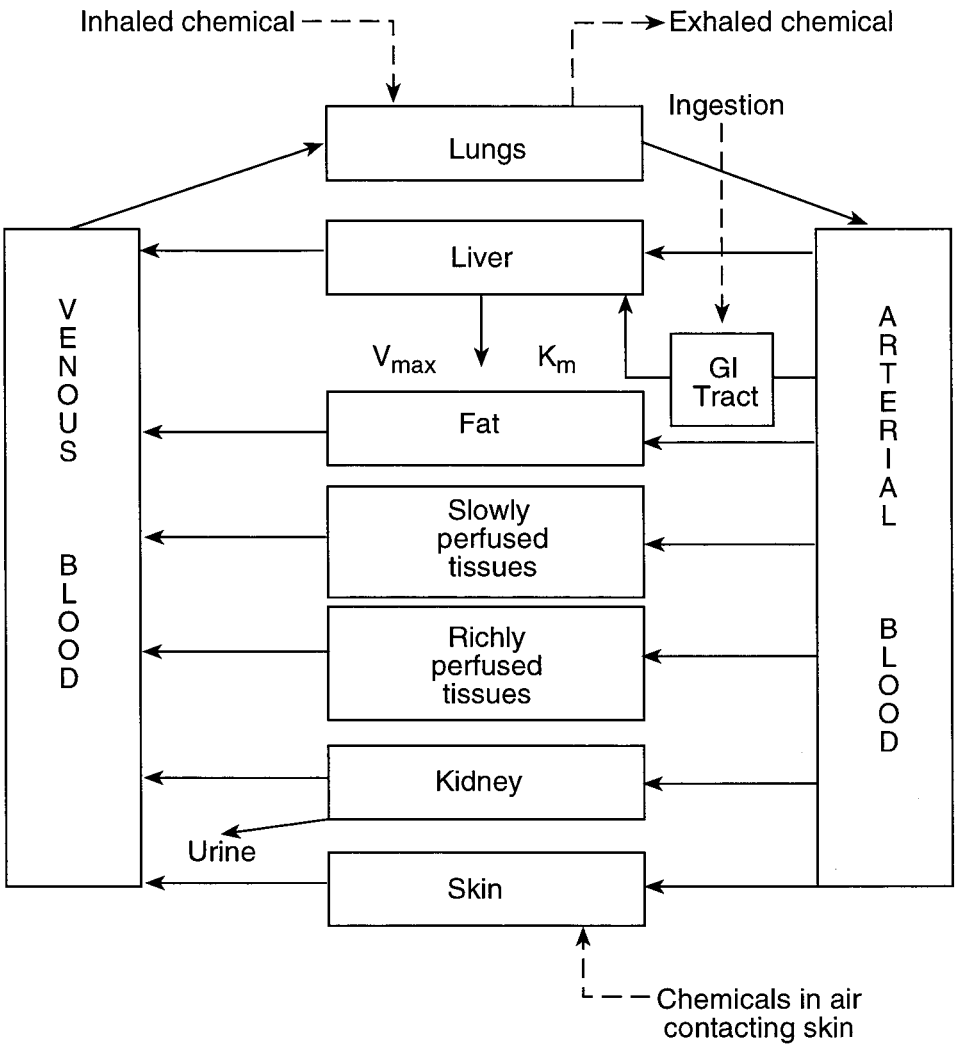
PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 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.

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



Source: adapted from Krishnan et al. 1994

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

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

A physiologically based pharmacokinetic (PBPK) analysis used data from a study with 8-week old Fischer 344 rats as a model to study the mechanism of protection by initial chlorfenvinphos exposure against the toxicity of a subsequent exposure. The analysis concluded that, contrary to expectation, the body burden of chlorfenvinphos decreased after the initial exposure upon subsequent challenge exposure. The model predicted that decreased body burden of oral chlorfenvinphos dose might be due to the decrease in the plasma concentration after a challenge with chlorfenvinphos. In the rat study on which the model is based, the acute oral toxicity of chlorfenvinphos was reduced by the oral pretreatment of rats with chlorfenvinphos after a subsequent challenge dose. This was accompanied by reduction in brain cholinesterase, liver, and plasma concentrations of chlorfenvinphos (by one-third and 4–10 times, respectively). Unbound fractions of chlorfenvinphos in blood and liver were estimated by the *in vitro* experiments and pretreatment did not change the unbound fraction of chlorfenvinphos. The authors stated that, according to the PBPK model, the decrease in body burdens of the oral chlorfenvinphos dose may be caused mainly by an increase in intrinsic clearance of chlorfenvinphos by the liver and a decrease in the partition coefficient of chlorfenvinphos between the emergent blood and he liver. The increase in the intrinsic clearance was suggested to be related to the metabolic induction of P-450 observed *in vitro*. Additionally, pretreatment decreased the absorption rate constant of the oral chlorfenvinphos dose. Essentially, this is responsible for the protection afforded against toxicity of subsequent exposure to chlorfenvinphos (Ikeda et al. 1992).

2.4 MECHANISMS OF ACTION

2.4.1 Pharmacokinetic Mechanisms

The level of activity of cholinesterases in the livers of mammalian species and the distribution of these enzymes have been suggested to be important factors in accounting for species specificity of some phosphate triester anticholinesterase agents, including chlorfenvinphos. These factors may account for the great variation in the toxicity of chlorfenvinphos among different animal species. The acute oral LD_{50} values of chlorfenvinphos for the rat, mouse, rabbit, and dog are 10, 100, 500, and 1,200 mg/kg, respectively. The relative conversion rates of chlorfenvinphos (by *O*-dealkylation) to the diester by liver slices from the rat, mouse, rabbit, and dog were 1, 8, 24, and 80 hours, respectively; these values correlate well with the published acute oral LD_{50} values for the species. The enzyme system responsible for this reaction was found to be microsomal and required molecular oxygen and $NADPH_2$

for activity. The activity of this enzyme system in isolated rat, mouse, and dog liver (washed) microsomes had rates of product formation of 0.02, 0.65, and 2.00 nmol (per mg of microsomal protein per minute), respectively. The activity of this enzyme system in isolated washed rabbit liver microsomes had a rate of product formation similar to that of other species used in this study.

The maximum specific activity of liver microsomes with respect to the dealkylation of chlorfenvinphos achieved in dieldrin-pretreated and phenobarbital-pretreated mice was 3.6 nmol at 1.6 mg/kg/day for 62 weeks and 6.0 nmol at 12 mg/kg/day for 40 weeks, respectively. The maximum specific activity of liver microsomes with respect to the dealkylation of chlorfenvinphos achieved in dieldrin- and phenobarbital-pretreated dogs was 4.1 nmol (per mg of microsomal protein per minute) at 2.0 mg/kg/day for 4 weeks and 9.2 nmol (per mg of microsomal protein per minute) at 20 mg/kg/day for 4 weeks, respectively. The activity of the enzyme system oxygen: $NADPH_2$ oxidoreductase (with chlorfenvinphos as substrate) in isolated washed monkey liver microsomes had a rate of product formation of 1.00 nmol (per mg of microsomal protein per minute). The maximum specific activity of liver microsomes with respect to the dealkylation of chlorfenvinphos achieved in dieldrin-pretreated monkeys given oral chlorfenvinphos doses of 0.03 mg/kg/day for 6.15 years was 1.8 nmol (per mg of microsomal protein per minute). It has been proposed that the mechanism of this reaction is mediated by oxidative dealkylation of chlorfenvinphos to the relatively nontoxic metabolite, 2-chloro-1-(2,4-dichlorophenyl) vinyl ethylhydrogen phosphate and acetaldehyde. The enzyme system was readily inducible, especially in the rat, by the administration of phenobarbital or dieldrin.

The maximum specific activity of liver microsomes with respect to the dealkylation of chlorfenvinphos achieved in dieldrin- and phenobarbital-pretreated rats was 12.4 nmol at 8 mg/kg/day for 4 weeks and 5.7 nmol at 14 mg/kg/day for 4 weeks, respectively. A 600-fold increase in specific activity was observed in the liver of dieldrin-pretreated rats; in rats pretreated with dieldrin at 200 ppm in the diet for 12 days, the acute LD_{50} for chlorfenvinphos was increased by a factor of 6–8. A quantitative study of the species distribution of phosphate esterases and glutathione *S*-alkyl transferase found that these enzymes are significantly less in the pig than all the other species studied, suggesting a significantly varied distribution among some mammalian species. The author of the study concluded that the distribution of these enzymes is an important factor in accounting for the species specificity of at least some anticholinesterase agents and that the glutathione-dependent alkyl transferase is predominantly a methyl transferase; the contribution of these two enzyme systems to the detoxification of any particular phosphate triester is dependent on the structure and solubility of the molecule (Donninger 1971).

These findings were confirmed by two other reports. In one, the oral LD_{50} values for rat, mouse, rabbit, and dog were 10–30, 150, 500, and >5,000 mg/kg, respectively; it can be >12,000 mg/kg in the rabbit. The difference in toxicity in rats and dogs was found to be due to rates of absorption and metabolism, bioavailability in blood, and rates of uptake by the brain and sensitivity of brain cholinesterase to the phosphorylating action of the compound. In studies using rats and dogs, the most important reaction in detoxification is the conversion of chlorfenvinphos to de-ethylchlorfenvinphos by oxidative de-ethylation. The reaction is catalyzed by a hepatic microsomal monooxygenase, probably cytochrome P-450. The relative rates of de-ethylation in liver slices were 1, 8, 24, and 88 for the rat, mouse, rabbit, and dog, respectively. Thus, an excellent inverse correlation between oral LD_{50} and rate of de-ethylation was established. Also evidence for the significance of the reaction *in vivo* was provided from experiments in which rats were protected 7-fold from the action of chlorfenvinphos by pre-treatment with dieldrin in the diet for 12 days. This treatment induces cytochrome P-450 microsomal monooxygenase and, thus, chlorfenvinphos de-ethylation. The de-ethylation was induced about 40-fold (measured *in vitro*) relative to cytochrome P-450 concentration. However, the author cautioned that species differences in toxicity response are often multi-factorial, and metabolism can be a minor component (Hutson and Millburn 1991).

In the second report, the metabolism of chlorfenvinphos in kidney subcellular fraction and in the serum of Fischer 344 rats (orally pretreated with chlorfenvinphos followed by an oral treatment with a similar chlorfenvinphos dose or 50 mg/kg phenobarbital in 24 hours), with or without the NADPH-generating system, was found to be negligible. Metabolism of chlorfenvinphos in the liver subcellular fraction without the NADPH-generating system was also practically negligible. However, when the NADPH-generating system was added to the liver subcellular fraction, chlorfenvinphos metabolism increased significantly (203% in the 9,000 g fraction and 178% in the microsomes) in the chlorfenvinphos-pretreated animals and in the phenobarbital-pretreated animals (565%). Additionally, chlorfenvinphos pretreatment increased cytochrome P-450 content (30%) in the hepatic microsomal fraction; phenobarbital pretreatment caused a 180% increase. Hepatic microsomal cytochrome b5 content and cytochrome P-450 reductase activity were also increased by chlorfenvinphos and phenobarbital pretreatment (121 and 130%, respectively, for chlorfenvinphos and 126 and 139%, respectively, for phenobarbital). Both chlorfenvinphos and phenobarbital pretreatment significantly increased protein content ($P<0.001$) in the microsomal fraction. Chlorfenvinphos treatment did not increase liver weight (relative to body weight). Increases were also noted in cytochrome P-450-linked activities such as aminopyrine *N*-demethylase (40%) and aniline hydroxylase (27%) content in the hepatic microsomal fraction and hexobarbital sleeping time and zoxazolamine paralysis time. Both chlorfenvinphos and phenobarbital are potent inducers of cytochrome P-450, which is involved in the metabolic detoxication of chlorfenvinphos. Thus, the

authors concluded that the increase in hepatic chlorfenvinphos metabolism may be due to the induction of the hepatic cytochrome P-450 system caused by the single oral short-term treatment. Also this induction may be one of the reasons for the decrease in plasma chlorfenvinphos concentration, which may be responsible for the reduction in toxicity of subsequent exposure to chlorfenvinphos (Ikeda et al. 1991).

Some of the major sites and enzyme systems involved in the detoxication of direct-acting neurotoxic organophosphates, like chlorfenvinphos, are NADPH-P-450, GSH transferase, FAD-monooxygenase and esterases; the most important of these is monooxygenase. The major activation step for microsomal monooxygenases is of critical importance for the toxicity of organophosphate compounds like chlorfenvinphos. According to this study, the relative rates of chlorfenvinphos *O*-dealkylation in rats, mice, rabbits, and dogs are 1, 8, 24, and 88, respectively. These rates correlated well with the oral LD_{50} values for these species, which were given as 10, 100, 500, and 12,000 mg/kg for rats, mice, rabbits, and dogs, respectively. According to the author, the potential for oxidative *O*-dealkylation in the P-450 pathway varies widely with species, with activity toward chlorfenvinphos correlating well with species selectivity. The author concluded that the biotransformation of delayed neurotoxicants will certainly influence relative potencies, but the distinction between a delayed neurotoxicant and the neurotoxicity of organophosphate compounds, which is mainly limited to acute effects, depends more heavily on pharmacodynamic than pharmacokinetic considerations. Thus, the pharmacokinetics of delayed neurotoxicants differ from the pharmacokinetics of neurotoxic organophosphates, like chlorfenvinphos (Hansen 1983).

2.4.2 Mechanisms of Toxicity

Most chlorfenvinphos toxicity results from the inhibition of cholinesterase activity in the central and peripheral nervous system when administered by the oral (Cupp et al. 1975; Pach et al. 1987) or inhalation route in acute-duration exposures in humans (Kolmodin-Hedman and Eriksson 1987), and when administered by the oral (Barna and Simon 1973; Osicka-Koprowska et al. 1984; Takahashi et al. 1991) or dermal route in acute-duration exposures in animals. Inhibition of cholinesterase activity results in accumulation of acetylcholine at muscarinic and nicotinic receptors leading to peripheral and central nervous system effects. These effects usually appear within a few minutes to a few hours after exposure depending on the extent of exposure. The enzyme is responsible for terminating the action of the neurotransmitter acetylcholine in the synapse of the pre- and post-synaptic nerve endings or in the neuromuscular junctions. On arrival of a nerve impulse at the synaptic gutter between the pre- and post-synaptic nerve endings or effector muscle fiber endplates, there is a

release of acetylcholine from the pre-synaptic terminals. At the post-synaptic nerve ending, acetylcholine acts as a chemical mediator to perpetuate the action potential. However, the action of -acetylcholine does not persist long as it is hydrolyzed by the enzyme, acetylcholinesterase, and rapidly removed.

As an anticholinesterase organophosphate, chlorfenvinphos inhibits the activity of the acetylcholinesterase enzyme by reacting with the esteratic sites of the enzyme to form a stable phosphorylated complex which is incapable of destroying acetylcholine at the synaptic gutter between the pre- and post-synaptic nerve endings in the central and peripheral nervous system or neuromuscular junctions of skeletal muscles, resulting in the accumulation of acetylcholine at these sites. This leads to continuous or excessive stimulation of cholinergic fibers in the post-ganglionic parasympathetic nerve endings, neuromuscular junctions of the skeletal muscles, resulting in hyperpolarization of nerve or muscle fibers and receptor desensitization until hydrolysis of the phosphorylated cholinesterase occurs. In some cases, a dealkylation and stabilization of the phosphorylated enzyme (“aging”) occurs such that hydrolysis can no longer take place and the enzyme is irreversibly inhibited. In such cases, return of acetylcholinesterase activity parallels the time required to resynthesize this enzyme.

In the parasympathetic system, stimulation of postganglionic fibers on the effector organs is mimicked by muscarine, and the receptors for the transmitters are called muscarinic receptors. They are found primarily in smooth muscle, the heart, and the exocrine glands. Stimulation of these receptors by inhibition of acetylcholinesterase activity produces signs and symptoms of cholinergic poisoning that include bronchoconstriction and increased bronchial secretions, increased salivation and lacrimation, exophthalmos, increased sweating, increased gastrointestinal tone and peristalsis, nausea, vomiting, abdominal cramps, diarrhea, hypotension and bradycardia that can lead to heart block, involuntary urination caused by contraction of the smooth muscle of the bladder, and constriction of the pupils or miosis.

At the autonomic ganglia and neuromuscular junctions, stimulation of transmission is mimicked by the action of nicotine, and the receptors are called nicotinic receptors. Inhibition of acetylcholinesterase activity leads to abnormal continuous or excessive stimulation of the receptor muscle fibers, causing weakness of the muscles, involuntary twitching, fasciculations, cramps, and eventual paralysis of the muscles. Paralysis of the respiratory muscles leads to respiratory failure and death. The central nervous system effects are due to accumulation of acetylcholine at various cortical, subcortical, and spinal levels (primarily in the cerebral cortex, hippocampus, and extrapyramidal motor system). Accumulation of acetylcholine in the central nervous system causes tension, anxiety, restlessness,

insomnia, headache, emotional instability, neurosis, excessive dreaming and nightmares, apathy, drowsiness, confusion, slurred speech, tremor, ataxia, convulsions, depression of respiratory and circulatory centers, and coma. The most likely cause of death in fatal organophosphate poisoning is paralysis associated with respiratory failure (Cupp et al. 1975; Klaassen et al. 1986; Shankar; Takahashi et al. 1991; Williams and Burson 1985).

The results of pretreatment of New Zealand white rabbits with 0.007 mg/kg oxotremorine, a direct agonist of muscarinic receptors, followed by treatment with 0.5 mg/kg chlorfenvinphos by hypothalamic infusion suggests that the chlorfenvinphos is neither an agonist nor antagonist of the muscarinic receptors in the rabbit hypothalamus. No overt changes in behavior or changes in hippocampal EEG were observed in the rabbits following infusion with 0.5 mg/kg chlorfenvinphos (Gralewicz et al. 1995).

Organophosphate-induced hypotension (reported for chlorfenvinphos only in rats receiving intravenous doses) (Takahashi et al. 1991) has been suggested to be due to factors other than inhibition of cholinesterase activity (Kojima et al. 1992). A study concluded that the alteration of brain and liver activities of the aromatic amino acid transferases may be due to the inhibitory effect of chlorfenvinphos on noradrenaline (norepinephrine) activity (Puzynska 1984). In other studies, chlorfenvinphos was shown to independently inhibit noradrenaline (norepinephrine) activity *in vivo* in rats rapidly (3 hours) at doses as low as 4 mg/kg (Brzezinski 1978; Osumi et al. 1975). On this basis, it has been postulated that chlorfenvinphos may also act via central noradrenergic mechanisms, disturbing the dynamic equilibrium between the rate of formation and utilization of noradrenaline (norepinephrine). This action via central noradrenergic mechanisms by chlorfenvinphos may be responsible for the changes in blood pressure observed in other studies after chlorfenvinphos intoxication (Brzezinski 1978).

It has also been suggested that the cholinergic action of organophosphates like chlorfenvinphos may interfere with the pathways controlling the secretory activity of the anterior pituitary lobe and the adrenal cortex whose hormones influence the activities of many enzymes, including aromatic amino acid transferases (Puzynska 1984). Interference with the secretory activity of the adrenal cortex may lead to a disruption in the normal activities of one or more components of the renal blood pressure regulatory systems (Klaassen et al. 1986).

2.4.3 Animal-to-Human Extrapolations

In one study, the rates of chlorfenvinphos de-ethylation by human liver microsomal fractions are 0.36 nmol/minute per mg protein (range 0.11–0.82) without induction and 1.03 nmol/minute per nmol

of cytochrome P-450 (range 0.42–1.78) with induction. The rates of chlorfenvinphos de-ethylation by liver microsomal fractions are 0.62 nmol/minute/mg protein (range 0.36–0.93) and 1.30 nmol/minute/nmol cytochrome P-450 (range 0.81–1.74) for uninduced rabbits. The rabbit is considered relatively resistant to the acute toxic action of chlorfenvinphos, with an LD_{50} of 500–1,000 mg/kg (provided in another study). These results demonstrate that human hepatic cytochrome P-450 is almost as active as that of rabbits. However, the authors stressed that these results refer to the total cytochrome P-450 complement of the cells and take no account of the several forms known to exist which differ from species to species, or of the environmental factors, and the other factors involved in the acute toxicity of chlorfenvinphos in humans. Based on the findings in this study, it appears that humans may de-ethylate chlorfenvinphos more like rabbits or mice than like rats (Hutson and Logan 1986).

2.5 RELEVANCE TO PUBLIC HEALTH

Overview

Chlorfenvinphos is an insecticide that was broadly used in the United States in both agriculture and control of pests in residential dwellings, gardens, and on household pets from 1963 until 1991, when all products containing chlorfenvinphos as an active ingredient were canceled (REFS 1995).

The absorption of chlorfenvinphos after inhalation exposure in humans or animals is unknown due to lack of data. Ingested and dermally contacted chlorfenvinphos is rapidly absorbed in humans and animals; the rate of absorption is dependent on the solvent in which the substance is dissolved. In humans, absorbed chlorfenvinphos is widely distributed and has been detected in compartments that include serum, cervical mucus, follicular and sperm fluids, and milk. It is extensively metabolized via oxidative dealkylation by liver microsomal fractions. Data from animal studies suggest that electrophilic metabolic intermediates or epoxides may be produced in the metabolism of chlorfenvinphos. There is no information on the elimination or excretion of chlorfenvinphos in humans and animals after inhalation or dermal exposures; however, ingested chlorfenvinphos is rapidly eliminated via the urine and feces, and expired air in animals. In humans, ingested chlorfenvinphos is rapidly removed from the blood and passed into the tissues; chlorfenvinphos has been detected in human sperm fluid and milk samples.

As an anticholinesterase organophosphate, the principal toxic effect of chlorfenvinphos is the inhibition of cholinesterase activity in the central and peripheral nervous system when administered by the oral

or inhalation route in acute-duration exposures in humans and the inhibition of cholinesterase activity in the central and peripheral nervous system when administered by the oral, or dermal route in acute-duration exposures. Inhibition of cholinesterase activity results in the accumulation of acetylcholine at acetylcholine receptors leading to cholinergic responses in the peripheral (muscarinic and nicotinic) and central nervous system and neuromuscular junctions. Severe inhibition of acetylcholinesterase activity often leads to cholinergic symptoms in humans and laboratory animals, which include excessive glandular secretions (salivation, lacrimation, rhinorrhea, miosis), exophthalmos, bronchoconstriction, vasodilation, hypotension, diarrhea, nausea, vomiting, urinary incontinence, and bradycardia. Tachycardia, mydriasis, fasciculations, cramping, twitching, muscle weakness, and muscle paralysis are associated with nicotinic receptor stimulation. Central nervous system toxicity may be mediated by either muscarinic or nicotinic receptors and includes respiratory depression, anxiety, insomnia, headache, apathy, drowsiness, dizziness, loss of concentration, confusion, tremors, convulsions, and coma. In non-fatal exposures, the effects are transient and recovery is rapid and complete following cessation of exposure. In sufficiently high doses, chlorfenvinphos exposure has resulted in death of humans, rats, and mice. These effects usually occur within a few minutes to a few hours after dosing, depending on the extent of exposure. In addition, chlorfenvinphos may interfere with the activity of noradrenaline (norepinephrine) in central adrenergic mechanisms in animals.

The greatest potential for significant exposure to this compound is found in occupational settings (i.e., manufacture and application of chlorfenvinphos). Currently, the most common exposure scenario for the general population comes from home use of imported foods and lanolin-containing pharmaceutical products. Workers involved in disposal of chlorfenvinphos-contaminated wastes are at a higher risk of exposure. Populations potentially at higher risk of exposure are: people living in the vicinity of plants where chlorfenvinphos was manufactured; people living near dairy farms, cattle or sheep holding areas, or poultry producing-facilities where chlorfenvinphos was used; and populations living near hazardous waste sites containing chlorfenvinphos. No association has been reported between chlorfenvinphos toxicity and low-level environmental contamination.

Minimal Risk Levels for Chlorfenvinphos

Inhalation MRLs.

Inhalation MRLs for acute-, intermediate-, or chronic-duration exposure to chlorfenvinphos have not been calculated because adequate data for developing these MRLs are not available. The available human reports involve mixed exposures. The available animal studies reported serious effects.

A human study reported immunological effects at a LOAEL of 0.21 mg/m^3 following prolonged occupational exposure to chlorfenvinphos by the inhalation route. However, the subjects of this study were also concurrently exposed to greater concentrations of other potentially immunotoxic substances such as formothion, sumithion, and malathion (Wysocki et al. 1987). In another human study, a group of nine gardeners (pesticide mixers) exposed to unknown concentrations of a mixture of pesticides (chlorfenvinphos, dimethoate, formothion, isofenphos), complained of headaches and had a mean difference (before and after exposure) of 0.56 nmol/mL for acetylcholinesterase and 2.67 nmol/mL for butyrylcholinesterase (Kolmodin-Hedman and Eriksson 1987). However, since these symptoms could also result from exposure to the other organophosphate pesticides in the mixture, the etiology for these symptoms is uncertain. Therefore the study was not useful for developing inhalation MRLs.

The available inhalation animal studies reported only serious effects (mortality, apnea, salivation, urination, exophthalmos, twitches, and tremors) (Takahashi et al. 1994; Tsuda et al. 1986) following exposure to chlorfenvinphos. Therefore, the data from these studies are not appropriate for use in the calculation of MRLs.

Oral MRLs.

- An MRL of 0.002 mg/kg/day has been developed for acute-duration oral exposure (14 days or less) to chlorfenvinphos.

This MRL for chlorfenvinphos is based on a LOAEL of 2.4 mg/kg/day for neurological effects (38% erythrocyte cholinesterase inhibition) in female rats (Barna and Simon 1973). In the study, two groups of adult female albino (Wistar) rats weighing 208 g were orally administered Birlane® (chlorfenvinphos) at dose of 0 or 2.4 mg/kg/day in the diet for 10 days. The study was designed to investigate the

effects of oral chlorfenvinphos on body weight increase, the gastrointestinal absorption of glucose, Na^+, and Ca^{2+}, and the effects of oral chlorfenvinphos on plasma and erythrocyte cholinesterase activity levels. Plasma cholinesterase activity was inhibited by 52%, and erythrocyte cholinesterase activity level was inhibited by 30% at a dose of 2.4 mg/kg/day (the only dose tested). Gastrointestinal absorption of glucose was increased by 30% over control values while Na^+ absorption was decreased by 32% below control values. Gastrointestinal absorption of Ca^{2+} and body weight increases were unaffected by chlorfenvinphos exposure. These changes in the gastrointestinal absorption of glucose, but Na^+ were not considered statistically significant ($P>0.05$) by the investigators.

The central nervous system is the principal target of chlorfenvinphos toxicosis. Chlorfenvinphos, an anticholinesterase organophosphate, inhibits cholinesterase activity in the central and peripheral nervous system in humans and animals (Cupp et al. 1975; Gralewicz et al.1990; Hunter 1969; Maxwell and LeQuesne 1982; Osumi et al. 1975; Osicka-Koprowska et al. 1984; Pach et al. 1987; Takahashi et al. 1991; Vestweber and Kruckenberg 1972). Chlorfenvinphos also inhibits noradrenaline (norepinephrine) activity in the central nervous system in animals (Brzezinski 1978; Osumi et al. 1975). Human subjects exposed to large acute doses of chlorfenvinphos exhibited severe cholinergic signs. These cholinergic signs were relieved by the administration of atropine and/or pralidoxime, indicating cholinesterase inhibition etiology (Cupp et al. 1975; Pach et al. 1987). In rats, low to moderate doses (2.4–30 mg/kg) of oral chlorfenvinphos significantly inhibited cholinesterase activities in a number of tissues: the brain, erythrocyte, and plasma (Barna and Simon 1973; Osicka-Koprowska et al. 1984; Puzynska 1984). An acute-duration oral study also found alterations in noradrenaline (norepinephrine) level in rat brain following exposure to chlorfenvinphos. A chlorfenvinphos dose of 13 mg/kg decreased noradrenaline (norepinephrine) levels in rat brains by 20%, as compared to control rats. According to the investigators, chlorfenvinphos accelerated the rate of NA disappearance from the brain (Brzezinski 1978).

Therefore, it is appropriate to base the acute oral MRL for chlorfenvinphos on cholinesterase inhibition.

It should be noted that a study by Osumi et al. (1975) which determined a NOAEL of 1 mg/kg/day and a LOAEL of 2 mg/kg/day for 38% inhibition of brain cholinesterase in rats was not used to calculate an acute oral MRL because it was deemed less appropriate due to the gavage (oral) route of administration of the test substance. An oral feeding study is preferred by ATSDR.

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

The MRL is based on a LOAEL of 1.5 mg/kg/day for adverse immunological/lymphoreticular effects in mice (Kowalczyk-Bronisz et al. 1992). In this study male and female inbred C57BL/6 mice and (C57BL/6 x DBA/2)F1 (BDF1/Iiw) hybrid mice (6–8 weeks old) were orally dosed with chlorfenvinphos (suspended in 1% methylcellulose solution) and evaluated for 5 days for the effect of chlorfenvinphos exposure on the mouse immune system. The mice were exposed to oral chlorfenvinphos doses of 0, 1.5, 3, or 6 mg/kg (0, 1/100, 1/50, or 1/25 LD_{50}) daily for 3 months; the control group was given 1% methylcellulose. Then exposed and control mice were immunized by intraperitoneal injections of 0.2 mL 10% sheep red blood cells. The IgM-PFC (plaque-forming or antibody-producing cells) number in spleen cell suspension was tested on day 4 after immunization and the procedure repeated 3 weeks after the exposure to chlorfenvinphos had been ceased. Exposed and control groups were subjected to immunological tests and hematological examinations. Lymphatic organs were histologically examined. A dose-related decrease in the number of hemolysin-producing cells was observed: plaque-forming cells (PFC) were 58% at the 6 mg/kg dose group and 85% at the 3 mg/kg dose level as compared to control values. Chlorfenvinphos treatment also caused reduction in the number of E rosette-forming cells by 30% at the 6 mg/kg dose level and by 25% at the 3 mg/kg dose level. Increases in Interlukin-1 (Il-1) activity and delayed-type hypersensitivity (DTH) reaction were observed 24 hours after challenge. Spleen colonies were stimulated, as evidenced by the increase of endogenous spleen colonies and exogenous spleen colonies (CFU-S). CFU-S increased 190% at the 1.48 mg/kg dose level; 137% at the 6 mg/kg dose level; 162% at 1.5 mg/kg dose level; and 70% at the 6 mg/kg dose level. When the IgM PFC number was tested 3 weeks later, after the exposure to chlorfenvinphos in the small dose (1.5 mg/kg), an increase (about 40%) in number of plaques was observed. There was a 50% reduction in thymus weight at the 1.5 mg/kg dose level, compared to controls; significant involution of the thymus was also noted.

In other studies, adverse immunologic/lymphoreticular effects have been associated with exposure to oral chlorfenvinphos. In an intermediate-duration dietary study with albino (Wistar) rats, there was a significant and irreversible reduction in relative spleen weight of female rats given $3 mg/kg/day chlorfenvinphos for 12 weeks (Ambrose et al. 1970). A study was undertaken to evaluate selected serological and cytoimmunological reactions in rabbits subjected to a long-term poisoning with subtoxic oral doses (10 mg/kg in a soya oil solution with a small amount of food) of chlorfenvinphos for 90 days. Chlorfenvinphos treatment significantly elevated serum hemagglutinin levels (16%) and

hemolysin activity (66%, P<0.05), and also increased the number of nucleated lymphoid cells producing hemolytic antibody to sheep erythrocytes, compared to controls (treated 906, P<0.05 and controls 618). Spleen cytomorphology changes, manifested mainly as transformation of primary follicles into secondary ones with well developed germinal centers, were also observed (Roszkowski 1978).

Therefore, it is appropriate to base the intermediate oral MRL for chlorfenvinphos on immunological effects.

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

This MRL for chlorfenvinphos was developed from a LOAEL of 0.7 mg/kg/day for adverse neurological effects in rats (Ambrose et al. 1970). In this study, four matched groups of weanling albino (Wistar) rats were culled to a narrow starting weight range and fed daily GC-4072 (technical chlorfenvinphos) doses of 0, 0.7, 2.1, 7, or 21 mg/kg/day (males) or 0, 0.8, 2.4, or 8, or 24 mg/kg/day (females) in the diet for 104 weeks. An additional group of non-littermate rats were administered 21 mg/kg/day (males) or 24 mg/kg/day (females) chlorfenvinphos for 104 weeks. Plasma and erythrocyte cholinesterase (ChE) activity levels were obtained from 4 rats of each sex per dose group at 1, 4, 8, and 12 weeks. At 13 weeks, 4 rats/sex/dose group were sacrificed for histopathologic examination. The rats in the 21 mg/kg/day (males) and 24 mg/kg/day (females) were sacrificed on the 95th week, and all other dose group animals were sacrificed on the end of the study (104 weeks). At each autopsy, relative organ weights were determined for heart and kidneys. All animals sacrificed in moribund condition, as well as those sacrificed at week 13, 95, and 104 weeks were examined grossly and microscopically, and organs (heart, lungs, liver, kidney, urinary bladder, spleen, stomach, small and large intestine, skeletal muscle, skin, bone marrow, pancreas, thyroid, adrenal, pituitary) from these animals were histopathologically examined. Chlorfenvinphos significantly decreased body weight gain of females at the 8 and 24 mg/kg/day dose groups from the 26th week until near the end of the study, although the decreased body weight gain became statistically insignificant at the end of the study. Increased relative liver weights were observed in males at the 7 mg/kg/day dose level, but no other signs of hepatopathology were reported. Compared to undosed controls, no consistent differences in body weight gains in males, survival of the test animals, food consumption, or mortality were evident at any dose level tested. No gross or microscopic histopathology was evident in any of the organs (heart, lungs, liver, kidney, urinary bladder, spleen, stomach, small and large intestine, skeletal muscle, skin, bone marrow, pancreas, thyroid, adrenal, pituitary) and tissues examined. No changes in organ-to-body weight were observed in the heart, kidney, spleen and testes.

Although the neurological effects of prolonged human exposure to low oral doses of chlorfenvinphos are not known due to a lack of studies, clinical reports of accidental and intentional acute exposure to relatively high doses of chlorfenvinphos-containing organic phosphate products indicate that neurological effects, mediated by cholinesterase inhibition (Cupp et al. 1975; Pach et al. 1987), may be the most sensitive toxicological consequences of human exposure to chlorfenvinphos. Similarly, chlorfenvinphos significantly inhibited both plasma and erythrocyte cholinesterase activities in Beagle dogs fed daily chlorfenvinphos doses of 0.3, 2, or 10 mg/kg/day (males), or 1.5, 10, or 50 mg/kg/day (females) in the diet (moist) for 104 weeks. Plasma cholinesterase activities were significantly inhibited at all dietary levels through week 39 of the study; 49% inhibition was noted at the 0.3 mg/kg/day (males) and 1.5 mg/kg/day (females) dose levels. Erythrocyte cholinesterase activity was significantly and consistently inhibited (36%) during the first 12 weeks only in the 10 mg/kg/day (males) and 50 mg/kg/day (females) dose levels (Ambrose et al. 1970).

Death. Data are not available to estimate the lethal dose of chlorfenvinphos by any route or for any duration of exposure in humans. However, one study reported the death of a 16-year-old victim following unintentional ingestion of an unspecified amount of chlorfenvinphos (Felthous 1978). In animal studies, the acute inhalation LC_{50} for rat is estimated as 130 mg/m^3 (Tsuda et al. 1986). The acute oral LD_{50} for technical chlorfenvinphos for both sexes of rat is variously estimated as 15.4 mg/kg (Hutson and Wright 1980); 22.8 mg/kg (Hutson and Logan 1986); 34.3 mg/kg (Ikeda et al. 1992); and 9.7 mg/kg/day (Ambrose et al. 1970). The acute oral LD_{50} values for male and female rats have been given as 23 mg/kg and 25.5 mg/kg, respectively (Puzynska 1984). Chlorfenvinphos appears to be less acutely toxic to mice than to other experimental animals, with estimated LD_{50} values of 148 mg/kg (male) and 109 mg/kg (female) (Kowalczyk-Bronisz et al. 1992). The LD_{50} for rabbits has been estimated to be 300 mg/kg (Ambrose et al. 1970) and 500–1,000 mg/kg (Hutson and Logan 1986); the estimated LD_{50} for dogs is 50.5 mg/kg/day (Ambrose et al. 1970).

The dermal LD_{50} values for undiluted chlorfenvinphos and emulsifiable concentrate for rabbits have been estimated as 400 and 1,087 g/kg, respectively (Ambrose et al. 1970). No reports of human deaths resulting from dermal exposure to chlorfenvinphos were located, but evidence from non-lethal human data and animal studies (Hunter 1969; Vestweber and Kruckenberg 1972) indicates that human lethality by this route of exposure is unlikely.

Systemic Effects.

Respiratory Effects. No studies were located regarding respiratory effects in humans following acute-, intermediate- or chronic-duration inhalation, oral, or dermal exposure to chlorfenvinphos. Respiratory effects were noted in two clinical reports of human ingestion: accidental ingestion of a mange-mite medication containing organic phosphate; and intentional ingestion in a suicide attempt of the preparation Enolofos®, which contains 50% chlorfenvinphos. In both cases, effects stemmed from central cholinergic disturbances (Cupp et al. 1975; Pach et al. 1987). Animal data indicate that acute-duration inhalation exposure to high doses of chlorfenvinphos (16–390 mg/m^3) may be accompanied by transient apnea (Takahashi et al. 1991, 1994). No effects on the respiratory system were noted in rats and dogs orally administered chlorfenvinphos at doses as high as 100 mg/kg/day (rats) or 50 mg/kg/day (dogs) for 12 weeks; or in rats at doses as high as 24 mg/kg/day for 104 weeks (Ambrose et al. 1970). Due to inadequate data, it is unknown whether human exposure to environmental concentrations of chlorfenvinphos could result in adverse respiratory effects.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans following acute-, intermediate-, or chronic-duration inhalation, oral, or dermal exposure to chlorfenvinphos. Animal data indicate that acute-duration inhalation exposure to high doses of chlorfenvinphos (16–390 mg/m^3) may be preceded by an initial hypotension followed by steadily increasing hypertension and abnormal cardiac conductivity (Takahashi et al. 1991, 1994). Evidence from animal studies also indicates that oral chlorfenvinphos is not directly toxic to the cardiovascular system, but may modulate the function of the cardiovascular system via its effect on the central nervous system (Ambrose et al. 1970; Klaassen et al. 1986; Puzynska 1984). Although this route of exposure may not be relevant to human exposure to chlorfenvinphos, Sprague-Dawley rats also exhibited similar signs at a dose of 16 mg/kg following intravenous administration of the chlorfenvinphos (Takahashi et al. 1991). Due to inadequate data, it is unknown whether human exposure to environmental concentrations of chlorfenvinphos could result in adverse cardiovascular effects.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans following acute-, intermediate-, or chronic-duration oral exposure to chlorfenvinphos. Acute- and intermediate-duration oral exposures to chlorfenvinphos increased gastrointestinal absorption of glucose, while decreasing the gastrointestinal absorption of Na^+; however, Ca^{2+} absorption was unaffected in adult Wistar rats. These changes in glucose and Na^+ absorption were not considered statistically significant ($P>0.05$) by the investigators (Barna and Simon 1973). Weanling albino (Wistar) rats of both sexes chronically administered daily dietary chlorfenvinphos doses of 21 mg/kg/day (males) or 24 mg/kg/day (females) exhibited no gross or microscopic histopathology in

the stomach or small and large intestine at autopsy (Ambrose et al. 1970). Based on the available information, human exposure to chlorfenvinphos at hazardous waste sites is not likely to result in any significant adverse gastrointestinal effects.

Hematological Effects. The hematological effects from inhalation exposure to chlorfenvinphos are not known due to lack of data in humans and laboratory animals. Similarly, the hematological effects of oral chlorfenvinphos exposure are not certain because of limited and inconclusive data in animals. Uncorroborated investigations conducted on 4 groups of male and female rabbits (13 each) at a dose of 10 mg/kg for 90 days to evaluate the serological effects of chlorfenvinphos reported significant increases of hemolysin and hemagglutinin serum titers as compared to controls. Hemagglutinin and hemagglutinin IgG titers were increased by 16 and 18%, respectively, while hemolysin and hemolysin IgG titers were elevated by 66 and 102%, respectively (Roszkowski 1978). However, no effects were observed on monitored hematological parameters in rats and Beagle dogs given oral chlorfenvinphos (up to 24 mg/kg/day for rats and 50 mg/kg/day for dogs) for 104 weeks (Ambrose et al. 1970). Due to the lack of data on the hematological effects of chlorfenvinphos in humans and scarcity of data in animals, the hematological effects of human exposure to chlorfenvinphos are uncertain.

Musculoskeletal Effects. No studies were found regarding musculoskeletal effects in humans or animals following inhalation or dermal exposure to chlorfenvinphos. In animal oral studies, no effects on the musculoskeletal system were noted in dogs orally administered chlorfenvinphos at doses as high as 50 mg/kg/day (females) for 12 weeks; or in rats at doses as high as 24 mg/kg/day (females) for 104 weeks (Ambrose et al. 1970). Therefore, adverse musculoskeletal effects in humans from exposure to chlorfenvinphos at hazardous waste sites are not likely.

Hepatic Effects. Based on the currently available data, chlorfenvinphos exposure at environmental levels is not likely to present risk of liver injury to humans. No hepatic effects were reported in humans from exposure to chlorfenvinphos by any route. Although chlorfenvinphos proved to be porphyrinogenic in tissue culture without induction and markedly porphyrinogenic with induction (Koeman et al. 1980), no such evidence was found in the currently available *in vivo* studies. The limited information on the hepatic effects of chlorfenvinphos indicates that the substance is not significantly hepatotoxic by the oral route in acute-, intermediate-, or chronic-duration exposures. While no changes in liver weight (relative to body weight) were reported in Fischer 344 rats given a single oral chlorfenvinphos dose of 15 mg/kg, P-450 activity was increased by 30%. Aminopyrine-*N*-demethylase and aniline hydroxylase activities were also increased by 40 and 27%, respectively

(Ikeda et al. 1991). Although intermediate oral administration of doses of chlorfenvinphos induced alterations in serum sorbitol dehydrogenase and brain and liver levels of aromatic amino acids transferases L-phenylalanine aminotransferase, L-tyrosine aminotransferase, and L-tryptophan aminotransferase in mature Wistar rats (Puzynska 1984), no gross or microscopic histopathology in liver tissues was evident in weanling albino (Wistar) rats administered daily dietary chlorfenvinphos doses of 90 mg/kg/day (males) or 100 mg/kg/day (females) or in mongrel dogs given daily dietary doses of 10 mg/kg/day (males) or 50 mg/kg/day (females) for 12 weeks. However, relative liver weights were significantly and irreversibly decreased in the rats at a dose of 2.7 mg/kg/day (males) or 3 mg/kg/day (females) (Ambrose et al. 1970). In chronic (104 weeks) feeding studies, increased relative liver weights were observed in males at a dose of 7 mg/kg/day following dietary administration of chlorfenvinphos to both sexes. No liver histopathological or adverse liver function effects were reported in Beagle dogs fed daily dietary chlorfenvinphos doses of 10 mg/kg/day (males) or 50 mg/kg/day (females) for 104 weeks (Ambrose et al. 1970).

Renal Effects. No studies were located regarding renal effects in humans or animals following acute-, intermediate-, or chronic-duration oral exposure to chlorfenvinphos. In one study, no gross or microscopic histopathology in kidney tissues was evident in weanling albino (Wistar) rats administered daily dietary chlorfenvinphos doses of 90 mg/kg/day (males) or 100 mg/kg/day (females), or in mongrel dogs given daily dietary doses of 10 mg/kg/day (males) or 50 mg/kg/day (females) for 12 weeks. However, relative kidney weights were significantly and irreversibly decreased in the rats at a dose of 2.7 mg/kg/day (males) or 3 mg/kg/day (females) (Ambrose et al. 1970). In chronic (104 weeks) feeding studies, no gross or microscopic histopathology in the kidney and urinary bladder tissues examined or changes in relative kidney weights were evident in this strain of rats following daily doses of 21 mg/kg/day (males) or 24 mg/kg/day (females) (Ambrose et al. 1970). No kidney histopathological or adverse kidney function effects were reported in Beagle dogs fed daily dietary chlorfenvinphos doses of 10 mg/kg/day (males) or 50 mg/kg/day (females) for 104 weeks (Ambrose et al. 1970). Renal function was not assessed in these studies. Due to the lack of adequate data on the renal effects on chlorfenvinphos in humans and animals, the renal effects of human exposure to chlorfenvinphos are not known.

Endocrine Effects. There are no reports of endocrine effects in humans exposed by acute-, intermediate-, or chronic-duration ingestion of chlorfenvinphos. No studies were located regarding endocrine effects in animals after intermediate- or chronic-duration oral exposure to this insecticide. Although a significant increase (>300%) of plasma corticosterone was observed at 1 and 3 hours, and

of plasma aldosterone from 1 to 6 hours after treatment of male Wistar rats with a single chlorfenvinphos dose of 6.15 mg/kg (50% LD_{50}) by stomach tube (Osicka-Koprowska et al. 1984), the toxicological significance of these findings and relevance to human health are unknown.

Dermal Effects. No studies were found regarding dermal effects in humans or animals following inhalation or dermal exposure to chlorfenvinphos. In animal oral studies, no adverse changes were seen in the skin of weanling albino (Wistar) rats of both sexes given oral doses of up to 100 mg/kg/day (female rats) chlorfenvinphos for 12 weeks (Ambrose et al. 1970). Similarly, no adverse changes were seen in the skin of weanling albino (Wistar) rats of both sexes chronically (104 weeks) given daily dietary chlorfenvinphos doses of 0.7, 2.1, 7, or 21 mg/kg/day (males) or 0.8, 2.4, 8, or 24 mg/kg/day (females) (Ambrose et al. 1970). Therefore, adverse dermal effects in humans from exposure to chlorfenvinphos at hazardous waste sites are not likely.

Ocular Effects. No studies were located regarding ocular effects in humans or animals following acute-, intermediate-, or chronic-duration inhalation, oral, or dermal exposure to chlorfenvinphos. Consequently, it is not known whether human exposure to environmental concentrations of chlorfenvinphos could result in adverse ocular effects.

Body Weight Effects. No studies were located regarding body weight effects in humans following acute-, intermediate-, or chronic-duration inhalation, oral, or dermal exposure to chlorfenvinphos. In animal studies, acute-duration exposure of adult rats to oral Birlane® (chlorfenvinphos) at a dose of 2.4 mg/kg/day in the diet for 10 days did not affect body weight increases. Body weight increases were also unaffected following oral doses of 0.8 mg/kg/day in the diet for 30 days (Barna and Simon 1973). Similarly, no body weight changes were seen in mongrel dogs exposed to dietary chlorfenvinphos at a dose of 10 mg/kg/day (males) or 50 mg/kg/day (females) for 12 weeks (Ambrose et al. 1970). However, oral exposure of weanling rats for the same duration was associated with a significant but slightly reversible depression on growth, observed at a dose of 9 mg/kg/day (males) or 10 mg/kg/day (females). In an accompanying chronic-duration oral study (104 weeks), chlorfenvinphos also significantly and reversibly decreased body weight gain of female weanlings at dose levels of $8 mg/kg/day. Beagle dogs given daily dietary chlorfenvinphos doses of 10 mg/kg/day (males) or 50 mg/kg/day (females) for the same duration (104 weeks) exhibited no significant changes in body weight (Ambrose et al. 1970). Based on the available information, human exposure to environmental concentrations of chlorfenvinphos is not likely to result in any significant adverse body weight effects.

Metabolic Effects. Occupational exposure to airborne chlorfenvinphos significantly lowered NBT-dye reduction in both stimulated and non-stimulated cells, and caused a significant decrease of the spontaneous E rosette formation (not influenced by exposure time) in the blood (early E rosettes, 52%; late E rosettes, 57%). The authors of this study concluded that this may be regarded as a probable mechanism by which organophosphoric chemicals interfere with metabolism and cause membrane damage in human cells. About half of the subjects in the study were smokers (Wysocki et al. 1987). In animal studies, Carworth Farm E strain male rats orally administered a single [^{14}C]chlorfenvinphos dose of 2.5 or 13.3 mg/kg in olive oil (with or without prior monooxygenase induction with dieldrin) exhibited minimal changes in the metabolic profiles (Hutson and Wright 1980). However, the gastro-intestinal absorption of glucose was increased by 30% over control values, while Na^+ absorption was decreased by 32% below control values in adult female albino (Wistar) orally administered Birlane® (chlorfenvinphos) at a dose of 2.4 mg/kg/day in the diet for 10 days, although, Ca^{2+} absorption was unaffected. Similarly, oral chlorfenvinphos increased glucose absorption 12% while decreasing Na^+ absorption by 23% at a dose of 0.8 mg/kg/day in the diet to this strain of rats for 30 days. Gastro-intestinal absorption of Ca^{2+} was, likewise, unaffected by chlorfenvinphos exposure in this intermediate exposure to oral chlorfenvinphos. The changes in glucose and Na^+ absorption were not considered statistically significant ($P>0.05$) by the investigators (Barna and Simon 1973). The LOAEL of 2.4 mg/kg/day from the 10-day dosing protocol of this study, based on adverse neurological effects in rats was used to derive an acute oral MRL of 0.002 mg/kg/day for chlorfenvinphos.

Evidence from rat studies indicates that the alteration of brain and liver activities of the aromatic amino acid transferases by chlorfenvinphos may be due to the inhibitory effect of the substance on noradrenaline (norepinephrine) activity, since noradrenaline (norepinephrine) has been shown to affect amino acid transferase (L-tyrosine aminotransferase) activity in another study (Puzynska 1984).

Based on the available information, human exposure to environmental concentrations of chlorfenvinphos is not likely to result in any significant adverse metabolic effects.

Other Systemic Effects. No studies were located regarding other systemic effects in humans following acute-, intermediate-, or chronic-duration inhalation, oral, or dermal systemic effects from exposure to chlorfenvinphos. In animal studies, no significant effect on food consumption was evident in weanling albino (Wistar) rats administered daily dietary chlorfenvinphos doses of 90 mg/kg/day (males) or 100 mg/kg/day (females) or in mongrel dogs given daily dietary doses of 10 mg/kg/day (males) or 50 mg/kg/day (females) for 12 weeks. Similarly, in chronic (104 weeks) feeding studies, no

significant effect on food consumption was evident in rats and Beagle dogs following daily doses of 21 mg/kg/day (males) or 24 mg/kg/day (females) and 10 mg/kg/day (males) or 50 mg/kg/day (females), respectively (Ambrose et al. 1970). Based on the available information, human exposure to environmental concentrations of chlorfenvinphos is not likely to result in any significant adverse effects on food consumption effects.

Immunological and Lymphoreticular Effects. Only one study was located that reported immunological effects in humans. In this report, occupational exposure to inhaled chlorfenvinphos for an average of 15 years was associated with damage to humoral mechanisms in humans. The subjects exhibited significant decrease of the spontaneous E rosette formation and lowered absolute lymphocyte count in the peripheral blood. However, the subjects were also concurrently exposed to greater concentrations of other potentially immunotoxic substances such as formothion, sumithion, and malathion (Wysocki et al. 1987). In animal studies, the gastrointestinal absorption of glucose was increased by 30% over control values in adult female albino (Wistar) rats orally administered Birlane® (chlorfenvinphos) at a dose of 0 or 2.4 mg/kg/day in the diet for 10 days. Similarly, gastrointestinal absorption of glucose was increased by 12% over control values following orally administered Birlane® (chlorfenvinphos) at a dose of 0 or 0.8 mg/kg/day in the diet to this strain of rats for 30 days. However, the changes in glucose and Na^+ absorption were not considered statistically significant ($P>0.05$) by the investigators (Barna and Simon 1973). It has been observed in other studies that an increased metabolic activity of neutrophils and monocytes during phagocytosis is accompanied by higher consumption of glucose and oxygen. Hydrogen peroxide is then derived from the pentose cycle, NAD-, and NADP-oxidase action (Kolanoski 1977 as cited in Wysocki et al. 1977). However, the relationship between increased gastrointestinal absorption of glucose and increased glucose utilization is not clear. In other animal studies, rabbits orally exposed to chlorfenvinphos for 90 days also exhibited significantly elevated serum hemagglutinin level (16%) and hemolysin activity (66%, $P<0.05$) as well as increased numbers of nucleated lymphoid cells producing hemolytic antibodies to sheep erythrocytes. Spleen cytomorphology changes, manifested mainly as transformation of primary follicles into secondary ones with well developed germinal centers, were also observed (Roszkowski 1978). Intermediate-duration dietary exposure of rats resulted in a significant and irreversible reduction in relative spleen weight of female rats given $3 mg/kg/day chlorfenvinphos for 12 weeks. However, no gross or microscopic histopathology was evident in the spleen and bone marrow tissues of the rats upon examination (Ambrose et al. 1970). No histopathological changes in the spleen or bone marrow or changes in absolute or relative spleen weights were noted in rats or Beagle dogs of both sexes given dietary chlorfenvinphos doses of 21 mg/kg/day (males) or 24 mg/kg/day (females), or 10 mg/kg/day (males) or 50 mg/kg/day (females), respectively, for 104 week (Ambrose et al. 1970). C57BL/6 mice and (C57BL/6 x DBA/2)F1 (BDF1/Iiw) hybrid mice (6–8 weeks old) orally exposed to

chlorfenvinphos for 90 days exhibited a reversible reduction in the number of E rosette-forming cells as well as a dose-related decrease in the number of hemolysin-producing cells, reduction in the number of plaque-forming cells, increases in Interlukin-1 activity and DTH reaction, stimulation of spleen colonies, and disturbance in humoral immune factors (immunoglobulins) at a LOAEL of 1.5 mg/kg (Kowalczyk-Bronisz et al. 1992). The LOAEL of 1.5 mg/kg/day, based on adverse immunolymphoreticular effects in this study, was used to derive an intermediate oral MRL of 0.002 mg/kg/day for chlorfenvinphos. While the existing human inhalation study and the animal oral studies provide some indication that chlorfenvinphos exposure is associated with immunological changes, these changes were not consistent with depressive effect on immune reactions. Thus, the changes reported in these studies may simply be immunological mobilizations of the organisms to xenobiotics as opposed to damage to the major histocompatibility complex. Consequently, it is not certain that human inhalation or oral exposure to chlorfenvinphos can result in immune dysfunction.

Neurological Effects. No studies were located regarding neurological effects in humans after acute- or intermediate-duration inhalation exposure to chlorfenvinphos, or after intermediate- or chronic-duration dermal exposure. Chlorfenvinphos inhibits cholinesterase activity in the central and peripheral nervous system of humans and animals (Ambrose et al. 1970; Barna and Simon 1973; Cupp et al. 1975; Gralewicz et al. 1989a, 1989b, 1990; Hunter 1969; Maxwell and LeQuesne 1982; Osicka-Koprowska et al. 1984; Pach et al. 1987; Takahashi et al. 1991; Vestweber and Kruckenberg 1972). It also interferes with noradrenaline (norepinephrine) activity in central adrenergic mechanisms in animals (Brzezinski 1978; Osumi et al. 1975). Inhibition of acetylcholinesterase activity results in accumulation of acetylcholine at muscarinic and nicotinic receptors leading to peripheral and central nervous system effects. These effects usually appear within a few minutes to a few hours after exposure, depending on the extent of exposure. In a human case report, a 16-year-old white male who mistakenly ingested a formulation (identified as Dermaton®) was hospitalized 90 minutes afterward with symptoms of abdominal cramps, nausea, vomiting, generalized weakness, cold dry skin, hypothermia, listlessness, constricted pupils, hypertension, respiratory distress, fine generalized muscular twitching, and apprehension. Plasma and erythrocyte activity levels were significantly inhibited. All vital signs returned to normal after gastric lavage and treatment with atropine and pralidoxime (Cupp et al. 1975).

The available information indicates that chlorfenvinphos has similar neurological effects in animals. The information indicates that the substance causes disruptions in the central and peripheral nervous system in rats and dogs following acute-, intermediate-, or chronic-duration exposures via the oral route at doses as low as 0.8 mg/kg/day (Ambrose et al. 1970; Barna and Simon 1973; Maxwell and

LeQuesne 1982; Osumi et al. 1975; Puzynska 1984; Takahashi et al. 1994). These disruptions are mediated by the inhibition of cholinesterase activity in the peripheral and central nervous tissue and are manifested as abnormal muscle reflex, muscle fasciculations, Straub tail reflex, twitches, convulsions, chromodacryorrhea, exophthalmos, gasping, lacrimation, prostration, salivation, sleep disturbances, diarrhea, emesis, and urination (Ambrose et al. 1970; Maxwell and LeQuesne 1982; Osumi et al. 1975; Puzynska 1984; Takahashi et al. 1991). Cholinesterase activity in the brain of male Wistar rats was unaffected 3 hours after oral administration of 1 mg/kg of chlorfenvinphos. However, at doses of $2 mg/kg, oral chlorfenvinphos produced a marked decrease in the brain cholinesterase activity to 18–38% of the control ($P<0.001$) value. The maximum inhibition occurred 3 hours after the administration, after which the cholinesterase activity increased gradually. Erythrocyte cholinesterase activity also decreased after 4 mg/kg of chlorfenvinphos; the lowest level (20%, $P<0.001$) was attained 3 hours after treatment (Osumi et al. 1975). This study was not used to calculate an acute oral MRL because it was deemed less appropriate due to the gavage (oral) route of administration. An oral feeding study is preferred for this purpose by the ATSDR MRL Workgroup. In a chronic-duration study, chlorfenvinphos significantly inhibited both plasma and erythrocyte cholinesterase activities in a dose-dependent manner in weanling albino (Wistar) rats fed daily chlorfenvinphos doses of 0.7, 2.1, 7, or 21 mg/kg/day (males) or 0.8, 2.4, 8, or 24 mg/kg/day (females) mg/kg/day in the diet for 104 weeks. Plasma and erythrocyte cholinesterase activities were inhibited by 48% in females and 45% in males in the first week of treatment and by 20% in females and 33% in males in the fourth week of treatment, respectively, at the lowest dose tested (0.7 mg/kg/day for males and 0.8 mg/kg/day for females). No gross or microscopic histopathology was evident in the brain tissue examined (Ambrose et al. 1970). The LOAEL of 0.7 mg/kg/day, based on adverse neurological effects in rats in this study, was used to derive a chronic oral MRL of 0.0007 mg/kg/day for chlorfenvinphos. In another study, all 36 Sprague-Dawley rats exposed to chlorfenvinphos doses of 10.5 mg/kg/day in the diet for 3–6 months exhibited repetitive muscle activity when given two electrical stimuli simultaneously. This is indicative of hyper stimulation due to depletion or inactivation of neuromuscular junction cholinesterase. The abnormality became more marked with time, even on constant dosing (Maxwell and LeQuesne 1982). The indications from this study may be useful in explaining electrophysiological abnormalities described in some workers chronically exposed to some organophosphorus compounds.

Besides its cholinergic action, there is limited evidence that chlorfenvinphos acts via central noradrenergic mechanisms in rats by accelerating the noradrenaline (norepinephrine) turnover in the brain *in vivo* by the release of noradrenaline (norepinephrine) from brain tissue stores (Brzezinski 1978).

On the basis of the existing evidence, human exposure to chlorfenvinphos is likely to result in neurological effects stemming from interference with both the central cholinergic and adrenergic mechanisms.

Reproductive Effects. No studies were located regarding reproductive effects in humans following acute-, intermediate-, or chronic-duration inhalation, oral, or dermal exposure to chlorfenvinphos. Reports from single-generation, intermediate- or chronic-duration rat and dog studies are largely negative for reproductive effects (Ambrose et al. 1970). However, these studies did not evaluate reproductive function. A 3-generation reproductive study in albino (Wistar) rats reported significant (50%) reduction in fertility in the F/2 generation at a LOAEL of 3 mg/kg/day (Ambrose et al. 1970). Although human data are lacking, the indications provided by the animal data suggest that adverse reproductive effects may result from prolonged human exposure to chlorfenvinphos at levels found at hazardous wastes sites.

Developmental Effects. No studies were located regarding developmental effects in humans following acute-, intermediate-, or chronic-duration inhalation exposure to chlorfenvinphos; or regarding acute-duration exposure of animals to chlorfenvinphos. A statistical model for hazard identification concluded that chlorfenvinphos is likely to interfere with development in rabbits and hamsters but not in rats. The model was essentially a database of 175 probable, suspected, unknown, and probably negative teratogenic or embryotoxic drugs and chemical compounds. For each of the compounds, including chlorfenvinphos, the results of any developmental toxicity testing in up to 14 animal species and any reports of mutagenicity or carcinogenicity were recorded. The compounds were categorized with respect to their human developmental toxicant effect: -1.0 testing negative, 0.0 not tested (unknown), 0.5 tested with equivocal results (suspicious), and 1.0 testing positive. Although the model did not evaluate the adverse reproductive effects potential of chlorfenvinphos in primates, mice, and dogs, it correctly classified the study compounds 63–91% of the time. The model had a sensitivity of 62–75%, and a positive predictive value of 75–100%. However, the model had a negative predictive value of 64–91%, indicating the model is not optimal for hazard identification (Jelovsek et al. 1989). In acute exposures, chlorfenvinphos inhibited respiratory efficiency of juvenile rats in a dose-dependent manner at a LOAEL of 29 mg/kg/day. At 300 mg/kg/day, chlorfenvinphos completely arrested respiration in these rats (Skonieczna et al. 1981). In a 3-generation rat study, chlorfenvinphos induced significant but slightly reversible body weight gains in female rats at a LOAEL of $8 mg/kg/day as well as increased pup mortality and reduced lactational index at a LOAEL of 2.7 mg/kg/day. However, no teratogenic effects were reported in offspring rats (Ambrose et al. 1970). In single-generation intermediate- and chronic-duration studies with juvenile rats in which the rats were given dietary chlorfenvinphos doses of 90 mg/kg/day (males) or 100 mg/kg/day (females) for 12 weeks, or 21 mg/kg/day (males) or 24 mg/kg/day (females) in the

diet for 104 weeks, no significant effects on development were reported (Ambrose et al. 1970). Although human data are lacking, the indications provided by the animal data suggest that adverse developmental effects may result from prolonged human exposure to chlorfenvinphos at levels found at hazardous wastes sites.

Genotoxic Effects. No studies were located regarding genotoxic effects of chlorfenvinphos in humans or animals following any route of exposure. Chlorfenvinphos was negative for mutagenicity in both base-pair change-type strains microorganisms (WP2 *hcr* of *Escherichia coli*; TA1535, TA1537, TA1538, TA98 of *Salmonella typhimurium*). In a mutagenicity study, the dose-response curve at doses of 0, 50, 500, and 5,000 µg/plate, for the mutagenic activity of chlorfenvinphos for the *S. typhimurium* strain TA100 was reduced by the S9 mix (metabolic activation). At present, no mutagenic pesticide whose activity decreases in the presence of the S9 mix is carcinogenic except captan (F-28) (Moriya et al. 1983). The mutagenic potency of chlorfenvinphos for strain TA100 has been reported as 0.038 revertants/nmol, indicative of a positive response (Dean 1972; Moriya et al. 1983; Vishwanath and Kaiser 1986). A mixture of 15 pesticides (containing 0.3% chlorfenvinphos) tested negative for mutagenicity in the *Salmonella* microsome assay, with or without metabolic activation with PCB-induced rat liver S9, at concentrations up to 500 µg/plate in the *Salmonella*-microsome assay. The mixture also failed to induce SCEs in human lymphocytes *in vitro* as well as *in vivo* mutagenicity in the micronucleus bone marrow assay in male Wistar rats at concentrations proportional to the ratio determined in foods ranging from 0.1 to 20 µg/mL (Dolara et al. 1993). In other large-scale screening programs which revealed microbial mutagenic activity in four new compounds (all fungicides), chlorfenvinphos (without metabolic activation) exhibited no mutation induction capacity in a rec-assay procedure (prescreening of DNA-damaging chemicals) utilizing strains of *Bacillus subtilis*, H17 Rec+ and M45 Rec-. Also, no mutation potential was evident in a reversion-assay (determination of mutation specificities) in which two tryptophan-requiring strains (auxotrophic) of *E. coli* (B/r try WP2 and WP2 *try hcr*) and four strains of *S. typhimurium* (TA1535, TA1536, TA1537, TA1538) were used. The *E. coli* auxotrophic strains and *Salmonella* TA1535 are reversible by base-pair change-type mutagens and the three *Salmonella* strains (TA1536, TA1537, and TA1538) are reversible by frameshift mutagens (Shirasu 1973; Shirasu et al. 1976).

There are no unequivocal data to indicate that chlorfenvinphos reacts directly with DNA *in vivo* or *in vitro* to produce mutations in either germ or somatic cells. In a study to determine the ability of vinyl phosphate esters, like chlorfenvinphos, to form methylated bases in DNA of calf thymus failed to detect 6-methyl guanine, a known mutagen. In both the reaction with dsDNA and ssDNA, 7-methyl guanine was the main methylation product. However, all methyl derivatives of adenine constituted

about 40% and 50% of all methylation products in the case of dsDNA and ssDNA, respectively. 3-Methylcytosine was the only methyl derivative of a pyrimidine identified (Wiaderkiewicz et al. 1986). In another study, tetrachlorvinphos (Gardona®) was evaluated for its potential to induce chromosomal aberrations and SCEs *in vitro* in a primary culture of Swiss mice spleen cells at concentrations of 0.25, 0.50, 1.0, or 2.0 µg/mL. After 4 hours of treatment, tetrachlorvinphos induced a high percentage of metaphases with chromosomal aberrations in the mouse spleen cells in a dose-dependent manner. According to the authors, the results indicate that tetrachlorvinphos in the tested concentrations is mutagenic in mouse spleen cell cultures (Amer and Aly 1992). In this study, a structural analog of chlorfenvinphos (tetrachlorvinphos) was used; therefore, the data are difficult to relate to chlorfenvinphos without extensive structure-activity relationship analysis. Data from these studies are shown on Tables 2-3 and 2-4.

Cancer There are no epidemiological or laboratory animal data to evaluate the carcinogenicity of chlorfenvinphos in humans by any route of exposure. However, a study to determine the ability of vinyl phosphate esters, like chlorfenvinphos, to form methylated bases in DNA of calf thymus failed to detect 6-methyl guanine, a known mutagen. In both the reaction with dsDNA and ssDNA, 7-methyl guanine was the main methylation product. However, all methyl derivatives of adenine constituted about 40% and 50% of all methylation products in the case of dsDNA and ssDNA, respectively. 3-Methylcytosine was the only methyl derivative of pyrimidine identified (Wiaderkiewicz et al. 1986). It is noteworthy that the production of electrophilic metabolic intermediates or epoxides in the metabolism of chlorfenvinphos, which could react with nucleophilic cellular components (DNA, RNA, and proteins) leading to carcinogenesis, was considered unlikely by a theoretical analysis (Akintonwa 1985).

In a mutagenicity study, the dose-response curve, at doses of 0, 50, 500, and 5,000 µg/plate, for the mutagenic activity of chlorfenvinphos for the *S. typhimurium* strain TA100 was reduced by the S9 mix (metabolic activation). At present, no mutagenic pesticide whose activity decreases in the presence of the S9 mix is carcinogenic except captan (F-28) (Moriya et al. 1983). A theoretical analysis that predicted the mammalian biotransformation products based on the recognition of the structure of chlorfenvinphos, understanding of Types I and II metabolism of foreign compounds, and mechanistic biochemistry, also acknowledged that cytochrome P-450 monooxygenase (an inducible enzyme) is the relevant enzyme which mediates the biotransformation of chlorfenvinphos. The author of this study postulated that the 2,4-dichlorobenzoyl glycine (2,4-dichlorohippuric acid) was produced in the rat by this mechanism. The production of electrophilic metabolic intermediates or epoxides in the metabolism of chlorfenvinphos, which could react

Table 2-3. Genotoxicity of Chlorfenvinphos *In Vivo*

End point	Species (Test System)	Exposure Route	Results	Reference
Mammalian systems:				
bone marrow micronucleus test	rat	Oral	–	Dolara et al. 1993

– = negative result; ± = weakly positive

Table 2-4. Genotoxicity of Chlorfenvinphos *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Reverse mutation				
Salmonella typhimurium TA1535, TA1537, TA1538, TA98	Gene mutation	–	–	Moriya et al 1983
S. typhimurium TA1535, TA1536, TA1537, TA1538	Gene mutation	–	–	Shirasu et al. 1976
S. typhimurium TA97, TA98, TA100, TA1530, TA1535	Gene mutation	–	–	Vishwanath and Kaiser 1986
S. typhimurium TA100	Gene mutation	±	+	Moriya et al 1983
S. typhimurium	Gene mutation	–	–	Dolara et al. 1993
Escherichia coli *B/r try* WP2 *and* WP2 *try hcr*	Gene mutation	–	–	Shirasu et al. 1976
E. coli WP2 *hcr*	Gene mutation		–	Shirasu 1973; Dean 1972
Bacillus subtilis H17 Rec+ and M45 Rec-	Gene mutation	–	–	Shirasu et al. 1976
Eukaryotic cells:				
Human peripheral blood lymphocytes	Chromosomal aberration	–	–	Dolara et al. 1993
Calf thymus	DNA binding	–	–	Wiaderkiewicz et al. 1986

- = negative result; + = positive result; ± = weakly positive

with nucleophilic cellular components (DNA, RNA, and proteins) leading to carcinogenesis, was considered unlikely by this theoretical approach (Akintonwa 1984).

In contrast, tetrachlorvinphos (Gardona®), a structural analog to chlorfenvinphos, was evaluated for potential to induce chromosomal aberrations and SCEs *in vitro* in a primary culture of Swiss mice spleen cells at concentrations of 0.25, 0.50, 1.0, or 2.0 µg/mL. After 4 hours of treatment, tetrachlorvinphos induced a high percentage of metaphases with chromosomal aberrations in the mouse spleen cells in a dose-dependent manner. The corresponding number of metaphases for tetrachlorvinphos doses of 0.25, 0.50, 1.0, and 2.0 µg/m were 150, 590, 590, 600, and 600. According to the authors, the results of this evaluation indicate that tetrachlorvinphos (in the tested concentrations) ismutagenic in mouse spleen cell cultures (Amer and Aly 1992). However, in both of these studies, structural analogs of chlorfenvinphos (methylbromophenvinphos and tetrachlorvinphos, respectively) were used; therefore, the data are difficult to relate to chlorfenvinphos without extensive structure-activity relationship analysis.

Although neither human epidemiological evidence or evidence from rodent cancer bioassays is available, the current theoretical evidence indicates that human exposure to chlorfenvinphos is not likely to present cancer risk.

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 chlorfenvinphos 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 (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by chlorfenvinphos 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 Chlorfenvinphos

Chlorfenvinphos is rapidly absorbed from the gastrointestinal tract and widely distributed throughout the body in humans (Pach et al. 1987; Wagner et al. 1990). Traces of unchanged chlorfenvinphos have been detected in animal urine following exposure (Hunter 1972; Szczepaniak and Sienkiewitcz 1980). Chlorfenvinphos undergoes biotransformation to a variety of polar metabolites, including 2-chloro-1-(2,4-dichlorophenyl) vinylethylhydrogen phosphate; 1-(2,4-dichlorophenyl) ethanol; 1-(2,4-dichlorophenyl) ethanediol; 2,4-dichloromandelic acid; and 2,4-dichlorobenzoyl glycine (Hutson and Wright 1980), which have been detected in animals. Analysis of urine samples for the presence of these metabolites represents a potentially preferable means of assessing exposure since this method is non-invasive. However, as an organophosphate, chlorfenvinphos is rapidly metabolized and excreted from the body; therefore, urinary metabolite analysis is useful only in the evaluation of recent exposures.

The major action resulting from human exposure to chlorfenvinphos is the inhibition of cholinesterase activity (see Section 2.4). Two pools of cholinesterases are present in human blood: -acetylcholinesterase in erythrocytes and pseudocholinesterase in plasma. Acetylcholinesterase, present in human erythrocytes, is identical to the enzyme present in neuromuscular tissue (the target of chlorfenvinphos action), while plasma pseudocholinesterase has no known physiological function. Inhibition of both forms of cholinesterase activities has been associated with exposure to chlorfenvinphos in humans and animals (Cupp et al. 1975; Gralewicz et al. 1989a, 1989b, 1990; Hunter 1969; Maxwell and LeQuesne 1982; Osicka-Koprowska et al. 1984; Pach et al. 1987; Takahashi et al. 1991; Vestweber and Kruckenberg 1972). Inhibition of erythrocyte, plasma, or whole blood cholinesterase activities may be used as a marker of exposure to chlorfenvinphos. However, inhibition of cholinesterase activity is a common action of anticholinesterase compounds, which include organophosphates, like chlorfenvinphos, and carbamate compounds. In addition, a wide variation in normal cholinesterase values exists in the general population, and there are no studies which report a quantitative association between cholinesterase activity levels and exposure to chlorfenvinphos in humans. Thus, the inhibition of cholinesterase activity is not a specific biomarker of effect for chlorfenvinphos exposure; it is indicative only of effect and is not useful for dosimetric analysis. It should be noted that changes in plasma cholinesterase (pseudocholinesterase) activity are considered a more sensitive biomarker of exposure for organophosphate exposure than changes in erythrocyte cholinesterase activity (Endo et al. 1988; Hayes et al. 1980). It has been suggested that in the absence of baseline values for cholinesterase activity, sequential post-exposure cholinesterase analyses be used to confirm a diagnosis of organophosphate (like chlorfenvinphos) poisoning (Coye et al. 1987).

This method of using the inhibition of cholinesterase activity as an indicator for exposure to organophosphate exposure lacks even greater specificity when used to assess exposure to compounds like chlorfenvinphos which are weak cholinesterase inhibitors. As a vinyl phosphate, chlorfenvinphos is metabolized to desethyl chlorfenvinphos which could be detected in urine as a specific biomarker of exposure to chlorfenvinphos or other vinyl phosphates. The method of detection involves conversion of urinary desethyl chlorfenvinphos to the more easily measurable methyl desethyl chlorfenvinphos with diazomethane. The analyses of 24-hour samples of urine from 14 male volunteers were made at 14-day intervals on four occasions (days 10, 24, 38, and 52) during exposure, and on one occasion, 15–16 days after cessation of exposure, using this novel detection method. The average daily excretion of *beta*-methyl desethyl chlorfenvinphos during exposure was 120 µg, which was 4.7% of the dose. In post-exposure urine, the concentrations of *beta*-methyl desethyl chlorfenvinphos were 0 in most cases, and no greater than 5–10 µg/day in the remainder. The excretion rate of *alpha*-methyl desethyl chlorfenvinphos was 15 ±5 µg/day during exposure, but

fell to 0 or <5 µg/day post-exposure. Thus, the higher level of excretion of desethyl chlorfenvinphos in the urine found in an acute dosing experiment was not maintained when the dose was diminished four-fold and administered daily. However, since desethyl chlorfenvinphos accounts for only about 5% of the dose at this low exposure level, its concentration in urine would lack sensitivity when used as an index of exposure to chlorfenvinphos (Hunter et al. 1972). Although this method of assessing chlorfenvinphos exposure may not be useful in low exposure conditions, the method could be used to evaluate exposure to high doses of chlorfenvinphos such as occurs in human acute poisoning cases. It has been suggested that the concentration of chlorfenvinphos (or its unique metabolites) in the blood may be a better index of exposure than inhibition of cholinesterase activity (Hunter 1968, 1969).

2.6.2 Biomarkers Used to Characterize Effects Caused by Chlorfenvinphos

Inhibition of erythrocyte, plasma, or whole blood cholinesterase activities in humans and animals that results from chlorfenvinphos exposure (Barna and Simon 1973; Brzezinski 1978; Cupp et al. 1975; Pach et al. 1987; Kolmodin-Hedman and Eriksson 1987; Osicka-Koprowska et al. 1984; Osumi et al. 1975; Takahashi et al. 1991) may be used as a marker of effect for chlorfenvinphos exposure. However, inhibition of cholinesterase activity is a common action of anticholinesterase compounds, which include organophosphates, like chlorfenvinphos, and carbamate compounds. In addition, a wide variation in normal cholinesterase values exists in the general population, and there are no studies which report a quantitative association between cholinesterase activity levels and exposure to chlorfenvinphos in humans. Thus, inhibition of cholinesterase activity is not a specific biomarker of effect for chlorfenvinphos exposure; it is indicative only of effect and not useful for chlorfenvinphos-specific dosimetric analysis.

It should be noted that plasma cholinesterase (pseudocholinesterase) activity is considered a more sensitive biomarker of effect for organophosphate exposure than erythrocyte cholinesterase activity (Endo et al. 1988; Hayes et al. 1980). It has been suggested that in the absence of baseline values for cholinesterase activity, sequential post-exposure cholinesterase analyses be used to confirm a diagnosis of organophosphate poisoning (Coye et al. 1987).

In combination with analysis of reductions in the level of cholinesterase activity, the manifestations of severe organophosphate (chlorfenvinphos) poisoning, clinically characterized by a collection of cholinergic signs and symptoms, which may include dizziness, fatigue, tachycardia or bradycardia, miosis, diarrhea, and vomiting (Chambers and Levi 1992; Cupp et al. 1975; Klaassen et al. 1986;

Takahashi et al. 1991; Williams and Burson 1985), are useful biomarkers of effect for identifying poisoned victims of organophosphates (chlorfenvinphos). Also, these manifestations are not specific to chlorfenvinphos but to anticholinesterase compounds (such as organophosphates and carbamate compounds) in general. A positive response to atropine treatment is considered a confirmation of organophosphate poisoning.

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 toxicity of chlorfenvinphos may be affected by other substances. Some chemicals may increase the toxicity of chlorfenvinphos in an additive manner. Chemical substances such as other anticholinesterase organophosphates, carbamates, or some pyrethroid insecticides that cause neurotoxicity are expected to act in an additive manner with chlorfenvinphos with respect to its potential to induce cholinergic toxicity.

As a direct-acting cholinesterase activity inhibitor (P=O type in contradistinction from P=S types), other chemicals may interfere with the toxicity of chlorfenvinphos indirectly by accelerating its metabolism to less toxic metabolites through their actions on drug-metabolizing enzymes, specifically, glutathione-S-transferase and P-450 or mixed function monooxygenases (Akintonwa 1984, 1985; Akintonwa and Itam 1988; Donninger 1971; Hansen 1983; Hutson and Logan 1986; Hutson and Millburn 1991; Hutson and Wright 1980). The duration and intensity of action of chlorfenvinphos is largely determined by the speed at which it is metabolized via oxidative *O*-dealkylation in the body by liver microsomal cytochrome P-450 or mixed function monooxygenases (MFO). More than 200 drugs, insecticides, carcinogens, and other chemicals are known to induce the activity of liver microsomal drug-metabolizing enzymes. The characteristic biological actions of these chemicals are highly varied. Although there is no relationship between their actions or structures and their ability to induce enzymes, most of the inducers are lipid-soluble at physiological pH. These inducers of the MFO system include the following classes of drugs: hypnotic and sedatives (barbiturates, ethanol, tetrahydrofuran, metyrapone); anesthetic gases (methoxyflurane, halothane); central nervous system stimulants (amphetamine); anticonvulsants (diphenylhydantoin); tranquilizers (meprobamate); antipsychotics (triflupromazine); hypoglycemic agents (carbutamide); anti-inflammatory agents (phenylbutazone); muscle relaxants (orphenadrine); analgesics (aspirin, morphine); antihistaminics (diphenhydramine); alkaloids (nicotine); polychlorinated aromatic hydrocarbons; insecticides

(chlordane, DDT, BHC, aldrin, dieldrin, heptachlorepoxide); pyrethrins; steroid hormones (testosterone, progesterone, cortisone); and polycyclic aromatic hydrocarbons (3-methylcholanthrene, 3,4-benzpyrene, α-naphthoflavone) (Akintonwa 1984; Hansen 1983; Hutson and Logan 1986; Hutson and Millburn 1991; Hutson and Wright 1980; Ikeda et al. 1991; Klaassen et al. 1986; Williams and Burson 1985). Thus, exposure to any of these enzyme inducers prior to, or concurrent with, exposure to chlorfenvinphos may result in accelerated biotransformation of chlorfenvinphos to its less toxic metabolites. Conversely, since chlorfenvinphos is active *per se*, concurrent exposure to chlorfenvinphos and MFO enzyme-inhibiting substances (e.g., carbon monoxide; ethylisocyanide; SKF 525A, halogenated alkanes, such as CCl_4; alkenes, such as vinyl chloride; and allylic and acetylenic derivatives) would increase the mammalian half-life and, thus, the toxicity of chlorfenvinphos (Akintonwa 1984; Akintonwa and Itam 1988; Donninger 1971; Hansen 1983; Hutson and Logan 1986; Hutson and Millburn 1991; Hutson and Wright 1980; Williams and Burson 1985).

Chlorfenvinphos exposure may interfere with the short-acting muscle relaxant succinylcholine used concurrently with anesthetics. The action of succinylcholine is terminated by means of its hydrolysis by plasma cholinesterase (Klaassen et al. 1986). Since plasma cholinesterase activity is strongly inhibited by chlorfenvinphos in humans (Cupp et al. 1975; Kolmodin-Hedman and Eriksson 1987; Pach et al. 1987; Takahashi et al. 1991; Vestweber and Kruckenberg 1972) and animals (Gralewicz et al. 1989a, 1989b, 1990; Kolmodin-Hedman and Eriksson 1987; Maxwell and LeQuesne 1982; Osicka-Koprowska et al. 1984; Pach et al. 1987; Takahashi et al. 1991; Vestweber and Kruckenberg 1972), it is expected that concurrent exposure to chlorfenvinphos may result in the prolongation of the action of succinylcholine leading to prolonged muscular paralysis.

2.8 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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

The magnitude of chlorfenvinphos toxicity, like the toxicity of any xenobiotic, is affected by the rate of its metabolic biotransformation to less toxic substances (Klaassen et al. 1986). Therefore, low xenobiotic metabolizing activity would result in greater toxicity. The newborn of several animal species, including humans, have an almost complete lack of ability to metabolize xenobiotics and may be more sensitive to chlorfenvinphos toxicity. Studies on experimental animals showed that starvation depressed P-450 activity due to actual loss of the enzyme protein (Boyd and Carsky 1969; Puzynska 1984). Thus, it is expected that dietary deficiency in protein would increase chlorfenvinphos toxicity by diminishing its metabolism in the liver. Hereditary factors may also contribute to population sensitivity to chlorfenvinphos. Atypical plasma cholinesterase with low activity is present in a small percentage of the human population. This is the result of an hereditary factor with 0.04% occurrence in the population. Since plasma cholinesterase activity is strongly inhibited by chlorfenvinphos (Cupp et al. 1975; Gralewicz et al. 1989a, 1989b, 1990; Kolmodin-Hedman and Eriksson 1987; Maxwell and LeQuesne 1982; Osicka-Koprowska et al. 1984; Pach et al. 1987; Takahashi et al. 1991; Vestweber and Kruckenberg 1972), it is expected that individuals who have atypical ChE (or low plasma cholinesterase activity) will be unusually sensitive to the muscle relaxant succinylcholine (Klaassen et al. 1986) and may suffer prolonged muscle paralysis if administered succinylcholine while exposed to chlorfenvinphos. Congenital low plasma cholinesterase activity may also increase subpopulation sensitivity to chlorfenvinphos exposure. This is because, after exposure, plasma cholinesterase acts as a depot for chlorfenvinphos due to its strong affinity for the substance (Davies and Holub 1980; Edson and Noakes 1960; Klemmer et al. 1978; Williams et al. 1959), thus decreasing the availability of the chlorfenvinphos dose to neuromuscular tissue, the target of chlorfenvinphos toxicity in the population with normal plasma cholinesterase levels. In individuals with congenital low plasma cholinesterase activity, less chlorfenvinphos is bound in the blood and more unbound chlorfenvinphos is in circulation to reach neuromuscular tissue, the target of chlorfenvinphos toxicity.

Individuals who have abnormally low tissue cholinesterase due to prior exposure to cholinesterase activity inhibitors are also exceptionally susceptible to the cholinesterase activity-inhibiting toxicity of chlorfenvinphos. These individuals may include those who are occupationally exposed to anticholinesterases, such as other cholinesterase activity inhibiting organophosphate or carbamate pesticides. In this regard, patients on medication that inhibit cholinesterase activity may also be unusually susceptible to the cholinesterase activity inhibiting toxicity of chlorfenvinphos.

2.9 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to chlorfenvinphos. 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 chlorfenvinphos. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following text provides specific information about treatment following exposures to chlorfenvinphos.

2.9.1 Reducing Peak Absorption Following Exposure

Organophosphate insecticides like chlorfenvinphos are rapidly absorbed after inhalation, ingestion, or dermal contact (Hutson and Wright 1980; Ikeda et al. 1991; Wagner et al. 1990). In oral exposures, emesis is not indicated because of the danger of aspiration of stomach contents by an obtunded patient. Gastric lavage, with a solution of 5% sodium bicarbonate or 2% potassium permanganate, may be indicated within the first 60 minutes after ingestion to get rid of unabsorbed chlorfenvinphos in the stomach (Cupp et al. 1975; Pach et al. 1987; Shankar 1967, 1978). Activated charcoal can also be used, but cathartics are not necessary due to the diarrhea induced by muscarinic activity. However, if diarrhea is not present in the patient, cathartics, mixed with activated charcoal, can be used.

Decontamination is the first step in reducing dermal or conjunctival absorption. This decontamination should begin immediately after the exposure is recognized. Contaminated clothing should be removed, and skin, hair, and nails should be washed with soap and plenty of water. Health care workers and emergency responders should be protected from secondary contamination, and clothes and other contaminated material should be treated as contaminated waste. Eyes should be irrigated with copious amounts of room-temperature water or saline, if available, for at least 15 minutes. If irritation, lacrimation, or especially pain, swelling, and photophobia persist after 15 minutes of irrigation, expert ophthalmologic care should be sought (Ellenhorn and Barceloux 1988).

If exposure is via inhalation, the exposed individual should be moved to fresh air and efforts should be directed toward the maintenance of an open airway, airway suctioning, endotracheal intubation. Artificial ventilation with supplemental oxygen may be helpful (Ellenhorn and Barceloux 1988).

2.9.2 Reducing Body Burden

Chlorfenvinphos is rapidly metabolized, with an estimated mammalian biological half-life of 12–15.13 hours (Akintonwa 1984; Akintonwa and Itam 1988; Donninger 1971; Hutson and Millburn 1991; Hutson and Wright 1980). Consequently, efforts at reducing body burdens of poisoned persons may not be critical to the outcome. Although hemoperfusion has been successfully used in one case of chlorfenvinphos poisoning (Pach et al. 1987), dialysis and hemoperfusion are not recommended in organophosphate poisonings because of the extensive tissue distribution of the absorbed doses (Mücke et al. 1970; Poklis et al. 1980). The use of P-450-inducing substances such as some antipsychotics (triflupromazine) and analgesics (aspirin, morphine) would tend to accelerate the metabolism of chlorfenvinphos, thereby decreasing its toxicity (Akintonwa 1984; Akintonwa and Itam 1988; Donninger 1971; Hutson and Millburn 1991; Hutson and Wright 1980; Klaassen et al. 1986; Williams and Burson 1985).

2.9.3 Interfering with the Mechanism of Action for Toxic Effects

As an anticholinesterase organophosphate, the principal toxic effects of chlorfenvinphos in humans and laboratory animals derive from inhibition of cholinesterase activity (Cupp et al. 1975; Gralewicz et al. 1989a, 1989b, 1990; Hunter 1969; Kolmodin-Hedman and Eriksson 1987; Maxwell and LeQuesne 1982; Osicka-Koprowska et al. 1984; Pach et al. 1987; Takahashi et al. 1991; Vestweber and Kruckenberg 1972). Severe inhibition of the activities of these enzymes results in accumulation of -acetylcholine at its sites of action, and excessive or interminable stimulation of both sympathetic and parasympathetic cholinergic receptors leading to muscarinic and nicotinic effects (Klaassen et al. 1986; Williams and Burson 1985).

Timely treatment of chlorfenvinphos poisoning cases with atropine and cholinesterase regeneration with pralidoxime and other oximes, significantly reduces the cholinergic effects (Cupp et al. 1975; Pach et al. 1987).

Pralidoxime acts by hydrolyzing phosphorylated acetylcholinesterase and is effective in counteracting the paralytic effects (muscular weakness and fasciculations) of anticholinesterase agents, such as chlorfenvinphos, if it is administered prior to the “aging” (See Mechanisms of Toxicity) of the phosphorylated enzyme (Williams and Burson 1985). Pralidoxime is most effective if started within the first 24 hours, preferably within 6–8 hours of exposure, prior to the irreversible phosphorylation of the enzyme (Shankar 1967, 1978; Schenker 1992).

Atropine is an anti-muscarinic agent which, in large doses, alleviates bronchoconstriction and reduces secretion in the oral cavity and the airway. Atropine also counters some of the central nervous system effects (Cupp et al. 1975; Pach et al. 1987). It is recommended that atropine be given immediately by intravenous injection at a dose of 2 mg and every 10–20 minutes thereafter at an intramuscular dose of 0.67 mg until evidence of "atropinization" or muscarinic blockade, such as flushing, dry mouth, dilated pupils, and tachycardia is seen (Shankar 1978). Atropine therapy is required for less than 24 hours because of the shorter duration of effect (Schenker et al. 1992). The most clinically important indication for continued atropine treatment is persistent wheezing (pulmonary rales) or bronchorrhea (Woo 1990). Pralidoxime acts to regenerate inhibited cholinesterase enzyme activity at all affected sites (Shankar 1967, 1978; Schenker 1992; Taitelman 1992).

2.10 ADEQUACY OF THE DATABASE

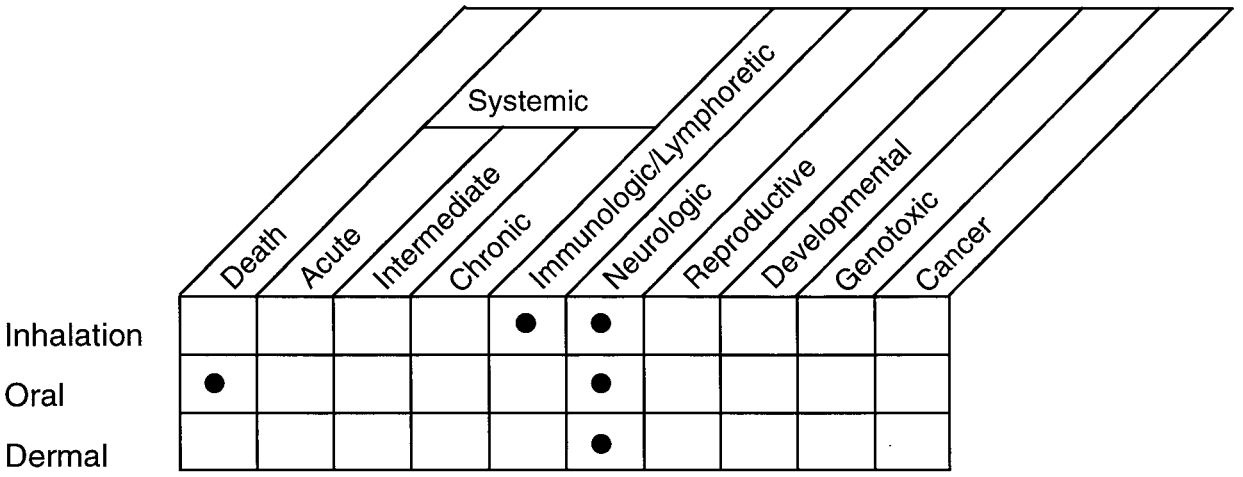
Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of chlorfenvinphos is available. Where adequate information is 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 chlorfenvinphos.

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.

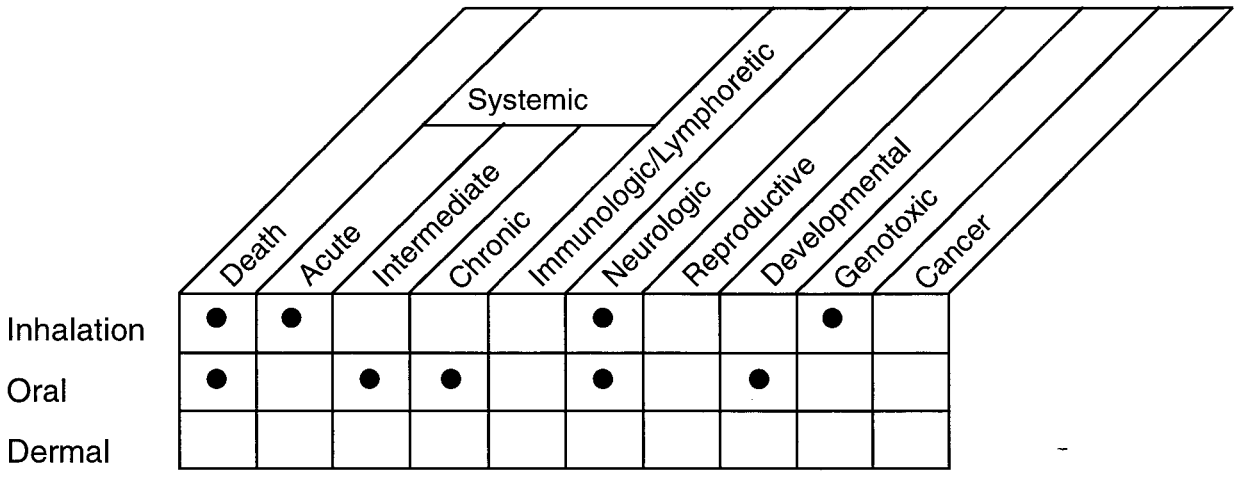
2.10.1 Existing Information on Health Effects of Chlorfenvinphos

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to chlorfenvinphos are summarized in Figure 2-5. The purpose of this figure is to illustrate the existing information concerning the health effects of chlorfenvinphos. 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, or should missing information in this figure be interpreted as a "data

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



Human



Animal

● Existing Studies

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.

The available information indicates that chlorfenvinphos is a toxic substance to most species of experimental animals, deriving its toxicity principally from the inhibition of cholinesterase activity. All three reports concerning the health effects of chlorfenvinphos in humans described individuals or group of individuals exposed either occupationally or in the home by accident. The route in the occupational exposure reports is believed to be dermal, although an occupational exposure was reported as inhalation. The route for the accidental exposure case was oral. Thus, Figure 2-5 reflects that information exists for oral and inhalation exposures in humans. However, all of the human reports on inhalation exposures are limited because of probable concurrent or sequential exposures to other substances of similar qualitative toxicity present in the environment (workplace or home), such as other organophosphate pesticides present as components of organophosphate-containing household products. In all cases, the doses at which these effects occurred in the human studies are not known and the purity of the material to which these subjects were exposed is questionable because of the accidental nature of the exposures, thus rendering evaluation of substance-relatedness to these reported toxicities uncertain. The availablehuman data, therefore, fail to fully characterize the human health effects from acute-, intermediate-, or chronic-duration inhalation exposures to chlorfenvinphos.

Information regarding the health effects of chlorfenvinphos following ingestion in laboratory animals is also limited due to a paucity of definitive studies. Only limited information is available on the health effects resulting from dermal exposures. In all health effects categories, acute-, intermediate-, and chronic-duration exposure data for inhalation exposure are limited for both humans and laboratory animals. Consequently, it was not possible to develop acute-, intermediate-, or chronic-duration inhalation MRLs for chlorfenvinphos. Furthermore, no information on the carcinogenic effects of chlorfenvinphos exposure is available for humans or laboratory animals by any route of exposure.

An acute oral MRL of 0.002 mg/kg/day has been derived for chlorfenvinphos from a LOAEL of 2.4 mg/kg/day, based on adverse neurological effects in rats (Barna and Simon 1973). An intermediate oral MRL of 0.002 mg/kg/day for chlorfenvinphos has been developed from a LOAEL of 2 mg/kg/day, based on adverse immunolymphoreticular effects in mice (Kowalczyk-Bronisz et al.

1992). A chronic oral MRL of 0.0007 mg/kg/day for chlorfenvinphos has been developed from a LOAEL of 0.7 mg/kg/day, based on adverse neurological effects in rats (Ambrose et al. 1970).

2.10.2 Identification of Data Needs

Acute-Duration Exposure. No data are available on the acute-duration effects of human inhalation exposure to chlorfenvinphos. The available animal inhalation studies reported only serious effects (mortality, apnea, salivation, urination, exophthalmos, twitches, and tremors) (Takahashi et al. 1994; Tsuda et al. 1986) following exposure to chlorfenvinphos. Therefore, the data from these studies are not appropriate for use in the derivation of an acute-duration inhalation MRL.

The information available on acute oral human exposures consists primarily of studies that reported interference with central cholinergic and adrenergic mechanisms (disturbances in cholinesterase and noradrenaline (norepinephrine) levels) and secondary effects resulting from these disturbances manifested as neurological symptoms and death in some cases. The adverse effects reported in humans included death (Felthous 1978) and neurological effects (Cupp et al. 1975; Kolmodin-Hedman and Eriksson 1987; Pach et al. 1987). In animals effects noted from acute oral exposure to chlorfenvinphos included death in rats (Ambrose et al. 1970; Hutson and Logan 1986; Hutson and Wright 1980; Ikeda et al. 1991, 1992; Puzynska 1984; Takahashi et al. 1991), mice (Hutson and Logan 1986; Kowalczyk-Bronisz et al. 1992; Wysocka-Paruszewska et al. 1980), dogs (Hutson and Logan 1986; Ambrose et al. 1970), and rabbits (Ambrose et al. 1970; Hutson and Logan 1986); systemic effects in rats, i.e., hepatic (Ikeda et al. 1991; Puzynska 1984), endocrine (Osicka-Koprowska et al. 1984), and metabolic (Barna and Simon 1973); and neurological effects in rats (Barna and Simon 1973; Brzezinski 1978; Ikeda et al. 1992; Osicka-Koprowska et al. 1984; Osumi et al. 1975; Puzynska 1984; Takahashi et al. 1991). Thus, the acute effects of oral chlorfenvinphos are relatively well-characterized, stemming principally from the inhibition of cholinesterase activity.

No effects were noted in humans dermally exposed to chlorfenvinphos for acute durations (Hunter 1968). In animals, death (Ambrose et al. 1970) and neurological effects (Vestweber and Kruckenberg 1972) have resulted from dermal exposure to chlorfenvinphos.

Additional studies via the inhalation and dermal routes of exposure would be helpful for establishing a dose-response relationship and for identifying thresholds for adverse effects for these routes of

exposure to chlorfenvinphos. This information is necessary for determining levels of significant exposure to chlorfenvinphos that are associated with adverse health effects for the protection of potentially exposed populations living near hazardous waste sites that contain chlorfenvinphos.

Intermediate-Duration Exposure. No information is available on the effects of human intermediate-duration exposure, by any route (inhalation, oral, dermal). No animal studies were available on intermediate-duration inhalation or dermal exposure to chlorfenvinphos.

Available information on the adverse effects resulting from intermediate-duration oral exposure of animals to chlorfenvinphos include death in rats and dogs (Ambrose et al. 1970); systemic effects in rats, i.e., renal (Ambrose et al. 1970) and metabolic (Barna and Simon 1973); immunological/lymphoreticular effects in rats (Ambrose et al. 1970: Kowalczyk-Bronisz et al. 1992; Roszkowski 1978); neurological effects in rats (Ambrose et al. 1970; Barna and Simon 1973; Maxwell and LeQuesne 1982) and dogs (Ambrose et al. 1970); reproductive effects in rats (Ambrose et al. 1970); developmental effects in rats (Ambrose et al. 1970). Additional available studies found no effects on mortality in rats and dogs (Ambrose et al. 1970); no significant systemic effects, i.e., respiratory, cardiovascular, gastrointestinal, musculoskeletal, hepatic, renal, endocrine, and body weight in dogs or respiratory, cardiovascular, gastrointestinal, hepatic, renal, endocrine, dermal effects in rats (Ambrose et al. 1970) endocrine system effects in mice (Kowalczyk-Bronisz et al. 1992), or body weight changes in rabbits (Roszkowski 1978) and rats (Barna and Simon 1973; Maxwell and LeQuesne 1982); no immunological/lymphoreticular effects in rats and dogs (Ambrose et al. 1970); and no reproductive effects in rats (Ambrose et al. 1970). Data from these studies sufficiently demonstrate that chlorfenvinphos interferes with the immunological/ lymphoreticular system. An intermediate oral MRL of 0.002 mg/kg/day for chlorfenvinphos has been developed from a LOAEL of 2 mg/kg/day, based on adverse immunolymphoreticular effects in mice (Kowalczyk-Bronisz et al. 1992).

Due to the lack of adequate human or animal data for effects from intermediate-duration inhalation exposure to chlorfenvinphos, no intermediate-duration inhalation MRL was derived for chlorfenvinphos. Additional studies via the inhalation and dermal routes of exposure would be helpful for establishing a dose-response relationships and for identifying thresholds for adverse effects for chlorfenvinphos exposure. This information is necessary for determining levels of significant exposure to chlorfenvinphos that are associated with adverse health effects for the protection of potentially exposed populations living near hazardous waste sites that contain chlorfenvinphos.

Chronic-Duration Exposure and Cancer. No controlled epidemiological studies regarding the systemic toxicities of chlorfenvinphos resulting from chronic-duration inhalation exposure are available. However, two retrospective epidemiological studies regarding the systemic toxicities of chlorfenvinphos resulting from chronic-duration inhalation exposures are available. Although the existing human studies reported immunological (Wysocki et al. 1987) and neurological effects (Kolmodin-Hedman and Eriksson 1987), the subjects in the Wysocki et al. (1987) report were also concurrently exposed to greater concentrations of other potentially immunotoxic substances such as formothion, sumithion, and malathion while the subjects in the Kolmodin-Hedman and Eriksson (1987) study were exposed to unknown concentrations of a mixture of potentially neurotoxic pesticides which included dimethoate, formothion, and isofenphos. Therefore, data from these studies were not useful for developing a chronic inhalation MRL for chlorfenvinphos.

There is no information on the effects of chronic human oral exposure to chlorfenvinphos. In animals the existing information on adverse effects from chronic oral exposure to chlorfenvinphos is limited to systemic effects, i.e., hepatic and body weight effects in rats (Ambrose et al. 1970); and neurological effects in rats and dogs (Ambrose et al. 1970). Additional available studies found no effects on mortality in rats and dogs (Ambrose et al. 1970); no significant systemic effects, i.e., cardiovascular, hematological, hepatic, renal, endocrine, or body weight in dogs, or respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, renal, endocrine, or dermal effects in rats (Ambrose et al. 1970); no immunological/ lymphoreticular effects in rats and dogs (Ambrose et al. 1970); and no reproductive effects in rats (Ambrose et al. 1970). Data from these studies sufficiently demonstrate that chlorfenvinphos is an anticholinesterase organophosphate. A chronic oral MRL of 0.0007 mg/kg/day for chlorfenvinphos has been developed from a LOAEL of 0.7 mg/kg/day, based on adverse neurological effects in rats (Ambrose et al. 1970).

No information on the effects of chronic-duration dermal exposure to chlorfenvinphos is currently available.

No epidemiological studies or chronic rodent cancer bioassays are available for assessing the carcinogenic potential of chlorfenvinphos. However, a study to determine the ability of vinyl phosphate esters (like chlorfenvinphos) to form methylated bases in DNA of calf thymus failed to detect 6-methyl guanine, a known mutagen. In both the reaction with dsDNA and ssDNA, 7-methyl guanine was the main methylation product. However, all methyl derivatives of adenine constituted about 40% and 50% of all methylation products in the case of dsDNA and ssDNA, respectively. 3-Methylcytosine was the

only methyl derivative of pyrimidine identified. An analog of chlorfenvinphos, methylbromo-phenvinphos, was used in this study (Wiaderkiewicz et al. 1986); therefore, the data are difficult to relate to chlorfenvinphos without extensive structure-activity relationship analysis. In a mutagenicity study, the dose-response curve, at doses of 0, 50, 500, 5,000 µg/plate for the mutagenic activity of chlorfenvinphos for the *S. typhimurium* strain TA100 was reduced by the S9 mix (metabolic activation). At present, no mutagenic pesticide for which activity decreases in the presence of the S9 mix is carcinogenic except captan (F-28) (Moriya et al. 1983).

Additional studies via the inhalation and dermal routes of exposure would be helpful for establishing a dose-response relationships and for identifying thresholds for adverse effects for prolonged exposure to chlorfenvinphos. This information is necessary for determining levels of significant exposure to chlorfenvinphos that are associated with adverse health effects for the protection of potentially exposed populations living near hazardous waste sites that contain chlorfenvinphos. Inhalation, oral, and dermal bioassays would be helpful to determine whether populations with long-term inhalation, oral, or dermal exposure (especially those living near hazardous waste sites or establishments where wastes containing chlorfenvinphos are released into the air or water) are at risk of developing cancers.

Genotoxicity. No studies were located regarding genotoxic effects of chlorfenvinphos in humans following inhalation, oral, or dermal exposure. Chlorfenvinphos was negative for mutagenicity in both base-pair change-type strains microorganisms (WP2 *hcr* of *E. coli*; TA1535, TA1537, TA1538, TA98 of *S. typhimurium*). In a mutagenicity study, the dose-response curve, at doses of 0, 50, 500, and 5,000 µg/plate, for the mutagenic activity of chlorfenvinphos for the *S. typhimurium* strain TA100 was reduced by the S9 mix (metabolic activation). At present, no mutagenic pesticide whose activity decreases in the presence of the S9 mix is carcinogenic except captan (F-28) (Moriya et al. 1983). The mutagenic potency of chlorfenvinphos for strain TA100 has been reported as 0.038 revertants/nmol, indicative of a positive response (Dean 1972; Moriya et al. 1983; Vishwanath and Kaiser 1986). A mixture of 15 pesticides (containing 0.3% chlorfenvinphos) tested negative for mutagenicity in the *Salmonella* microsome assay, with or without metabolic activation with PCB-induced rat liver S9, at concentrations up to 500 µg/plate in the *Salmonella*-microsome assay. The mixture also failed to induce SCEs in human lymphocytes *in vitro* as well as *in vivo* mutagenicity in the micronucleus bone marrow assay in male Wistar rats at concentrations proportional to the ratio determined in foods (range = 0.1–20 µg/mL) (Dolara et al. 1993). In other large-scale screening programs, which revealed microbial mutagenic activity in four new compounds (all fungicides), chlorfenvinphos (without metabolic activation) exhibited no mutation induction capacity in a rec-assay procedure (prescreening of DNA-damaging chemicals) utilizing strains of *B. subtilis*, H17 Rec+ and

M45 Rec-. Also, no mutation potential was evident in a reversion-assay (determination of mutation specificities) in which two tryptophane-requiring strains (auxotrophic) of *E. coli* (B/r *try* WP2 and WP2 *try hcr*) and four strains of *S. typhimurium* (TA1535, TA1536, TA1537, TA1538) were used. The *E. coli* auxotrophic strains and *Salmonella* TA1535 are reversible by base-pair change-type mutagens and the three *Salmonella* strains (TA1536, TA1537, and TA1538) are reversible by frameshift mutagens (Shirasu 1973; Shirasu et al. 1976).

There are no unequivocal data to indicate that chlorfenvinphos reacts directly with DNA *in vivo* or *in vitro* to produce mutations in either germ or somatic cells. In a study to determine the ability of vinyl phosphate esters (like chlorfenvinphos) to form methylated bases in DNA of calf thymus, DNA failed to detect 6-methyl guanine, a known mutagen. In the reaction both with dsDNA and ssDNA, 7-methyl guanine was the main methylation product. However, all methyl derivatives of adenine constituted about 40% and 50% of all methylation products in the case of dsDNA and ssDNA, respectively. 3-Methylcytosine was the only methyl derivative of pyrimidine identified (Wiaderkiewicz et al. 1986). In another study, tetrachlorvinphos (Gardona®) was evaluated for potential to induce chromosomal aberrations and SCEs *in vitro* in a primary culture of Swiss mice spleen cells at concentrations of 0.25, 0.50, 1.0, or 2.0 µg/mL. Tetrachlorvinphos induced a high percentage of metaphases with chromosomal aberrations in the mouse spleen cells after 4 hours of treatment in a dose-dependent manner. According to the authors, the results indicate that tetrachlorvinphos in the tested concentrations are mutagenic in mouse spleen cell cultures (Amer and Aly 1992). In both of these studies, structural analogs of chlorfenvinphos (methylbromophenvinphos and tetrachlorvinphos, respectively) were used; therefore, the data are difficult to relate to chlorfenvinphos without extensive structure-activity relationship analysis. These limited data suggest that chlorfenvinphos might not be genotoxic. Thus, the existing information on the mutagenic potential of chlorfenvinphos is equivocal. Additional genotoxicity assays in microorganisms and mammalian cells (*in vivo* and *in vitro*) will be helpful in determining if the substance is clastogenic or can cause mutation in somatic or germ cells. This information is necessary to determine whether potentially exposed populations, especially those living near hazardous waste sites, are at risk of developing genetic diseases.

Reproductive Toxicity. No studies were located regarding reproductive effects in humans following acute-, intermediate-, or chronic-duration inhalation, oral, or dermal exposure to chlorfenvinphos. Animal studies regarding reproductive effects after acute-, intermediate-, or chronic- inhalation or dermal exposure to chlorfenvinphos, or acute-duration oral exposure to chlorfenvinphos are also lacking. A 3-generation reproductive study in albino (Wistar) rats orally exposed to chlorfenvinphos

reported significant (14%) reduction in fertility and decrease in maternal body weight gains at a LOAEL of 2.7 mg/kg/day (Ambrose et al. 1970). Single-generation studies, in which rats and dogs were exposed to chlorfenvinphos for intermediate- or chronic-durations found no histopathology or changes in relative weights of the testes and ovaries of the tested animals (Ambrose et al. 1970). However, these studies did not evaluate reproductive function. Consequently, additional reproductive toxicity studies in animals exposed to chlorfenvinphos via inhalation, oral, or dermal route would be helpful in evaluating the potential for chlorfenvinphos to cause adverse reproductive effects in humans. This information is necessary to determine whether potentially exposed populations, especially those living near hazardous waste sites, are at risk of developing reproductive diseases.

Developmental Toxicity. No studies were located regarding developmental effects in humans following acute-, intermediate-, or chronic-duration inhalation, oral, or dermal exposure to chlorfenvinphos. Animal studies regarding developmental effects after acute-, intermediate- or chronic-duration inhalation and dermal exposure to chlorfenvinphos are also lacking. The limited information on the developmental toxicity of oral chlorfenvinphos exposure indicates that the substance may interfere with the normal development of rats by inhibiting cellular respiration (Skonieczna et al. 1981) and interfering with maternal body weight gains, and reducing lactational index and offspring survivability in the developing rodents (Ambrose et al. 1970). A statistical model for hazard identification developed for use in predicting the developmental toxicity of 175 chemicals, including chlorfenvinphos, produced equivocal results concerning the developmental toxicity of chlorfenvinphos to the rat, rabbit, and hamster. The model was essentially a database of 175 probable, suspected, unknown, and probably negative teratogenic or embryotoxic drugs and chemical compounds. For each of the compounds, including chlorfenvinphos, the results of any developmental toxicity testing in up to 14 animal species and any reports of mutagenicity or carcinogenicity were recorded. The compounds were categorized with respect to their human developmental toxicant effect: -1.0 testing negative, 0.0 not tested (unknown), 0.5 tested with equivocal results (suspicious), and 1.0 testing positive. However, the model had a sensitivity of 62–75%, a positive predictive value of 75–100%, and a negative predictive value of 64–91%, indicating that it is not optimal for hazard identification (Jelovsek et al. 1989). Additional information on the developmental effects in animals exposed to chlorfenvinphos via the inhalation, oral, or dermal route would be helpful in evaluating the potential for chlorfenvinphos to cause developmental toxicity in humans. This information is necessary to determine whether offspring of potentially exposed populations, especially those living near hazardous waste sites, are at risk of developmental adverse effects.

Immunotoxicity. No studies were located regarding immunological and lymphoreticular effects in humans following acute- or intermediate-duration inhalation exposure to chlorfenvinphos, or regarding immunological and lymphoreticular effects in humans following acute-, intermediate-, or chronic-duration oral exposure to chlorfenvinphos. No studies were available regarding immunological and lymphoreticular effects in animals following acute-, intermediate-, or chronic-duration inhalation or dermal exposure to chlorfenvinphos.

Only one study was located that reported immunological effects in humans. In this report, occupational exposure to inhaled chlorfenvinphos for an average of 15 years was associated with damage to humoral mechanisms in humans. This study is not suitable for assessing the immunological and lymphoreticular effects of human exposure to chlorfenvinphos because the subjects of this study were also concurrently exposed to greater concentrations of other potentially immunotoxic substances such as formothion, sumithion, and malathion (Wysocki et al. 1987). In animal studies, intermediate-duration dietary exposure of rats resulted in a significant and irreversible reduction in relative spleen weight of female rats given $3 mg/kg/day chlorfenvinphos for 12 weeks. However, no gross or microscopic histopathology was evident in the spleen and bone marrow tissues of the rats upon examination (Ambrose et al. 1970). A chronic study in dogs and rats did not note any histopathological changes in the spleen or bone marrow or changes in absolute or relative spleen weights in Wistar rats or Beagle dogs of both sexes given dietary chlorfenvinphos doses of 21 mg/kg/day (males) or 24 mg/kg/day (females), or 10 mg/kg/day (males) or 50 mg/kg/day (females), respectively, for 104 week (Ambrose et al. 1970). In other animal studies, C57BL/6 mice and (C57BL/6 x DBA/2)F1 (BDF1/Iiw) hybrid mice (6–8 weeks old) orally exposed to chlorfenvinphos for 90 days exhibited a reversible reduction in the number of E rosettes-forming cells as well as a dose-related decrease in number of hemolysin producing cells; reduction in the number of plaque-forming cells; increases in Interlukin-1 activity and DTH reaction; stimulation of spleen colonies; and disturbance in humoral immune factors (immunoglobulins) at a LOAEL of 1.5 mg/kg (Kowalczyk-Bronisz et al. 1992).

Rabbits orally exposed to chlorfenvinphos for 90 days also exhibited significantly elevated serum hemagglutinin level (16%) and hemolysin activity (66%, $P<0.05$) as well as increased number of nucleated lymphoid cells producing hemolytic antibody to sheep erythrocytes. Spleen cytomorphology changes, manifested mainly as transformation of primary follicles into secondary ones with well developed germinal centers, were also observed (Roszkowski 1978). While the existing human inhalation study and the animal oral studies provide some indication that chlorfenvinphos exposure is associated with immunological changes, these changes were not consistent with depressive effect on immune reactions. Thus, the changes reported in these studies

may simply be immunological mobilizations of the organisms to xenobiotics in contradistinction from immune system damage. Consequently, additional TIER II animal immunotoxicity testing (cell-mediated immunity, cell surface marker profile immunopathology, humoral immunity, cytolytic macrophage function, and bone marrow tests) via the inhalation, oral, or dermal route for chlorfenvinphos would be helpful to more fully assess the potential of chlorfenvinphos to cause immunotoxicity in humans. This information is necessary to determine whether potentially exposed populations, especially those living near hazardous waste sites, are at risk of developing immunological diseases.

Neurotoxicity. No studies were located regarding neurological effects in humans after acute- or intermediate-duration inhalation exposure to chlorfenvinphos; or following acute-, intermediate-, or chronic-duration oral exposure; or after intermediate- or chronic-duration dermal exposure. In humans, chlorfenvinphos inhibits cholinesterase activity in the central and peripheral nervous system when administered by the oral (Cupp et al. 1975; Pach et al. 1987) or inhalation route in acute-duration exposures (Kolmodin-Hedman and Eriksson 1987). Inhibition of cholinesterase activity results in accumulation of acetylcholine at muscarinic and nicotinic receptors leading to peripheral and central nervous system effects. These effects usually appear within a few minutes to a few hours after exposure depending on the extent of exposure. In a human case report, a 16-year-old white male who mistakenly ingested a formulation (identified as Dermaton®) was hospitalized 90 minutes afterward with symptoms of abdominal cramps, nausea, vomiting, generalized weakness, cold dry skin, hypothermia, listlessness, constricted pupils, hypertension, respiratory distress, fine generalized muscular twitching, and apprehension. Plasma and erythrocyte activity levels were significantly inhibited. All vital signs returned to normal after gastric lavage and treatment with atropine and pralidoxime (Cupp et al. 1975).

No studies were located regarding neurological effects in animals after acute- or intermediate-duration inhalation or dermal exposure to chlorfenvinphos. The available information indicates that chlorfenvinphos has similar neurological effects in animals. In animals, chlorfenvinphos inhibits cholinesterase activity in the blood and neuromuscular tissue by the oral route in acute-duration exposures in rats at doses as low as 2 mg/kg (Barna and Simon 1973; Osicka-Koprowska et al. 1984; Osumi et al. 1975; Puzynska 1984; Takahashi et al. 1991); in rats following intermediate-duration exposures via the oral route at doses as low as 9 mg/kg/day (Ambrose et al. 1970; Maxwell and LeQuesne 1982); and in dogs following chronic-duration exposure via the oral route at doses as low as 0.7 mg/kg/day (Ambrose et al. 1970). These disruptions in cholinesterase activity resulted in cholinergic responses manifested as abnormal muscle reflex, muscle fasciculations, Straub tail reflex, twitches, convulsions, chromodacryorrhea, exophthalmos, gasping,

lacrimation, prostration, salivation, sleep disturbances, diarrhea, emesis, and urination (Ambrose et al. 1970; Maxwell and LeQuesne 1982; Osumi et al. 1975; Puzynska 1984; Takahashi et al. 1991). In one of these studies, all 36 Sprague-Dawley rats exposed to chlorfenvinphos doses of 10.5 mg/kg/day in the diet for 3–6 months exhibited repetitive muscle activity when given two simultaneous stimuli. This abnormality became more pronounced with time, even on constant dosing (Maxwell and LeQuesne 1982). The findings from this study may be useful in explaining electrophysiological abnormalities described in some workers chronically exposed to some organophosphorus compounds.

Intraperitoneal administration of daily doses of 100, 150, 200, or 300 mg/kg/day chlorfenvinphos to White Leghorn hens for 10 days or until death resulted in cholinergic signs. Typical cholinergic signs, including inability to stand, salivation, and retching (as well as some deaths) were observed immediately after the administration of any dose level of chlorfenvinphos, with or without atropine coadministration. No signs of delayed neurotoxicity or evidence of neurological lesions suggestive of demyelination or neural damage were observed in the brain and sciatic nerve tissues examined (Ambrose 1970). Although all doses of chlorfenvinphos elicited cholinergic responses (leg weakness, salivation, and retching) from hens given 100, 150, 200, or 300 mg/kg by intraperitoneal injection, no signs of delayed neurotoxicity was evident after 20 days of observation (Ambrose et al. 1970).

Besides its cholinergic action, chlorfenvinphos also acts via central noradrenergic mechanisms in rats by accelerating the noradrenaline (norepinephrine) turnover in the brain *in vivo* by the release of noradrenaline (norepinephrine) from brain tissue stores (Brzezinski 1978).

Although the available toxicity information in humans and animals is sufficient to establish that short- and long-term exposure to chlorfenvinphos results in adverse neurological effects, additional animal studies by the inhalation, oral, and dermal routes will be helpful in the more accurate assessment of the exposure levels at hazardous waste sites at which these effects are likely to occur.

Epidemiological and Human Dosimetry Studies. Although the available epidemiological studies sufficiently identify inhibition of cholinesterase activity as the characteristic and most critical effect of human exposure to chlorfenvinphos, these studies inadequately identify the dose at which this effect occurs (Cupp et al. 1975; Kolmodin-Hedman and Eriksson 1987; Pach et al. 1987; Wysocki et al. 1987). Well-conducted acute-, intermediate-, and chronic-duration (for effects other than inhibition of cholinesterase activity) human dosimetry studies are not available. Therefore, well conducted

retrospective epidemiological studies would be helpful in determining and quantifying the effects (including neurologic, immunologic, and reproductive effects) of inhalation, oral, or dermal chlorfenvinphos exposure on human health; such studies would also provide useful data regarding the carcinogenic potential of chlorfenvinphos in humans, especially people living around hazardous waste sites or establishments where wastes containing chlorfenvinphos are released into the air or water, and people who are occupationally exposed to chlorfenvinphos for long periods of time.

Biomarkers of Exposure and Effect.

Exposure. The major action resulting from human exposure to chlorfenvinphos is the inhibition of acetylcholinesterase activity (see Section 2.4). Two pools of cholinesterases are present in human blood: acetylcholinesterase in erythrocytes and neuromuscular tissue and butyrylcholinesterase (pseudocholinesterase) in plasma. Acetylcholinesterase, present in human erythrocytes, is identical to the enzyme present in neuromuscular tissue (the target of chlorfenvinphos action). While plasma cholinesterase (pseudocholinesterase or butyrylcholinesterase) has no known physiological function, it has been suggested that it scavenges acetylcholine that gets into the plasma. Inhibition of the activity of both forms of cholinesterase has been associated with exposure to chlorfenvinphos in humans and animals (Cupp et al. 1975; Gralewicz et al. 1989a, 1989b, 1990; Hunter 1969; Kolmodin-Hedman and Eriksson 1987; Maxwell and LeQuesne 1982; Osicka-Koprowska et al. 1984; Pach et al. 1987; Takahashi et al. 1991; Vestweber and Kruckenberg 1972). Inhibition of erythrocyte, plasma, or whole blood cholinesterase may be used as a marker of exposure to chlorfenvinphos. However, inhibition of cholinesterase activity is a common action of anticholinesterase compounds, which include organophosphates like chlorfenvinphos, and carbamate compounds. In addition, a wide variation in normal cholinesterase values exists in the general population, and there are no studies which report a quantitative association between cholinesterase activity levels and exposure to chlorfenvinphos in humans.

Chlorfenvinphos undergoes biotransformation to a variety of polar metabolites, including 2-chloro-1-(2,4-dichlorophenyl) vinylethylhydrogen phosphate; 1-(2,4-dichlorophenyl) ethanol; 1-(2,4-dichloro-phenyl) ethanediol; 2,4-dichloromandelic acid; and 2,4-dichlorobenzoyl glycine (Hutson and Wright 1980), which have been detected in animals. Analysis of blood samples for the presence of these metabolites represents a potential means of assessing exposure. Analysis of urine samples for metabolic products provides a non-invasive method for detecting exposure. As an organophosphate, chlorfenvinphos is rapidly metabolized and excreted from the body; therefore, urinary metabolite analysis is useful only in the evaluation of recent exposures (Hutson and Wright 1980). There are no

studies which report a quantitative association between metabolite levels and exposure to chlorfenvinphos in humans. Therefore, these biomarkers are only indicative of exposure and are also not useful for dosimetric analysis. The inhibition of cholinesterase activity method as a measure of organophosphate exposure lacks even greater specificity when used to assess exposure to compounds which are weak inhibitors of cholinesterase activity, like chlorfenvinphos (Hunter et al. 1972). As a vinyl phosphate, chlorfenvinphos is metabolized to desethyl chlorfenvinphos, which could be detected in urine as a specific biomarker of exposure to chlorfenvinphos, or other vinyl phosphates (Akintonwa 1984, 1985; Akintonwa and Itam 1988; Donninger 1971; Hansen 1983; Hutson and Logan 1986; Hutson and Millburn 1991; Hutson and Wright 1980). The method of detection involves conversion of urinary desethyl chlorfenvinphos to the more easily measurable methyl desethyl chlorfenvinphos with diazomethane. This method is a more specific biomarker for chlorfenvinphos exposure and has been successfully used as such in a case of 14 male volunteers exposed to chlorfenvinphos for 53 days. However, since desethyl chlorfenvinphos accounts for only about 5% of the dose at this low exposure level, its concentration in urine would lack sensitivity when used as an index of exposure to chlorfenvinphos (Hunter et al. 1972). Although this method of assessing chlorfenvinphos exposure may not be useful in low exposure conditions, the method could be used to evaluate exposure to high doses that occur in human acute poisoning cases. Further research in the adaptation of this method for low-dose exposure would be useful.

Effect. Inhibition of the activities of erythrocyte, plasma, or whole blood cholinesterase in humans and animals that results from chlorfenvinphos exposure (Cupp et al. 1975; Gralewicz et al. 1989a, 1989b, 1990; Hunter 1969; Kolmodin-Hedman and Eriksson 1987; Maxwell and LeQuesne 1982; Osicka-Koprowska et al. 1984; Pach et al. 1987; Takahashi et al. 1991; Vestweber and Kruckenberg 1972) may be used as a marker of effect for chlorfenvinphos exposure. However, the inhibition of cholinesterase activity is a common action of anticholinesterase compounds, which include organophosphates like chlorfenvinphos, and carbamate compounds. In addition, a wide variation in normal cholinesterase values exists in the general population, and there are no studies which report a quantitative association between cholinesterase activity levels and exposure to chlorfenvinphos in humans. Thus, the inhibition of cholinesterase activity is not a specific biomarker of effect for chlorfenvinphos exposure; it is indicative only of effect and is not useful for chlorfenvinphos-specific dosimetric analysis. In combination with analysis of reductions in the level of cholinesterase activity, the manifestations of severe organophosphate (chlorfenvinphos) poisoning, clinically characterized by a collection of cholinergic signs and symptoms (which may include dizziness, fatigue, tachycardia or bradycardia, miosis, and vomiting) (Chambers and Levi 1992; Cupp et al. 1975; Klaassen et al. 1986; Pach et al. 1987; Takahashi et al. 1991; Williams and Burson 1985), are useful biomarkers of effect for identifying victims of organophosphates (chlorfenvinphos) poisoning. These manifestations, however,

are also not specific to chlorfenvinphos, but to anticholinesterase compounds, such as organophosphates and carbamate compounds, in general. A study conducted in rats reported that 1–6 hours after administration, chlorfenvinphos (13 mg/kg) decreased the noradrenaline (norepinephrine) level in rat brain by 20% as compared to controls (Brzezinski 1978). A similar study in Wistar rats found a 16% transient reduction in brain noradrenaline (norepinephrine) 3 hours after oral dosing with 4 mg/kg chlorfenvinphos (Osumi et al. 1975). Further research on the adrenergic effects of chlorfenvinphos exposure might provide a more specific biomarker for chlorfenvinphos when used in combination with inhibition of cholinesterase activity and clinical signs of interference with the central cholinergic mechanism.

Absorption, Distribution, Metabolism, and Excretion. No studies were located regarding the absorption of chlorfenvinphos after inhalation exposure; or regarding the distribution or metabolism of chlorfenvinphos after inhalation or dermal exposure; or regarding excretion of chlorfenvinphos after inhalation or dermal exposure in humans. In humans, dermally applied chlorfenvinphos was absorbed in a concentration-dependent manner with rates of 0.06–1.43 mg/cm^2/hour. Concentrations of intact chlorfenvinphos of <0.7–22 µg/L were found in the blood of volunteers 8 hours later (Hunter 1969). The rates of chlorfenvinphos de-ethylation by liver microsomal fractions are 0.36 nmol/minute per mg protein (range 0.11–0.82) without induction and 1.03 nmol/minute per nmol of cytochrome P-450 (range 0.42–1.78) with induction (Hutson and Logan 1986). Chlorfenvinphos levels of 13.66, 1.69, 2.02, and 1.89 µg/kg were detected in 4 of the 11 samples of cervical mucus in environmentally exposed persons. Chlorfenvinphos levels of 0.42 µg/kg were detected in 1 of the 10 sperm fluid samples and 1 of the 10 human milk samples, respectively (Wagner et al. 1990). A serum concentration of 300 ng/mL chlorfenvinphos was reported for a 29-year-old patient who had attempted suicide by ingesting about 50 mL of the preparation Enolofos®, which contains 50% chlorfenvinphos. The authors of this report surmised that orally absorbed chlorfenvinphos is widely and rapidly distributed (Pach et al. 1987).

No studies were located regarding the absorption of chlorfenvinphos after inhalation or dermal exposure; or regarding the distribution or metabolism of chlorfenvinphos after inhalation, oral, or dermal exposure; or regarding excretion of chlorfenvinphos after inhalation or dermal exposure in animals. The available animal studies indicate that orally administered chlorfenvinphos is minimally absorbed and metabolized in rats (Hutson and Wright 1980). The metabolism of oral doses of the substance is mediated by hepatic microsomal monooxygenase (cytochrome P-450) via oxidative dealkylation (Donninger 1971; Hutson and Millburn 1991; Ikeda et al. 1991). Thirteen metabolites of chlorfenvinphos have been identified in animal studies or predicted from theoretical biotransformation as justified by the known structure of chlorfenvinphos and understanding of biochemical reactions of

monooxygenation, reduction, hydrolysis, glucuronidation, glutathione-S-transferase conjugation, and amino acid conjugation. The 13 metabolites identified or predicted are: 2-chloro-1-(2',4'-dichlorophenyl) vinyldiethyl phosphate; acetaldehyde; 2-chloro-1-(2',4'-dichlorophenyl) vinylethylhydrogen phosphate; 2,4-dichlorophenacylchloride; 2-chloro-1-(2',4'-dichlorophenyl) ethanol; 2,4-dichloromandelic acid; 2,4-dichloromandelic acid ester glucuronide; 2,4-dichloroacetophenone; 1-(2',4'-dichlorophenyl) ethanol; 1-(2',4'-dichlorophenyl) ethanediol; 1-(2',4'-dichlorophenyl) ethanediol-2-glucuronide; 1-hydroxy-1-(2',4'-dichlorophenyl) acetyl glycine; and 1-(2',4'-dichlorophenyl) ethanediol (Akintonwa 1984, 1985; Akintonwa and Itam 1988; Hunter et al. 1972; Hutson and Millburn 1991; Hutson and Wright 1980). Orally absorbed chlorfenvinphos is eliminated mainly in the urine in rats in 0-32 hours (Hutson and Wright 1980). Additional studies in animals, designed to measure the rate of inhalation, gastrointestinal, and dermal absorption, distribution, and excretion of chlorfenvinphos would be useful in extrapolating the toxicokinetics of chlorfenvinphos in humans, especially those living around hazardous waste sites.

Comparative Toxicokinetics. Chlorfenvinphos inhibits cholinesterase activity in the central and peripheral nervous system, resulting in cholinergic symptoms as reported in several human poisoning incidents (Cupp et al. 1975; Hunter 1969; Pach et al. 1987; Kolmodin-Hedman and Eriksson 1987). However, the purity of the material to which these subjects were exposed is questionable, and the doses at which these effects occurred are unknown because of the accidental nature of the exposures. Therefore, it is difficult to determination whether the adverse effects reported in these human studies are attributable to exposure to technical chlorfenvinphos. Although information is available that indicates that dermally applied chlorfenvinphos was absorbed in a concentration-dependent manner (with rates of 0.06–1.43 mg/cm^2/hour), inhibiting cholinesterase in a dose-dependent in the human subjects (Hunter 1969), information on the toxicokinetics of chlorfenvinphos in humans is limited to serum levels following ingestion (Pach et al. 1987).

Similarly, chlorfenvinphos inhibits cholinesterase activity in the central and peripheral nervous system in animals (Gralewicz et al. 1989a, 1989b, 1990; Kolmodin-Hedman and Eriksson 1987; Maxwell and LeQuesne 1982; Osicka-Koprowska et al. 1984; Pach et al. 1987; Takahashi et al. 1991; Vestweber and Kruckenberg 1972).

The level of activity of cholinesterases in the livers of mammalian species and the distribution of these enzymes have been suggested to be important factors in accounting for species specificity of some phosphate triester anticholinesterase agents, including chlorfenvinphos. These factors may account for the great variation in the conversion of chlorfenvinphos to less toxic metabolites and, consequently, the

toxicity of chlorfenvinphos among different animal species. The relative rates of conversion of chlorfenvinphos (by *O*-dealkylation) to the diester by liver slices from the rat, mouse, rabbit, and dog are 1, 8, 24, and 80 hours, respectively; evidently correlating with the published acute oral LD_{50} values for the species. A quantitative study of the species distribution of phosphate esterases and glutathione *S*-alkyl transferase found that these enzymes are significantly less in the pig than all the other species studied (Donninger 1971; Hansen 1983; Ikeda et al. 1991). The ease of absorption, bioavailability in blood, and rates of uptake by the brain and sensitivity of brain cholinesterase to the phosphorylating action of the compound may be additional factors in species sensitivity to the toxicity of chlorfenvinphos as demonstrated in the dog and rabbit (Hutson and Millburn 1991).

Additional comparative studies regarding the absorption, distribution, and excretion of chlorfenvinphos after inhalation, oral, or dermal exposure in animals would be useful in species or route-route extrapolation. This information could be used to determine an appropriate animal model for the evaluation of the toxicokinetics of chlorfenvinphos.

Methods for Reducing Toxic Effects. Although dialysis and hemoperfusion are currently not recommended in organophosphate poisonings because of the extensive tissue distribution of the absorbed doses (Mücke et al. 1970; Poklis et al. 1980), hemoperfusion has been successfully used in one chlorfenvinphos poisoning treatment (Pach et al. 1987). Further studies are necessary in view of the relatively few clinical observations concerning the use of hemoperfusion in the treatment of acute poisoning caused by vinyl phosphate compounds like chlorfenvinphos.

2.10.3 Ongoing Studies

No information on ongoing studies in humans or laboratory animals for chlorfenvinphos was located.