3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of methoxychlor. 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.

The health effects of methoxychlor have been investigated mainly in studies using technical grade methoxychlor, although a few studies have used laboratory grade (98% pure) or recrystallized (>99% pure) preparations. Technical grade methoxychlor is the grade used in industrial preparations and typically contains about 80–90% methoxychlor, with the remainder being composed of >50 chemically-related compounds (IARC 1979; Lamoureux and Feil 1980). Please refer to Chapter 4 for more detailed information on the composition of methoxychlor. Some *in vivo* studies suggest that technical grade methoxychlor is approximately 2–4 times more potent with respect to reproductive and developmental effects than is pure methoxychlor (Bitman and Cecil 1970; Tullner 1961). This is because several of the contaminants in technical grade methoxychlor are directly estrogenic (Kupfer and Bulger 1987b), whereas pure methoxychlor is proestrogenic and requires metabolic activation before exhibiting estrogenic activity (Bulger et al. 1978d; Kupfer and Bulger 1979). Some of the estrogenic contaminants of technical grade methoxychlor are the same as those formed by metabolism *in vivo* (Bulger et al. 1985). It is important to recognize that because of the biological activity of these contaminants, dose-response relationships obtained using technical grade methoxychlor may not be directly applicable to pure methoxychlor. For this reason, the chemical purity of the methoxychlor used in key quantitative studies is provided. *Since most studies employ technical grade methoxychlor, the text refers to purity only when other grades are used.*

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELS have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

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

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

3.2.1 Inhalation Exposure

In air, methoxychlor is generally associated with particulate matter, and inhalation exposure to methoxychlor can occur by inspiration of methoxychlor-laden dust. Although the health effects resulting from inhalation exposure to methoxychlor have not been extensively investigated, there are a few reports that describe health effects following this type of exposure.

3.2.1.1 Death

A single case study reported the death of a 49-year-old male after an acute inhalation exposure to a pesticide mixture containing methoxychlor and captan (Ziem 1982). The exposure level was not reported. Death occurred 6 months after exposure and was attributed to aplastic anemia. Because only a single case was described and because the exposure was to a mixture of pesticides, it is not possible to deduce the role of methoxychlor in this outcome.

No studies were located regarding death in animals after inhalation exposure to methoxychlor.

3.2.1.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, musculoskeletal, hepatic, renal, endocrine, dermal, or ocular effects in humans or animals after inhalation exposure to methoxychlor.

Hematological Effects. A single case study reported the development of aplastic anemia in a 49-year-old male after an acute inhalation exposure to a pesticide mixture containing methoxychlor and captan (Ziem 1982). Symptoms of fatigue and bruising developed several weeks after exposure. The authors stated that although there are published case studies of aplastic anemia in humans following exposure to structurally-related pesticides such as dichlorodiphenyltrichloroethane (DDT) and lindane, no other reports of methoxychlor-induced aplastic anemia were located. Due to the limited database and the exposure to other chemicals (captan) in this study, a causal relationship between methoxychlor and aplastic anemia cannot be established. It is possible that this illness had an etiology unrelated to the pesticide exposure.

Body Weight Effects. There is no information regarding body weight effects of methoxychlor in humans. One animal study showed a 24% reduction in weight gain in rats intermittently exposed to 360 or 430 mg/m^3 methoxychlor for 4–5 weeks (Haag et al. 1950). However, the vehicle used to deliver the methoxychlor (Pyrax plus 3% Santo-Cel) was toxic and resulted in several deaths in the controls; therefore, the results of this study are difficult to interpret.

3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans or animals after inhalation exposure to methoxychlor.

3.2.1.4 Neurological Effects

A single case study described neurological effects in a 21-year-old male acutely exposed (15–20 minutes) to a pesticide mixture that contained 15% methoxychlor and 7.5% malathion (Harell et al. 1978). The subject noted blurred vision and nausea 8–9 hours after exposure. He was admitted to the hospital

36 hours after exposure in a state of dehydration with severe abdominal cramps and diarrhea. Approximately 4 days later, he experienced dizziness and complete deafness followed by difficulty moving the extremities, hypoesthesias, parasthesias in the limbs, bilateral foot drop, and leg pain. There was no improvement in any of these neurological effects 6 years after exposure. The authors noted that neither methoxychlor nor malathion typically produced such profound effects, and attributed the special susceptibility of this individual to a deficiency in the enzyme responsible for the detoxification of malathion. Whether methoxychlor contributed to the effects is not known.

Only one study was located regarding the neurological effects of inhalation exposure to methoxychlor-laden dust in animals. Intermittent exposure (2 hours/day, 5 days/week for 4 weeks) of rabbits to 430 mg/m^3 methoxychlor produced hind leg paralysis and disseminated nodules in the cerebral cortex in one out of two animals (Haag et al. 1950). Neurological effects were not observed in the two control animals. The results from this study are limited by the small number of animals tested, and by the possibility that the observed paralysis in the affected animal was the result of a disease that is endemic to rabbits (Haag et al. 1950). Thus, no firm conclusions can be made on the neurotoxicity of methoxychlor in animals after inhalation exposure.

3.2.1.5 Reproductive Effects

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

3.2.1.6 Developmental Effects

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

3.2.1.7 Cancer

A single epidemiological study was located on the potential association between methoxychlor exposure and occurrence of leukemia in farmers (Brown et al. 1990). In this study, 11/578 cases of leukemia and 16/1,245 controls were found to have been occupationally exposed to methoxychlor. After adjustment for vital status, age, state, tobacco use, family history of lymphopoietic cancer, high risk occupations and high risk exposures, an odds ratio of 2.2 was calculated. This increase was statistically significant;

however, it is difficult to make firm conclusions on the carcinogenicity of methoxychlor in humans based on a single study.

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

3.2.2 Oral Exposure

3.2.2.1 Death

No studies were located regarding death in humans after oral exposure to methoxychlor.

Reported acute LD_{50} values for recrystallized and technical grade methoxychlor range from 3,460 to 7,000 mg/kg in male and female rats (Cannon Laboratories 1976; Hodge et al. 1950; Smith et al. 1946) and 2,900 mg/kg in mice (Coulston and Serrone 1969). A more recent study reported an acute LD_{50} in male and female Wistar rats of 2,828 mg/kg for methoxychlor that contained 88% *p,p'* isomer and 12% *o,p'* isomer, and an acute LD_{50} of 1,782 mg/kg for a methoxychlor formulation that contained only 25% methoxychlor (*p,p'* isomer) (Dikshith et al. 1990). Mortality following a single exposure to 1,000, 2,000, 4,000, or 8,000 mg/kg of the mixed isomer methoxychlor was 0, 25, 100, and 100%, respectively, in male rats, and 25, 50, 50, and 75%, respectively, in female rats (Dikshith et al. 1990). Two out of 17 pregnant rabbits died following exposure to 251 mg/kg/day methoxychlor on days 7–19 of gestation (Kincaid Enterprises 1986). Exposure to 790–4,200 mg/kg/day recrystallized and technical grade methoxychlor in feed for 4–16 weeks produced significant increases in mortality in rats (Davison and Cox 1976; Haag et al. 1950; Hodge et al. 1950). Mortality rates were higher in female Wistar rats (0–25%) than in males (0–10%) exposed to 100–1,000 mg/kg/day methoxychlor for 90 days (Dikshith et al. 1990). Mortality was low in male and female Wistar rats that received 200–800 mg/kg/day of a formulation containing 25% methoxychlor for 90 days (Dikshith et al. 1990). Increases in mortality were also reported in dogs exposed to 2,000 mg/kg/day for 8–24 weeks (Tegeris et al. 1966). In dogs and rats, death was preceded by neurological effects (tremors, convulsions), as discussed below. LOAEL values for the lethal effects of methoxychlor are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.2.2 Systemic Effects

No studies were located regarding respiratory, musculoskeletal, renal, endocrine, dermal, ocular, body weight, or metabolic effects in humans after oral exposure to methoxychlor. Data on the remaining

systemic effects after methoxychlor ingestion are available from a single study in humans. The highest NOAEL values and all LOAEL values from each reliable study for the systemic effects of methoxychlor in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1. These studies are discussed below.

Respiratory Effects. A single study reported pulmonary edema in rabbits exposed to 200 mg/kg/day methoxychlor by gavage for 1–3 weeks, but the authors attributed this effect to possible gavage error resulting in aspiration of the chemical; no control group was included in the study (Smith et al. 1946). Chronic exposure of rats to 77 mg/kg/day recrystallized methoxychlor (Deichmann et al. 1967) or 107 mg/kg/day technical grade methoxychlor (NCI 1978) or of mice to 599 mg/kg/day technical grade methoxychlor (NCI 1978) did not produce any significant histopathological effects in the respiratory tract. These data are too limited to draw firm conclusions, but suggest that the lung is not especially sensitive to ingested methoxychlor.

Cardiovascular Effects. A single case report documented the ingestion of approximately 125 mL of a commercial product that contained methoxychlor (about 15 mg of methoxychlor) by a 62-year-old man in an attempted suicide (Thompson and Vorster 2000). Testing of a serum sample collected at the time of admission to the hospital showed a methoxychlor level of 0.67 µg/mL serum. His blood pressure was very low (58/40) and his pulse rate was high (88 beats per minute). After initiation of treatment, his blood pressure recovered to 110/70.

In animals, one study reported the development of fatty hearts in two of four rabbits administered lethal doses of methoxychlor (200 mg/kg/day) for 1–3 weeks (Smith et al. 1946). However, no gross or histopathological changes in the heart were noted in rats or mice following chronic oral exposure to 107 or 599 mg/kg/day methoxychlor, respectively (NCI 1978). Although no firm conclusion can be

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
	ACUTE EXPOSURE								
	Death								
1	Rat	Once (GO)					3460	(LD50)	Cannon Laboratories 1976 TG
2	Rat (Wistar)	1x (GO)					2828	(LD50)	Dikshith et al. 1990 mixed isomers
3	Rat	Once (GO)					5000	(LD50)	Hodge et al. 1950 RC TG
4	Mouse (NS)	Once (G)					2900	(LD50)	Coulston and Serrone 1969 TG
5	Rabbit (New Zealand)	Gd 7-19 1x/d (GW)					251	(2/17 deaths)	Kincaid Enterprises 1986 TG
	Systemic								
6	Rat	1x/d Gd 6- 15 (GO)	Bd Wt		50	(reduced maternal body weight gain)			Khera et al. 1978 TG
7	Rat	Once (GO)	Hepatic	640					Morgan and Hickenbottom 1979 TG
8	Rabbit	Gd 7-19 1x/d (GW)	Hepatic	5	35.5	(pale/mottled appearance of the liver)			Kincaid Enterprises 1986 TG
			Bd Wt	5	35.5	(anorexia and 14.4% decreased body weight gain)			
	Neurological								
9	Rat (Wistar)	Once (GO)			2500	(decreased locomotor activity)	3000	(tremors)	Cannon Laboratories 1976 TG

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
ACUTE EXPOSURE									
Reproductive									
10	Rat	Gd 6-15 (F)					97.3	(dose-related increases in post-implantation loss)	Culik and Kaplan 1976 TG
11	Rat (Holtzman)	8d 1x/d (GO)		50 F	75 F	(induction of uterine decidualization)			Cummings 1993 98% pure
12	Rat	1x/d 8 d (GO)		100	200	(decreased uterine receptivity to implantation)			Cummings and Gray 1987 LG
13	Rat	1x/d 3-8 d (GO)			100	(decreased serum progesterone)	200	(decreased number of implantation sites and uterine weight; increased number of resorptions)	Cummings and Gray 1989 LG
14	Rat	Gd 1-8 1x/d (GO)		25	50	(decreased serum progesterone)	250	(decreased number of implantation sites)	Cummings and Laskey 1993 LG
15	Rat	Gd 1-3 1x/d (GO)			100	(accelerated embryo transport into uterus)	200	(decreased number of implantations)	Cummings and Perreault 1990 LG
16	Rat	1x/d 5-76 d, starting at weaning at 21 ppd (GO)			25	(precocious vaginal opening and estrus as early as 3 d after exposure started)			Gray et al. 1989 TG
17	Mouse	1x/d 5 d/wk 2 wk (GO)			25	(persistent vaginal cornification)			Martinez and Swartz 1991 TG 50%
18	Rabbit	Gd 7-19 1x/d (GW)		5			35.5	(increased frequency of abortion and late resorptions)	Kincaid Enterprises 1986 TG

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
ACUTE EXPOSURE							
Developmental							
19	Rat	Gd 6-15 (F)			40.8 (increased incidence of wavy ribs)		Culik and Kaplan 1976 TG
20	Rat	1x/d Gd 14-20 (GO)		30			Gellert and Wilson 1979 NS
21	Rat	1x/d Gd 6-15 (GO)				200 (increased percent dead, resorbed, or anomalous fetuses)	Khera et al. 1978 TG
22	Rabbit	Gd 7-19 1x/d (GW)		5	35.5 (10-11% decreased fetal body weight and percentage of male fetuses)		Kincaid Enterprises 1986 TG
INTERMEDIATE EXPOSURE							
Death							
23	Rat (Sherman)	16 wk (F)				1200 (4/12 died)	Davison and Cox 1976 TG
24	Rat	4 wk (F)				917 (6/7 females died)	Haag et al. 1950 RC
25	Rat	45 d (F)				4200 (16/20 died)	Hodge et al. 1950 TG

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
INTERMEDIATE EXPOSURE							
	Systemic						
26	Rat (Sprague-Dawley)	Gd 14 - ppd 42 1x/d (GO)	Hemato	50 M	150 M (24% increase in relative spleen weight)		Chapin et al. 1997 95% pure
			Hepatic	50 M	150 M (13% increase in relative liver weight)		
			Renal	50 M	150 M (16% increase in relative kidney weight)		
			Endocr	50 M	150 M (36% increase in relative and absolute adrenal gland weight)		
			Bd Wt	5	50 (10% decrease in body weight gain)		
27	Rat (Sprague-Dawley)	16 wk (F)	Hepatic	90	1200 (66% increased relative liver weight; decreased total and relative vitamin A content)		Davison and Cox 1976 TG
			Bd Wt	90	1200 (22% decreased body weight gain)		
28	Rat (Long- Evans)	309d 1x/d (GO)	Hepatic		200 M (37% decrease in absolute liver weight)		Gray et al. 1999 90% purity (TG)
			Renal		200 M (37% decrease in absolute kidney weight)		
			Endocr		200 M (17% decrease in absolute pituitary weight; 60% increase in relative adrenal gland weight)		
			Bd Wt			200 M (37% decrease in body weight gain)	

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
INTERMEDIATE EXPOSURE							
29	Rat (CD)	28d; 1x/d (GO)	Hepatic		20 F (decreased serum albumin)		Okázaki et al. 2001 LG
			Renal	100	500 (basophilic tubules; dilatation of renal tubules and casts)		
	Immuno/ Lymphoret						
30	Rat (CD)	28d; 1x/d (GO)		100 M	500 M (atrophy of the thymus)		Okazaki et al. 2001 LG
	Neurological						
31	Rat Long-Evans, Sprague-Dawl F344	1x/d 55-66 d (GO)			400 F (increased and acyclic running wheel activity)		Gray et al. 1988 TG
	Reproductive						
32	Rat (Wistar)	1x/d 70 d for males 5 wk for females (GO)				100 (infertility; degeneration of the testis and ovaries).	Bal 1984 TG
33	Rat (Sprague-Dawley)	Gd 14 - ppd 42; 1x/d (GO)		5 M	5[b] F (precocious vaginal opening)	50 F (reduced fertility; 68% reduction in number of implants; cystic ovaries, few corpora lutea; irregular estrus cycles)	Chapin et al. 1997 95% pure
34	Rat (Sprague-Dawley)	21d 1x/d (G)			250 F (slows the regression of endometriosis)		Cummings and Metcalf 1995b NS
35	Rat	1x/d 7 d/wk 8 wk (GO)			25 M (elevated pituitary prolactin levels)		Goldman et al. 1986 TG

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
INTERMEDIATE EXPOSURE							
36	Rat	1x/d 55-66 d (GO)				400 (persistent vaginal cornification; atrophy of ovary and uterus)	Gray et al. 1988 TG
37	Rat	1x/d 56-66 d (GO)				100 (80% decreased in fertility when treated males and females mated; decreased number of liver pups per litter; decreased caudal epididymal sperm count)	Gray et al. 1989 TG
38	Rat	1x/d 59-76 d (GO)			25 M (elevated levels of prolactin in the pituitary) 25 F (precocious vaginal opening)		Gray et al. 1989 TG
39	Rat	1x/d 76-99 d (GO)			100 M (elevated prolactin, FSH and TSH in the pituitary)	100 F (40% decreased fertility when treated females were mated with untreated males; decreased number of live pups per litter; ovarian atrophy and histopathology)	Gray et al. 1989 TG
40	Rat (Long- Evans)	309d 1x/d (GO)				200 M (delayed puberty; decreased testes weight, fertility, and sperm counts; altered mating behavior)	Gray et al. 1999 90% purity (TG)
41	Rat (Sprague-Dawley)	6-9 wk (F)			60 (decreased ovary weight)	150 (decreased ovary weight; increased uterus weight; decreased mating frequency and fertility in females)	Harris et al. 1974 TG

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
INTERMEDIATE EXPOSURE							
42	Rat (Long- Evans)	12 wk (F)				300 (persistent vaginal cornification; decreased mating frequency)	Harris et al. 1974 TG
43	Rat (Long- Evans)	6 wk (F)				60 (precocious vaginal opening in females; decreased mating frequency and fertility in males and females)	Harris et al. 1974 TG
44	Rat	30-45 d (F)		140		1400 (testicular atrophy)	Hodge et al. 1950 TG
45	Rat (Long- Evans)	15d 1x/d GO			50 F (precocious vaginal opening)		Laws et al. 2000 TG 95%
46	Rat (CD)	28d; 1x/d (GO)		20		100 M (atrophy of seminiferous tubules and Leydig cells in testes; decreased sperm; cell debris in lumen of epididymis; atrophy of mammary acinus) 100 F (abnormal estrous cyclicity)	Okazaki et al. 2001 LG
47	Mouse	1x/day 5 d/wk 4 wk (GO)			50 (persistent vaginal cornification; decreased ovary weight)		Martinez and Swartz 1991 TG
48	Mouse	1x/d 5 d/wk 4 wk (GO)			100 (increased lipid in interstitial and thecal cells of the ovary)		Martinez and Swartz 1992 TG 50%

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL: Less Serious (mg/kg/day)	LOAEL: Serious (mg/kg/day)	Reference Chemical Form
INTERMEDIATE EXPOSURE							
Developmental							
49	Rat (Sprague-Dawley)	Gd 14 - ppd 21 1x/d (GO)			5 (decreased click response in M and F; decreased approach stimulus in M; no dose-response relationship)	50 F (decreased fertility; 68% reduction in number of implants)	Chapin et al. 1997 95% pure (LG)
50	Rat	1x/d 59-76 d (GO)				50 (precocious vaginal opening; 45.3% decreased fertility in offspring; acyclic estrus; pituitary abnormalities)	Gray et al. 1989 TG
51	Rat	6-9 wk (F)			60 (precocious vaginal opening in female offspring)		Harris et al. 1974 TG
52	Rat (Sprague-Dawley)	9 wk (F)				60 (reduced fertility in males and female offspring)	Harris et al. 1974 TG
CHRONIC EXPOSURE							
Systemic							
53	Rat (Osborne-Mendel)	24-27 mo (F)	Resp	77			Deichman et al. 1967 RC
			Gastro	77			
			Hepatic	77			
			Renal	77			

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
CHRONIC EXPOSURE							
54	Rat (albino)	1 yr (F)	Resp	917			Haag et al. 1950 RC
			Cardio	917			
			Gastro	917			
			Musc/skel	917			
			Hepatic	917			
			Renal	917			
			Dermal	917			
			Ocular	917			
			Bd Wt			197 M (>10% decrease in body weight)	

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
CHRONIC EXPOSURE							
55	Rat (Osborne-Mendel)	78 wk (F)	Resp	107			NCI 1978 TG
			Cardio	107			
			Gastro	107			
			Hemato	107			
			Musc/skel	107			
			Hepatic	107			
			Renal	107			
			Dermal	107			
			Ocular	107			
			Bd Wt		107 F (decreased body weight gain)		

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
CHRONIC EXPOSURE							
56	Mouse (B6C3F1)	78 wk (F)	Resp	599			NCI 1978 TG
			Cardio	599			
			Gastro	599			
			Hemato	599			
			Musc/skel	599			
			Hepatic	599			
			Renal	599			
			Dermal	599			
			Ocular	599			
			Bd Wt		172 F (12% decrease in body weight)		
	Immuno/ Lymphoret						
57	Rat (Osborne-Mendel)	78 wk (F)		107			NCI 1978 TG
58	Mouse (B6C3F1)	78 wk (F)		599 M			NCI 1978 TG
	Neurological						
59	Rat (Osborne-Mendel)	78 wk (F)		107 F			NCI 1978 TG
60	Mouse	78 wk (F)		599 M			NCI 1978 TG

Levels of Significant Exposure to Methoxychlor - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	CHRONIC EXPOSURE						
	Reproductive						
61	Rat (CD)	3 generations (F)		18		92 F (decreased fertility)	Haskell Laboratories 1966 TG

a The number corresponds to entries in Figure 3-1

b Used to derive a minimal risk level (MRL) of 0.005 mg/kg/day; dose divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

Figure 3-1. Levels of Significant Exposure to Methoxychlor - Oral

Acute (≤14 days)

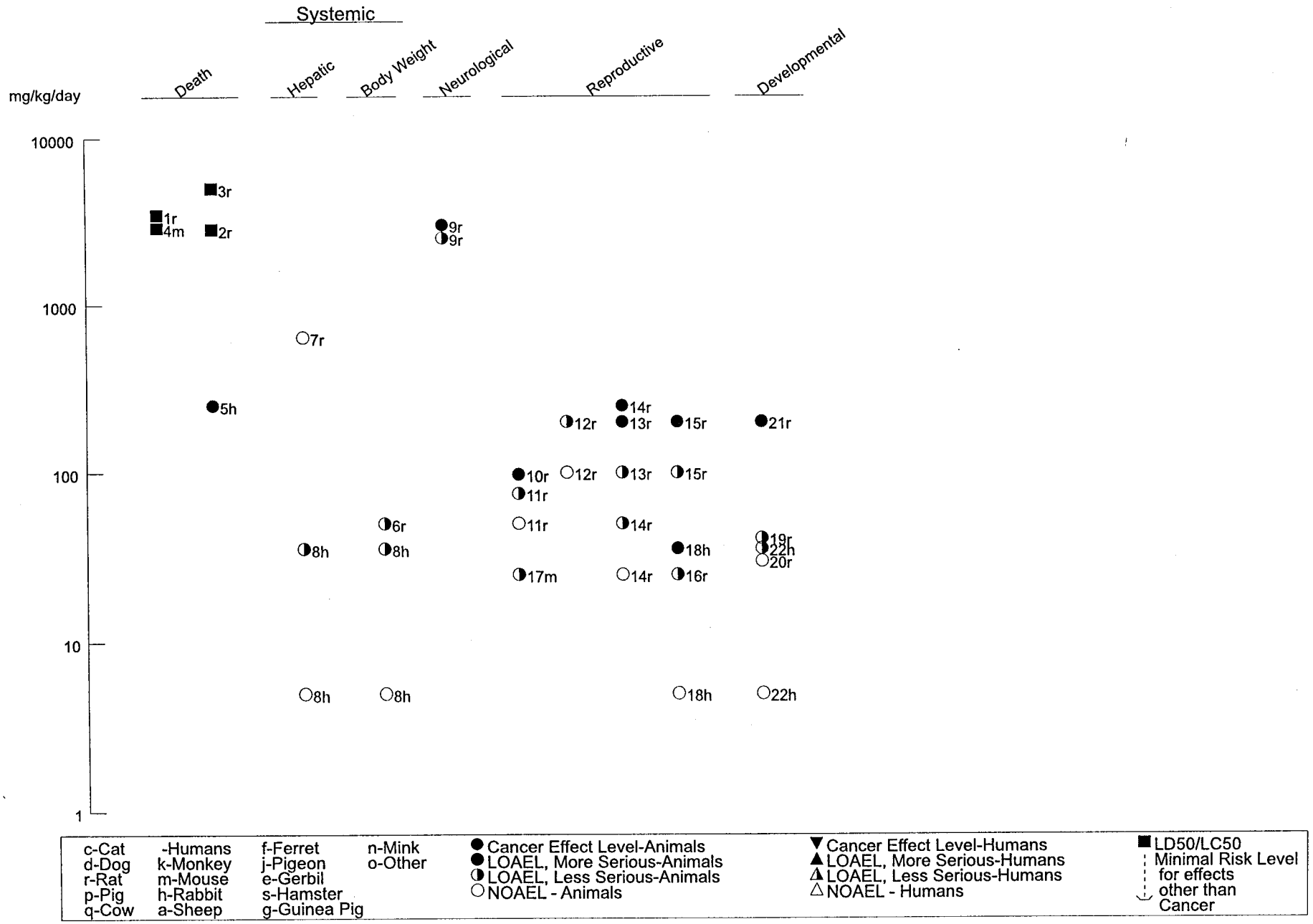


Figure 3-1. Levels of Significant Exposure to Methoxychlor - Oral (*continued*)

Intermediate (15-364 days)

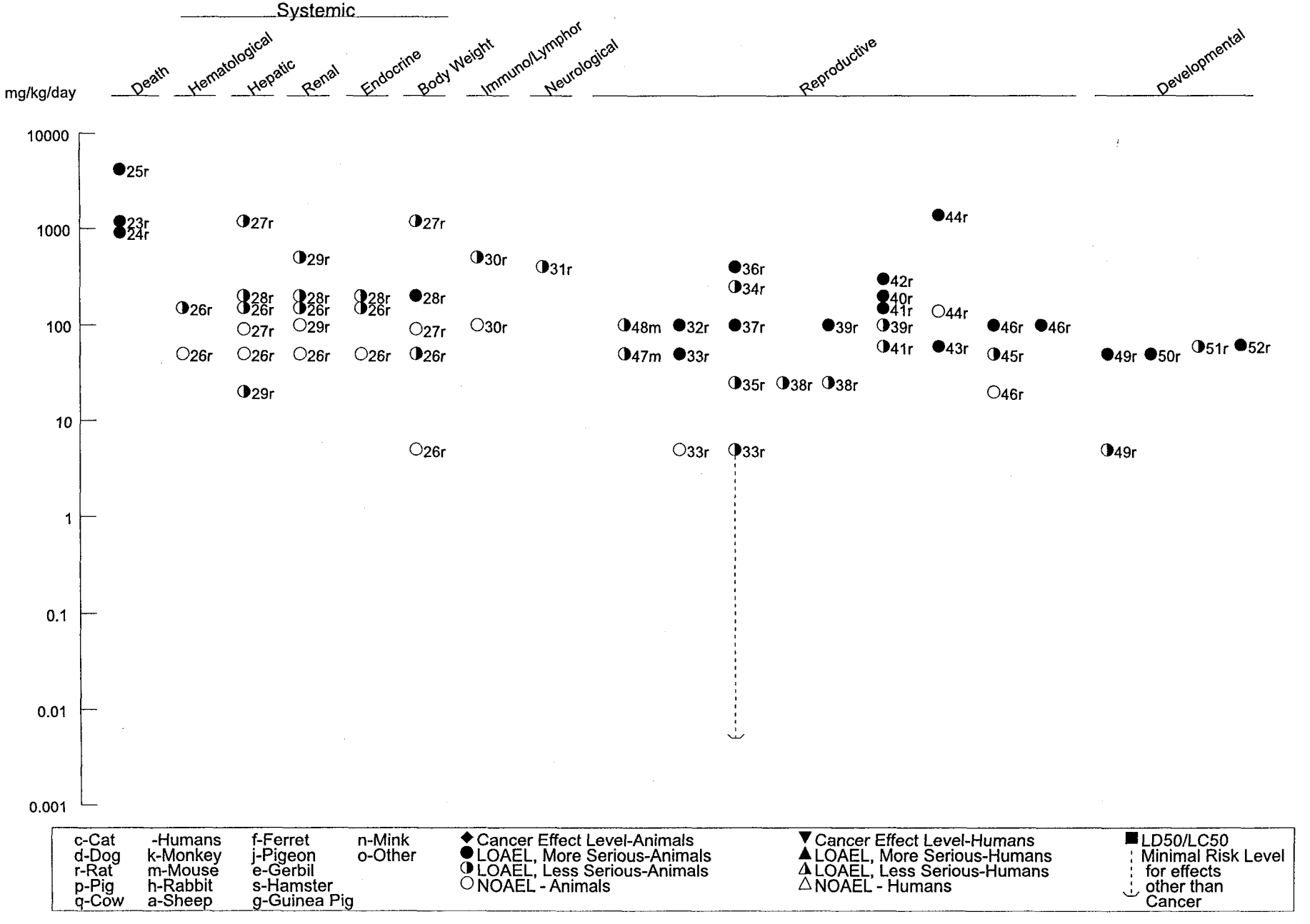
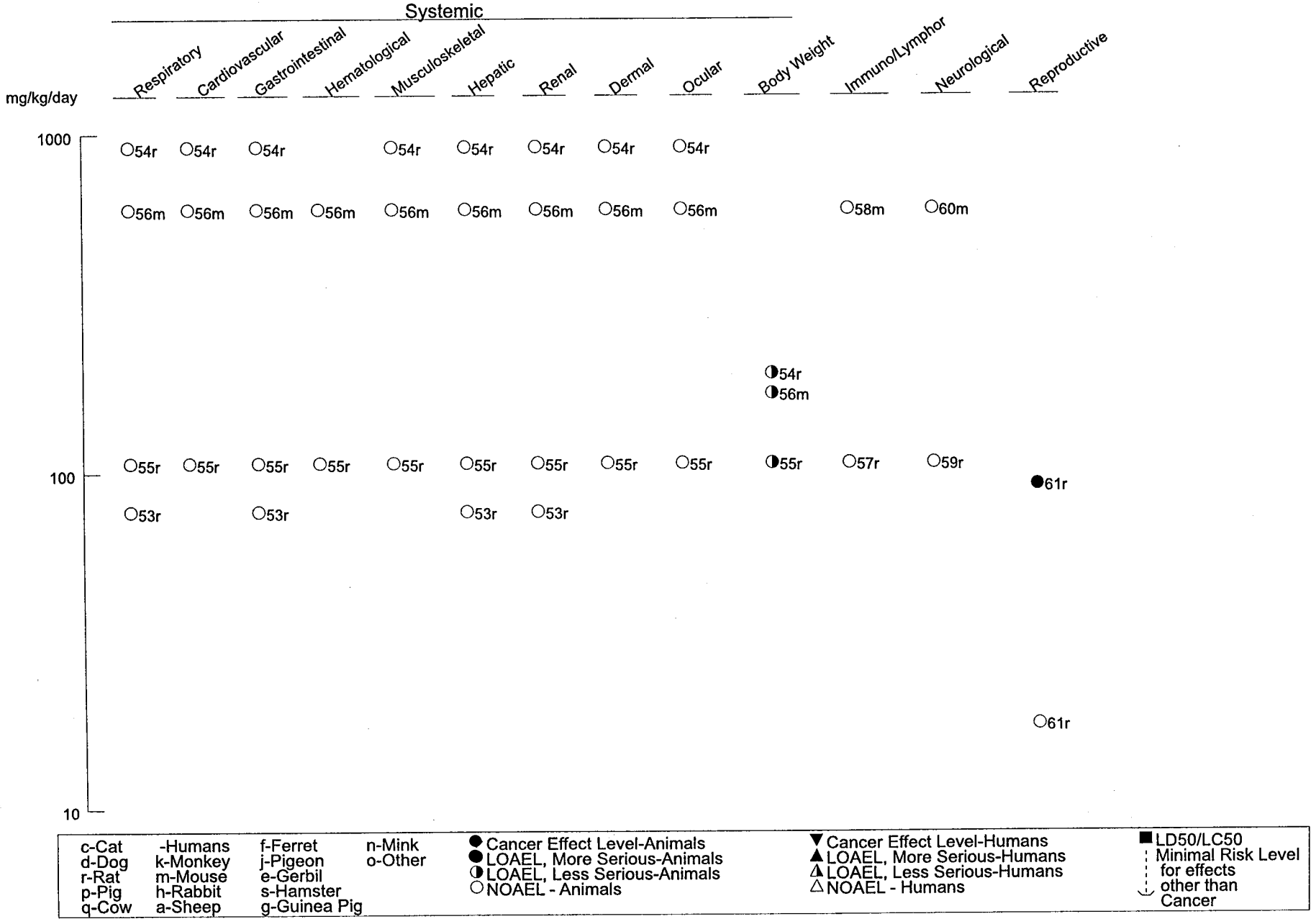


Figure 3-1. Levels of Significant Exposure to Methoxychlor - Oral (*continued*)

Chronic (≥365 days)



drawn regarding cardiovascular effects of methoxychlor, the data suggest that the heart is not a primary target organ for methoxychlor.

Gastrointestinal Effects. No histological evidence of injury to the small intestine was detected in biopsy samples from four men and four women who ingested 2 mg/kg/day of methoxychlor for 6 weeks (Wills 1969).

Diarrhea was noted in rats given acutely lethal doses (7,000 mg/kg) of methoxychlor (Smith et al. 1946). However, no histopathological changes of the gastrointestinal tract were noted following chronic oral exposure of rats to 77 mg/kg/day recrystallized methoxychlor (Deichmann et al. 1967) or 107 mg/kg/day technical grade methoxychlor (NCI 1978), or mice to 599 mg/kg/day technical grade methoxychlor (NCI 1978). These data suggest that the gastrointestinal tract is not a sensitive target organ for methoxychlor.

Hematological Effects. Only one study was located regarding the hematological effects of methoxychlor in humans after oral exposure. Electron microscopy of bone marrow biopsy samples and chemical analysis of the blood from four male and four female volunteers exposed to 2 mg/kg/day for 6 weeks revealed no significant changes (Wills 1969).

Rabbits administered lethal doses of methoxychlor (200 mg/kg/day) for 1–3 weeks developed hemosiderosis of the spleen, but no controls were included in the study (Smith et al. 1946). Rats exposed to 150, but not 50, mg/kg/day methoxychlor for 35 days had increased relative spleen weights (Chapin et al. 1997). However, no gross or histopathological changes were noted on the hematopoietic system (bone marrow, spleen) in rats or mice exposed to 107 or 599 mg/kg/day methoxychlor in the feed for 78 weeks (NCI 1978). No changes in red blood cell, white blood cell, or hemoglobin levels were observed in rats exposed to up to 1,000 mg/kg/day methoxychlor for 90 days (Dikshith et al. 1990). These data are insufficient to evaluate the hematological effects of methoxychlor.

Musculoskeletal Effects. In animals, no histopathological effects were detected in the skeletal muscles or bones of rats or mice following chronic oral exposure to doses of 107 or 599 mg/kg/day methoxychlor, respectively (NCI 1978). This suggests the musculoskeletal system is not an important target for methoxychlor.

Hepatic Effects. Only one study was located regarding the effects of methoxychlor on the liver in humans after oral exposure. Electron microscopy of liver biopsy samples from 4 men and 4 women exposed for 6 weeks to 2 mg/kg/day revealed no histopathological changes (Wills 1969). In addition, there were no changes in serum enzyme levels such as glutamate-oxaloacetate aminotransferase (SGOT; also called aspartate aminotransferase or AST), glutamate-pyruvate aminotransferase (SGPT; also called alanine aminotransferase or ALT), and alkaline phosphatase (AP) that are indicative of liver injury. Based on these limited data, the liver of humans does not appear to be affected by low doses of methoxychlor for intermediate durations. The hepatic effects of chronic oral exposure to low doses of methoxychlor have not been studied in humans.

Several studies in animals have described effects on the liver following oral exposure to methoxychlor, but the effects were usually mild or moderate, and effects were generally observed only at high doses (lethal or near lethal). In male rats given a single oral dose of 640 mg/kg methoxychlor, increased hepatic levels of glucose-6-phosphatase and decreased levels of glycogen phosphorylase and lactate were detected, but there were no observable histopathological changes (Morgan and Hickenbottom 1979). These enzymatic changes suggest that methoxychlor may promote the utilization of liver glycogen (Morgan and Hickenbottom 1979). The livers of pregnant rabbits administered 35.5 mg/kg/day methoxychlor on days 7–19 of gestation were pale and mottled in appearance (Kincaid Enterprises 1986). This effect was not noted in rabbits administered 5.01 mg/kg/day methoxychlor. "Fatty degeneration" of the liver (not otherwise described) was noted in two out of four rabbits given lethal doses of methoxychlor (200 mg/kg/day) for 1–3 weeks; no controls were included in this study (Smith et al. 1946). Levels of serum enzymes (SGOT or AST, SGPT or ALT, AP) were elevated in dogs receiving 2,000, but not 1,000, mg/kg/day methoxychlor in feed for 8–24 weeks (Tegeris et al. 1966). Rats that received 100–1,000 mg/kg/day methoxychlor for 90 days had small, but statistically significant, nondose related changes in serum and/or liver enzyme activities (GOT, GPT, and AP) and serum protein levels (Dikshith et al. 1990). Significant increases in liver weight were reported in rats administered lethal doses of methoxychlor (500–1,200 mg/kg/day for 13–16 weeks) (Davison and Cox 1976; Dikshith et al. 1990). Rats fed nonlethal doses of methoxychlor (60 mg/kg/day) for 9 weeks, spanning mating through weaning, had enlarged livers and elevated liver vitamin A concentrations prior to mating, but no significant differences from controls following weaning (Harris et al. 1974). Although reductions in liver weight have also been reported in animals receiving nonlethal doses of methoxychlor for intermediate or chronic durations (Cecil et al. 1974; Deichmann et al. 1967; Gray et al. 1989, 1999), these reductions were generally associated with decreases in body weight gain and are not considered adverse. No histopathological changes were found in the liver of rats or mice chronically exposed to 107 or

599 mg/kg/day methoxychlor, respectively (NCI 1978), or rats exposed to 77 mg/kg/day (Deichmann et al. 1967). Taken together, these data suggest that high doses of methoxychlor may cause hepatic effects in some animals, but that the liver is not a key target organ for methoxychlor.

Because DDT (a structural analogue of methoxychlor) is known to cause vitamin A depletion in the livers of exposed animals, a number of studies have investigated the effects of methoxychlor on the vitamin A content of the liver. A significant reduction in total liver vitamin A content was observed in rats receiving doses of 9 mg/kg/day or more for 16 weeks (Davison and Cox 1976), and vitamin A concentration (µg/100 mg liver), but not total vitamin A, was significantly reduced in rats exposed to 60 mg/kg/day for 9 weeks (Harris et al. 1974). No significant change in vitamin A content was reported in rats administered 5 mg/kg/day methoxychlor for 8–16 weeks (Cecil et al. 1974; Phillips and Hatina 1972). In an *in vitro* assay, methoxychlor did not react with human transthyretin, a carrier protein for vitamin A and thyroid hormones (Van den Berg et al. 1991). However, no microsomal activation system was employed to determine if methoxychlor metabolites could bind transthyretin. Decreases in the vitamin A content of liver are not necessarily adverse by themselves, so the significance of this effect is uncertain.

Renal Effects. Adverse effects on the kidneys were reported in animals administered lethal or near-lethal doses of methoxychlor. Renal nephrosis was observed in four out of four rabbits administered 200 mg/kg/day methoxychlor for 1–3 weeks, but the significance is unclear since no controls were included (Smith et al. 1946). Basophilic tubules, dilatation of renal tubules, and casts were seen in rats exposed to 500, but not 100, mg/kg/day for 28 days; no further description of the lesions was provided (Okazaki et al. 2001). Cystic tubular nephropathy was reported in rats exposed to 861 mg/kg/day methoxychlor in the feed for 33–55 days (Tullner and Edgcomb 1962). Cystic tubular nephropathy was accompanied by elevated blood urea nitrogen (BUN) in pigs exposed to 1,000 mg/kg/day methoxychlor in the feed for 24 weeks (Tegeris et al. 1966). No histopathological changes were detected in rats and mice exposed to 107 or 599 mg/kg/day, respectively, in the feed for 78 weeks (NCI 1978) or in rats exposed to up to 1,000 mg/kg/day for 90 days (Dikshith et al. 1990). One study showed a 37% reduction in kidney weight in rats treated with 200 mg/kg/day for 4 weeks, but this was associated with a 37% reduction in body weight and therefore, may not be adverse (Gray et al. 1999). Although limited, these data suggest that high doses may injure the kidneys, but that renal effects are not of major concern at lower doses.

Endocrine Effects. Only limited data were located regarding the effects of methoxychlor on endocrine glands in animals following oral exposure. A decrease in absolute pituitary weight was seen in rats exposed to 200–400 mg/kg/day for 44 weeks or 100–200 mg/kg/day for 11–16 weeks (Gray et al. 1989, 1999). This was, however, associated with a reduction in body weight and, in most cases, a slight increase in relative pituitary weight. In these same studies, Gray et al. (1989) observed an increase in absolute and relative adrenal gland weight in male and female rats exposed to 100–200 mg/kg/day for 11–16 weeks. No treatment-related histopathological effects were seen. No histopathological effects or changes in adrenal gland weight were seen in male or female rats that received up to 1,000 mg/kg/day methoxychlor for 90 days (Dikshith et al. 1990). Increased relative and absolute adrenal gland weights were seen in male rats exposed to 150 mg/kg/day methoxychlor from gestation day 14 through postnatal day 42 (Chapin et al. 1997). This effect was not seen in female rats or in male rats at 5 or 50 mg/kg/day. No histopathological data were reported. Adrenal gland weight returned to normal (compared to controls) with cessation of methoxychlor administration (Chapin et al. 1997).

In female laboratory animals, exposure to methoxychlor results in a number of effects involving the reproductive tract and the major hormone-producing glands that regulate it. Atrophic and degenerative changes in the ovaries were seen in female mice and rats exposed to 25–400 mg/kg/day methoxychlor for intermediate durations (Bal 1984; Chapin et al. 1997; Gray et al. 1988, 1989; Harris et al. 1974; Martinez and Swartz 1991). Decreased serum progesterone levels were observed in female rats acutely exposed to 50–100 mg/kg/day laboratory grade methoxychlor, but not to 25 mg/kg/day (Cummings and Gray 1989; Cummings and Laskey 1993). Ultrastructural changes in ovarian cells of mice exposed to 100 mg/kg/day for 4 weeks included an accumulation of lipid in the interstitial and thecal cells (Martinez and Swartz 1992). It has been postulated that methoxychlor disrupts the feedback regulation of pituitary hormones that normally exert an effect on the uterus and other estrogen-sensitive tissues (Martinez and Swartz 1992).

Oral exposure to methoxychlor also produces a number of effects in male laboratory animals. A 21% decrease in ventral prostate weight was observed in male rats exposed to 154 mg/kg/day for 90 days (Shain et al. 1977). Decreased weight of the testes was observed in male rats and mice exposed to methoxychlor at 50–1,400 mg/kg/day for intermediate durations (Bal 1984; Gray et al. 1989, 1999; Hodge et al. 1950; Tullner and Edgcomb 1962), and regression of the seminiferous epithelium was seen in mice exposed to 60 mg/kg/day for 50 days or more (Wenda-Rozewicka 1983). A dose-related inhibition of testes development, fewer mature germ cells, and fewer germ cells of each type were observed in the testes of male rats exposed to 50 or 150 mg/kg/day methoxychlor from gestation

day 14 through postnatal day 42 (Chapin et al. 1997). Decreased caudal epididymal sperm counts were observed in rats exposed to 50–400 mg/kg/day for intermediate-duration exposures (Gray et al. 1989, 1999). Exposure to 25–50 mg/kg/day methoxychlor produced increased levels of prolactin in the pituitary of male rats (Goldman et al. 1986; Gray et al. 1989). The hypothalamus of rats exposed to 50 mg/kg/day developed elevated levels of gonadotropin releasing hormone (GnRH) (Goldman et al. 1986). Serum levels of thyroid stimulating hormone (TSH) were decreased in rats following exposure to 100–200 mg/kg/day methoxychlor (Gray et al. 1989). Serum T_3 (triiodothyronine) levels were increased in female rats at 100 and 500 mg/kg/day, and T_3 and TSH levels were increased in male rats exposed to 500, but not 100, mg/kg/day for 28 days (Okazaki et al. 2001). Serum luteinizing hormone (LH) levels were decreased in female rats exposed to 100, but not 20, mg/kg/day methoxychlor, and serum FSH and prolactin levels were increased and testosterone levels were decreased in male rats exposed to 500, but not 100, mg/kg/day (Okazaki et al. 2001). Serum LH, prolactin, testosterone, and interstitial fluid testosterone were unaffected in male rats exposed to 200–400 mg/kg/day for 44 weeks (Gray et al. 1999).

Effects of methoxychlor on the endocrine system are likely related to methoxychlor-associated reproductive disturbances. For example, decreased serum progesterone levels (Chapin et al. 1997; Cummings and Gray 1989; Cummings and Laskey 1993) may result from the estrogenic effects of methoxychlor on the ovaries that cause decreased follicular and corpus luteum development (Chapin et al. 1997). The effects on the ovaries may be caused directly by methoxychlor metabolites or may result from effects on the pituitary and hypothalamus, which alter the release of regulatory hormones that affect the reproductive and accessory sex glands (Chapin et al. 1997; Martinez and Swartz 1992). There is a high level of interdependence between the hypothalamus, pituitary, and peripheral endocrine glands; therefore, feedback mechanisms between the peripheral endocrine glands and the pituitary and hypothalamus and between the pituitary and the hypothalamus are probably also involved in the alteration of hormone levels by methoxychlor. Effects of methoxychlor on the reproductive system are discussed in more detail in Section 3.2.2.5 Reproductive Effects, and mechanisms for effects on the endocrine and reproductive systems are discussed in Section 3.5.2 Mechanisms of Toxicity and Section 3.6 Toxicities Mediated Through the Neuroendocrine Axis.

Dermal Effects. Gross and microscopic examination of the skin did not reveal any treatment related effects in rats or mice chronically orally exposed to 107 or 599 mg/kg/day methoxychlor, respectively (NCI 1978). These data are too sparse to permit a firm conclusion to be made on the dermal effects of methoxychlor, but suggest that the skin is not a target system.

Ocular Effects. No studies were located regarding ocular effects in humans after oral exposure to methoxychlor. No ocular effects were seen in rats or mice chronically exposed to 107 or 599 mg/kg/day methoxychlor, respectively (NCI 1978).

Body Weight Effects. Several animal studies suggest that oral exposure to methoxychlor can lead to decreased body weight. Acute exposure of rats to 40.8–400 mg/kg/day or intermediate exposure to 25–400 mg/kg/day resulted in decreased growth (Chapin et al. 1997; Culik and Kaplan 1976; Gray et al. 1989, 1999; Khera et al. 1978). A 14.4% decrease in body weight gain was noted in pregnant rabbits administered 35.5 mg/kg/day methoxychlor, but not 5.01 mg/kg/day methoxychlor (Kincaid Enterprises 1986). Rats exposed to 100–1,000 mg/kg/day methoxychlor for 90 days had a 5–43% decrease in body weight gain (Dikshith et al. 1990). In dogs exposed to 1,000 mg/kg/day for 8–24 weeks, decreased body weight was accompanied by an absence of body fat in normal depot areas (Tegeris et al. 1966). Chronic exposure of rats to 107 mg/kg/day (NCI 1978) or 229 mg/kg/day (Haag et al. 1950) or mice to 599 mg/kg/day (NCI 1978) produced a decrease in body weight gain. Since many of these studies also reported depressed food intake in methoxychlor-treated animals, it is possible that this is responsible, in part, for the observed decreases in body weight gain. However, in studies in which pair-fed controls were used, a 22% decreased body weight was observed in rats fed diets containing 861 mg/kg/day for 33–55 days (Tullner and Edgcomb 1962) or 1,200 mg/kg/day for 16 weeks (Davison and Cox 1976). These data indicate that body weight gain may be adversely affected by relatively high doses of methoxychlor.

Metabolic Effects. No studies were located regarding metabolic effects in animals after oral exposure to methoxychlor.

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after oral exposure to methoxychlor. In animals, no adverse histological effects were detected in the thymus, spleen, or lymph nodes of rats chronically exposed to 107 mg/kg/day or mice chronically exposed to 599 mg/kg/day (NCI 1978). However, histological examination may not be sensitive enough to detect changes in immune system function. In male (but not female) rats exposed to 5 or 50 mg/kg/day methoxychlor from gestation day 14 through postnatal day 42, a respective 35 or 42% decrease in plaque-forming cells/spleen was observed, indicating a possible attenuation of the primary immune response (Chapin et al. 1997). The plaque-forming cell assay quantitates the production of a specific antibody following administration of an

antigen. The physiological functioning of a number of immune system components, including B cells, T helper cells, and macrophages, are reflected in this assay. Thymus weights were decreased by about 15 and 30% in rats exposed to 50 and 150 mg/kg/day, respectively, from gestation day 14 through postnatal day 42 (Chapin et al. 1997). No histological data were provided. Spleen weight, splenic natural killer cell activity, splenic lymphoproliferative response, and splenic cell-surface phenotypes did not differ from controls. The study authors suggested that the observed effects on plaque-forming cell numbers in males may have been anomalous because no such effects were observed in females and no changes were observed in males in related end points such as splenic lymphoproliferative response (Chapin et al. 1997). However, atrophy of the thymus was also observed in male, but not female, rats exposed to 500, but not 100, mg/kg/day methoxychlor for 28 days (Okazaki et al. 2001). Relative thymus weights were lower in female rats exposed to 500, but not 100, mg/kg/day, but the difference did not reach statistical significance (Okazaki et al. 2001). No histological abnormalities were noted. These data suggest that high exposure levels of methoxychlor may affect certain immunological parameters in rodents and that male rats may be more sensitive to the immunological effects of methoxychlor than females. Considering the available data, the immune system does not appear to be a primary target for methoxychlor.

3.2.2.4 Neurological Effects

A single case report documented the ingestion of approximately 125 mL of a commercial product that contained methoxychlor (about 15 mg of methoxychlor) by a 62-year-old man in an attempted suicide (Thompson and Vorster 2000). The man showed no response to pain or verbal stimuli and had pale skin with profuse sweating. Testing of a serum sample collected at the time of admission to the hospital showed a methoxychlor level of 0.67 µg/mL serum.

Neurological effects have been observed in some animals exposed to methoxychlor. Large acute doses of 1,000 mg/kg methoxychlor or more administered to rats produced neurological effects such as decreased locomotor activity, tremors, lacrimation, salivation, nasal bleeding, dyspnea, diarrhea, convulsions, and paralysis (Cannon Laboratories 1976; Dikshith et al. 1990). In dogs, exposure to 1,000–4,000 mg/kg/day methoxychlor for 8–24 weeks produced a dose-dependent increase in neurological effects including apprehension, nervousness, increased salivation, tremors, convulsions, and death (Tegeris et al. 1966). When rats with liver damage (induced by carbon tetrachloride) were exposed to methoxychlor, they exhibited tremors similar to those described above for dogs (Lehman 1952). Assuming that one of the effects of the liver injury is decreased metabolism of methoxychlor, this observation suggests that the

parent compound (rather than a metabolite) may be mainly responsible for these types of neurological effects. This idea is consistent with the observation that DDT (a poorly metabolized analogue of methoxychlor) also produces similar neurological signs (Agency for Toxic Substances and Disease Registry 1994). An increased incidence (significance not reported) of hunched posture and rough fur was reported in rats exposed to 22–107 mg/kg/day methoxychlor in the feed for 78 weeks (NCI 1978). No changes in brain weight or histopathology were noted in rats or mice chronically exposed to 107 or 599 mg/kg/day methoxychlor, respectively (NCI 1978) or in rats exposed to up to 1,000 mg/kg/day for 90 days (Dikshith et al. 1990).

Exposure to methoxychlor has also produced behavioral changes in animals. Doses of 200 or 400 mg/kg/day administered for 55–66 days produced an increased running wheel activity in ovariectomized and intact female rats, respectively (Gray et al. 1988). In rats, running wheel activity is regulated by estrogen and is synchronous with the 5-day estrus cycle, being greater during proestrus than during estrus and diestrus. Ovariectomy reduces running wheel activity and abolishes cyclicity. Administration of progesterone to methoxychlor-treated ovariectomized rats significantly lowered running wheel activity (Gray et al. 1988). Because progesterone blocks the synthesis of estrogen receptors in the central nervous system and reproductive tract but does not lower running wheel activity induced by nonestrogenic mechanisms, the authors concluded that the observed inhibition of methoxychlor-induced running wheel activity by progesterone was evidence of an estrogenic effect of methoxychlor on the central nervous system.

The administration of 200 mg/kg/day methoxychlor for 2 weeks also increased receptivity to mating in ovariectomized hamsters (Gray et al. 1988). Behavioral estrus remained in more than half of the hamsters at 1 week post-exposure. Although the magnitude of the effects of methoxychlor on mating receptivity in ovariectomized hamsters was not as great as that observed with estradiol (1.0 mg/kg), the effects persisted longer following cessation of exposure (Gray et al. 1988). Nine of 14 hamsters exposed to methoxychlor for 13 days displayed lordosis behavior, and15/15 hamsters exposed to weekly administration of estrogen displayed lordosis behavior. Neurobehavioral effects were also observed following exposure of rats to 5, 50, or 150 mg/kg/day methoxychlor from gestation day 14 through postnatal 21 (Chapin et al. 1997). Male rats treated with 150 mg/kg/day, but not 5 or 50 mg/kg/day, showed an increased handling reactivity. Non-dose-related but statistically significant changes were seen for sensorimotor parameters: locomotor activity (increased in females in the 50 mg/kg/day group, but not at 5 or 150 mg/kg/day), click response (decreased in males at 5 and 150 mg/kg/day, but not 50 mg/kg/day, and in females at 5 mg/kg/day, but not 50 or 150 mg/kg/day), and approach stimulus (lower in males at 5 mg/kg/day, but

not 50 or 150 mg/kg/day). Temporary but statistically significant changes that occurred at various times during the study included increased urination in the open field in females at 50 and 150 mg/kg/day, but not 5 mg/kg/day on postpartum day 66, increased defecation in females at 150 mg/kg/day, but not 5 or 50 mg/kg/day on postpartum day 31, and decreased hindlimb grip strength in females at 50 mg/kg/day, but not 5 or 150 mg/kg/day on postpartum days 47 and 66. There were no differences between the controls and treatment groups in the passive avoidance/cognitive function tests.

An increase in urine-marking behavior was observed in adult male offspring of mouse dams exposed to 0.02 mg/kg/day on gestation days 11–17 (vom Saal et al. 1995). This experiment is also described in Parmigiani et al. (1998). Parmigiani et al. (1998) also observed a statistically significantly increased incidence of aggressive behavior (infanticide) toward unrelated pups in adult male offspring of mice treated with 1.8 mg/kg/day during gestation days 11–17, but not at lower (0.02 or 0.18 mg/kg/day) or higher (18.2 or 90.0 mg/kg/day) exposure levels. Exposure to 0.02–182 µg/kg/day of the potent estrogen, DES, had no effect on this behavior. However, limitations in the study designs of vom Saal et al. (1995) and Parmigiani et al. (1998) make these studies difficult to interpret, (please refer to Appendix A for a more detailed discussion of the study limitations). Aggressive grooming behavior was altered in a non-monotonic fashion in male mice orally exposed *in utero* to 0.02, 0.2, or 2.0 mg/kg/day methoxychlor (Palanza et al. 1999). This behavior was statistically significantly decreased in the 0.02 mg/kg/day group, and was increased and decreased in the 0.2 and 2.0 mg/kg/day groups, respectively, but without statistical significance. This change in behavior was only seen on day 39, but not on day 54, postpartum. Unlike the neurological effects (tremors, convulsions) discussed above, these behavioral effects of methoxychlor (increased running wheel activity and receptivity to mating, urine-marking, infanticide), which have been shown previously to be influenced by estrogen or testosterone, are most likely attributable to one or more of the metabolites or contaminants of methoxychlor that exhibit estrogenic or anti-androgenic activity.

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

3.2.2.5 Reproductive Effects

A single study was located regarding effects of oral exposure to methoxychlor on reproductive tissues in humans. Electron microscopic examination of biopsy samples from the testes of men exposed to 2 mg/kg/day (three men) for 6 weeks revealed no histopathological changes from controls (three men)

(Wills 1969). In addition, no effects on menstrual cycles were noted in similarly dosed women at 0.5, 1.0, or 2.0 mg/kg/day (four per exposure group).

The reproductive effects of methoxychlor have been well studied in animals. These studies indicate that the reproductive system is a sensitive target of methoxychlor toxicity in both males and females. Effects associated with methoxychlor exposure include histopathological changes in the reproductive organs and accessory glands, impaired pubertal development and reproductive function, and altered hormone levels. These effects are due to the estrogenic activity of both the *O*-demethylated metabolites of methoxychlor and some of the *O*-demethylated contaminants of technical grade methoxychlor (Bulger et al. 1985). Information regarding the reproductive effects of methoxychlor in male and female animals is presented separately below.

Reproductive Effects in Female Animals. Methoxychlor adversely affects the development of the female reproductive system. The day of vaginal opening and first estrus was significantly earlier in female rats exposed to 25–100 mg/kg/day methoxychlor beginning on postpartum day 21 and extending through mating, gestation, and lactation (Gray et al. 1989). In these experiments, vaginal opening occurred at an average age of 32 days in controls, but in exposed groups, occurred as early as 3 days after beginning exposure. Average age at vaginal opening ranged from 24 to 26 days in the exposed groups. The onset of estrus cyclicity was accelerated in animals exposed to 25 mg/kg/day and normal in animals exposed to 50–100 mg/kg/day. The onset of estrus cyclicity was delayed in animals dosed with 200 mg/kg/day. Similarly, precocious vaginal opening and estrus were noted in female rats exposed *in utero*, during lactation and postweaning to 5–150 mg/kg/day (Chapin et al. 1997; Harris et al. 1974). In female rats exposed to 5, 50, or 150 mg/kg/day during gestation days 14–21, via lactation during postpartum days 1–7, and then directly via oral gavage through postpartum day 42 (dams were no longer treated after postpartum day 7 and pups stayed with the dams until postpartum day 21), vaginal opening was accelerated by 2–7 days (35.2, 30.8, and 33.4 days postpartum in the 5, 50, and 150 mg/kg/day groups, respectively, compared to 37.4 days for the controls) (Chapin et al. 1997). Female rats exposed *in utero* throughout gestation to 0, 60, or 150 mg/kg/day methoxychlor and maintained on the same oral dose postnatally had earlier vaginal opening than controls (23 and 19 days postpartum in the 60 and 150 mg/kg/day groups, respectively, compared to 39 days for the controls) (Harris et al. 1974). Fertility was significantly impaired in females exposed to 50 or 150 mg/kg/day, but not to 5 mg/kg/day, between gestation day 14 and postnatal day 42 (Chapin et al. 1997). Incidences for females that became pregnant after cohabitating with untreated males were 13/15 (control), 11/15 (5 mg/kg/day), 3/15 (50 mg/kg/day),

and 0/15 (150 mg/kg/day) (Chapin et al. 1997). Descriptions of developmental effects can be found in greater detail in Section 3.2.2.6 Developmental Effects.

Methoxychlor produces gross and histopathological changes in the mature female reproductive system after oral exposure. An accumulation of lipid was observed in ovarian interstitial and thecal cells of mice exposed to 100 mg/kg/day for 4 weeks (Martinez and Swartz 1992). Cellular hypertrophy of the uterine epithelial cells was observed in adult female mice exposed to 50 or 100 (but not 2, 10, or 20) mg/kg/day methoxychlor (as Marlate, a 50% mixture; contaminants unknown) 5 days/week for 4 weeks; this effect was similar to the response to estradiol (Swartz et al. 1994). Electron microscopic examination revealed dose-related ultrastructural changes, including one or more of the following at all dose levels: elongated microvilli, decreased numbers of microvilli, increased vacuoles, dilated endoplasmic reticulum and Golgi complexes, enlarged and disrupted mitochondria, and irregular apical cell surface. A marked uterotrophic effect (as indicated by a 2–3-fold increase in uterine weight) was observed in ovariectomized mice exposed to 16.7 mg/kg/day and in ovariectomized plus adrenalectomized rats and hypophysectomized rats exposed to 95.2 mg/kg/day for 3 days (Tullner 1961). From the time of vaginal opening until mating, increased vaginal cornification and a decreased percentage of vaginal smears with leukocytes was observed in female rats acutely exposed to 50–200 mg/kg/day pre- and postnatally (Chapin et al. 1997) or beginning on postpartum day 21 (Gray et al. 1989) or exposed to 400 mg/kg/day for intermediate durations (Gray et al. 1988). An enlarged uterus was observed in rats exposed to 150 mg/kg/day for 6 weeks (Harris et al. 1974) or 100 or 500 mg/kg/day for 28–31 days (Okazaki et al. 2001) and in pigs exposed to 1,000 mg/kg/day for 24 weeks (Tegeris et al. 1966). However, absolute uterine weight was reduced by 35% (and relative uterine weight by 27%) in intact virgin female rats exposed to 150 mg/kg/day from gestation day 14 through postnatal 42 (Chapin et al. 1997). This effect was even more pronounced on the pregnant uterus (21–50% reduction in weight of empty uterus at exposures of 5–50 mg/kg/day) (Chapin et al. 1997). Mice treated initially with ENU (a uterine cancer initiator) and then administered 390 mg/kg/day methoxychlor for 26 weeks had no increased incidence of tumors, but had increased absolute and relative uterine weights (Mitsumori et al. 2000). Mammary gland hyperplasia was also observed in rats exposed to 500, but not 100, mg/kg/day for 28–31 days (Okazaki et al. 2001) or pigs exposed to 1,000 mg/kg/day for 24 weeks (Tegeris et al. 1966), and decreased ovarian weight was reported in adult rats exposed to 500, but not 100, mg/kg/day methoxychlor for 90 days (Dikshith et al. 1990). Intermediate-duration exposures to 25–500 mg/kg/day methoxychlor produced atrophic changes in the ovaries of exposed female mice and rats (Bal 1984; Chapin et al. 1997; Gray et al. 1988, 1989; Harris et al. 1974; Martinez and Swartz 1991; Okazaki et al. 2001). Some of these effects mimic those of estrogen, but some differ from estrogenic effects. Methoxychlor (60–300 mg/kg/day) and estradiol

benzoate (0.015–0.075 mg/kg/day) resulted in decreased mating frequency and ovarian weight, increased uterine weight, and precocious vaginal opening, whereas only methoxychlor caused increased estrus (Harris et al. 1974). In adult ovariectomized female rats in which endometriosis had been surgically induced, administration of 250 mg/kg/day methoxychlor for 3 weeks resulted in maintenance of endometriotic sites, similar to that seen following administration of estrone (1 µg/rat), and also similar to intact rats (endogenous estrogen present) in which endometriosis had been induced (Cummings and Metcalf 1995b). Ovariectomized vehicle control rats (no endogenous or exogenous estrogen present) showed a substantial regression in size of endometriotic sites. These data indicate that estrogens maintain, or prevent regression of, endometriotic sites, and that sufficient levels of methoxychlor produce results similar to those of estrogen.

Oral exposure to methoxychlor adversely affects reproductive function in mature females by interfering with estrus cyclicity and decreasing fertility. Female rats exposed to 500, but not 100, mg/kg/day methoxychlor for 28–31 days had significantly fewer estrus cycles and remained in estrus longer than controls (Okazaki et al. 2001). Rats that received 100 mg/kg/day methoxychlor showed a nonsignificant decrease in the number of estrus cycles (Okazaki et al. 2001). Persistent vaginal cornification (sometimes referred to as persistent estrus) was observed in female mice acutely exposed to 25 mg/kg/day (Martinez and Swartz 1991) and in female rats exposed to 300–400 mg/kg/day for intermediate durations (Gray et al. 1988; Harris et al. 1974). Female pigs exposed to 1,000 mg/kg/day for 24 weeks failed to come into estrus during the exposure period (Tegeris et al. 1966). Female mice exposed to a wide range of doses of methoxychlor (0.02, 0.2, 2, 20, or 100 mg/kg/day) on gestational days 11–17 showed no decrease in number of pups/litter, and in fact showed an increase at 0.2 mg/kg/day (Palanza et al. 2001). The study authors interpreted the non-monotonic response as an overall lack of effect on number of pups/litter. Female rats exposed to 100 mg/kg/day methoxychlor beginning on postpartum day 21 showed a 40% decrease in fertility and live pups/litter when mated with untreated males and an 80% decrease when mated with similarly treated males (Gray et al. 1989). Higher doses (200 mg/kg/day) produced infertility in 100% of the animals. Females that did not become pregnant had no implantation sites indicating that the effect occurs prior to implantation. Significant decreases in fertility or complete infertility occurred in female rats following intermediate-duration exposure to 100 mg/kg/day (Bal 1984) or to 50 or 150 mg/kg/day (Chapin et al. 1997). With chronic-duration exposure, decreases in fertility were noted in the second and third generation male and female rats exposed to 79 and 92 mg/kg/day methoxychlor, respectively, for three generations (Haskell Laboratories 1966); no effects were seen in rats exposed to 18 mg/kg/day for 3 generations. These decreases were predominantly attributed to effects on female animals rather than male animals. No effects on fertility were noted in rats similarly treated with

16 (males) or 18 (females) mg/kg/day methoxychlor. In female rats, a decreased mating frequency and a decreased fertility in those that mated were noted following intermediate-duration exposure to 60–150 mg/kg/day (Harris et al. 1974). An increased number of resorptions have been consistently reported in female rats following acute- and intermediate-duration exposure to 35.5–200 mg/kg/day laboratory grade and technical grade methoxychlor (Culik and Kaplan 1976; Cummings and Gray 1989; Cummings and Perreault 1990; Gray et al. 1989; Harris et al. 1974; Kincaid Enterprises 1986).

The effects of methoxychlor on uterine decidualization (the buildup of the uterine lining necessary for implantation and pregnancy) vary with exposure level. Acute exposures to 100–250 mg/kg/day laboratory grade methoxychlor in female rats reportedly inhibit the decidual growth of the uterus (Cummings and Gray 1987, 1989; Cummings and Laskey 1993; Cummings and Perreault 1990). Further investigation by Cummings (1993) showed that methoxychlor acts as an estrogen to induce uterine decidualization only over a narrow exposure range. The artificially induced decidual cell response in the rodent mimics the implantation of the blastocyst and induces the development of the uterine decidual tissue. This response assay was used by Cummings (1993) to investigate the estrogenicity of methoxychlor and its mechanism of reducing fertility in female rats. Estrogen and progesterone are required to induce the decidualization of the uterus. In ovariectomized female rats (used because no endogenous estrogen or progesterone is present and pseudopregnancy can be initiated with exogenous estrone and progesterone administration), exposure to low doses (5 or 50 mg/kg/day) of laboratory grade methoxychlor plus 2 mg progesterone for 8 days produced the same degree of decidualization as in controls (progesterone alone). Higher doses (75 or 100 mg/kg/day) of methoxychlor plus progesterone produced maximal decidualization similar to estrone (0.004 mg/kg/day) plus progesterone. Decidualization response decreased with further increased methoxychlor dose (500 mg/kg/day) plus progesterone (Cummings 1993). Such a decrease in decidualization might contribute to preimplantation loss. Methoxychlor also has been shown to accelerate embryo transport into the uterus, which may further contribute to increases in preimplantation loss (Cummings and Laws 2000; Cummings and Perreault 1990). These findings provide insight into the possible mechanisms by which methoxychlor reduces fertility in female rats.

Exposure to methoxychlor produces changes in a number of hormone levels in female animals after oral exposure. Decreased serum progesterone levels were observed in female rats acutely exposed to 50–500 mg/kg/day laboratory grade methoxychlor, but not to 25 mg/kg/day (Cummings and Gray 1989; Cummings and Laskey 1993). Serum estradiol levels were not altered (Cummings and Laskey 1993). When ovaries from methoxychlor-treated rats were removed and incubated with human chorionic

gonadotropin (to stimulate steriod production and release), ovarian progesterone concentration was unaffected, but ovarian estradiol and testosterone concentrations were significantly reduced (Cummings and Laskey 1993). Pituitary levels of prolactin were decreased in intact female rats but increased in ovariectomized rats exposed to 400 mg/kg/day for intermediate-durations (Gray et al. 1988). In female rats dosed with 100 mg/kg/day methoxychlor in oil by gavage for 4 weeks, a number of ultrastructural changes were noted in the ovarian cells, including the accumulation of lipid (Martinez and Swartz 1992). Similar effects were seen in rats exposed to 1 mg/kg/day estradiol-17β. The authors suggested that these cells retained their ability to synthesize lipids but have lost the ability to convert lipids to steroid hormones. The changes in hormone levels discussed above may also be an important mechanism by which methoxychlor can affect female reproduction. Martinez and Swartz (1992) speculated that methoxychlor causes a feedback inhibition of pituitary hormone secretions resulting in a lack of stimulation of ovarian cells to produce hormones that would have otherwise exerted an effect on the uterus and other estrogen-sensitive tissues.

An intermediate-duration oral MRL of 0.005 mg/kg/day was derived based on the LOAEL of 5 mg/kg/day for accelerated onset of puberty (i.e., precocious vaginal opening) in immature female rats exposed to methoxychlor *in utero*, during lactation, and after weaning (Chapin et al. 1997), as described in the footnote in Table 3-1.

Reproductive Effects in Male Animals. Methoxychlor adversely affects the development of the male reproductive system. Preputial separation was significantly delayed in a dose-dependent manner (days 40–42 in controls, day 43.8 in the 100 mg/kg/day methoxychlor group, days 50–53 in the 200 mg/kg/day, day 68 in the 300 mg/kg/day group, and day 74 in the 400 mg/kg/day group) in male rats exposed to 100–400 mg/kg/day beginning on postpartum day 21 for 56 days to 10 months (Gray et al. 1989, 1999), suggesting that sexual maturity was delayed. Male fertility in rats exposed to methoxychlor from gestation day 14 to postpartum day 42 was significantly impaired at 150 mg/kg/day, but not at 5 or 50 mg/kg/day; only 2/15 150 mg/kg/day rats successfully impregnanted untreated females, compared with 13/15 in the control group (Chapin et al. 1997). Gray et al. (1989) also observed a slight decrease in testes weight and caudal epididymal sperm count in male offspring of rats exposed to 50–200 mg/kg/day methoxychlor for 59 days. Welshons et al. (1999) observed a substantial increase in prostate weight in adult male mice exposed *in utero* (gestation days 11–17) to 0.02 and 2.0 mg/kg/day, and seminal vesicle weight was increased in a dose-dependent manner. Preputial gland, testes, and adrenal gland weights were not significantly affected. Lateral, but not ventral, prostate weight was increased by 44%, and inflammation was evident in male rats exposed to 50 mg/kg/day methoxychlor from gestation

day 18 through postpartum day 5 (Stoker et al. 1999). In a simultaneous experiment, lateral prostate inflammation was also seen in male rats exposed to estradiol-17β from gestation day 18 through postpartum day 5 (dams received 134 µg on gestation day 18–22, then pups received 6.7 µg on postpartum days 1–5), but lateral prostate weight was decreased by 59% (Stoker et al. 1999). Testis weight was not affected. Mating frequency and fertility in rats were decreased in male offspring of rats exposed to 60 mg/kg/day methoxychlor (Harris et al. 1974). In addition to structural and functional effects, urine-marking behavior and infanticide behavior toward unrelated pups were altered in male offspring of mice exposed to 0.02–91 mg/kg/day methoxychlor *in utero* (Parmigiani et al. 1998; vom Saal et al. 1995). These reproductive/developmental effects are discussed in more detail in Section 3.2.2.6 Developmental Effects.

Oral exposure to methoxychlor can produce gross and histopathological changes in the mature male reproductive system. Decreased weight of the testes was observed in male rats and mice exposed to 50–1,400 mg/kg/day for intermediate-durations (Bal 1984; Dikshith et al. 1990; Gray et al. 1989, 1999; Hodge et al. 1950; Tullner and Edgcomb 1962; Wenda-Rozewicka 1983). In addition, a decreased ventral prostate weight was observed in male rats exposed to 154 mg/kg/day for 90 days (Shain et al. 1977) or 100 mg/kg/day for 28 days (Okazaki et al. 2001). Atrophy of the dorso-lateral prostate was also seen in rats following exposure to 500 mg/kg/day for 28 days (Okazaki et al. 2001). Three studies reported a decreased caudal epididymal sperm count in rats exposed to 50–500 mg/kg/day for intermediate-duration exposures (Gray et al. 1989, 1999; Okazaki et al. 2001). No gross or histopathological changes were noted in the testes of male mice acutely exposed to 60 mg/kg/day (Wenda-Rozewicka 1983) or 200–400 mg/kg/day (Gray et al. 1999). A dose-related inhibition of testes development, fewer mature germ cells, and fewer germ cells of each type were observed in the testes of male rats exposed to 5, 50, or 150 mg/kg/day from gestation day 14 through postnatal day 42 (Chapin et al. 1997). Additionally, testis, epididymis, seminal vesicle, and prostate weights were decreased by 23–85% in males exposed to 50 or 150 mg/kg/day (Chapin et al. 1997) or to 100 or 500 mg/kg/day for 28 days (Okazaki et al. 2001). Decreases in prostate weight have also been seen following exogenous estradiol administration (Rajfer and Coffey 1978; Stoker et al. 1999; vom Saal et al. 1997).

Exposure to methoxychlor by the oral route adversely affects reproductive function in mature male animals. Fertility was decreased by 80% when males exposed to 100 mg/kg/day from postpartum day 21 until necropsy (on postpartum days 77–87) were mated with similarly treated females, compared to only a 50% decrease when untreated males were mated with treated females (Gray et al. 1989). Since pseudopregnancies were not observed in females that were mated with methoxychlor-treated males, the

authors concluded that treated males failed to provide sufficient copulatory stimulation to induce changes in the females necessary for pregnancy (Gray et al. 1989). Fertility was decreased by 50–75% when males exposed to 200–400 mg/kg/day methoxychlor from postpartum day 21 until necropsy at 11 months of age were mated with untreated females (Gray et al. 1999). Decreased fertility was also reported in male mice exposed to 60 mg/kg/day for intermediate durations (Wenda-Rozewicka 1983) and in male rats exposed to 150 mg/kg/day from gestation day 14 through postnatal day 42 (Chapin et al. 1997). In addition, mating frequency and fertility in those that mated were significantly reduced in the male rats exposed *in utero*, during lactation, and postweaning to 60 mg/kg/day (Harris et al. 1974) or 200–400 mg/kg/day (Gray et al. 1999). These data collectively indicate that methoxychlor can decrease fertility in male animals and that the reduced fertility in male animals may be due to impaired mating behavior, inadequate stimulation of the female animal, or deficits in other reproductive parameters (decreased sperm count, testicular atrophy).

Methoxychlor also produces changes in hormone levels in male animals after oral exposure. Changes in hormone levels often accompany or precede the reproductive effects discussed above. Exposure to 25–50 mg/kg/day methoxychlor produced increased levels of prolactin, follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH) in the pituitary of male rats (Goldman et al. 1986; Gray et al. 1989; Stoker et al. 1999); serum prolactin levels were non-statistically significantly increased at 200 mg/kg/day and serum FSH levels were not affected (Gray et al. 1989). Prolactin levels in adult male rats were not affected by estradiol administration during gestation (gestation days 18–22) and postpartum days 1–5 (Stoker et al. 1999). The hypothalamus of rats exposed to 50 mg/kg/day developed elevated levels of gonadotropin releasing hormone (GnRH) (Goldman et al. 1986). Serum levels of several hormones (TSH, testosterone, progesterone) were decreased in rats as a result of exposure to doses of 100 mg/kg/day laboratory grade or technical grade methoxychlor (Cummings and Gray 1989; Gray et al. 1989), although serum LH, prolactin, testosterone, and interstitial fluid testosterone were unaffected in male rats exposed to 200–400 mg/kg/day for 44 weeks (Gray et al. 1999). Similarly, reduced levels of testosterone were reported in the interstitial fluid and epididymis of male rats exposed to 100 mg/kg/day from postpartum day 21 to postpartum day 77 (Gray et al. 1989). Effects of methoxychlor on the endocrine system are likely related to some of the histopathological, functional, and behavioral changes described above (see Section 3.5.2, Mechanisms of Toxicity for further discussion).

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

3.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to methoxychlor.

In animals, exposure to methoxychlor during gestation can produce signs of maternal and fetal toxicity. The incidence of offspring with wavy ribs was significantly increased in rats exposed to 40.8 mg/kg/day or more methoxychlor on days 6–15 of gestation. This effect was also noted in rats administered 17.8 mg/kg/day, however, the increase (2–3-fold) was not statistically significant (Culik and Kaplan 1976). Acute doses of 50 mg/kg/day or more caused a decreased body weight in pregnant rats, whereas doses of 200 mg/kg/day produced an increased percentage of dead, resorbed, or anomalous fetuses and decreased fetal weight (Khera et al. 1978). The anomalies also consisted primarily of wavy or extra ribs. The skeletal effects were judged by the authors to be due to delayed development of alkaline phosphatase activity and to arrested calcium deposition in the ribs due to the toxicity of methoxychlor, and were not considered to be true signs of teratogenicity. Fetal body weights were decreased by 10% when pregnant rabbits were administered 35.5 mg/kg/day methoxychlor on days 7–19 of gestation (Kincaid Enterprises 1986). In addition the percentage of offspring which were male was significantly decreased. These effects were not observed in rabbits administered 5.01 mg/kg/day methoxychlor (Kincaid Enterprises 1986).

Methoxychlor can adversely affect the reproductive development of rats and mice exposed *in utero*, during lactation, or after weaning. Effects of postweaning exposure on reproductive development are discussed in Section 3.2.2.5 Reproductive Effects. No effects were detected on reproductive development in male and female rats acutely exposed *in utero* (gestation days 14–20) to 30 mg/kg/day methoxychlor (Gellert and Wilson 1979). Female offspring of rats exposed for intermediate durations to 25 mg/kg/day methoxychlor from weaning through gestation and lactation exhibited precocious vaginal opening, and at 50 mg/kg/day also exhibited abnormal estrus cycling and pituitary abnormalities (Gray et al. 1989). Precocious vaginal opening and delayed prepuce separation were also seen in female and male offspring, respectively, of rats exposed to 50 or 150 mg/kg/day methoxychlor from gestation day 14 through postnatal day 7, followed by direct exposure of the pups through postnatal day 42 (Chapin et al. 1997). Male offspring had slightly decreased testes weight and caudal epididymal sperm count compared to controls, but these changes were not statistically significant (Gray et al. 1989).

A substantial and statistically significant increase in prostate weight (61% at 0.02 mg/kg/day and 51% at 2.0 mg/kg/day) was seen in adult (9.5 months of age) male offspring of CF-1 mice exposed to 0, 0.02, and

2.0 mg/kg/day methoxychlor on gestation days 11–17 (Welshons et al. 1999), and seminal vesicle weight was increased by 20% in the 2.0 mg/kg/day group. Although no positive control was included in this study, the methoxychlor-induced prostate enlargement was greater than the enlargement observed in previous studies by the same investigators examining effects from gestational exposure to other estrogen receptor ligands (estradiol or DES) (vom Saal et al. 1997). There were no statistically significant exposure-related changes in body weight or weights of preputial glands, testes, or adrenals. These findings are in agreement with studies showing increased prostate weight in adult mice exposed to slightly elevated levels of estrogen perinatally (Nonneman et al. 1992; Timms et al. 1999; vom Saal 1989; vom Saal et al. 1997) and are consistent with the estrogenic activity of some methoxychlor metabolites. Only a small number of mice were assessed (one pup/litter from five to six litters) in this study. A more detailed analysis of Welshons et al. (1999) is presented in the Acute MRL Worksheet in Appendix A of this profile. Additionally, prostate weight in adult mice has been shown to be affected by intrauterine position of the fetus. Male mice that are between two female mice during *in utero* development have about a 35% higher serum level of estradiol due to diffusion from the adjacent females and have larger, heavier adult prostates than male mice that develop between two male mice (Nonneman et al. 1992; Timms et al. 1999; vom Saal 1989). Low levels of exogenous estrogen and estrogenic substances can also result in increased adult prostate weights (Rajfer and Coffey 1978; vom Saal et al. 1997). Higher levels of these same substances result in decreased adult prostate weights (Rajfer and Coffey 1978; vom Saal et al. 1997; Welshons et al. 1999). The exposure-response curve for this effect of estrogens takes on an inverted U-shape. This type of dose-response curve has also been reported for prostate weight following exposure to DES (vom Saal et al. 1997). U-shaped curves are recognized curvilinear dose-response relationships in toxicological and epidemiological studies, especially at low doses in the threshold region of response (Davis and Svendsgaard 1990). Inflammation, and a corresponding weight increase, of the lateral prostate was observed in male offspring of rats exposed to 50 mg/kg/day methoxychlor from day 18 of gestation to postpartum day 5 (Stoker et al. 1999). No difference was noted in body weight, ventral prostate weight, testes weight, testosterone level, mean serum prolactin, or prostate deoxyribonucleic acid (DNA) levels. Reductions in testes, epididymis, seminal vesicle, and ventral prostate weights were seen in male offspring of rats exposed to 50 or 150 mg/kg/day from gestation day 14 through postnatal day 7, followed by direct exposure of the pups through postpartum day 42 (Chapin et al. 1997). Some *in vitro* data also suggest that methoxychlor and its metabolite, bis-hydroxy methoxychlor (HPTE), can alter rat embryonic testis development (Cupp and Skinner 2001).

Breeding of rats (exposed *in utero* and through lactation to 50 mg/kg/day methoxychlor, but not exposed thereafter) resulted in a 30% decrease in fertility and a significant reduction in the number of pups/female

and litters/female (Gray et al. 1989). Treated F1 females mated with treated F1 males had 0, 0, 80, and 100% decreases in fertility in the 25, 50, 100, and 200 mg/kg/day groups, respectively. Treated F1 females mated with untreated F1 males had 50 and 0% fertility, respectively, compared to 83% for controls. The fertility of treated males was not reduced when they were mated with untreated females. These data suggest that the decrements in fertility in the offspring were most likely attributed to effects in the female. Similar results were reported by Harris et al. (1974) in which pregnant female rats were exposed to 60–300 mg/kg/day for 6 weeks prior to mating and throughout gestation and lactation. After weaning, pups were maintained on the same treatment as the dams. The onset of puberty was accelerated in female offspring exposed to 60 mg/kg/day. Both males and females exhibited a decreased mating frequency and a decreased fertility in those animals that mated.

A number of reproductive abnormalities were observed in male and female rats that were exposed to 50 or 150 (but not 5) mg/kg/day from gestation day 14 through postpartum day 42 (Chapin et al. 1997). Female rats in both exposure groups had highly irregular or absent estrus cycles, and there was a severe reduction in the number of females that conceived when mated to nontreated males. In females that did conceive, a severe reduction in the number of implants was seen. This was accompanied by an increased incidence of endometrial squamous metaplasia, endometrial hyperplasia, vaginal cornification (an estrogenic response), vaginal epithelial hyperplasia, underdeveloped mammary tissue, and atrophied ovaries with little or no detectable follicular development and few or no corpora lutea. The serum estrogen:progesterone ratio was elevated, which the authors attributed to a lack of progesterone due to absence of ovulation and corpora lutea formation, and the FSH level was decreased. Males exposed to 50 or 150 mg/kg/day from gestation day 14 through postnatal day 42 also displayed reproductive deficits including significantly decreased ability to impregnate untreated females. Fewer nontreated females had detectable sperm in their vagina when mated with male offspring exposed to 150 mg/kg/day, and sperm motility, epididymal sperm count, and testicular spermatid numbers were reduced in these male rats (Chapin et al. 1997).

The offspring of mice (F1a) exposed to 50 mg/kg/day methoxychlor on days 6–15 of gestation and through lactation had an increased incidence of atretic follicles (Swartz and Corkern 1992). In addition, a second (unexposed) litter (F1b) produced following weaning of the F1a offspring exhibited precocious vaginal opening. It is unclear why the F1a exposed litter did not experience precocious vaginal opening and by what mechanism this effect was produced in the unexposed F1b offspring.

Effects on neurobehavioral development were also observed following exposure of rats to 5, 50, or 150 mg/kg/day from gestation day 14 through postnatal 21 (Chapin et al. 1997). Male rats treated with 150 mg/kg/day showed an increased handling reactivity. Non-dose-related but statistically significant changes were seen for sensorimotor parameters: locomotor activity (increased in females in the 50 mg/kg/day group), click response (decreased in males and females at 5 mg/kg/day and males at 150 mg/kg/day), and approach stimulus (lower in males at 5 mg/kg/day). Temporary but statistically significant changes that occurred at various times during the study included increased urination in the open field in females at 50 and 150 mg/kg/day, increased defecation in females at 150 mg/kg/day, and decreased hindlimb grip strength in females at 50 mg/kg/day. There were no differences between the controls and treatment groups in the passive avoidance/cognitive function tests. Male and female rats exposed *in utero*, via lactation, and then directly in the feed to 98, but not 9.8, mg/kg/day had increased intake of a sodium solution (an estrogen-responsive behavior) on postpartum days 69–75 (Ferguson et al. 2000).

A series of recent studies examined behavioral effects in adult mice after exposure to low levels of methoxychlor or other estrogenic agents during critical *in utero* developmental periods. A dose-dependent increase in urine-marking behavior was observed in adult male offspring of mice exposed to 0.02–91 mg/kg/day methoxychlor on gestation days 11–17 (vom Saal et al. 1995; also reported in Parmigiani et al. 1998). However, only two male pups/litter were tested, and each pup was tested only one time. It was also difficult to assess the accuracy of the measurement of the number of urine marks and to know how reproducible the results are, and there was no indication of what, if any, statistical methods were used to evaluate the results. The low level of exposure in this study was considered by the authors to be more reflective of the physiological levels at which hormonal effects from steroid receptor binding alone occur, without more wide ranging toxic effects caused by high levels of the (estrogenic) chemical. Urine-marking behavior is known to be influenced by testosterone levels and the male mouse's social status, and urine marking also influences the social and reproductive behaviors of other mice, both male and female, that come in contact with deposited urine (Parmigiani et al. 1998). Therefore, changes in urine-marking behavior may disrupt reproductive behaviors. This group of investigators showed that *in utero* exposure to low levels of other estrogenic chemicals, such as diethylstilbestrol (DES) and *o,p*-DDT, also increased the rate of depositing urine marks by mature male mice when they were placed in novel environments (Palanza et al. 1999). An increase in aggressive behavior (infanticide) toward unrelated pups was also observed in adult male offspring of mice treated with 1.8 (but not 0.02, 0.18, 18.2, or 91) mg/kg/day methoxychlor during gestation days 11–17 (Parmigiani et al. 1998). This behavior was not affected by 0.02–182 µg/kg/day DES. The significance of these results were difficult to

determine due to the lack of biological information on the strain of mouse used in the study ("house mouse"), the small number of pups tested per litter (two males and two females), and the lack of dose-response (the effect was only seen at one middle dose). These socio-sexual behaviors (urine-marking and infanticide) in mice have been proposed to be influenced by similar neuroendocrine and genetic mechanisms that may not always exhibit monotonic dose-response relationships (Palanza et al. 1999; Parmigiani et al. 1998).

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

3.2.2.7 Cancer

No studies were located regarding carcinogenic effects in humans after oral exposure to methoxychlor.

Evidence concerning the carcinogenicity of methoxychlor from animal studies is mainly negative, although this is somewhat controversial. The National Cancer Institute (NCI) conducted a study in which mice and rats were fed up to 599 and 107 mg/kg/day methoxychlor, respectively, for 78 weeks. The types and incidences of tumors in methoxychlor-fed animals did not differ significantly from control animals (NCI 1978). NCI concluded that methoxychlor was not carcinogenic to mice or rats under the conditions of this assay. Recrystallized methoxychlor was not carcinogenic to rats when administered at doses of 77 mg/kg/day for 24–27 months, either alone or in combination with other chemicals (aldrin, aramite, DDT, and thiourea) (Deichmann et al. 1967). Similar results were reported by Radomski et al. (1965) with doses of 4 mg/kg/day. Hodge et al. (1952) reported that the tumors observed in methoxychlor-fed rats exposed to up to 80 mg/kg/day for 2 years occurred at a similar frequency as controls. Pituitary tumors were observed in the female rats exposed *in utero* to 50 mg/kg/day (Gray et al. 1989), but data regarding tumor incidence were not provided in this study. Adult female mice heterozygous for the *p53* allele that received a single intraperitoneal injection of N-ethyl-N-nitrosourea (ENU) to induce uterine tumor formation showed no enhancement of endometrial stromal sarcoma or atypical hyperplasia induction, whereas ethinylestradiol enhanced the development of ENU-induced uterine tumors (Mitsumori et al. 2000).

The data from the cancer studies discussed above (Deichmann et al. 1967; Hodge et al. 1952; NCI 1978; Radomski et al. 1965), in addition to data from a number of unpublished studies by the FDA, were

examined and interpreted in a series of reports by Reuber (1979a, 1979b, 1980). Reuber (1979a) re-examined the histological slides from an unpublished FDA study, and reported an increased incidence of liver carcinomas (3/9 females and 3/8 males, compared to none observed in controls) in Osborne-Mendel rats fed 100 mg/kg/day for 2 years. In addition, Reuber reported an incidence of 1/10 and 5/10 for ovarian tumors in female rats fed 5 and 25 mg/kg/day, respectively, for 2 years, although data concerning ovarian tumor incidence in control animals or in animals at higher doses was not provided. The incidence of other tumors was similar in both treated and control animals (Reuber 1979a). In a re-analysis of data from another unpublished FDA study, Reuber (1979b) reported an increased incidence of testicular tumors (27/51) in male Balb/c mice exposed to 97.5 mg/kg/day methoxychlor in the feed for 2 years compared to an incidence of 8/71 in controls. In addition, the tumors observed in treated animals were reported to be larger, less differentiated and more invasive than those observed in control animals (Reuber 1979b). Based on the available data, Reuber concluded that methoxychlor produces liver tumors in mice and rats, and possibly in dogs (Reuber 1980). In addition, he concluded that methoxychlor is carcinogenic to the testes of male mice, bone of female mice, and the ovaries of female rats. However, there is considerable disagreement between Reuber and the original authors in the interpretation of the histopathological data. EPA (1987b) concluded that the differences observed between Reuber's interpretation and those of the original authors may be due in part to the use of inappropriate control data in Reuber's analysis and the difficulty in distinguishing histopathological lesions as regenerative hyperplasia, hyperplastic nodules, benign tumors, and malignant tumors.

Based on a review of all the available data, EPA has classified methoxychlor as a Group D carcinogen, not classifiable as to human carcinogenicity (IRIS 2000). Similarly, IARC (1987) has classified methoxychlor as a Group 3 carcinogen (not classifiable as to its carcinogenicity to humans) and NCI (1978) concluded there was insufficient evidence to classify methoxychlor as a carcinogen.

3.2.3 Dermal Exposure

3.2.3.1 Death

No studies were located regarding death in humans after dermal exposure to methoxychlor. Only two studies were located regarding the lethality of methoxychlor in animals after dermal exposure. One out of three rabbits died after intermediate-duration exposure to 3 mL/kg/day of a 30% solution (900 mg/kg/day) of recrystallized methoxychlor in dimethyl phthalate (Haag et al. 1950). No deaths were observed in animals exposed to lower doses. No decrease in survival occurred in mice dermally exposed to

recrystallized methoxychlor at doses of 10 mg/day, 1 day/week for 2 years (Hodge et al. 1966). LOAEL values for the lethal effects of methoxychlor are recorded in Table 3-2.

Table 3-2 Levels of Significant Exposure to Methoxychlor - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL				Reference Chemical Form
				Less Serious		Serious		
INTERMEDIATE EXPOSURE								
Death								
Rabbit	1x/d 5 d/wk 13 wk					3 mL/kg/day 30%	(1/3 died after 1.4 weeks)	Haag et al. 1950 RC
Systemic								
Rabbit	1x/d 5 d/wk 13 wk	Hepatic	1 mL/kg/day 30%	2 mL/kg/day 30%	(fatty degeneration)			Haag et al. 1950 RC
		Bd Wt	1 mL/kg/day 30%	2 mL/kg/day 30%	(decreased body weight gain)			
CHRONIC EXPOSURE								
Mouse C3H/AnF	1x/d 1 d/wk 104 wk	Dermal	10 mg/day					Hodge et al. 1966 RC
		Bd Wt	10 mg/day					

Bd Wt = body weight; d = day; wk = week(s); x = times(s)

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, renal, endocrine, ocular, or body weight effects in humans or animals after dermal exposure to methoxychlor. Limited data are available on hepatic, dermal/ocular, and other systemic effects in animals. The highest NOAEL values and all LOAEL values from each reliable study for the systemic effects of methoxychlor in each species and duration category are recorded in Table 3-2. These studies are discussed below.

Hepatic Effects. Fatty degenerative changes in liver were observed in one of three rabbits exposed to 1 mL/kg/day of a 30% solution (300 mg/kg/day) of recrystallized methoxychlor in dimethyl phthalate, and in 4 of 6 rabbits administered higher doses (2–3 mL/kg/day; equivalent to 600–900 mg/kg/day), 5 days/week for 13 weeks (Haag et al. 1950). The authors state that no apparent effects from dimethyl phthalate were observed.

Dermal Effects. No gross or histopathological changes were observed in the skin of mice exposed to dermal doses of up to 10 mg/day recrystallized methoxychlor in acetone, 1 day/week for 2 years (Hodge et al. 1966).

3.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans or animals after dermal exposure to methoxychlor.

3.2.3.4 Neurological Effects

No studies were located regarding neurological effects in humans after dermal exposure to methoxychlor.

Neurological effects of methoxychlor in animals after dermal exposure were observed in a single study. Disseminated nodules and petechial hemorrhages were noted in the brains of rabbits exposed dermally to 1 mL/kg/day or more of a 30% solution (300 mg/kg/day) of recrystallized methoxychlor in dimethyl phthalate, 5 days/week for 13 weeks (Haag et al. 1950). Similar effects were not observed in control animals. These lesions were accompanied by foreleg paralysis in two out of six animals exposed to the higher doses (2–3 mL/kg/day). The authors suggested the effects may be manifestations of an underlying

disease that is endemic to rabbits and was somehow potentiated by exposure to methoxychlor. The foreleg paralysis seen in two of the rabbits may have been related to the lesions of the central nervous system.

3.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after dermal exposure to methoxychlor. Although no quantitative data were provided, Tullner (1961) reported marked uterine stimulation in immature female mice dusted with an insecticide containing methoxychlor. Inhalation and oral exposures to the dust were likely to have occurred as well.

3.2.3.6 Developmental Effects

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

3.2.3.7 Cancer

No studies were located regarding carcinogenic effects in humans after dermal exposure to methoxychlor.

Two studies were located that investigated the carcinogenic potential of methoxychlor in animals after dermal exposure. Dermal exposure of mice to 10 mg/day recrystallized methoxychlor, 1 day/week for 2 years did not produce any treatment-related tumors (Hodge et al. 1966). However, in this study, only the skin underwent histopathological examination. Other tissues were only examined grossly. Another study examined the effects of topically applied methoxychlor on skin tumor-promotion in mice (Dwivedi and Tabbert 1994). Application of 300 nmol methoxychlor in 100 µL acetone to the shaved back skin (previously initiated with 7,12-dimethylbenz[*a*]anthracene (DMBA)) of mice twice a week for 20 weeks did not result in papilloma development, while 100% of the mice treated with DMBA followed by 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA, a known tumor promoter) developed tumors (Dwivedi and Tabbert 1994). Methoxychlor also did not induce ornithine decarboxylase (ODC) activity in mouse skin (Dwivedi and Tabbert 1994).

3.3 GENOTOXICITY

Numerous *in vivo* and *in vitro* studies have assessed the genotoxic potential of methoxychlor, and the results of these studies are presented in Tables 3-3 and 3-4, respectively.

The genotoxicity of methoxychlor has been well studied *in vitro* and to a lesser extent *in vivo*. Methoxychlor does not appear to be genotoxic in prokaryotic test systems. Negative results were obtained in mutagenicity tests using many different strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538, G46, C3076, and D3052), with or without metabolic activation by rat liver microsomal fractions (Grant et al.1976; Mortelmans et al. 1986; Probst et al. 1981; Waters et al. 1980). Negative results were also reported for tests of differential toxicity in DNA repair-proficient and repair-deficient strains of *Bacillus subtilis* or *Escherichia coli* and for mitotic recombination in *Saccharomyces cerevisiae* (Probst et al. 1981; Waters et al. 1980). When selected contaminants of methoxychlor were tested for their mutagenic potential, only 3,6,11,14-tetramethoxydibenzo(g,p)chrysene produced an increased mutation frequency (Grant et al. 1976), however, most of the known contaminants of methoxychlor were not tested in this study. This compound is a relatively minor contaminant of technical methoxychlor comprising only 0.0005% (5 ppm) by weight (Mitchell et al. 1978).

Mixed results have been obtained in genotoxicity tests using mammalian cells *in vitro*. Methoxychlor did not induce unscheduled DNA synthesis in human lung fibroblasts (Waters et al. 1980) or rat hepatocytes (Probst et al. 1981). In cultured bovine uterine epithelial and stromal cells, DNA synthesis (thymidine incorporation) was inhibited at high methoxychlor concentrations and stimulated at low methoxychlor concentrations (Tiemann et al. 1996). Single-stranded DNA breaks were not induced in human or rat testicular cells by methoxychlor (Bjørge et al. 1996b). Methoxychlor did not produce mutations at the thymidine kinase (TK) locus in human lymphoma cells, with or without metabolic activation (Caspary et al. 1988). However, in mouse lymphoma cells, metabolically activated methoxychlor induced increases in mutation frequencies at the TK locus (Caspary et al. 1988; Mitchell et al. 1988; Myhr and Caspary 1988). Mixed results were reported on ability of methoxychlor to induce neoplastic transformations. A dose-dependent increase in neoplastic transformations were reported in cultured mouse fibroblasts (Dunkel et al. 1981), but not in Syrian hamster embryo cells or virally infected rat embryo cells (Dunkel et al. 1981; Pienta 1980). The reason for differing results in mouse lymphoma cells compared to human lymphoma cells, and in mouse fibroblasts compared to Syrian hamster embryo cells and rat embryo cells is uncertain, but may represent important species differences. In the absence of

Table 3-3. Genotoxicity of Methoxychlor *In Vivo*

Species (test system)	End point	Results	Reference
Mammalian cells:			
Mouse hepatic cells	Single-stranded DNA breaks	–	Umegaki et al. 1993
Mouse bone marrow cells	Chromosome aberration	–	Degraeve and Chollet 1984
Mouse sperm cells	Chromosome aberration	–	Degraeve and Chollet 1984
Nonmammalian cells:			
Drosophila melanogaster	Sex-linked recessive lethal mutation	–	Waters et al. 1980
D. melanogaster	Sex-linked recessive lethal mutation	–	Benes and Sram 1969
D. melanogaster	Sex-linked recessive lethal mutation	–	Valencia 1981

– = negative result; + = positive result

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

Species test system)	End point	With activation	Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium	Reverse mutation	–	–	Mortelmans et al. 1986
S. typhimurium	Reverse mutation	–	–	Grant et al. 1976
S. typhimurium	Reverse mutation	–	–	Waters et al. 1980
S. typhimurium	Reverse mutation	–	–	Probst et al. 1981
Escherichia coli	Reverse mutation	–	–	Waters et al. 1980
E. coli	Reverse mutation	–	–	Probst et al. 1981
E. coli	Differential toxicity[a]	–	No data	Waters et al. 1980
Bacillus subtilis	Differential toxicity[a]	–	No data	Waters et al. 1980
Eukaryotic organisms:				
Saccharomyces cerevisiae	Mitotic recombination	–	–	Waters et al. 1980
Mammalian cells:				
Human testicular cells	Single-stranded DNA breaks	No data	–	Bjørge et al. 1996b
Human lung fibroblasts	Unscheduled DNA synthesis	–	–	Waters et al. 1980
Human lymphoma cells	Mutation of TK locus	–	–	Caspary et al. 1988
Mouse lymphoma cells	Mutation at TK locus	+	–	Caspary et al. 1988
Mouse lymphoma cells	Mutation at TK locus	+	–	Myhr and Caspary 1988
Mouse lymphoma cells	Mutation at TK locus	+	–	Mitchell et al. 1988
Chinese hamster cells	Inhibition of metabolic cooperation	No data	+	Kurata et al. 1982
Syrian hamster embryo cells	Neoplastic transformation	–	–	Pienta 1980
Syrian hamster embryo cells	Neoplastic transformation	No data	–	Dunkel et al. 1981

Table 3-4. Genotoxicity of Methoxychlor *In Vitro (continued)*

Species (test system)	End point	With activation	Without activation	Reference
Mouse fibroblasts	Neoplastic transformation	No data	+	Dunkel et al. 1981
Rat embryo cells (virus-infected)	Neoplastic transformation	No data	–	Dunkel et al. 1981
Rat hepatocytes	Unscheduled DNA synthesis	No data	–	Probst et al. 1981

[a] Comparison between DNA repair proficient and repair deficient strains
– = negative result; + = positive result

a metabolic activation system, methoxychlor inhibited metabolic cooperation in Chinese hamster cells, an activity possibly associated with tumor promoters (Kurata et al. 1982). Exposure to methoxychlor did not induce morphological transformation in Syrian hamster embryo cells (Pienta 1980).

In vivo genotoxicity studies generally yielded negative results (Table 3-3). In mice intraperitoneally injected with 10 mg/kg methoxychlor, the frequency of chromosomal aberrations was not increased in bone marrow cells or sperm cells (Degraeve and Chollet 1984). Single-stranded DNA breaks were not increased in hepatic cells of mice injected intraperitoneally with 170 mg/kg/day for 5 days (Umegaki et al. 1993). No increased frequency of sex-linked recessive lethal mutations were noted in *Drosophila melanogaster* exposed to methoxychlor (Benes and Sram 1969; Valencia 1981; Waters et al. 1980).

No studies were located regarding genotoxic effects in humans or animals after inhalation, oral, or dermal exposure to methoxychlor.

3.4 TOXICOKINETICS

No data were located concerning the toxicokinetics of methoxychlor in humans following any route of exposure, or in animals following inhalation exposure. Studies in animals indicate that methoxychlor is well absorbed by the gastrointestinal tract and to a lesser extent by the skin. However, some of the data from animal studies come from ruminant animals, which may have limited relevance to humans and other nonruminant species. Once in the bloodstream, methoxychlor appears to distribute to most tissues of the body, with highest levels usually found in fat. Methoxychlor is metabolized rapidly by the liver and neither the parent compound nor the metabolites tend to accumulate in fat or other tissue. The metabolism of methoxychlor has been fairly well studied *in vitro* and *in vivo* in animals and with human liver microsomes. Both sets of data indicate that methoxychlor undergoes demethylation to form phenolic derivatives, with dechlorination and dehydrochlorination reactions occurring to a lesser extent. Most of the ingested dose of methoxychlor is eliminated in the feces via biliary excretion of metabolites. Urinary excretion contributes to a lesser extent (approximately 10% of the total administered dose as indicated in mouse studies). The toxicokinetics of methoxychlor in humans is expected to be similar to the toxicokinetics of methoxychlor observed in animals.

3.4.1 Absorption

No quantitative studies were located regarding absorption of methoxychlor in humans after exposure by any route. There is no information regarding age-related differences in rate or extent of absorption of methoxychlor in animals or humans after exposure by any route. Thus, it is unknown whether absorption of methoxychlor by children differs from that by adults.

3.4.1.1 Inhalation Exposure

No studies were located regarding absorption of methoxychlor in animals after inhalation exposure.

3.4.1.2 Oral Exposure

Observations of adverse health effects in animals following oral exposure provide indirect evidence that ingested methoxychlor is absorbed by the gastrointestinal tract (see Section 3.2.2 Health Effects: Oral Exposure). Data from studies examining fecal and urinary excretion of radioactivity after oral administration of radiolabeled methoxychlor to mice and goats indicate that methoxychlor is rapidly and efficiently absorbed by the gastrointestinal tract.

In mice administered single doses of 50 mg/kg recrystallized, radiolabeled methoxychlor in oil by gavage, 90% of the dose was excreted as metabolites in the feces (85% polar metabolites) and 10% was excreted in urine (63.8% polar metabolites) within 48 hours (Kapoor et al. 1970). Assuming that the fecal metabolites (primarily demethylated, dechlorinated, and dehydrochlorinated compounds) resulted from biliary excretion of absorbed material and not from degradation of unabsorbed parent by enteric bacteria, gastrointestinal absorption of methoxychlor appears to exceed 90% in mice.

In two lactating female goats administered single oral doses of 3.6 or 11.6 mg/kg laboratory grade, radiolabeled methoxychlor encapsulated in gelatin, 40.5 and 67.5% of the doses were excreted in the feces within 3 days, respectively, and 58.4 and 27.1% were excreted in the urine, respectively (Davison et al. 1982). Metabolites (demethylated, dechlorinated, and dehydrochlorinated products of methoxychlor) were estimated to represent 70 and 81% of radioactivity in the feces, respectively. Assuming that metabolites in feces resulted from biliary excretion of absorbed material (and that the remaining radioactivity in feces was not absorbed), the data indicate that at least 87 and 82% of the respective administered doses were absorbed. In a bile-cannulated, castrated male goat given an oral dose

of 25.6 mg/kg radiolabeled methoxychlor, 7.8, 35.2, and 44.4% of the radioactivity was excreted within 3 days in the bile, feces, and urine, respectively (Davison et al. 1983). The profile of metabolites in the collected bile was reported to be similar to the metabolite profile in the feces collected from the female goats, but Davison et al. (1983) did not report any chemical analysis of the feces collected from the bile-cannulated goat. The data provide support that methoxychlor is rapidly and efficiently absorbed by the mammalian gastrointestinal tract. However, interpretation of the goat studies is limited, because only one animal was tested per dose, bile cannulation may have influenced absorption, and ruminant toxicokinetic properties are not always relevant to nonruminant mammals.

3.4.1.3 Dermal Exposure

Two studies in animals suggest dermal absorption of methoxychlor may range from 5 to 20%. In one study, a single dermal dose of 200 mg laboratory grade methoxychlor in dichloromethane was applied to the shaved backs of two goats. Three days later, 5–8% of the dose was recovered in the carcass, urine, and feces (Davison et al. 1983). In the second study, four cows were dermally exposed to a single dose of 5 g methoxychlor in an emulsion (Skaare et al. 1982). The levels of methoxychlor detected in milk were comparable to the levels in milk from cows given an intravenous dose of 1 g methoxychlor. The authors concluded that approximately 20% of the dermal dose was absorbed. Because of differences in skin, dermal absorption by goats and cows may not be a good model for dermal absorption by humans.

3.4.2 Distribution

There are no studies regarding distribution of methoxychlor in humans after exposure by any route, nor any studies examining possible age-related differences in distribution of methoxychlor in animals or humans after exposure by any route. Thus, it is unknown whether distribution of methoxychlor and metabolites in children differs from that in adults.

There are no studies directly examining whether methoxychlor and metabolites cross the placenta in humans or animals. Subtle effects on the development of reproductive organs have been observed in male and female offspring of rodents exposed to oral doses of methoxychlor during gestation (Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974); however, it should be noted that exposure was not exclusively during gestation in any of these studies. It is unclear if these effects result directly from methoxychlor and metabolites passing through the placenta or indirectly from a disturbance of maternal physiological processes.

3.4.2.1 Inhalation Exposure

No studies were located regarding distribution in animals after inhalation exposure to methoxychlor.

3.4.2.2 Oral Exposure

In animals, methoxychlor preferentially distributes to the fat but does not appear to persist. Furthermore, metabolic adaptation may enhance elimination. In rats exposed to 1.25, 5, or 25 mg/kg/day methoxychlor for 4–18 weeks, fat levels of methoxychlor were reported to be nondetectable, 1–7 mg/kg, and 14–36 mg/kg, respectively (Kunze et al. 1950). Levels of methoxychlor in fat peaked during the first 9 weeks of exposure, after which time a gradual decline was noted during the last 9 weeks of exposure (Kunze et al. 1950). Methoxychlor was not detected in the fat following the 2-week recovery period. In female rats exposed to 50, 125, or 250 mg/kg/day in the feed for 6 weeks, the levels of methoxychlor detected in abdominal fat were 21, 68, and 61 mg/kg, respectively (Harris et al. 1974). The levels of methoxychlor in fat were 34 and 140 mg/kg in female rats when exposure to 50 and 125 mg/kg/day was continued through pregnancy and weaning (exposure duration=12 weeks) (Harris et al. 1974). The authors speculated that the higher levels of methoxychlor in fat of female rats after pregnancy and weaning was due to a lower fat content in these animals. However, exposure duration (12 vs. 6 weeks) may have been a factor as well. Levels of methoxychlor in the fat of sheep exposed to 6 and 49 mg/kg/day for 18 weeks peaked at 7.8 mg/kg by week 10, and at 24 mg/kg by week 6, respectively (Reynolds et al. 1976). A steady decline of methoxychlor in fat was noted after week 10 in the low dose group, suggesting that metabolic adaption enhanced elimination (Reynolds et al. 1976). Methoxychlor was not detected in fat from either group following a 12–14-week recovery period. In dogs administered 20 or 100 mg/kg/day methoxychlor for 1 year, the levels of methoxychlor in the fat were 8.9 and 85 mg/kg, respectively (Hodge et al. 1952). The levels of methoxychlor in the fat of rats exposed to 1.25, 10, or 80 mg/kg/day for 2 years were 3.7, 2.3–6.8, and 11–22.7 mg/kg, respectively (Hodge et al. 1952). In rats receiving 80 mg/kg/day, the highest levels of methoxychlor detected in the kidneys, liver, and brain were less than 4.2, 0.5, and 0.2 mg/kg, respectively (Hodge et al. 1952).

Three days after oral administration of 3.6 or 11.6 mg/kg radiolabeled methoxychlor to lactating goats, radioactivity was detected in adrenals, brain, gall bladder, heart, kidneys, and liver (Davison et al. 1982), thus indicating that absorbed methoxychlor and metabolites are widely distributed by the blood. However, the radioactivity in these tissues represented <1% of the administered dose. These observations

are consistent with other information indicating that methoxychlor is rapidly metabolized and excreted from the body.

Methoxychlor and/or methoxychlor metabolites have been detected in milk following oral exposure of animals to methoxychlor during lactation. Accumulation in milk and/or elimination through lactation do not appear to be predominant dispositional fates for methoxychlor, but transfer of methoxychlor and methoxychlor metabolites to suckling rat pups via milk has been demonstrated. In lactating female goats given oral doses of 3.6 or 11.6 mg/kg radiolabeled methoxychlor, radioactivity in milk collected for 3 days was below limits of detection for one goat and represented only 0.065% of the dose given to the other goat (Davison et al. 1982). In female rats fed 5, 50, or 150 mg/kg/day methoxychlor during late gestation and early lactation, milk levels of methoxychlor at postnatal day 7 were 25, 128, and 221% of plasma methoxychlor levels, respectively (Chapin et al. 1997). Mono-hydroxy methoxychlor and bis-hydroxy methoxychlor, two major metabolites of methoxychlor, showed similar patterns of concentration in the milk with increasing methoxychlor exposure level. The data suggest that methoxychlor and metabolites concentrate in milk, relative to maternal plasma levels, after intermediate-duration dose levels $50 mg/kg/day. Mean plasma levels of methoxychlor in suckling pups were <5 (below detection limit), 12.4, 37.8, and 59.9 ng/mL in the 0-, 5-, 50-, and 150-mg/kg/day groups, respectively (Chapin et al. 1997). Mono-hydroxy methoxychlor was detected only in 150-mg/kg/day pup plasma (6.2 ng/mL), and bis-hydroxy methoxychlor was detected in the 50- and 150-mg/kg/day pup plasma (6.4 and 11.5 mg/kg/day). Pup plasma was not drawn for analysis until 27–30 hours after dams received the last dose; therefore, measured methoxychlor and metabolites may not have been indicative of peak body burden.

No studies were located that examined whether preconceptional or pregestational exposure of females to methoxychlor would result in exposure to the developing embryo/fetus or neonate. The evidence that methoxychlor is rapidly metabolized and eliminated from the body (e.g., all radioactivity from radiolabeled oral doses of 50 mg/kg methoxychlor was excreted by mice in feces and urine within 48 hours [Kapoor et al. 1970]) suggests, however, that it is unlikely that methoxychlor at low background exposure levels would be stored in maternal tissues and subsequently mobilized during pregnancy or lactation. It is unknown what mechanism produced the results reported by Swartz and Corkern (1992) (discussed in Section 3.2.2.6 Developmental Effects); in this experiment, females from a litter produced after maternal exposure had ceased exhibited precocious puberty, but earlier offspring of these same dams exposed during gestation did not.

3.4.2.3 Dermal Exposure

A single study was located concerning the distribution of methoxychlor in animals after dermal exposure. Three days after two goats were administered a single dermal dose of 200 mg methoxychlor, low levels of methoxychlor (<0.3 mg/kg tissue) were detected in the skin, muscle, liver, fat, and kidneys (Davison et al. 1983). Less than 0.1% of an applied dermal dose of methoxychlor was excreted in the milk of cows after 30 days (Ivey et al. 1983; Skaare et al. 1982). Interpretation of data from these studies is limited since only two goats were tested, and goats and cows may not be good models for dermal exposures in humans.

3.4.2.4 Other Routes of Exposure

Further evidence, albeit indirect, that methoxychlor and/or metabolites can be excreted in milk is provided by observations of morphological changes in the reproductive tract of 15-day-old female offspring of lactating mice given 14 daily intraperitoneal injections of 1, 2, or 5 mg technical-grade methoxychlor in sesame oil (30, 60, or 150 mg/kg/day) on postnatal days 1–14 (Appel and Eroschenko 1992). Exposure-related, statistically significant changes included increased reproductive tract weight, increased thickness of epithelia of vagina and uterine horns, and increased incidence of mucified and/or cornified vagina. These stimulatory effects on development of the female reproductive tract are consistent with the estrogenic activity of methoxychlor metabolites.

The estrogenic activity of methoxychlor administered intraperitoneally has also been demonstrated in adult ovariectomized ND4 Swiss Webster mice (Eroschenko et al. 2000). Three daily intraperitoneal doses of 0.125 mg (4 mg/kg/day) methoxychlor resulted in an increase in uterine epithelial height of approximately the same magnitude as three intraperitoneal doses of 25 ng (0.9 µg/kg/day) of estradiol 17-β. However, the potency of methoxychlor was much less than estradiol (approximately 4,400 times less) and the range of effects elicited by methoxychlor was different from those elicited by estradiol. While estradiol increased reproductive tract weight and uterine luminal fluid albumin content, methoxychlor did not, and in fact appeared to reduce the stimulatory response of sensitive uterine cells to estradiol. The study authors speculated that these differences may be due to the different affinities of methoxychlor for the various forms of ER (ERα and ERβ) compared to estradiol and its possible interaction with other receptors that have not yet been elucidated.

Estrogenic effects have also been observed in female mice following subcutaneous administration. Methoxychlor (10 mg/kg/day) administered for 5 days resulted in a significant increase in uterine weight,

similar to the effects of estradiol-17β (Al-Jamal and Dubin 2000). However, this effect was eliminated by co-administration with raloxifene (a selective estrogen receptor modulator), whereas the uterine effects of estradiol-17β were not. This supports other studies (Eroschenko et al. 2000; Gaido et al. 1999; Ghosh et al. 1999) that have suggested that the estrogenic effects of methoxychlor may be mediated via different interactions with the estrogen receptors or different mechanisms than estradiol-17β. Increased uterine weight was also seen in immature mice administered methoxychlor ($50 mg/kg) or its estrogenic metabolite HPTE ($100 mg/kg) subcutaneously for 1 or 3 days (Newbold et al. 2001). Further examination of the uterine tissue showed increases in uterine epithelial cell height, uterine gland number, cell proliferation, and estrogen-inducible protein production (Newbold et al. 2001).

Methoxychlor administered subcutaneously to adult male rats resulted in a seemingly complicated alteration of serum and hypothalamic hormone levels (Lafuente et al. 2000). Prolactin release from the hypothalamus is affected by circadian rhythm. Methoxychlor exposure resulted in increased median serum prolactin levels and absolute pulse amplitude and in decreased relative pulse amplitude, but did not affect the frequency or duration of prolactin peaks or the half-life of prolactin in serum (Lafuente et al. 2000). Decreases in serum testosterone and luteinizing hormone (LH) were also noted in this study. Testosterone can stimulate prolactin release; therefore, the increase in prolactin does not appear to be mediated by testosterone. The study authors speculated that the alteration in prolactin release may be a consequence of direct effects of methoxychlor on the hypothalamus (Lafuente et al. 2000). Dopamine, which is an inhibitory neuromodulator of prolactin release, was increased in the anterior hypothalamus and decreased in the median eminence, which suggests a decrease in dopamine release; this could explain the increased prolactin release (Lafuente et al. 2000).

3.4.3 Metabolism

Figure 3-2 presents a summary of methoxychlor metabolic pathways. Following Figure 3-2 is a key to alternative chemical names for methoxychlor metabolites. There are no data to suggest that metabolism of methoxychlor is dependent on the way it enters the body, so the metabolism of methoxychlor is discussed below without reference to route of exposure.

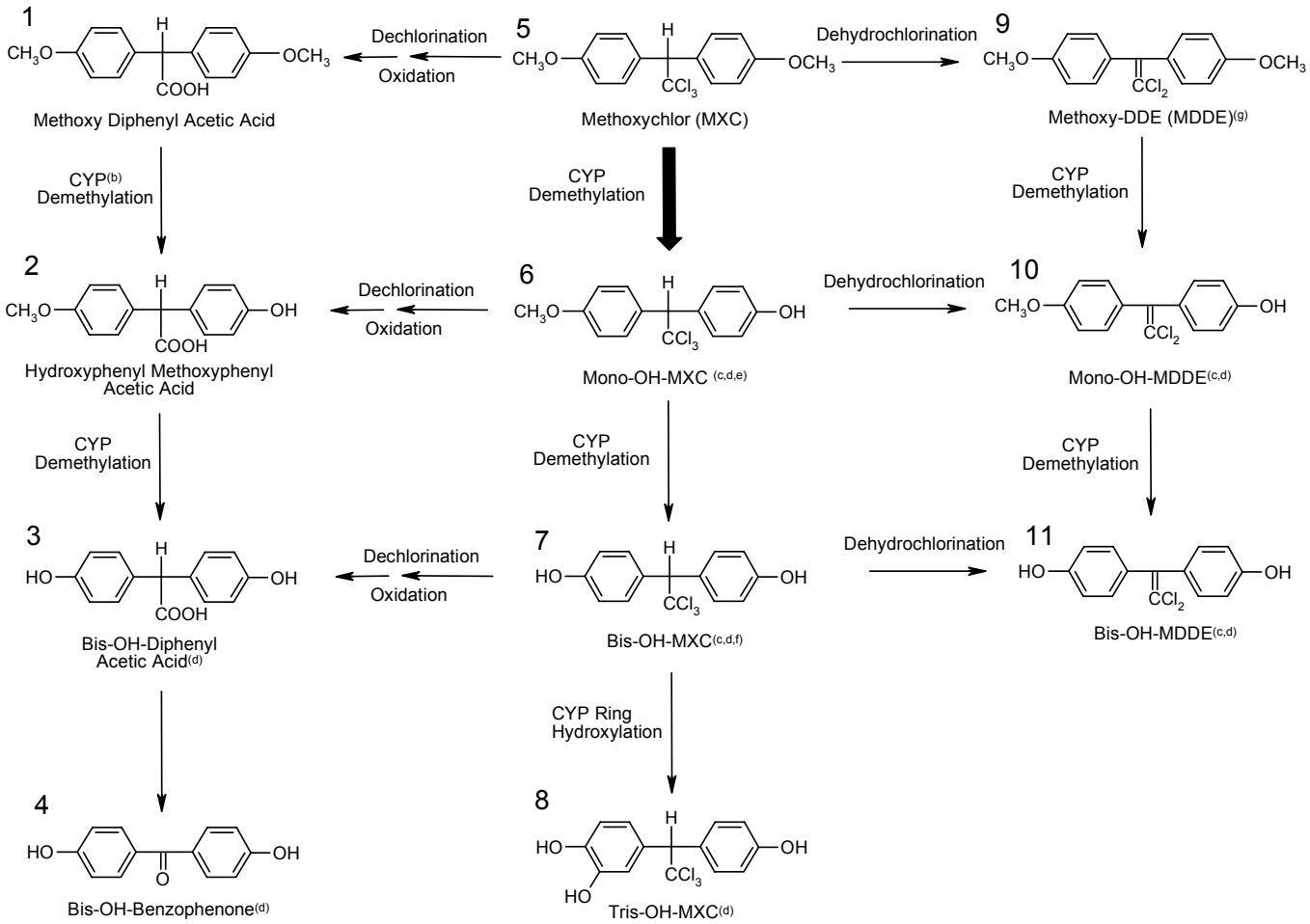
Methoxychlor is metabolized mainly in the liver. In *in vivo* studies in which rats were exposed to carbon tetrachloride to induce liver damage, the metabolism and excretion of methoxychlor were reduced, whereas tissue retention and toxicity were increased (Lehman 1952; Weikel and Laug 1958).

The primary pathway by which methoxychlor is metabolized in the liver is sequential demethylation reactions to yield mono- and bis-hydroxy methoxychlor. Alternative names for these metabolites are 2-(*p*-methoxyphenyl)-2-(*p*-hydroxyphenyl)-1,1,1-trichloroethane and 2,2-bis (*p*-hydroxyphenyl)-1,1,1-trichloroethane (sometimes abbreviated as HPTE). Dechlorinated metabolites such as bis-hydroxydiphenyl acetic acid and bis-hydroxybenzophenone have also been identified (see Figure 3-2).

Methoxychlor and 5 metabolites were identified by thin layer chromatography in urine and feces collected from mice for up to 11 days after administration of single oral doses of 50 mg/kg/day radiolabeled, recrystallized methoxychlor in oil (Kapoor et al. 1970). Mono-hydroxy methoxychlor and bis-hydroxy-methoxychlor, the major metabolites, accounted for approximately 30 and 23% of the administered dose, respectively. Bis-hydroxy-diphenylacetic acid and bis-hydroxy-benzophenone, presumably formed via dechlorination and subsequent oxidation as shown in Figure 3-2, accounted for about 11% of the administered radioactivity. Nonmetabolized methoxychlor and methoxy diphenyl dichloroethylene (bis-OH-MDDE) accounted for about 8 and 1% of the administered radioactivity, respectively (Kapoor et al. 1970). The latter metabolite has been proposed to be formed by dechlorination of bis-hydroxy methoxychlor (i.e., HPTE), or by alternative pathways in which dechlorination preceeds demethylation (see Figure 3-2). About 27% of administered radioactivity was not recovered in the thin layer chromatography procedure used in this analysis.

In goats administered single doses of 3.6–25.6 mg/kg, most of the dose was metabolized to demethylated, dechlorinated, and dehydrochlorinated derivatives of methoxychlor, although a ring hydroxylated species

Figure 3-2. Proposed Metabolic Pathways of Methoxychlor[a]



(a) Adapted from Kapoor et al. 1970; Kupfer and Bulger 1987b; Kupfer et al. 1990
(b) CYP = cytochrome P-450
(c) Estrogenic compound
(d) Metabolite identified in excreta
(e) Mono-OH-MXC = 2-(p-Methoxyphenyl)-2-(p-hydroxyphenyl)-1,1,1-trichloroethane
(f) Bis-OH-MXC = 2,2-Bis(p-hydroxyphenyl)-1,1,1-trichloroethanol (HPTE)
(g) MDDE = Methoxydiphenyldichloroethylene

Figure 3-2 Proposed Metabolic Pathways of Methoxychlor Key to Metabolite Chemical Names

1. Bis(4-methoxyphenyl)acetic acid
   Methoxy Diphenyl Acetic Acid

2. α-(4-hydroxyphenyl)-α-(4-methoxyphenyl)acetic acid
   Hydroxyphenyl Methoxyphenyl acetic acid

3. Bis(4-hydroxyphenyl)acetic acid
   Bis-OH-Diphenyl acetic acid
   CASRN: 40232-93-7

4. 4,4-Dihydroxybenzophenone
   Bis-OH-Benzophenone
   CASRN: 611-99-4

5. 1,1,1-Trichloro-2,2-bis(4-methoxyphenyl)ethane
   Methoxychlor (MXC)
   CASRN: 72-43-5

6. 1,1,1-Trichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethane
   Mono-OH-MXC
   2-(p-Methoxyphenyl)-2-(p-hydroxyphenyl)-1,1,1-trichloroethane

7. 1,1,1-Trichloro-2,2-bis(4-hydroxyphenyl)ethane
   Bis-OH-MXC
   2,2-Bis(p-hydroxyphenyl)-1,1,1-trichloroethanol (HPTE)
   CASRN: 2971-36-0

8. 1,1,1-Trichloro-2-(3,4-dihydroxyphenyl)-2-(4-hydroxyphenyl)ethane
   Tris-OH-MXC

9. 1,1-Dichloro-2,2-bis(4-methoxyphenyl)ethene
   Methoxy-DDE (MDDE)
   Methoxydiphenyldichloroethylene
   CASRN: 2132-70-9

10. 1,1-Dichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethene
    Mono-OH-MDDE

11. 1,1-Dichloro-2,2-bis(4-hydroxyphenyl)ethene
    Bis-OH-MDDE
    CASRN: 14868-03-2

was also identified in the urine as a minor metabolite (Davison et al. 1982, 1983). Most of the urinary metabolites were conjugated with glucuronic acid.

In vitro studies using human and rat liver microsomal preparations confirm the generation of phenolic compounds via demethylation by hepatic cytochrome P450 (CYP) enzymes (Bulger et al. 1978d, 1985; Dehal and Kupfer 1994; Elsby et al. 2001; Kishimoto and Kurihara 1996; Kupfer et al. 1990; Kurihara and Oku 1991; Stresser and Kupfer 1997, 1998; Stresser et al. 1996). Early studies demonstrated that methoxychlor produced type I spectral changes when added to CYP enzymes from rats, mice, rabbits, sheep, and houseflies (Donovan et al. 1978; Kulkarni et al. 1975; Tsujita and Ichikawa 1993), indicative of substrate binding to CYP enzymes.

Several partially purified CYP isozymes from rat liver microsomes can demethylate methoxychlor: CYP2C6 and 2A1 showed lower K_m and V_{max} values than values for CYP2B1 and CYP2B2, indicating that the former have a higher affinity, but lower capacity, for methoxychlor than the latter (Kishimoto et al. 1995); thus, which pair of enzymes is likely to be performing most of methoxychlor metabolism will depend on the amount of methoxychlor available to the liver. Antibodies to CYP2B1 and CYP2C6 were used with rat liver microsomes and purified preparations of CYP2C6 to provide evidence that CYP2C6 and another unidentified CYP isozyme may represent the most important isozymes in the initial demethylation of methoxychlor in rats (Kishimoto and Kurihara 1996).

Repeated exposure to methoxychlor appears to induce hepatic CYP enzymes involved in its metabolism. Intraperitoneal injection of mature female rats with 200 mg/kg/day methoxychlor twice daily for 4 days increased western blot-detected hepatic levels of CYP2B1, 2B2, and 3A proteins by 3-, 2.8-, and 1.6-fold compared with controls, but did not change levels of CYP2E1 (Li et al. 1995). Injection of immature female rats with 300 mg/kg/day for 4 days increased levels of CYP2B1, 2B2, and 3A proteins by 9.0-, 7.8-, and 5.1-fold without inducing levels of CYP2E1 (Li et al. 1995). In these experiments, methoxychlor caused a markedly greater increase in the amount of CYP2B and 3A proteins than in actual enzyme activity. A recent *in vitro* study indicated that induction of the hepatic CYP2B enzyme by methoxychlor and its metabolites may be mediated via the constitutive androstane receptor (CAR), which initiates transcription of CYP2B RNA and results in increased CYP2B enzyme (Blizard et al. 2001).

In vitro studies with human liver microsomes and human recombinant CYP isozymes indicate that multiple CYP isozymes are involved in the demethylation of methoxychlor in humans (Stresser and Kupfer 1998). Incubation of pooled liver microsomes from three human subjects with saturating levels of

methoxychlor (25 μM) and NADPH for up to 125 minutes produced mono-hydroxy methoxychlor, bis-hydroxy methoxychlor, and tris-hydroxymethoxychlor consistent with the central demethylation and ring hydroxylation reactions noted in Figure 3-2 (Stresser and Kupfer 1998). Rates of formation were highest for mono-hydroxy methoxychlor followed by bis-hydroxy methoxychlor and tris-hydroxy methoxychlor. Rates of mono-demethylation at non-saturating concentrations of 1 μM using liver preparations from 14 individuals varied 80-fold, whereas rates from preparations from 26 individuals assayed at saturating concentrations varied 23-fold. Rates of formation of mono- and bis-hydroxy methoxychlor with human liver microsomes were generally lower than rates with rat liver microsomes, but some human liver samples displayed higher rates. At nonsaturating methoxychlor concentrations, demethylation by human microsomes was strongly inhibited by CYP2C19 inhibitors, tranylcypromine (also inhibits CYP2A6), and S-mephenytoin (substrate for CYP2C19). Moderate inhibition was produced by tolbutamide (substrate for CYP2C9), furafylline (inhibitor of CYP1A2), sulfaphenazole (inhibitor of CYP2C9), and coumarin (substrate of CYP2A6), whereas weak inhibition was produced by quinidine (substrate of CYP3A4) and ketoconazole (inhibitor of CYP3A4). The involvement of multiple CYP isozymes was supported by the observation of biphasic enzyme kinetics in Eadie-Hofstee plots of methoxychlor demethylation rates with human liver microsomes. Recombinant CYP2C19 expressed in lymphoblast cells was more active in demethylating methoxychlor than similarly expressed 1A2, and human cDNA-expressed CYP2C19, purified from bacterial lysates, catalyzed methoxychlor demethylation at rates 4-fold higher than rates for CYP2C9 and CYP2C18. Stresser and Kupfer (1998) proposed that CYP2C19 and CYP1A2 may be the major CYP demethylases for methoxychlor, but noted that other forms, including CYP2A6, CYP2C9, and CYP2B6, are likely to be major contributors, especially in individuals with low levels of CYP2C19 or CYP1A2.

Ring hydroxylation of methoxychlor or of hydroxy-methoxychlor derivatives (see Figure 3-2 for formation of tris-hydroxy methoxychlor from bis-hydroxy methoxychlor) may involve a different set of CYP isozymes than CYP-catalyzed demethylation. Studies using nine human cDNA-expressed CYP isozymes in microsomes from lymphoblastoid cells (CYP1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2D6, 2E1, and 3A4) indicated that significantly increased ring hydroxylation of methoxychlor was catalyzed only by CYP1A2 or CYP2B6 (Stresser and Kupfer 1997). Ring hydroxylation of mono-hydroxy methoxychlor in this system was catalyzed by five isozymes (CYP1A2, 2B6, 2D6, 2E1, and 3A4), with CYP3A4 displaying the highest rates of ring hydroxylation (Stresser and Kupfer 1997). Ring hydroxylation of methoxychlor or bis-hydroxy methoxychlor (ortho to the methoxy or hydroxy moities) was shown to be enhanced in liver microsomes from phenobarbital-induced rats compared with microsomes from non-induced rats and to be markedly inhibited by anti-CYP2B monoclonal antibodies (Stresser et al. 1996).

These data suggest that CYP2B isozymes may be most important in catalyzing ring hydroxylation of methoxychlor and hydroxy-methoxychlor derivatives in rats.

Activation of Methoxychlor to Estrogenic Metabolites. The rapid demethylation of methoxychlor decreases its neurotoxicity and leads to a rapid elimination from the body (Lehman 1952), making it significantly less toxic than its structural analogue, DDT. However, this detoxification pathway also is thought to act as an activation pathway for reproductive and developmental effects. Data from *in vitro* and *in vivo* rat studies indicate that the phenolic metabolites of methoxychlor resulting from demethylation (and contaminants in technical grade and laboratory grade methoxychlor) are responsible for most of the estrogenic activity rather than methoxychlor itself (Bulger et al. 1978b,1978d; Charles et al. 2000; Sumida et al. 2001). Highly purified methoxychlor did not interfere with *in vitro* binding of estradiol-17β to 8S estrogen receptors in rat uterine cytosol, but the demethylated metabolite, 2,2-bis-(*p*-hydroxyphenyl)-1,1,1-trichloroethane (i.e., HPTE), was a potent inhibitor of this binding (Bulger et al. 1978b). In this assay system, technical grade methoxychlor, and even laboratory grade (99% pure), inhibited estradiol binding to cytosolic estrogen receptors, indicating that some contaminants in methoxychlor preparations have estrogenic activity (Bulger et al. 1978b). In ovariectomized rats, intraperitoneal injection of 3 or 10 mg laboratory grade methoxychlor in corn oil significantly increased activities of uterine ornithine decarboxylase activity (ODC, a sensitive marker of estrogenic activity) within 7 hours by 15- or 136-fold, respectively, and significantly increased relative uterine weight by 1.5-fold (i.e., 53% increase), at the higher dose only (Bulger et al. 1978b). Injection of lower doses of HPTE, 0.1 or 0.5 mg per rat, significantly increased ODC activities by 17- or 607-fold, respectively; the 0.5 mg dose level also significantly increased relative uterine weight by 1.3-fold (Bulger et al. 1978b).

Two estrogen receptors, α and β, have recently been identified with overlapping and differential roles in mediating estrogenic responses in mammals. The complexity of the mechanism(s) by which methoxychlor metabolites induce estrogenic responses has also been recently demonstrated by results showing that HPTE acts as an agonist for estrogen receptor α expressed in human hepatoma cells (i.e., similarly to estradiol-17β, it induced gene expression mediated by estrogen receptor α) and acts as an antagonist for estrogen receptor β (i.e., it abolished estradiol-17β-induced gene expression mediated by estrogen receptor β) (Gaido et al. 1999). This was further supported by studies showing differential expression of certain ER-related mRNAs in tissues of mice treated with HPTE or estradiol-17β, depending on ERα and ERβ content of the tissues (Waters et al. 2001).

The dehydrochlorination of HPTE to methoxydiphenyldichloroethylene (bis-OH-MDDE) (see Figure 3-2) also acts as an activation pathway, since bis-OH-MDDE was even more active than HPTE in *in vitro* assays of binding to rat uterine cytosol receptors and in *in vivo* rat assays for induction of uterine ODC activity and increase in relative uterine weight following intraperitoneal exposure (Bulger et al. 1985). Although the enzyme responsible for catalyzing the dehydrochlorination of methoxychlor and its demethylated metabolites has not been studied, it is probably the same glutathione-requiring dehydrochlorinase which catalyzes the conversion of DDT to 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene (DDE) (Hodgson and Levi 1987). Data were not located regarding the estrogenic activity of methoxy diphenylacetic acid and methoxy benzophenone, so it is not known whether dechlorination and oxidation of methoxychlor to these products act as activating or detoxifying pathways.

Age and Metabolism. Although there is no direct information regarding age-related differences in metabolism of methoxychlor in children, multiple CYP enzymes are involved in methoxychlor metabolism. It is likely that CYPs 2C19, 1A2, 2B6, 2C9, 2A6, and perhaps 2D6, 2E1, and 3A4 play a role in phase I human metabolism (Hong et al. 1987; Lacroix et al. 1997; Leeder and Kearns 1997; Mote et al. 1990; Ratenasavanh et al. 1991; Rich et al. 1990; Sonnier and Cresteil 1998; Treluyer et al. 1997; Vieira et al. 1996; Yang et al. 1994). Many of these phase I enzymes are likely to have overlapping roles. Although nothing is known about phase II metabolism in humans, Davison et al. (1982, 1983) found glucuronic acid conjugates of methoxychlor metabolites in goats; these conjugates are a product of glucuronosyltransferase and it is possible that this enzyme may be involved in human methoxychlor metabolism as well. Although it is unknown whether an overall age-related difference in methoxychlor metabolism would be observed *in vivo* in humans, there is some information that the following enzymes may be developmentally regulated: CYP2C19 (Leeder and Kearns 1997), CYP1A2 (Leeder and Kearns 1997; Rich et al. 1990), CYP2C9 (Ratenasavanh et al. 1991; Treuler et al. 1997), CYP2D6 (Leeder and Kearns 1997; Sonnier and Cresteil 1998), CYP2E1 (Hong et al. 1987; Vieira et al. 1996), CYP3A4 (Lacroix et al. 1997; Leeder and Kearns 1997; Mote et al. 1990; Yang et al. 1994), and glucuronosyltransferase (Leeder and Kearns 1997). In contrast, other researchers have found that CYPs 2A6, 2C9, 2D6, 2E1, and 3A are expressed at the same levels in perinatal (37 weeks of gestation) to geriatric human livers (72 years) (Ratenasavanh et al. 1991; Tateishi et al. 1997).

Metabolites and Covalent Adducts. *In vitro* studies indicate that methoxychlor can be metabolized to reactive intermediates capable of forming covalent adducts with cellular proteins. Studies with rat liver microsomes demonstrated that methoxychlor undergoes CYP mediated activation and the resultant reactive metabolites covalently bind to hepatic microsomal proteins (Bulger et al. 1983). Adduct

formation requires the presence of NADPH and oxygen, and is inhibited by metyrapone, SKF-525A, hexobarbital and ethylmorphine, indicating that the activation of methoxychlor is catalyzed by the cytochrome P450 enzyme system. Liver microsomes from rats pretreated with phenobarbital, an inducer of CYP2B1 and 2B2, exhibited enhanced covalent binding to microsomal proteins, and antibodies against phenobarbital-induced CYP isozymes inhibited covalent binding, indicating that the major portion of this activity was facilitated by catalysis by CYP2B1 and/or CYP2B2 (Bulger and Kupfer 1989, 1990). Comparative studies with liver microsomal preparations from humans and rats indicate that the mechanism of methoxychlor covalent modification of microsomal proteins is similar in the two species (Bulger and Kupfer 1989). Free radical scavengers act to inhibit adduct formation without affecting the production of polar (demethylated) metabolites (Bulger et al. 1983). Although the formation of methoxychlor adducts has not been well characterized, these adducts contain intact methoxy groups, suggesting that this reaction occurs independently from demethylation reactions (Bulger and Kupfer 1990). The phenolic metabolites of methoxychlor, as well as the dehydrochlorinated product MDDE and its phenolic derivatives, are also capable of undergoing CYP mediated covalent binding. It was postulated that the formation of a reactive intermediate involves modification of the side chain, possibly through homolytic cleavage of the C-H or C-Cl bond (Bulger and Kupfer 1990). The relevance of protein adduct formation to mechanisms by which methoxychlor produces health effects is uncertain, but may be related to possible inactivation of CYP3A by tris-hydroxy methoxychlor (Li et al. 1993).

Comparative Toxicokinetics. The available data comparing rat and human liver microsomal metabolism of methoxychlor indicate qualitative similarities as well as some indications of quantitative differences. Rat liver microsomes were observed to have a higher capacity than human liver microsomes to metabolize methoxychlor to covalent binding intermediates (Bulger and Kupfer 1990). *In vitro* data also indicate that covalent binding of methoxychlor to human liver microsomes is similar across age and sex, whereas in rats, covalent binding in mature males is much higher than in mature females and immature males and females (Bulger and Kupfer 1989). Another comparison found that *in vitro* rates of demethylation of methoxychlor and mono-hydroxy methoxychlor with human liver microsomes were generally higher in rat liver microsomes than in human liver microsomes (Stresser and Kupfer 1998).

3.4.4 Elimination and Excretion

Direct information regarding possible age-related differences in the rate, extent, or route of elimination of methoxychlor and metabolites was not located.

3.4.4.1 Inhalation Exposure

No studies were located regarding excretion in humans or animals after inhalation exposure to methoxychlor.

3.4.4.2 Oral Exposure

In comparison to DDT (a pesticide which is relatively persistent and slowly eliminated), animal studies indicate that ingested methoxychlor is excreted rapidly, predominantly in the feces and to a lesser extent in the urine. Approximately 90% of an oral dose of 50 mg/kg recrystallized methoxychlor was recovered in the feces of mice within 48 hours, and 10% was excreted in the urine (Kapoor et al. 1970). Only 7–8% of the material in the feces was excreted as the parent compound. In contrast, only 1.02 and 4.3% of the administered radioactivity was recovered in feces and urine collected from mice within 24 hours and 11 days, respectively, of administering single oral doses of 12.5 mg/kg radiolabeled DDT.

Assuming that the fecal metabolites (primarily demethylated, dechlorinated, and dehydrochlorinated compounds) did not result from degradation of unabsorbed parent compound by enteric bacteria, these data suggest that biliary excretion of metabolites contributes significantly to methoxychlor clearance. Supporting these observations are those that methoxychlor metabolites identified in bile collected from a bile-cannulated male goat were similar to those in feces collected from lactating female goats given 3.6 or 11.6 mg/kg methoxychlor (Davison et al. 1982, 1983). In the female goats, 40.5 and 67.5% of administered doses were excreted in the feces within 3 days, respectively, and metabolites accounted for 70 and 81% of radioactivity in the feces, respectively (Davison et al. 1982).

3.4.4.3 Dermal Exposure

No studies were located regarding excretion in humans after dermal exposure to methoxychlor. Three days after applying a single dose of 200 mg laboratory grade methoxychlor to the shaved backs of goats, 0.37–0.91% of the dose was excreted in the feces and 0.53–0.72% of the dose was excreted in the urine (Davison et al. 1983).

3.4.4.4 Other Routes of Exposure

Parenteral exposure studies in animals indicate that biliary excretion of methoxychlor accounts for a significant fraction of fecal excretion. In bile-duct cannulated rats given a single intravenous dose of radiolabeled methoxychlor, the radiolabel was first detected in the bile within 1 minute (Weikel 1957). Fifty percent of the dose was excreted in the bile after 4 hours. The urinary excretion of label in bile-cannulated rats was only 0.1–0.2% compared to 5–10% in noncannulated rats, suggesting that the appearance of urinary metabolites was largely due to material that was reabsorbed in the gut (i.e., enterohepatic circulation).

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

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

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

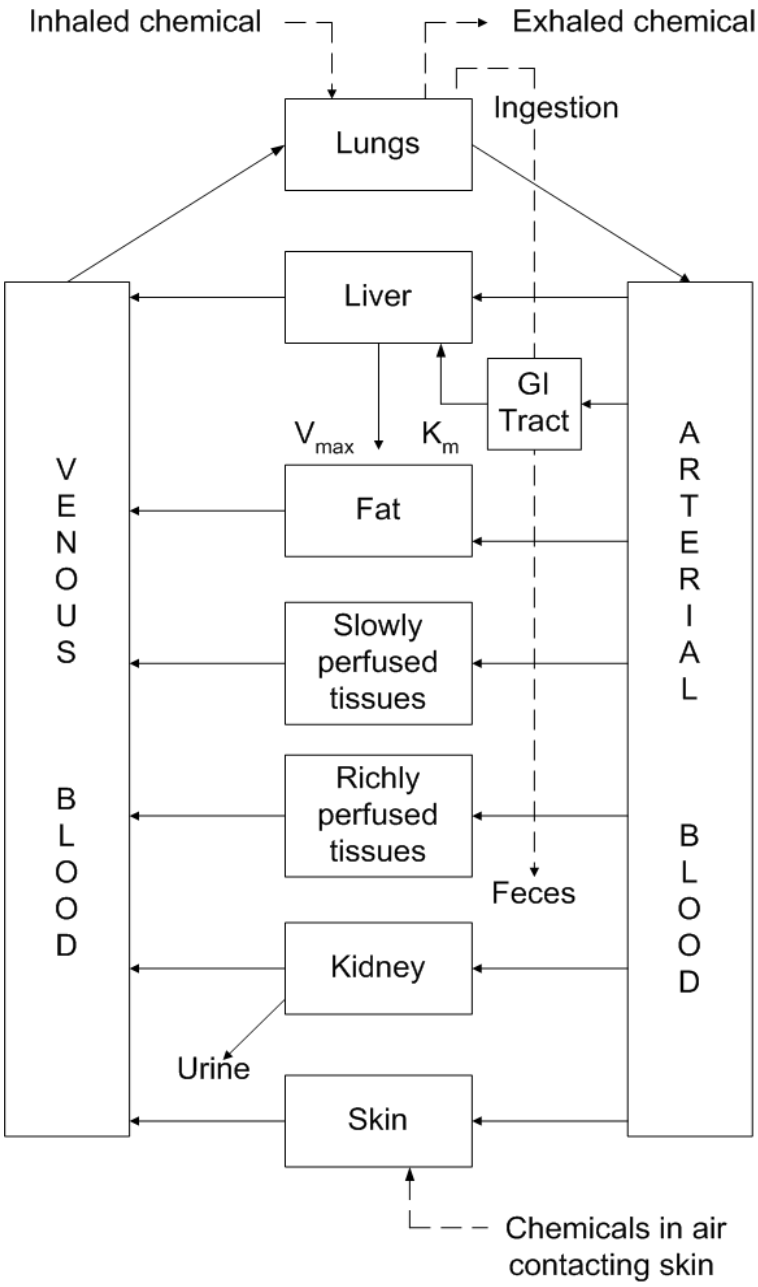
PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in 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 3-3 shows a conceptualized representation of a PBPK model.

No studies were located regarding PBPK models for methoxychlor.

3.5 MECHANISMS OF ACTION

As discussed in Section 3.2, the chief effects of methoxychlor in animals are on the reproductive system. Although reproductive effects have mainly been observed in animals following oral exposures, it is likely that these types of effects could occur following inhalation and dermal exposures as well, if absorption of

Figure 3-3. 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.

equivalent amounts occurred. For this reason, the mechanism of action for methoxychlor is discussed below without reference to route of exposure.

3.5.1 Pharmacokinetic Mechanisms

Absorption. No studies were located regarding the mechanism of absorption of methoxychlor in humans or animals by any route.

Distribution. No studies were located regarding the mechanism of distribution of methoxychlor in humans or animals after exposure by any route.

Metabolism. Methoxychlor is metabolized to estrogenic compounds in animals and humans by hepatic CYP enzymes (Dehal and Kupfer 1994; Kishimoto and Kurihara 1996; Stresser and Kupfer 1997, 1998; Stresser et al. 1996). In rats, *in vivo* and *in vitro* studies have shown that metabolism of methoxychlor to its mono-, bis-, tris-, and ring-hydroxy metabolites is mediated by CYP2C6, 2A1, 2B1, 2B2, and 3A (Kishimoto and Kurihara 1996; Li et al. 1995). The K_m values for partially purified preparations of CYP2C6, 2A1, 2B1, and 2B2 were 0.36, 0.38, 1.07, and 2.34 μM, respectively, and the V_{max} values were 0.40, 0.36, 1.20, and 1.22 mol/mol P450/minute, respectively (Kishimoto et al. 1995). Studies examining inhibition by antibodies to CYP2B1 and CYP2C6 of methoxychlor demethylation in rat liver microsomes and purified preparations of CYP2C6 provided evidence that CYP2C6 and another unidentified CYP isozyme make important contributions in the initial demethylation of methoxychlor in rats (Kishimoto and Kurihara 1996). Ring hydroxylation of methoxychlor and hydroxy methoxychlor derivatives in rats involves CYP2B isozymes, based on observations of inhibition of *in vitro* activity by anti-CYP2B monoclonal antibodies and enhanced ring hydroxylation activity in liver microsomes from phenobarbital-pretreated rats (Stresser et al. 1996).

Results from *in vitro* studies with human liver microsomes (discussed in Section 3.4.3 Metabolism) have led to the proposal that CYP2C19 and CYP1A2 may be the major CYP isozymes catalyzing the demethylation of methoxychlor and its mono-hydroxy derivative, and that other CYP forms, including CYP2A6, 2C9, and 2B6, may make major contributions, especially in individuals with low levels of CYP2C19 or CYP1A2 (Stresser and Kupfer 1998). Nonlinear regression analysis of data from insect cells overexpressing human CYP2C19 or 1A2 showed standard Michaelis-Menten kinetics for a one-enzyme model. The K_m (μM) values for cytochrome CYP2C19 and 1A2 were similar (0.43 and 0.51), but the V_{max} (nmol/minute/nmol P450) for 2C19 (26.8) was twice that for 1A2 (12.4), indicating greater

intrinsic clearance of methoxychlor by CYP2C19 (Stresser and Kupfer 1998). Similar analysis of data from human liver microsomes (containing multiple CYPs) showed a fit for a two-enzyme system, even though there were wide variations for V_{max} (20–576 pmol/minute/mg) and K_m (0.35–12 μM) between samples (Elsby et al. 2001; Stresser and Kupfer 1998). The discrepancy in kinetic values is probably an indication of individual genetic variability in expression of CYP enzymes (Shimada et al. 1994). Studies with human cDNA-expressed CYP isozymes suggest that ring hydroxylation of methoxychlor or its hydroxy derivatives may involve a different set of CYP isozymes than CYP-catalyzed demethylation, including CYP1A2, 2B6, 2D6, 2E1, and 3A4 (Stresser and Kupfer 1997).

Excretion. No studies were located regarding the mechanism of excretion of methoxychlor in humans or animals after exposure by any route.

3.5.2 Mechanisms of Toxicity

The primary effects of methoxychlor in animal models involve the reproductive system, and result from the interaction of methoxychlor and its metabolites with estrogen receptors, and possibly androgen receptors, although the data indicating androgen receptor binding are limited. Many of the reproductive effects of methoxychlor are similar to those caused by estrogen, and are believed to be due to the ability of the mono- and bis-hydroxy derivatives of methoxychlor and MDDE to act as estrogen analogues (Bulger et al. 1978a, 1978b, 1978c, 1978d, 1985; Ousterhout et al. 1981). Androgens and estrogens are very lipid soluble and thus, they diffuse easily through the cell membrane into the cytosol and nucleus (DeFranco 1999). Once a steroid binds to its receptor and the hormone-receptor complex reaches the nucleus, it binds to hormone response elements in the enhancers, silencers, or promoters upstream of the genes controlled by the steroid in question. The hormone-receptor complex acts as a transcription factor to either stimulate or repress transcription of RNA from the steroid responsive gene. The resulting RNA transcript is spliced to form messenger RNA, which in turn directs the synthesis of specific proteins that direct the characteristic steroid hormone responses (Fregly and Luttge 1982). Some ancillary models for immediate action of steroid hormones have recently been proposed and involve either cross-talk interactions with, or possibly direct binding to, other hormone and growth factor cell surface receptors, and cell surface receptor mediated uptake of serum carrier proteins bound to steroid hormones (Chen and Farese 1999).

In vitro studies have shown that methoxychlor itself does not bind to the estrogen receptor (Bulger et al. 1978a, 1978b; Matthews et al. 2000; Ousterhout et al. 1981) but that the mono-hydroxy and bis-hydroxy

derivatives of methoxychlor and MDDE do bind to the receptor and cause nuclear translocation with the following order of potency: bis-OH-MDDE >> bis-OH-methoxychlor > mono-OH-MDDE > mono-OH-methoxychlor >> MDDE = methoxychlor (inactive) (Bulger et al. 1985). A similar hierarchy (bis-OH-MDDE > bis-OH-methoxychlor >> MDDE > methoxychlor) has been observed *in vivo* for the induction of uterine ornithine decarboxylase and increased uterine weight (Bulger et al. 1985). It was long thought that estrogen mediated its effects by binding to a single receptor, estrogen receptor α (ERα). Recently, however, a second estrogen receptor, ERβ, has been discovered (Kuiper et al. 1996; Mosselman et al. 1996). Although bis-hydroxy methoxychlor has been shown to bind to ERα and ERβ (Gaido et al. 1999, 2000), none of the earlier (before 1996) studies examining the difference in estrogen receptor binding affinity between methoxychlor and its metabolites differentiated between ERα and ERβ.

It has not been fully established what effects are mediated by each estrogen receptor. However, there appears to be tissue-specific distribution of the two receptors (Kuiper et al. 1997), which may allow for tissue-specific effects by estrogens. In tissues where both receptors are expressed, ligand binding to the receptors results in heterodimer formation (an ERα receptor pairs up with an ERβ receptor) (Cowley et al. 1997; Pace et al. 1997; Pettersson et al. 1997), which may result in different patterns of gene regulation than seen with homodimeric pairing (an ERα with an ERα or an ERβ with an ERβ). Additionally, each different estrogenic compound might act as an estrogen agonist at one receptor type and an estrogen antagonist at the other receptor type. This is apparently the case with the bis-hydroxy metabolite of methoxychlor, which has been shown to be an ERα agonist and an ERβ antagonist in some *in vitro* assay systems (Gaido et al. 1999, 2000).

Adding further to the mechanistic complexity, Ghosh et al. (1999) have suggested that methoxychlor acts through a third, as yet unelucidated, mechanism, not necessarily via an ER. Ghosh et al. (1999) showed that methoxychlor induced an increase in mRNA of two estrogen-responsive genes (lactoferrin, LF, and glucose-6-phosphate dehydrogenase, G6PD) in uteri of ovariectomized wild-type and ovariectomized ERα-knockout mice. Induction of LF and G6PD mRNA by methoxychlor was slightly greater in wild-type mice than in ERα-knockout mice at 15–30 mg/kg/day. Since no functional ERα receptors were present in the ERα knockout mice, the involvement of ERβ was indicated. However, when an estrogen inhibitor (ICI 182,780) was added, which should have eliminated methoxychlor interaction with ERβ, methoxychlor still induced an increase in LF and G6PD mRNA at a slightly lower level than without inhibitor in wild-type mice and at the same level in ERα knockout mice. In the same experiment, estradiol-17β resulted in large increases in LF and G6PD mRNA in wild-type mice, but in low levels of LF and G6PD mRNA (similar to control mice) in ERα-knockout mice (the authors point out that the

concentration of ERβ in uterus of the wild-type and ERα-knockout mice was very low). When an inhibitor was added, no increase in LF and G6PD mRNA was induced by estradiol-17β in wild-type mice. Thus, the "estrogenic" activity of methoxychlor was not attenuated by the absence of functional ERα receptors or the presence of an estrogen inhibitor effective at ERβ, suggesting that an additional mechanism of toxicity may exist besides interaction with ERα or ERβ.

A study that examined the effects of methoxychlor, DDE, and estradiol-17β on steroidogenesis and FSH responsiveness in ovarian cells provides additional support for a non-estrogenic mechanism of endocrine disruption by methoxychlor (Chedrese and Feyles 2001). In CHO-FSH-R cells (a Chinese hamster ovary cell line genetically modified to express the FSH receptor) exposed to DDE in the presence of FSH, DDE inhibited FSH-stimulated cAMP synthesis, which likely resulted in the observed decrease in progesterone synthesis and in decreased activity or synthesis of steroidogenic enzymes. Methoxychlor did not affect FSH-stimulated cAMP synthesis, but did inhibit estradiol-17β-stimulated progesterone synthesis in primary culture pig granulosa cells (Chedrese and Feyles 2001). Since progesterone is required for normal ovulation and implantation, the study authors speculated that this mechanism may partially explain the detrimental effects of methoxychlor on reproduction (Chedrese and Feyles 2001). The involvement of an ER is unlikely because no metabolic activation system was included, and methoxychlor, unlike its metabolite HPTE, does not interact with the ERs to any great extent (Bulger et al. 1978a, 1978b, 1985; Matthews et al. 2000; Ousterhout et al. 1981).

In contrast, the results of another *in vitro* study indicated that purified methoxychlor may have weak intrinsic estrogenicity (Elsby et al. 2001). A yeast estrogenicity assay was used that did not incorporate a microsomal metabolic activation system. The yeast had the DNA sequence of the human ERα integrated into the genome and contained transfected expression plasmids with the yeast 3-phosphoglycerate kinase promoter, estrogen responsive sequences, and a β-galactosidase reporter gene. Binding of an active ligand to the ER initiated transcription of the reporter gene, secretion of β-galactosidase into the medium, and ultimately, a color change in the medium from yellow to red. Methoxychlor was at least 100,000 times less potent than estradiol-17β and about 100 times less potent than the methoxychlor metabolite HPTE (Elsby et al. 2001).

An *in vitro* assay in human breast cancer MCF-7 cells examined the estrogenicity of methoxychlor and other xenoestrogens, as well as estradiol-17β and DES, by quantitatively assaying the induction or repression of four endogenous estrogen-regulated marker genes (pS2, transforming growth factor β3, monoamine oxidase A, and α1-antichymotrypsin) (Jørgensen et al. 2000). Methoxychlor induced pS and

α1-antichymotrypsin and repressed monoamine oxidase A and transforming growth factor β3, as did estradiol-17β, but methoxychlor was at least 100,000 times less potent than estradiol-17β (10^{-13}–10^{-11} M for estradiol-17β and 10^{-6}–10^{-5} M for methoxychlor). These estrogenic activities of methoxychlor were apparently mediated through the ERα since no ERβ was detected in MCF-7 cells.

Methoxychlor, like estradiol-17β, stimulated an increase in uterine peroxidase activity (a marker of estrogen action) (Cummings and Metcalf 1994). Also similar to estradiol-17β, methoxychlor stimulation of peroxidase was inhibited by actinomycin D (an RNA synthesis inhibitor) and cycloheximide (a protein synthesis inhibitor), indicating a similar mechanism of action. Estrogen-induced protein synthesis, an early effect of estradiol in the immature rat uterus and indicator of receptor occupancy and mRNA synthesis, was stimulated by 99% pure methoxychlor or estrone in an identical manner (Cummings and Metcalf 1995a). Estrogen-induced protein synthesis by methoxychlor and estrone was inhibited similarly by actinomycin D and cycloheximide. Methoxychlor induced the secretion of proteins in mature ovariectomized mice (Rourke et al. 1991) and neonatal mice (Eroschenko and Rourke 1992). Although the proteins secreted by methoxychlor were similar to those observed following estradiol treatment, some differences were noted, particularly so for neonatal mice. It was postulated by the study authors that posttranslational alterations to the estrogen receptors might be responsible for these differences (Rourke et al. 1991). These observations strongly support the view that the estrogenic effects of methoxychlor are mediated via binding of *O*-demethylated metabolites to estrogen receptors resulting in protein synthesis. Secretion of altered or different proteins into the uterine fluid during implantation and pregnancy could alter the essential uterine environment which may ultimately be responsible for decrements in fertility.

There are a number of physiological mechanisms by which the estrogenic effects of *O*-demethylated metabolites or contaminants of methoxychlor can interfere with reproduction. In female animals, possible mechanisms for decreased fertility include decreased mating frequency (Harris et al. 1974) decreased decidualization and decreased uterine receptivity to implantation (Cummings and Gray 1987, 1989), accelerated tubal transport of fertilized ova (Cummings and Perrault 1990), complete inhibition of implantation and altered preimplantation embryonic development and transport in mice (Hall et al. 1997; seen after intraperitoneal methoxychlor administration), and atresia of preovulatory follicles with reduced corpus luteum formation (Gray et al. 1989). In male animals, possible mechanisms for decreased fertility include decreased mating frequency (Gray et al. 1999; Harris et al. 1974), inadequate cervical stimulation of female animals to induce events necessary for implantation (Gray et al. 1989), and decreased Leydig cell function (Akingbemi et al. 2000; Gray et al. 1989).

Many of the effects described above are probably mediated by a direct effect of methoxychlor metabolites on estrogen-sensitive tissues. Alternatively, some of these changes may result from the effects of methoxychlor metabolites on the endocrine system. For example, methoxychlor has been shown to cause increased levels of gonadotropin releasing hormone in the hypothalamus (Goldman et al. 1986), elevated levels of prolactin, and thyroid stimulating hormone in the pituitary (Goldman et al. 1986; Gray et al. 1989), and reduced levels of thyroid-stimulating hormone, testosterone, and progesterone in the serum (Cummings and Gray 1989; Gray et al. 1989). These methoxychlor-induced effects on the endocrine system may be partially responsible for methoxychlor-induced effects such as uterine and mammary gland hyperplasia (Tegeris et al. 1966) and gonadal atrophy (Bal 1984; Gray et al. 1988; Hodge et al. 1950; Tullner and Edgcomb 1962), and could also be important in impaired reproduction associated with methoxychlor exposure (Bal 1984; Gray et al. 1989; Harris et al. 1974).

Some of the reproductive effects seen in male rodents following methoxychlor exposure are similar to those produced by either estrogens or antiandrogens (Gray et al. 1999). Delayed puberty and reduced accessory sex gland size can be caused by an estrogen or an antiandrogen. Estrogenic compounds cause a decrease in LH secretion from the pituitary and in serum testosterone and an increase in pituitary tumors and hyperprolactinemia, whereas antiandrogens enhance LH secretion from the pituitary, cause an increase in serum testosterone, and have no effect on prolactin or on pituitary tumorigenesis (Gray et al. 1999). In male rats dosed with 0, 200, 300, or 400 mg/kg/day methoxychlor from weaning (postpartum day 21) through 11 months of age, preputial separation was delayed in a dose-dependent manner, pituitary size was decreased at all exposure levels, and there were no statistical differences from controls in serum LH, prolactin, and testosterone (Gray et al. 1999). These results suggest that methoxychlor might interact with the androgen receptor (AR) as an antagonist; however, it should be noted that in some *in vitro* assays, estradiol-17β can itself act as a weak androgen antagonist (Kelce et al. 1995). Only one study has investigated the potential androgen antagonism of methoxychlor and its metabolites; this experiment was not a direct receptor binding assay. The methoxychlor metabolite HPTE was a weak AR antagonist of dihydrotestosterone in HepG2 human hepatoma cells transiently transfected with the human androgen receptor and a reporter gene linked to an androgen responsive promoter; methoxychlor itself showed even less androgen antagonism in this experiment (Maness et al. 1998). *p,p'*-DDE, an isomer of the methoxychlor structural analog DDT, is also an AR antagonist of dihydrotestosterone (Danzo 1997; Kelce et al. 1995, 1997; Maness et al. 1998). It is thought that androgen antagonism explains some of the reproductive and developmental effects seen in male rats exposed to *p,p'*-DDE, including reduced anogenital distance, retention of thoracic nipples, delayed puberty, and reduced accessory sex organ weights (Kelce et al. 1997; Loeffler and Peterson 1999; You et al. 1998). Some of these effects (delayed

puberty and reduced accessory sex organ weights) are also seen following exposure to methoxychlor (Bal 1984; Gray et al. 1989, 1999; Hodge et al. 1950; Shain et al. 1977; Tullner and Edgcomb 1962). More exhaustive studies regarding methoxychlor metabolite binding to the AR are necessary to be able to determine the contribution of androgen antagonism to the reproductive and developmental effects resulting from methoxychlor exposure.

While the reproductive effects of methoxychlor appear to be due to the estrogenic nature of the *O*-demethylated metabolites, parent methoxychlor may be responsible for the neurotoxic effects observed at high exposure levels in animals (Cannon Laboratories 1976; Tegeris et al. 1966). The chief reason for suspecting this is that methoxychlor is a close structural analogue of DDT (differing in that the methoxy groups of methoxychlor are replaced by chlorine groups in DDT), and high levels of DDT are known to be neurotoxic in animals (Agency for Toxic Substances and Disease Registry 1994). DDT prevents the deactivation of the sodium gate after neuron activation and membrane depolarization (Brown et al. 1981; Coats 1990; Wu et al. 1975), resulting in hyperexcitability of the nerve. Other molecular mechanisms may contribute to DDT induced hyperexcitability of the nerve; these include prolonging the action potential by preventing the full opening of the potassium gates (Narahashi and Haas 1967) and interference with two specific neuronal adenosine triphosphatases (ATPases) thought to be involved in controlling sodium, potassium, and calcium fluxes through the nerve membrane (Matsumura and Patil 1969; Matsumura 1985). *In situ* studies have shown that methoxychlor (and some other DDT structural analogs) increase and prolong the depolarizing afterpotential of nerves, similar to DDT (Wu et al. 1975). It is important to note that if the neurotoxic effects (tremors, convulsions) of methoxychlor are indeed attributable to the parent compound, then *O*-demethylation to the phenolic metabolites represents a detoxification pathway for neurotoxicity. This is consistent with the fact that this type of neurotoxicity has only been noted at doses high enough to surpass metabolic capacity (Cannon Laboratories 1976; Tegeris et al. 1966) or in animals in which hepatic metabolism has been impaired (Lehman 1952).

3.5.3 Animal-to-Human Extrapolations

Some species differences in sensitivity to methoxychlor have been observed. Rabbits appear to be more sensitive than rats to short-term exposure to methoxychlor (Kincaid Enterprises 1986; Smith et al. 1946). All rats administered a single gavage dose of 5,000 mg/kg methoxychlor survived, whereas 7 of 13 rats given 7,000 mg/kg died (Smith et al. 1946). Four of 4 rabbits died following the administration of 4–15 daily doses of 200 mg/kg/day methoxychlor (Smith et al. 1946). Fatty degeneration of the liver and nephrosis were observed in some or all of the rabbits that died following exposure to 200 mg/kg/day.

Two of 17 pregnant rabbits died within 10 days of receiving 13 daily oral exposures to 251 mg/kg/day (Kincaid Enterprises 1986).

Recent studies in mice show effects that may be mediated through the estrogen receptor (increased prostate weight and altered urine-marking behavior) at low exposure levels (0.02 mg/kg/day) (vom Saal et al. 1995; Welshons et al. 1999). No other studies were located that examined similar end points in other species exposed to similarly low levels of methoxychlor; therefore, it is unclear whether there are significant differences between species in susceptibility to these effects of methoxychlor.

Several *in vitro* studies have indicated the presence in human hepatic microsomes of enzymatic activities similar to those in rats that are responsible for the metabolism of methoxychlor to its estrogenic metabolites (Dehal and Kupfer 1994; Stresser and Kupfer 1998). Therefore, there is some indication that rats may be an adequate animal model for the estrogenic effects of methoxychlor in humans. Rates of formation of mono- and bis-hydroxy methoxychlor from methoxychlor with human liver microsomes were generally lower than rates with rat liver microsomes, but some human liver samples displayed higher rates than the rates for rat microsomes (Stresser and Kupfer 1998).

Another *in vitro* study examined the relative binding affinities (RBA) of methoxychlor, its metabolite HPTE, and other estrogenic substances (compared to estradiol-17β) to bacterially expressed estrogen receptor fusion proteins from humans (ERα), mouse (ERα), chicken, green anole (a lizard), and rainbow trout (Matthews et al. 2000). The ER fusion proteins contained three of the six domains of the receptor, including the ligand binding domain, fused to glutathione-*S*-transferase. Methoxychlor did not bind to the human or mouse receptor proteins (displaced <10% of radiolabeled estradiol-17β), bound only weakly to the chicken and green anole receptor proteins (displaced 10–50% of radiolabeled estradiol-17β), and had an RBA of 0.95 with the rainbow trout receptor protein. HPTE had RBAs of 1.2 with the human and mouse receptor proteins, 4.8 with the chicken and green anole receptor proteins, and 14 with the rainbow trout receptor protein. These data indicate some species differences in receptor binding, but support the mouse as an adequate model for the study of estrogenic effects of methoxychlor mediated through ER binding.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Colborn and Thomas (1992) and again by Colborn (1993), was also used in 1996 when Congress mandated the Environmental Protection Agency (EPA) to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), which in 1998 completed its deliberations and made recommendations to EPA concerning *endocrine disruptors.* In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

There is ample evidence, *in vitro* and *in vivo*, that methoxychlor has estrogenic properties. Estrogens, both endogenous and exogenous, or environmental, mediate many of their effects by binding to estrogen receptors (ER) in the cytoplasm of target tissue cells. Estrogens are very lipid soluble and diffuse easily through the cell membrane into the cytosol, where they bind to empty estrogen receptors. The occupied

receptor is translocated to the nucleus, where the receptor-estrogen complex binds to hormone response elements upstream of the gene or genes controlled by the estrogen and induces or inhibits the production of specific proteins that then produce a characteristic effect(s). The effect may be seen in the tissue in which the protein was synthesized or in a distant tissue following secretion and transport of the newly synthesized protein through the bloodstream. For many years, it was thought that estrogens mediated their effects by binding to a single receptor, estrogen receptor α (ERα). However, a second estrogen receptor, ERβ, has recently been discovered (Kuiper et al. 1996; Mosselman et al. 1996). It has not been fully established what effects are mediated by each receptor. However, there appears to be tissue-specific distribution of the two receptors (Kuiper et al. 1997), which may allow for tissue-specific effects by endogenous and exogenous estrogens. This adds complexity to the effects of estrogenic compounds because each compound may, theoretically, interact with each receptor as an estrogen agonist or antagonist. The estrogenicity of methoxychlor derives primarily from its metabolites, mono- and bis-hydroxy methoxychlor. Bis-hydroxy methoxychlor is known to bind to ERα and ERβ and is thought to be an ERα agonist and an ERβ antagonist (Gaido et al. 1999, 2000). Recent data by Ghosh et al. (1999) indicate that methoxychlor may also act via another, as yet unknown, mechanism not mediated by either ERα or ERβ. Estrogenic mechanisms are discussed in more detail in Section 3.5.2 Mechanisms of Toxicity.

There are no data showing estrogenic effects of methoxychlor in humans. However, there are numerous *in vivo* animal studies showing the estrogenic nature of methoxychlor and its metabolites. Below is a brief overview of the effects of exposure to methoxychlor on the endocrine and reproductive systems. A more detailed discussion of these studies can be found in Section 3.2.

Exposure to methoxychlor has been shown to affect the reproductive systems of male and female mammals in ways that mimic or antagonize the effects of estrogen. Effects have been observed following acute, intermediate, and chronic duration exposures and over a wide range of exposure levels. Effects have been seen following exposures *in utero*, during lactation, or post-weaning. There is also a wide range of effects, encompassing the structural, functional, and behavioral realms, in a number of different species. In female mammalian species, sexual maturity was affected, as demonstrated by precocious vaginal opening and delayed onset of estrus cyclicity in rats and mice exposed to 5–200 m/kg/day (Appel and Eroschenko 1992; Chapin et al. 1997; Cooke and Eroschenko 1990; Eroschenko 1991; Eroschenko and Cooke 1990; Gray et al. 1989; Harris et al. 1974; Swartz and Corkern 1992). A number of structural and functional effects were also seen, including mammary gland hyperplasia in pigs exposed to 1,000 mg/kg/day (Tegeris et al. 1966) and ovarian atrophy in rats and mice exposed to 25–400 mg/kg/day

(Bal 1984; Chapin et al. 1997; Gray et al. 1988, 1989; Harris et al. 1974; Martinez and Swartz 1991), gross and cellular ultrastructural alterations of the lining of the uterus in mice exposed to 50–100 mg/kg/day (Swartz et al. 1994), persistent estrus in rats and mice exposed to 25–400 mg/kg/day (Gray et al. 1988; Harris et al. 1974; Martinez and Swartz 1991), decreased mating frequency rats exposed to 60–160 mg/kg/day (Harris et al. 1974), decreased fertility in rats and mice exposed to 35.5–200 mg/kg/day (Bal 1984; Chapin et al. 1997; Culik and Kaplan 1976; Gray et al. 1989; Harris et al. 1974; Haskell Laboratories 1966; Kincaid Enterprises 1986; Wenda-Rozewicka 1983), decreased live offspring in rats exposed to 100–150 mg/kg/day (Chapin et al. 1997; Gray et al. 1989), and increased number of resorptions of fetuses in rats and rabbits exposed to 17.8–400 mg/kg/day (Culik and Kaplan 1976; Khera et al. 1978; Kincaid Enterprises 1986). Serum progesterone and pituitary prolactin hormone levels were also affected in rats exposed to 400 mg/kg/day (Gray et al. 1988), and cellular biochemistry of ovarian cells was altered (an accumulation of lipid in the interstitial and thecal cells) in mice exposed to 100 mg/kg/day (Martinez and Swartz 1992).

Similar types of effects are seen in male animals. Delayed preputial separation indicated delayed sexual maturity. Structural and functional effects seen included decreased testes, ventral prostate, epididymis, and seminal vesicle weights in rats exposed to 50–1,400 mg/kg/day (Bal 1984; Chapin et al. 1997; Goldman et al. 1986; Gray et al. 1989, 1999; Hodge et al. 1950; Shain et al. 1977; Tullner and Edgcomb 1962), increased adrenal gland, seminal vesicle, and prostate weights in rats exposed to 0.02–400 mg/kg/day (Chapin et al. 1997; Gray et al. 1989, 1999; Stoker et al. 1999; Welshons et al. 1999), inhibition of testes development in rats exposed to 5–150 mg/kg day (Chapin et al. 1997), decreased caudal epididymal sperm count in rats exposed to 50–400 mg/kg/day (Gray et al. 1989, 1999), decreased mating frequency in rats exposed to 60–400 mg/kg/day (Gray et al. 1999; Harris et al. 1974), decreased copulatory stimulation of females necessary for pregnancy in male rats exposed to 100 mg/kg/day (Gray et al. 1989), and decreased fertility in rats exposed to 35.5–300 mg/kg/day (Bal 1984; Chapin et al. 1997; Culik and Kaplan 1976; Gray et al. 1989; Harris et al. 1974; Haskell Laboratories 1966; Kincaid Enterprises 1986; Wenda-Rozewicka 1983). Hormone levels were also affected in males, with increased levels of pituitary prolactin, FSH, TSH, and hypothalamic GnRH in rats exposed to 25–50 mg/kg/day (Goldman et al. 1986; Gray et al. 1989), decreased levels of serum TSH in rats exposed to 100–200 mg/kg/day (Cummings and Gray 1989; Gray et al. 1989), decreased serum testosterone and progesterone in mice and rats exposed to 33–100 mg/kg/day (Amstislavsky et al. 1999; Cummings and Gray 1989; Gray et al. 1989), and decreased interstitial fluid and epididymide testosterone in rats exposed to 100 mg/kg/day (Gray et al. 1989). Some hormone alterations occurred at methoxychlor

exposure levels below those causing reproductive effects and may have contributed to or caused those effects.

It is thought that most, if not all, of the above endocrine disruptive effects are mediated by the interaction of the bis-hydroxy metabolite of methoxychlor (HPTE) with one or both of the estrogen receptors and/or the androgen receptor. It is also possible that tissue toxicity or some other indirect route of influence by methoxychlor or its metabolites resulted in some of these effects. Numerous *in vitro* studies have verified that methoxychlor binds to ERs, although with much lower affinity than estradiol. Methoxychlor has been shown to bind ER (probably both ERα and ERβ) from MCF-7 cells (a human breast cancer cell line) (Dodge et al. 1996), rabbit uterine cells (Danzo 1997), bovine uterine endometrial explants (Tiemann et al. 1996), GT1 cells (a murine immortal hypothalamic neuron cell line) (Roy et al. 1999), and MtT/E-2 cells (a estradiol-17β-responsive rat pituitary cell line) (Maruyama et al. 1999). Methoxychlor has also been shown to bind to human ERα and ERβ expressed in Sf9 (insect) cells (Kuiper et al. 1998). The methoxychlor metabolite bis-OH-methoxychlor (or HPTE) has been shown to bind to human and rat ERα and ERβ expressed in human hepatoma (HepG2) cells (Gaido et al. 1999, 2000), and to be an ERα agonist and an ERβ antagonist. Methoxychlor and HPTE can also weakly antagonize dihydrotestosterone at the human androgen receptor (AR) (expressed in HepG2 cells) (Maness et al. 1998). In male rats exposed to 200 mg/kg/day methoxychlor from weaning (postpartum day 21) through 11 months of age, preputial separation was delayed, pituitary size was decreased, and there were no statistical differences from controls in serum LH, prolactin, and testosterone (Gray et al. 1999); these effects are typical of antiandrogenic, but not estrogenic, compounds. The binding affinity and potency of methoxychlor was generally observed to be 1,000–100,000 times less than estradiol (Dodge et al. 1996; Kuiper et al. 1998). However, it is thought that estrogen is bound to serum proteins (>95%) *in vivo* that render it less available for migration into cells and receptor binding (Nagel et al. 1999; vom Saal et al. 1995). The fact that methoxychlor is not bound by these proteins must be taken into account when using *in vitro* assays in serum-free media to simulate *in vivo* exposures (Nagel et al. 1999; vom Saal et al. 1995). Receptor binding of methoxychlor is discussed in more detail in Section 3.5.2, Mechanisms of Toxicity.

Effects on Wildlife. Whether methoxychlor causes estrogenic effects in wildlife has been investigated in a number of experiments. Although methoxychlor has certainly produced effects in fish, amphibians, and sea urchins, only a subset of these effects seem to be due to estrogenic activity. The mechanism for these other effects is not known.

The estrogenic activity of methoxychlor has been demonstrated in catfish. In catfish, estrogen receptors have been found in liver, brain, and testes. The liver is the primary target tissue of estrogen in fish and is the site of the greatest ER concentration. Interaction with fish liver ER stimulates the synthesis of vitellogenin (the precursor to egg yolk) and also stimulates the synthesis of more ER. Nimrod and Benson (1997) tested methoxychlor, the *O*-demethylated metabolite of methoxychlor and several other environmentally relevant compounds for their ability to compete with estradiol for binding to catfish ER *in vitro*. Methoxychlor was only about 1/6,700 as potent and *O*-demethylated methoxychlor was 1/1,000 as potent as estradiol. Even though *O*-demethylated methoxychlor was not a very strong catfish ER ligand, it was the most potent environmentally relevant compound tested in this study, including *o,p'*-DDT and *o,p'*-DDE, which had extremely low binding ability. When injected into catfish intraperitoneally, methoxychlor did not induce a detectable estrogenic response, as measured by vitellogenin synthesis (Nimrod and Benson 1997). Curiously, after depletion of the liver enzyme thought to be responsible for methoxychlor metabolic transformation, serum vitellogenin and estradiol increased (Schlenk et al. 1997).

Methoxychlor causes subtlely different effects from estradiol in trout. Trout exposed every third day to 0, 0.5, 1, 2, or 4 mg/L methoxychlor (laboratory grade) by immersion for 2 hours followed by rinsing from 6 days prior to hatching through 24 days post-hatching showed increased mortality. Mortality following estradiol exposure was 3 times that of methoxychlor-induced mortality (Krisfalusi et al. 1998a). Further experiments showed that methoxychlor caused increased mortality at day 16 post-hatch regardless of number of treatments, whereas estradiol caused increased mortality after 10 treatments regardless of developmental time. Therefore, methoxychlor does not appear to be acting by the same mechanism as estradiol. Also noted in this study was that the skin of methoxychlor-treated fish was lighter than controls by 6 days post-hatching, and the skin of estradiol-treated fish was darker than controls between days 9 and 12 post-hatching, again indicating different mechanisms. The authors speculated that the lighter skin may be due to decreased synthesis and/or release of melanophore stimulating hormone from the pituitary and that the darker skin may be due to toxicity. Fish treated with methoxychlor also showed a dose-dependent decrease in weight. Estradiol caused weight reduction as well, but in a non-dose-dependent manner. A dose-dependent increase in mortality and decrease in growth in trout was also observed (Krisfalusi et al. 1998b). Methoxychlor treatment did not disrupt male sex differentiation or early testicular development in these trout.

Methoxychlor did not produce estrogenic effects in Japanese medaka (a teleost, or bony fish) at doses comparable to an effective dose of estradiol (Nimrod and Benson 1998). In Japanese medaka, no

developmental or reproductive toxicity was seen following exposure to 0, 0.2, 0.6, or 2.3 µg/L laboratory grade methoxychlor in the water for the first month after hatching. Parameters monitored included ovarian or testicular size, sex ratio, fertility, viability of eggs, and hatchability of eggs. These results contrast with the definitive effects that estradiol causes in these fish. Medaka are susceptible to phenotypic sex reversal when exposed to estrogens and androgens through the diet during early life stages. Exposure to 0.01. 0.12, or 1.66 µg/L estradiol as above resulted in 100% female populations. Fish (female) in the 1.66 µg/L estradiol group also had lower fecundity than controls.

In vitro experiments using cultured carp hepatocytes showed that methoxychlor was 1,000-fold less potent than estradiol. The order of estrogenic potency, as measured by vitellogenin induction, of compounds tested was methoxychlor > *o,p*-DDT > chlordecone ≈ bisphenol-A ≈ 4-t-pentylphenol (Smeets et al. 1999). When cells were exposed simultaneously to estradiol and methoxychlor, estradiol antagonized the effects of methoxychlor. Methoxychlor did not appear to be metabolized to more estrogenic metabolites in carp by CYP1A2 (one of the CYP liver enzymes in mammals responsible for metabolizing methoxychlor to more estrogenic compounds), as induction of this enzyme did not enhance the estrogenicity of methoxychlor.

In salamanders, methoxychlor sometimes causes subtlely different effects from estradiol and other times completely different effects. Salamander eggs exposed to up to 10 mg/L laboratory-grade methoxychlor experienced no increase in mortality through post-hatching day 10 (Ingermann et al. 1997). Exposures as low as 0.1 mg/L resulted in precocious hatching and reduced startle response. The effects of methoxychlor were compared to estradiol in salamanders (Ingermann et al. 1999). Salamander eggs were exposed to 0, 0.2, 1.0, or 5.0 µM laboratory grade methoxychlor, recrystallized methoxychlor, HPTE (the estrogenic metabolite of methoxychlor in mammals), estradiol, or deoxycorticosterone (DOC, an adrenal steroid) until 10 days post-hatching. Laboratory grade and recrystallized methoxychlor were equally potent at causing precocious hatching and a blunted startle response, suggesting that contaminants are not responsible for these effects. Only the highest exposure level of estradiol resulted in precocious hatching and an altered startle response. The startle response in estradiol-treated hatchlings frequently involved swimming in circles, whereas the startle response in methoxychlor-treated hatchlings involved a shorter straight distance traveled (never circles). Exposure of eggs and hatchlings to HPTE resulted in no effect on day of hatch or startle response. DOC had no effect on hatch time and resulted in reduced startle response only at the highest exposure level. The effects of laboratory grade and recrystallized methoxychlor and HPTE on maturation of frog oocytes *in vitro* were compared to the effects of estradiol (Pickford and Morris 1999). Both grades of methoxychlor caused a highly significant inhibition of

progesterone-induced germinal vesicle breakdown (GVBD, necessary for oocyte maturation), while estradiol and HPTE had no effect. This indicates that methoxychlor is the compound responsible for this effect and that this effect is not estrogenic in nature. Failure of ICI 182,780 (an ER inhibitor) to alter the effect of methoxychlor on oocyte maturation indicates that it is not mediated through the ER. The mechanism of this effect by methoxychlor also does not appear to involve the progesterone receptor, as neither methoxychlor nor HPTE exhibited any competitive binding affinity for this receptor.

In sea urchins, exposure of sperm to 3 ppm laboratory grade methoxychlor for 10 or 30 minutes caused a 30 or 100% reduction in fertilization, respectively (Mwatibo and Green 1997). Similar exposure of eggs did not result in a decrease in fertilization, but 5.5% of the embryos resulting from these eggs showed abnormal, stunted or absent gut, malformed spicules (part of the skeleton), and abnormal shape. Embryos from sperm exposed to methoxychlor for 10 minutes showed a slight, but statistically insignificant, increase in the same anomalies. The mechanism of the effect was not examined.

Taken together, these data indicate that methoxychlor has endocrine disruptive effects in fish, amphibian, and sea urchin fertility, growth, and development. Sometimes the effects of methoxychlor parallel those of estradiol and sometimes there are either subtle or drastic differences from the effects of estradiol. Thus, it is likely that not all of the effects of methoxychlor in aquatic wildlife are mediated through estrogen receptors. There may be many different mechanisms involved in the varied effects and the different species.

3.7 CHILDREN'S SUSCEPTIBILITY

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

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

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

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

There is no direct information on the toxicity of methoxychlor in children or on effects in adults that were exposed as children. Animal data indicate that the primary target of methoxychlor is the reproductive system and that methoxychlor can both affect adult animals and influence the development of the reproductive system in males and females. Thus, there is concern about whether sufficient exposure to methoxychlor might potentially affect the developing reproductive system of fetuses, children, and adolescents. Although there is no direct evidence that children are more susceptible to health effects from methoxychlor exposure than adults, lower doses of methoxychlor were generally required to produce reproductive effects in developing animals than in adults.

Numerous studies have shown that adult animals, exposed during development or as adults, exhibited effects attributed to the "estrogenic" activity of methoxychlor, indicating that metabolism of methoxychlor to its estrogenic metabolites occurs in developing and adult animals. These estrogenic effects included altered hormone levels, abnormal histology, and decreased fertility (Bal 1984; Chapin et al. 1997; Gray et al. 1989, 1999; Harris et al. 1974; Martinez and Swartz 1991). The exact mechanisms of disruption of normal reproductive function may differ between developmental and adult exposure. The reproductive organs and tissues of adult animals are already developed and capable of responding appropriately to normal hormone levels. Methoxychlor disrupts the normal hormone balance by interacting with estrogen, and possibly androgen receptors, which causes abnormal reproductive responses. Methoxychlor probably also directly affects tissues containing these receptors. Exposure to methoxychlor during critical periods of reproductive development may cause abnormal or under- or over-development of certain reproductive organs or tissues, as well as an altered tissue distribution of these same receptors, so that the organs/tissues have no capability to respond to hormones in a normal fashion, even if hormone levels return to normal following cessation of exposure.

There is ample information from animal studies to indicate that the developing organism is susceptible to the effects of methoxychlor and its metabolites. Wavy ribs have been observed in the offspring of female rats exposed to $7.8 mg/kg/day methoxychlor on gestation days 6–15 (Culik and Kaplan 1976). Khera et al. (1978) also noted wavy or extra ribs and decreased fetal weight, as well as an increased percentage of dead and resorbed fetuses, in offspring of female rats exposed to 200 mg/kg/day during gestation. Body weights of fetuses from female rabbits receiving 35.5 (but not 5.01) mg/kg/day methoxychlor on days 7–19 of gestation were decreased by 10% and the percentage of male offspring was decreased (Kincaid Enterprises 1986).

Methoxychlor also causes numerous functional changes in the adult and developing reproductive system of animals. Unlike its structural cousin, DDT, methoxychlor is metabolized rapidly to its mono- and bis-hydroxy derivatives. Therefore, the neurotoxicity associated with DDT is not seen following methoxychlor exposure (except at extremely high exposure levels). However, methoxychlor and its metabolites, especially bis-hydroxy methoxychlor, or HPTE, are estrogenic and can bind to estrogen receptors, enhancing or attenuating the effects of endogenous estrogens (Bulger et al. 1978d; Gaido et al. 1999, 2000; Kuiper et al. 1998; Kupfer and Bulger 1979, 1987b; Mosselman et al. 1996). Estrogen receptors are found in many tissues in males and females, including mammary gland, uterus, vagina, ovary, testes, epididymis, prostate, thymus, bone, central nervous system, pituitary, hypothalamus, and cardiovascular system (Kuiper et al. 1998). When the delicate balance of endogenous estrogen levels is disrupted, transient or permanent functional and/or structural abnormalities may occur in a variety of organs or tissues, especially in the reproductive system. Exposure to methoxychlor during critical stages of development or in the adult animal has been shown to result in a number of reproductive effects. Effects associated with methoxychlor exposure include histopathological changes in the reproductive organs and accessory glands (Bal 1984; Chapin et al. 1997; Gray et al. 1988, 1989; Martinez and Swartz 1991, 1992; Stoker et al. 1999; Swartz et al. 1994; Tegeris et al. 1966), impaired pubertal development (Chapin et al. 1997; Gray et al. 1989, 1999; Harris et al. 1974) and reproductive function (Bal 1984; Chapin et al. 1997; Gray et al. 1988, 1989; Harris et al. 1974; Haskell Laboratories 1966; (Wenda-Rozewicka 1983), and altered hormone levels (Cummings and Gray 1989; Cummings and Laskey 1993; Goldman et al. 1986; Gray et al. 1988, 1989; Martinez and Swartz 1992; Stoker et al. 1999). There is a wide range of doses reported in the literature that cause effects in developing animals. The great majority of studies report effects from *in utero*, neonatal, and adult exposure beginning at around 5–150 mg/kg/day (Bal 1984; Chapin et al. 1997; Gray et al. 1989, 1999; Harris et al. 1974; Martinez and Swartz 1991). Reproductive effects in female mice and rats from *in utero* and postnatal exposures include precocious vaginal opening (Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974), delayed onset of estrus (Gray et al. 1989), altered vaginal and uterine histology (Chapin et al. 1997; Gray et al. 1988, 1989), altered ovarian histology (Bal 1984; Chapin et al. 1997; Gray et al. 1989, 1999; Harris et al. 1974; Martinez and Swartz 1991), and decreased fertility (Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974). In male animals, delayed preputial separation, decreased testes, epididymis, seminal vesicle, prostate weight, and decreased fertility have been observed following developmental exposure (Chapin et al. 1997; Gray et al. 1999; Harris et al. 1974). A few studies have shown that *in utero* exposures as low as 0.02 mg/kg/day caused changes in sex accessory organs and changes in neurobehavioral and sociosexual behavioral parameters (Palanza et al. 1999; Parmigiani et al. 1999; vom Saal et al. 1999; Welshons et al. 1999). For

a more detailed discussion of the reproductive and developmental effects of methoxychlor, see Sections 3.2.2.5 Reproductive Effects and 3.2.2.6 Developmental Effects.

There are no studies directly examining whether methoxychlor and metabolites cross the placenta in humans or animals. Subtle effects on the development of reproductive organs have been observed in male and female offspring of rodents exposed to oral doses of methoxychlor during gestation (Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974); however, it should be noted that exposure was not exclusively during gestation in any of these studies. It is unclear if these effects result directly from methoxychlor and metabolites passing through the placenta or indirectly from a disturbance of maternal physiological processes. Levels of methoxychlor and its metabolites were determined in breast milk and plasma of female rats orally administered 5, 50, or 150 mg/kg/day methoxychlor during gestation and early lactation (Chapin et al. 1997). Breast milk levels of methoxychlor and mono- and di-hydroxy methoxychlor were very high compared to plasma levels (25–215% higher). Levels in pup serum were much lower (1.3–5.9% of dam plasma levels), but were still detectable at most exposure levels. Pup serum levels may actually reach much higher levels, since more than 24 hours elapsed between administration of the last dose of methoxychlor to the dam and collection of pup blood for analysis. Therefore, children may be exposed to significant levels of methoxychlor and metabolites via breast milk if their mother is orally exposed to sufficient amounts of methoxychlor. One study analyzed human breast milk for the presence of methoxychlor and found none (Hooper et al. 1997); however, two other studies did detect methoxychlor in human breast milk (Campoy et al. 2001a, 2001b). Unlike its structural cousin, DDT, methoxychlor is metabolized and eliminated from the body rapidly. Some methoxychlor is distributed to tissues throughout the body, but does not appear to persist, even in fat. Therefore, it is unlikely that methoxychlor from pre-conception exposure of the mother would be available for mobilization during pregnancy and lactation. However, puzzling, unexplained data suggest that it is possible that previous exposure of the mother to methoxychlor results in damage to her reproductive system in some way that causes future offspring to experience reproductive anomalies, although the evidence of such occurrences in animals is very limited. Swartz and Corkern (1992) noted precocious vaginal opening in female offspring of a second unexposed pregnancy (F1b) in mice. It is unclear why the F1a exposed litter did not experience precocious vaginal opening and by what mechanism this effect was produced in the unexposed F1b offspring.

The primary pathway by which methoxychlor is metabolized in the liver (the main site of methoxychlor metabolism) is sequential demethylation reactions to yield the estrogenic mono- and bis-hydroxy methoxychlor. *In vitro* studies with human liver microsomes and human recombinant cytochrome P450

(CYP) isozymes indicate that multiple CYP isozymes are involved in the demethylation of methoxychlor in humans (Stresser and Kupfer 1998). This study indicates that CYP2C19 and CYP1A2 may be the major CYP demethylases for methoxychlor, but that other forms, including CYP2A6, CYP2C9, and CYP2B6, are likely to be major contributors, especially in individuals with low levels of CYP2C19 or CYP1A2. Other CYP enzymes that may play a role include CYP2D6, CYP2E1, and CYP3A4. Many of these phase I enzymes are likely to have overlapping roles. Although nothing is known about phase II metabolism in humans, Davison et al. (1982, 1983) found glucuronic acid conjugates of methoxychlor metabolites in goats; these conjugates are a product of glucuronosyltransferase, and it is possible that this enzyme may be involved in human methoxychlor metabolism as well. Although it is unknown whether an overall age-related difference in methoxychlor metabolism would be observed *in vivo* in humans, there is some information that the following enzymes may be developmentally regulated: CYP2C19 (Leeder and Kearns 1997), CYP1A2 (Leeder and Kearns 1997; Rich et al. 1990), CYP2C9 (Ratenasavanh et al. 1991;Treluyer et al. 1997), CYP2D6 (Leeder and Kearns 1997; Sonnier and Cresteil 1998), CYP2E1 (Hong et al. 1987; Vieira et al. 1996), CYP3A4 (Lacroix et al. 1997; Leeder and Kearns 1997; Mote et al. 1990; Yang et al. 1994), and glucuronosyltransferase (Leeder and Kearns 1997). While CYP2C19 and CYP1A2 are not present in appreciable levels in human fetal liver, their activities increase to adult levels by 4 months to >1 year of age (Leeder and Kearns 1997; Ratenasavanh et al. 1991; Sonnier and Cresteil 1998). In contrast, other researchers have found that CYPs 2A6, 2C9, 2D6, 2E1, and 3A are expressed at the same levels in perinatal (37 weeks of gestation) to geriatric human livers (72 years) (Ratenasavanh et al. 1991; Tateishi et al. 1997).

There are no PBPK models for children, fetuses/pregnant women, infants/lactating women, or humans at any other stage of development.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

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

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself 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 methoxychlor are discussed in Section 3.8.1.

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

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

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Methoxychlor

Methods exist for determining the levels of methoxychlor and its metabolites in biological media (tissues, fluids, and excreta) (LeBel and Williams 1986; Mes 1981; Steinberg et al. 1989), although data from human samples are sparse and detectable levels of methoxychlor have generally not been reported. A more detailed discussion of the methods used to detect methoxychlor in biological and environmental samples can be found in Chapter 7 Analytical Methods. In a human population exposed to heavy agricultural spraying of pesticides (area sprayed 16–30 times per year), serum levels of methoxychlor were generally below the level of detection (detection limit of 0.24–4.07 mg/L) (Steinberg et al. 1989).

Similar results were also obtained with adipose tissue obtained from human autopsies (LeBel and Williams 1986).

Data from animal studies indicate that blood and tissue (fat) levels of methoxychlor do not remain elevated for very long after exposure, owing to its rapid metabolism to more polar, readily excretable metabolites (Davison et al. 1982; Hodge et al. 1952; Kapoor et al. 1970; Kunze et al. 1950; Reynolds et al. 1976). Because methoxychlor is rapidly metabolized, detection of these metabolites (e.g., the mono- and bis-hydroxy derivatives) may serve as a better biomarker of exposure than the parent compound, especially in excreta such as feces (Kapoor et al. 1970). Methoxychlor and its metabolites have been measured in the milk of lactating rats (exposed to 5, 50, or 150 mg/kg/day from gestation day 14 through lactation day 7) and were found to concentrate in the milk with increasing methoxychlor exposure level (Chapin et al. 1997). The data also suggest that methoxychlor and metabolites concentrate in milk, relative to maternal plasma levels, after intermediate-duration dose levels $50 mg/kg/day. Plasma levels of methoxychlor and its metabolites (mono-hydroxy methoxychlor and di-hydroxy methoxychlor) in suckling rat pups also increased with increasing dose of methoxychlor (Chapin et al. 1997). Metabolites in dam milk and pup plasma were below the level of detection (50 and 5 ng/mL, respectively) at the 5 mg/kg/day dose level. Pup plasma was not drawn for analysis until 27–30 hours after dams received the last dose; therefore, measured methoxychlor and metabolite levels may not have been indicative of peak body burden. Because of the relatively rapid clearance of these metabolites, measurements would probably only be useful in detecting recent exposures (within the past 24 hours). In fact, in one study that tested for methoxychlor in human breast milk, none was detected among regional populations in Kazakstan (Hooper et al. 1997). However, two other studies have detected methoxychlor in human milk samples (Campoy et al. 2001a, 2001b).

Only one study was found in which concentrations of methoxychlor and methoxychlor metabolites were measured in biological fluids at doses at which health effects were being observed in animals (Chapin et al. 1997). Precocious vaginal opening, decreased absolute ovarian weight, decreased absolute and relative uterine weight, and decreased serum FSH levels during estrus were observed in female offspring of rat dams exposed to $5 mg/kg/day from gestation day 14 to postpartum day 7 (Chapin et al. 1997). Exposure of dams was discontinued at postpartum day 7 and pups were dosed directly through postpartum day 42. The 5 mg/kg/day dose used in the Chapin et al. (1997) study is one of the lowest doses at which health effects have been observed; only a few studies (Palanza et al. 1999; Parmigiani et al. 1999; vom Saal et al. 1995; Welshons et al. 1999) have observed health effects at lower doses.

3.8.2 Biomarkers Used to Characterize Effects Caused by Methoxychlor

In animals, the primary target of methoxychlor-induced toxicity is the reproductive system and endocrine-related end points. Reproductive effects include accelerated development of the female reproductive system and delayed development of the male reproductive system in young animals and gonadal atrophy or hypertrophy in adult animals (Bal 1984; Chapin et al. 1997; Gray et al. 1988, 1989, 1999; Hodge et al. 1950; Martinez and Swartz 1991, 1992; Tullner 1961; Tullner and Edgcomb 1962; Welshons et al. 1999). These effects may cause a decrease in fertility in either sex. In some cases, the onset of these effects may be significantly delayed from the time of exposure.

Although none of the above reproductive effects have been observed in human studies or case reports, it is possible that clinical examination of the reproductive organs of exposed humans might reveal some of the changes observed in animals. In exposed human females, a Pap smear may reveal increased cornification of the vaginal epithelium, which is seen in response to methoxychlor exposure in female rats (Gray et al. 1989). Changes in the menstrual cycle might also occur (Gray et al. 1988, 1989; Martinez and Swartz 1991; Tegeris et al. 1966), although this was not observed in a small (n=four females) experimental study in humans (Wills 1969). In exposed human males, measurement of sperm count might reveal decreases which have been reported in exposed male rats (Bal 1984; Gray et al. 1989), although tissue biopsies revealed no adverse effects on the testes of male subjects (n=4) administered doses of up to 2 mg/kg/day for 6 weeks (Wills 1969). The serum levels of certain hormones, including progesterone, prolactin, and testosterone were decreased in methoxychlor-fed animals, therefore, monitoring hormone level changes in the serum of potentially exposed humans might provide a convenient biomarker for exposure to methoxychlor (Bal 1984; Goldman et al. 1986; Gray et al. 1989). However, none of these potential biomarkers have been validated or are specific for exposures to methoxychlor since other estrogenic compounds (other DDT analogs, chlordecone, polychlorinated biphenyls (PCBs), 3,9-hydroxybenz[a]anthracene, and cyclosiloxanes) may also produce similar effects (Bulger and Kupfer 1985).

Additional information concerning biomarkers for effects on the reproductive system can be found in the National Research Council Report on Biologic Markers in Reproductive Toxicology (NAS/NRC 1989), and on the neurological system in the Office of Technology Assessment Report on Identifying and Controlling Poisons of the Nervous System (OTA 1990).

The neurological system may also be affected by methoxychlor. Neurological effects such as decreased locomotor activity, tremors, apprehension, nervousness, and convulsions may be observed following large acute doses of methoxychlor. DDT, a poorly metabolized analogue of methoxychlor, has been shown to cause similar effects (Agency for Toxic Substances and Disease Registry 1994). In people with compromised liver function, neurological signs may occur at lower methoxychlor exposure levels.

3.9 INTERACTIONS WITH OTHER CHEMICALS

The joint toxic actions of binary mixtures of methoxychlor and other pesticides (including organophosphates and other chlorinated hydrocarbons) on acute lethality were examined in mice (Keplinger and Deichmann 1967). After determining oral LD_{50} values for the individual compounds, binary mixtures (with components at equitoxic doses based on LD_{50} values) were administered to the mice at the same dose ranges as the individual compounds. Based on the assumption of joint additive action, an expected LD_{50} value was calculated for each mixture and compared with the observed LD50. The ratio of expected:observed LD_{50} values for the methoxychlor/DDT mixture (0.66) indicated a less than additive action (i.e., mutual protection). Ratios for the methoxychlor/aldrin (0.81), methoxychlor/diazinon (0.82), methoxychlor/malathion (0.84), methoxychlor/toxaphene (0.92), and methoxychlor/aramite (1.25) mixtures were close to one, indicating joint additive action. Ratios for the methoxychlor/parathion (1.51), methoxychlor/delnav (1.96), methoxychlor/dieldrin (2.06), and methoxychlor/chlordane (2.26) mixtures were suggestive of greater than additive joint action (i.e., potentiation or synergism). In rats fed diets containing a mixture of methoxychlor, Aramite, DDT, and thiourea for 2 years, no treatment-related synergisms or antagonisms were observed on mortality, food consumption, weight gain, or tumor incidence (Radomski et al. 1965). Similar results were reported by Deichmann et al. (1967) in rats fed diets containing the same chemical mixture at higher concentrations.

When methoxychlor was administered orally to rats previously treated with carbon tetrachloride, DDT-like neurological symptoms were observed (Lehman 1952). In addition, methoxychlor was found to accumulate in the fat and liver in amounts approximately 15–19 times the levels observed in control animals. Carbon tetrachloride is known to inactivate certain hepatic enzymes (CYPs or cytochrome P450s) which metabolize xenobiotics, thereby increasing their retention. These data suggest that carbon tetrachloride and other chemicals which inhibit the metabolism of methoxychlor may increase the risk of neurotoxicity.

In mice receiving 25 mg/kg/day methoxychlor along with 12 mg/kg/day bromfenvinphos for 6 weeks, inflammatory infiltrations of the liver were larger and denser than observed in animals receiving bromfenvinphos alone (Zaleska-Freljan et al. 1983). Small changes were observed in the kidneys at similar frequencies and severities in both treatment groups. Although it was not investigated in this study, methoxychlor usually does not produce effects of the liver by itself at such low doses. Thus, the results of this study suggest that there may be an interaction between methoxychlor and bromfenvinphos in producing hepatic effects.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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

As noted earlier, neurotoxic effects were reported following exposure of rats with liver damage to methoxychlor (Lehman 1952). This suggests that individuals who have hepatic damage or who otherwise have their *O*-demethylation metabolic pathway compromised may be more susceptible to the DDT-like neurotoxic effects of methoxychlor, but this has not been studied in humans.

There is no information on the effects of methoxychlor in human fetuses, children, or adolescents. By extrapolation from animal studies, developing fetuses and young children may be the most susceptible human population to the reproductive effects of methoxychlor because the estrogenic and possibly antiandrogenic activity of methoxychlor metabolites may interfere with normal development of the reproductive tract. The offspring of nursing mothers exposed to methoxychlor may be susceptible since methoxychlor has been detected in human milk samples (Campoy et al. 2001a, 2001b) and animal studies indicate that methoxychlor and/or its biologically active metabolites can be released in milk (Chapin et al. 1997; Davison et al. 1982). Acute exposures to methoxychlor during critical periods of development may adversely affect the reproductive system. Such effects may not appear until later in life (onset of puberty or adulthood/childbearing).

A more detailed discussion of children's susceptibility can be found in Section 3.7 Children's Susceptibility.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

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

Ellenhorn MJ, Barceloux DG. 1988. Medical toxicology: Diagnosis and treatment of human poisoning. New York, NY: Elsevier, 1078–1080.

Haddad LM, Winchester JF. 1990. Clinical management of poisoning and drug overdose. Second edition. Philadelphia, PA: W.B. Sanders Company, 1084–1085.

3.11.1 Reducing Peak Absorption Following Exposure

Data regarding the reduction of methoxychlor absorption in humans after inhalation exposure were not located. Oral absorption of methoxychlor can be reduced with gastric lavage, activated charcoal, sodium sulfate, and cathartics (Ellenhorn and Barceloux 1988; Haddad and Winchester 1990). Since many commercial formulations of organochlorine insecticides contain organic solvents, emesis is not usually recommended due to the hazard of solvent aspiration (Ellenhorn and Barceloux 1988). In addition, oils should usually not be used as cathartics since they may enhance the absorption of methoxychlor (Haddad and Winchester 1990).

Dermal absorption of methoxychlor can be reduced by removing contaminated clothing and thoroughly washing the exposed skin with a mild soap (Ellenhorn and Barceloux 1988; Haddad and Winchester 1990). Oils should not be used as a cleansing agent since they may facilitate dermal absorption (Haddad and Winchester 1990).

3.11.2 Reducing Body Burden

Since animal studies indicate that methoxychlor is rapidly metabolized and cleared from the body, methods for reducing body burden are not expected to be especially effective in reducing human exposures. Activated charcoal is sometimes administered in serial doses to minimize enterohepatic recirculation of persistent chemicals (Ellenhorn and Barceloux 1988). Although a study in rats did establish that methoxychlor undergoes enterohepatic recirculation, the extent to which this occurred was minimal (5–10% of the dose) (Weikel 1957). Thus, it is not likely that the administration of activated charcoal will facilitate the excretion of methoxychlor to any significant extent, but this has not been studied.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Methods for managing the seizures and convulsions which may occur following large exposures to methoxychlor or other organochlorine pesticides include the intravenous administration of Diazepam, Valium, or phenobarbital. Patients should be monitored for the possibility of cardiopulmonary arrest (Ellenhorn and Barceloux 1988; Haddad and Winchester 1990).

Methods for interfering with the reproductive effects of methoxychlor were not located. These effects are presumed to be due to the estrogenic action of the phenolic metabolites of methoxychlor. Therefore, it would seem that by blocking the metabolism of methoxychlor to these derivatives, the reproductive effects would also be blocked. However, such an approach might actually increase the risk of other effects that are due to the parent compound. For example, limited data suggest that the neurotoxicity of methoxychlor is most likely due to the parent molecule (Lehman 1952) which may prevent the deactivation of the sodium gate after neuron activation and membrane depolarization (Brown et al. 1981; Coats 1990; Wu et al. 1975). More information is needed on the mechanism of action of methoxychlor in producing effects on the reproductive system (direct or indirect action) before methods for interfering with the mechanism can be determined.

3.12 ADEQUACY OF THE DATABASE

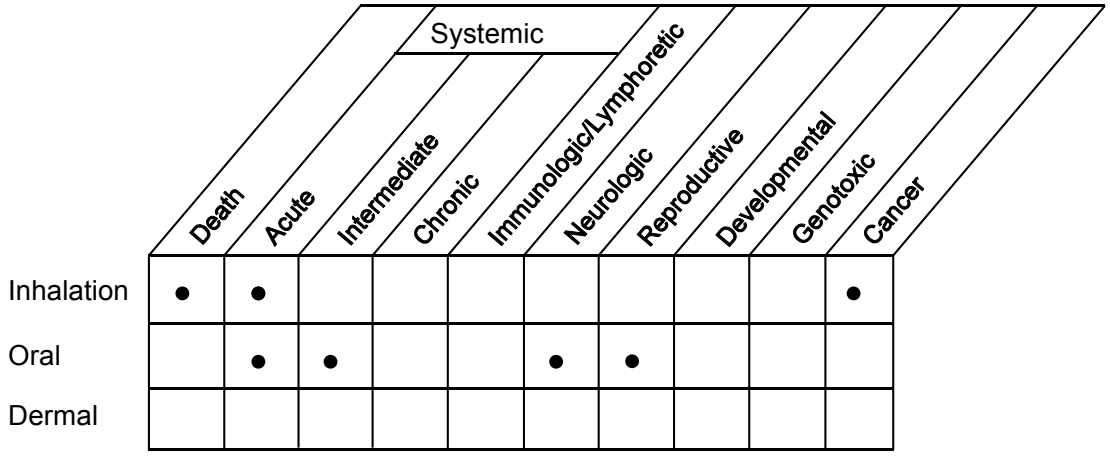
Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of methoxychlor is available. Where adequate information is not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

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

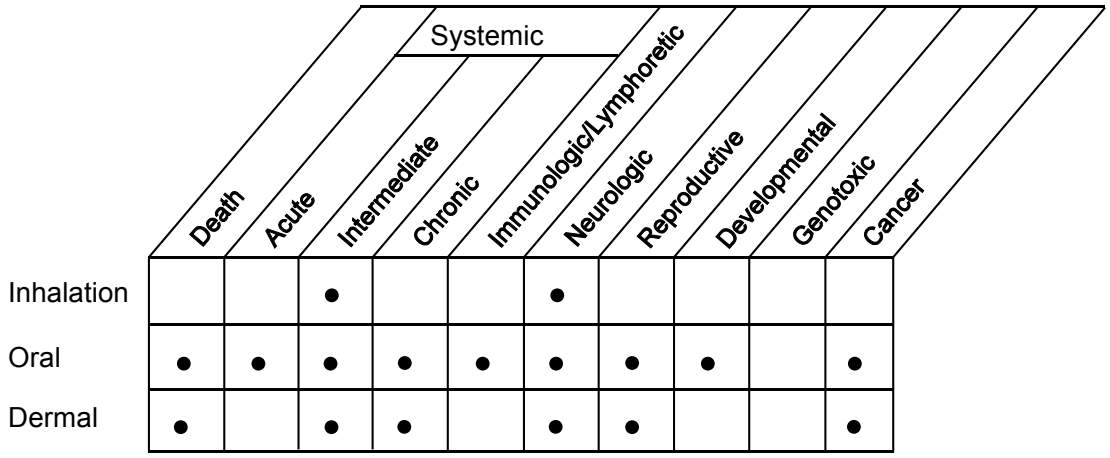
3.12.1 Existing Information on Health Effects of Methoxychlor

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to methoxychlor are summarized in Figure 3-4. The purpose of this figure is to illustrate the existing information concerning the health effects of methoxychlor. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be

Figure 3-4. Existing Information on Health Effects of Methoxychlor



Human



Animal

● Existing Studies

interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989b), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

As seen in Figure 3-4, information in humans is limited to a single clinical study that investigated systemic and reproductive effects following intermediate-duration oral exposure to methoxychlor. A single epidemiological study was located regarding cancer and occupational exposure to methoxychlor. Oral studies in animals provide information on death, systemic effects for acute, intermediate, and chronic exposures, neurological effects, developmental effects, reproductive effects, and cancer. Limited data are available from animal studies regarding systemic effects for intermediate and chronic exposures, neurological effects, and cancer for dermal exposure to methoxychlor. Limited data are also available for intermediate-duration exposures and neurological effects for inhalation exposure to methoxychlor.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. No data were located regarding the acute toxicity of methoxychlor in humans after inhalation, oral, or dermal exposure. Likewise, no data were located regarding acute toxicity in animals following inhalation or dermal exposure.

Acute oral studies in mice and rats identified LD_{50} values of 2,900–7,000 mg/kg/day (Coulston and Serrone 1969; Hodge et al. 1950; Smith et al. 1946). The cause of death was not determined in these studies. Acute oral exposure of animals to sublethal doses of methoxychlor indicate that reproductive development is the most sensitive target of methoxychlor (Cummings and Gray 1987, 1989; Cummings and Perreault 1990; Gray et al. 1989; Khera et al. 1978; Kincaid Enterprises 1986; Martinez and Swartz 1991; Parmigiani et al. 1998; Tullner 1961; vom Saal et al. 1995; Welshons et al. 1999). Precocious vaginal opening was noted in female rats administered oral doses of 25 mg/kg/day (Gray et al. 1989). The data from animal studies are insufficient to derive an acute oral MRL. A number of candidate MRL studies were considered. Although Gray et al. (1989) observed precocious vaginal opening (early puberty) in rats at 25 mg/kg/day, this study did not test doses as low as the current intermediate-duration MRL study (Chapin et al. 1997). The same effect, precocious vaginal opening, was demonstrated at 5 mg/kg/day administered from gestation day 14 to postnatal day 42 (Chapin et al. 1997), so there is some question as to whether the premature puberty would have occurred at a lower acute dose than the

25 mg/kg/day dose observed in the Gray et al. (1989) study. There were several hypothesis-generating studies at extremely low doses that were not definitive enough to use for MRL derivation; they are discussed further in both the Developmental Toxicity data needs subsection and Appendix A. These acute oral studies identified LOAEL values of 0.02–1.8 mg/kg/day for reproductive/developmental effects in mice (Parmigiani et al. 1998; vom Saal et al. 1995; Welshons et al. 1999). Further oral studies that better define the threshold dose for reproductive effects in rats, mice, and in other species would be useful.

Studies which identify the inhalation or dermal exposure levels which produce reproductive and developmental effects in animals may be valuable for predicting effects in persons acutely exposed to methoxychlor. Studies examining the effects of dermal and inhalation exposure to methoxychlor in other organ systems would also prove useful. Better pharmacokinetic information on the extent of inhalation and dermal absorption would help determine the importance of more acute duration toxicological data from these routes. Data from farmers and pesticide workers who have been exposed acutely to methoxychlor via these routes would also be useful to determine whether the acute effects observed in animals also occur in humans.

Studies are also needed that examine the effects of exposure to pesticide mixtures that contain methoxychlor.

Intermediate-Duration Exposure. Data regarding the toxicity of methoxychlor in humans exposed via the inhalation and dermal routes for intermediate durations are lacking. A very small study in humans (four males and four females) reported that oral exposure to 2 mg/kg/day for 6 weeks caused no adverse effects on the liver, small intestines, bone marrow, or testes (Wills 1969). A number of oral studies in animals indicate that the reproductive system is a target of methoxychlor toxicity. In females, reported effects of intermediate-duration oral exposure to methoxychlor include: precocious vaginal opening in rats exposed to 25–100 mg/kg/day (Gray et al. 1989; Harris et al. 1974); increased vaginal cornification and decreased number of vaginal smears with leukocytes in rats exposed to 50–400 mg/kg/day (Chapin et al. 1997; Gray et al. 1988, 1989); effects on uterine weight in rats and pigs exposed to 150–1,000 mg/kg/day (Chapin et al. 1997; Harris et al. 1974; Tegeris et al. 1966); atrophy and degeneration of the ovaries in mice and rats exposed to 25–400 mg/kg/day (Bal 1984; Chapin et al. 1997; Gray et al. 1988, 1989; Harris et al. 1974; Martinez and Swartz 1991); persistent vaginal cornification in rats and mice, and a lack of estrus in pigs exposed to 25–1,000 mg/kg/day (Gray et al. 1988; Harris et al. 1974; Martinez and Swartz 1991; Tegeris et al. 1966); decreased mating frequency in rats exposed to

60–150 mg/kg/day (Harris et al. 1974); decreased fertility in rats exposed to 35.5–200 mg/kg/day (Bal 1984; Chapin et al. 1997; Culik and Kaplan 1976; Gray et al. 1989; Harris et al. 1974; Kincaid Enterprises 1986); and altered hormone levels in the serum or pituitary of rats exposed to 100–400 mg/kg/day (Cummings and Gray 1989; Gray et al. 1988). A single multigeneration study reported decreased fertility in female rats of the second and third generations exposed to 92 but not to 18 mg/kg/day methoxychlor (Haskell Laboratories 1966). In males, the effects of intermediate-duration oral exposure to methoxychlor include: delayed preputial separation (an indicator of puberty) in rats exposed to 100–400 mg/kg/day (Gray et al. 1989, 1999); decreased testes weight, ventral prostate weight, and caudal epididymal sperm count in rats exposed to 50–1,400 mg/kg/day (Bal 1984; Gray et al. 1989, 1999; Hodge et al. 1950; Shain et al. 1977; Tullner and Edgcomb 1962); decreased mating frequency in rats exposed to 60 mg/kg/day (Harris et al. 1974) or 200–400 mg/kg/day (Gray et al. 1999); decreased fertility in rats and mice exposed to 60–100 mg/kg/day (Gray et al. 1989; Harris et al. 1974; Wenda-Rozewicka 1983); and altered pituitary and serum hormone levels in rats exposed to 25–100 mg/kg/day (Goldman et al. 1986; Gray et al. 1989). These types of effects are typical of exposures to estrogen, and are consistent with the observation that phenolic metabolites and contaminants of methoxychlor exhibit estrogenic activity *in vitro* and *in vivo* (Bulger et al. 1985).

These data are sufficient to derive an MRL for intermediate-duration oral exposures. A LOAEL of 5 mg/kg/day was identified for accelerated onset of puberty (i.e., precocious vaginal opening) in immature female rats exposed to methoxychlor *in utero*, during lactation, and after weaning (Chapin et al. 1997). However, a NOAEL was not identified in this study. Further oral studies would be helpful to: define the threshold dose for reproductive effects and narrow down the critical developmental window during which this effect can be produced.

Data regarding inhalation and dermal exposures to methoxychlor are limited. They include permanent neurological effects (Harell et al. 1978) and death (Ziem 1982) following exposure to pesticide mixtures containing methoxychlor and other pesticides; partial paralysis and disseminated nodules in the brains of rabbits exposed to methoxychlor dermally or via inhalation (Haag et al. 1950; the authors attributed these effects to a disease endemic to rabbits that was potentiated by exposure to methoxychlor); fatty degenerative changes of the liver in rabbits (Haag et al. 1950); death in rabbits (Haag et al. 1950); and uterine stimulation in mice following exposure to a pesticide mixture containing methoxychlor (Tullner 1961). No deaths or histopathological changes in the skin were noted in mice receiving intermittent dermal exposure to methoxychlor for 2 years (Hodge et al. 1966). Effects on the reproductive system have not been adequately investigated following inhalation or dermal exposures to methoxychlor. Studies

that identify reliable NOAELs and LOAELs for reproductive effects in animals exposed to methoxychlor via inhalation for intermediate-duration exposures would be valuable for predicting effect levels in humans occupationally exposed to methoxychlor. Information from farmers, pesticide workers, or persons who live near pesticide facilities or hazardous waste sites who are exposed to methoxychlor for intermediate-durations may serve to indicate whether the effects observed in animals also occur in humans at similar response levels. Better pharmacokinetic information on the extent of inhalation and dermal absorption would help determine the importance of more intermediate duration toxicological data from these routes.

The results of several studies indicated that methoxychlor exposure might result in alterations of the vitamin A content of the liver (Davison and Cox 1976; Harris et al. 1974), while others found no effect (Cecil et al. 1974; Phillips and Hatina 1972). An *in vitro* assay showed that methoxychlor itself does not interact with human transthyretin (a carrier protein for vitamin A and thyroid hormones), but did not include experiments with a microsomal activation system to determine the interaction of methoxychlor metabolites with transthyretin (Van den Berg et al. 1991).

Chronic-Duration Exposure and Cancer. There are no data regarding the chronic toxicity of methoxychlor in humans for inhalation, oral, or dermal exposures. Chronic oral exposure studies have been performed in animals (Deichmann et al. 1967; NCI 1978). No histopathological changes were detected in the lungs, gastrointestinal tract, liver, kidneys, brain, or pituitary of Osborne-Mendel rats chronically exposed to 77 mg/kg/day recrystallized methoxychlor (Deichmann et al. 1967). Similarly, histopathological changes were not observed in the lungs, heart, gastrointestinal tract, bone, liver, kidneys, skin, lymph nodes, thymus, brain, testes, ovaries, and uterus of B6C3F1 mice chronically exposed to 599 mg/kg/day methoxychlor or Osborne-Mendel rats chronically exposed to 107 mg/kg/day (NCI 1978). No histopathological changes were noted in the skin of C3H/AnF mice dermally exposed to 10 mg/day recrystallized methoxychlor, 1 time/week, for 2 years (Hodge et al. 1966). A single multigeneration study reported decreased fertility in female rats exposed to 92 mg/kg/day, but not to 18 mg/kg/day, and not in males exposed to up to 79 mg/kg/day (Haskell Laboratories 1966). These studies do not support the derivation of a chronic oral MRL, since most of the studies did not evaluate reproductive function, which is likely to be the most sensitive target. Derivation of a chronic oral MRL based on the NOAEL of 18 mg/kg/day (Haskell Laboratories 1966), would result in a value greater than MRLs derived for acute and intermediate durations, and therefore is not possible. Additional multigeneration studies in animals which investigate the chronic toxicity of methoxychlor with particular reference to reproductive and developmental effects in both sexes from inhalation, oral, or dermal

exposures to methoxychlor would provide valuable information. This information would be useful in establishing reliable NOAELs and LOAELs for the purposes of estimating risk to humans who live near hazardous waste sites or are occupationally exposed to methoxychlor. Epidemiological studies on agricultural or pesticide workers exposed to methoxychlor would also serve to indicate whether the effects observed in animals also occur in humans. Since animal studies indicate that the offspring of females that are exposed to methoxychlor either prior to or during pregnancy may also be affected, follow-up studies on the offspring of these exposed human populations may also prove to be valuable.

One epidemiological study reported that the risk of leukemia was slightly increased in farmers exposed to methoxychlor (Brown et al. 1990). Further epidemiological studies on agricultural and pesticide workers would be valuable to determine if a relationship between cancer and methoxychlor exposure exist. No increased tumor incidence was detected in the lungs, gastrointestinal tract, liver, kidneys, brain, or pituitary of Osborne-Mendel rats chronically exposed to 77 mg/kg/day recrystallized methoxychlor (Deichmann et al. 1967). Similarly, increased tumor incidences were not observed in the lungs, heart, gastrointestinal tract, bone, liver, kidneys, skin, lymph nodes, thymus, brain, testes, ovaries, and uterus of B6C3F1 mice chronically exposed to 599 mg/kg/day methoxychlor or Osborne-Mendel rats chronically exposed to 107 mg/kg/day (NCI 1978). No increased tumor incidence was noted in the skin of C3H/AnF mice dermally exposed to 10 mg/day recrystallized methoxychlor, 1 time/week, for 2 years (Hodge et al. 1966). Since prolonged exposure to elevated levels of estrogenic compounds have been linked to cancers in the reproductive tract of women and their female offspring, multigenerational oral animal studies focused on evaluation of tumor incidences in estrogen-sensitive tissues for parental and subsequent generations may provide information needed to assess the possible carcinogenicity of methoxychlor in humans.

Data regarding chronic-duration inhalation and dermal exposures are very limited. They include a possible link to leukemia seen in a single very small epidemiological study of farmers exposed via inhalation (Brown et al. 1990); and a lack of gross or histopathological changes in the skin of mice and lack of tumor formation in mice (gross examination) exposed dermally for 2 years (Hodge et al. 1966). Effects on the reproductive system have not been adequately investigated following chronic inhalation or dermal exposures to methoxychlor. Studies that identify reliable NOAELs and LOAELs for reproductive effects in animals exposed to methoxychlor via inhalation for chronic-duration exposures would be valuable for predicting effect levels in humans occupationally exposed to methoxychlor. Additional information from farmers, pesticide workers, or persons who live near pesticide facilities or hazardous waste sites who are exposed to methoxychlor for chronic durations may serve to indicate whether the

effects observed in animals also occur in humans at similar response levels. Better pharmacokinetic information on the extent of inhalation and dermal exposure would help determine the importance of more chronic-duration toxicological data from these routes.

Genotoxicity. The mutagenicity of methoxychlor has been well studied *in vitro* and there are sufficient data to indicate that methoxychlor is not mutagenic in prokaryotic systems (Grant et al. 1976; Mortelmans et al. 1986; Probst et al. 1981; Waters et al. 1980). However, *in vitro* studies in mammalian cells have yielded conflicting results. DNA synthesis (thymidine incorporation) was inhibited at high methoxychlor concentrations and stimulated at low methoxychlor concentrations in cultured bovine uterine epithelial and stromal cells (Tiemann et al. 1996). Several studies in mouse lymphoma cells with metabolic activation indicate that methoxychlor can induce mutations at the TK locus (Caspary et al. 1988; Mitchell et al. 1988; Myhr and Caspary 1988). However, mutations of this nature were not noted in human lymphoma cells (Caspary et al. 1988). Further *in vitro* studies using human lymphoma cells would confirm whether an important species difference exists between human and mouse lymphoma cells for this end point. Single-stranded DNA breaks were not induced in human or rat testicular cells by methoxychlor (Bjørge et al. 1996b). Several *in vivo* studies have shown that intraperitoneal exposure of mice does not increase the frequency of chromosomal aberrations (Degraeve and Chollet 1984) or single-stranded DNA breaks (Umegaki et al. 1993), and that methoxychlor does not increase sex-linked recessive lethal mutations in *Drosophila melanogaster* (Benes and Sram 1969; Valencia 1981; Waters et al. 1980). Additional *in vivo* studies that utilize human lymphocytes from workers or farmers exposed to methoxychlor would be useful in evaluating whether chromosomal aberrations develop in humans exposed to methoxychlor.

Reproductive Toxicity. There is no information regarding the reproductive effects of methoxychlor in humans after inhalation or dermal exposures, or in animals after inhalation or dermal exposures. In a single very small human study (16 males and 16 females), tissue biopsies revealed no adverse effects on the testes of male subjects (3 males at 2 mg/kg/day) or on the menstrual cycles of female subjects (4 per exposure level) administered doses of up to 2 mg/kg/day for 6 weeks (Wills 1969).

However, oral studies in animals indicate that the reproductive system, particularly the developing reproductive system, is the primary target of methoxychlor toxicity. In females, oral exposure to methoxychlor caused changes in pituitary and serum hormone levels in rats (Cummings and Gray 1989); early onset of puberty in rats (Anderson et al. 1994; Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974); atrophy of the ovaries in rats and mice (Anderson et al. 1994; Bal 1984; Gray et al. 1988, 1989;

Harris et al. 1974; Martinez and Swartz 1991), abnormal estrus in rats, mice, and pigs (Gray et al. 1988; Martinez and Swartz 1991; Tegeris et al. 1966); an enlarged uterus in rats and pigs (Cummings 1993; Harris et al. 1974; Tegeris et al. 1966); decreased fertility in rats (Bal 1984; Chapin et al. 1997; Culik and Kaplan 1976; Gray et al. 1989; Harris et al. 1974; Haskell Laboratories 1966; Kincaid Enterprises 1986); and decreased number of corpora lutea, decreased number of live pups, and increased resorptions in mice (Swartz and Eroschenko 1998). In addition, dermal exposure to methoxychlor had a uterotrophic effect in immature female mice (Tullner 1961) and injection with methoxychlor accelerated the onset of puberty in newborn female mice (Martinez and Swartz 1991).

In males, methoxychlor exposure produced changes in pituitary and serum hormone levels in rats (Fail et al. 1994; Goldman et al. 1986; Gray et al. 1989); delayed puberty in rats (Anderson et al. 1994; Chapin et al. 1997; Gray et al. 1999); testicular atrophy in rats (Bal 1984; Gray et al. 1999; Hodge et al. 1950; Shain et al. 1977; Tullner and Edgcomb 1962) and mice (Wenda-Rozewicka 1983); decreased prostate weight in rats (Chapin et al. 1997; Shain et al. 1977; Tullner and Edgcomb 1962); decreased fertility in rats and mice (Gray et al. 1989, 1999; Harris et al. 1974; Wenda-Rozewicka 1983); and altered sexual behavior in mice and rats (Gray et al. 1999; Parmigiani et al. 1999; vom Saal et al. 1995). In addition, testicular atrophy was noted in newborn male mice intraperitoneally injected with methoxychlor (Martinez and Swartz 1991). A study reporting early onset of puberty (precocious vaginal opening) in rats (Chapin et al. 1997) has been utilized to derive an intermediate duration oral MRL value. However, no NOAEL was identified in this study. Further oral studies that better define the threshold dose for reproductive effects in rats, mice, and in other species, and examine the similarities and differences in response between species would be useful. Further data needs relating to the developing reproductive system are discussed in the Developmental Toxicity subsection.

In vitro competitive binding assays have shown that methoxychlor itself does not bind to the estrogen receptor (Bulger et al. 1978a, 1978b; Ousterhout et al. 1981), but that the mono-hydroxy and bis-hydroxy derivatives of methoxychlor do bind to the receptor and cause nuclear translocation. An additional study has shown that bis-hydroxy methoxychlor binds to the ERα and ERβ subtypes of the estrogen receptor (Gaido et al. 1999). However, not all effects resulting from methoxychlor exposure mimic those of estrogen. Some effects (delayed preputial separation, decreased pituitary size, and a lack of effect on serum LH, prolactin, and testosterone) could be explained by androgen antagonism (Gray et al. 1999). Only one study has investigated the potential androgen antagonism of methoxychlor and its metabolites; this experiment was not a direct receptor binding assay. The methoxychlor metabolite bis-hydroxy methoxychlor (HPTE) was a weak AR antagonist of dihydrotestosterone in HepG2 human hepatoma cells

transiently transfected with the human androgen receptor and a reporter gene linked to an androgen responsive promoter; methoxychlor showed even less antagonism in this experiment (Maness et al. 1998). While it seems clear that methoxychlor metabolites mediate at least some of their effects via estrogen and androgen receptors, it is not entirely clear whether the effects are mediated directly in the tissues in which the receptor binding takes place, or if some effects are mediated indirectly through feedback mechanisms with the hypothalamus and pituitary and resulting altered hormone levels. Changes in pituitary prolactin, TSH, and FSH levels have been noted in rats exposed to methoxychlor (Goldman et al. 1986; Gray et al. 1988, 1989). Studies are needed that examine the effects of methoxychlor on the hypothalamus-pituitary-gonadal axis and all related hormones and the distribution of the ER and AR within the axis to better understand the varied effects of methoxychlor on development and reproduction.

Induction of two estrogen-responsive genes (lactoferrin [LF] and glucose-6-phosphate dehydrogenase, [G6PD]) in uteri of mice was not attenuated by an absence of ERα (in ERα knockout mice) or by an ER inhibitor (which would eliminate ERβ availability) (Ghosh et al. 1999). This suggests that not all of the effects from methoxychlor are mediated through a known ER. Additional studies are needed investigating this effect and incorporating different estrogen-responsive endpoints in mice and rats to clarify this alternative mechanism resulting in estrogen-like effects. There is also evidence in a non-mammalian species (frog) that methoxychlor acts via a non-ER mechanism (Pickford and Morris 1999). Methoxychlor caused a highly significant inhibition of progesterone-induced germinal vesicle breakdown (GVBD, necessary for oocyte maturation), while estradiol and HPTE had no effect. Studies investigating the mechanism of this effect of methoxychlor in frogs might also provide important information.

Animal studies which describe the long-term consequences of methoxychlor exposure during gestation, early postnatal life, or adulthood on reproductive parameters and function in both sexes would also be useful. Animal studies are also needed to identify reliable NOAELs and LOAELs for the inhalation and dermal routes of exposure. Studies in farmers, pesticide workers, or persons living at or near hazardous waste sites who are exposed to methoxychlor may serve to indicate whether the effects observed in animals also occur in humans. More information is needed regarding which metabolites, contaminants, and degradation products of methoxychlor are estrogenic, and therefore, of health concern to the public.

One study reported a decrease in the percentage of male offspring of rabbit dams exposed orally to methoxychlor (Kincaid Enterprises 1986). Other studies have not reported this effect. It is unclear what the mechanism for this alteration in sex ratio is. The same study also reported increased incidences of

dead and resorbed fetuses (Kincaid Enterprises 1986). It would be interesting to know if there is a sex-specific fetotoxic effect of methoxychlor or if sex ratio is really affected by methoxychlor.

Developmental Toxicity. No data were located regarding the developmental effects of methoxychlor in humans for inhalation, oral, or dermal routes of exposure or in animals for inhalation and dermal routes of exposure. Although oral is by far the predominant route of exposure, studies focusing on developmental effects resulting from inhalation and dermal exposure may be necessary to assess the possible impact of such exposures.

Oral studies in rats and mice indicate that exposure to methoxychlor during gestation can produce decreased number of implants, decreased fetal body weight, increased frequency of dead or resorbed fetuses, or fetuses with wavy ribs (Chapin et al. 1997; Culik and Kaplan 1976; Khera et al. 1978; Kincaid Enterprises 1986; Swartz and Eroschenko 1998).

Methoxychlor can also cause numerous functional changes in the reproductive system of both adult and developing animals. Methoxychlor accelerated the onset of puberty in female rats exposed for intermediate durations *in utero*, during lactation, and/or postweaning (Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974; Swartz and Corkern 1992). Additional studies examining this effect following shorter exposures, during gestation, and beginning at weaning (separate experiments), at doses at or below the lowest LOAEL (5 mg/kg/day in Chapin et al. 1997) are necessary to more closely define the critical interval and lowest level of exposure at which this occurs. Chapin et al. (1997) observed a reduction in testes, epididymis, seminal vesicle, and ventral prostate weights in male offspring of rats exposed to 50 or 150, but not 5 mg/kg/day from gestation day 14 through postnatal day 7, followed by direct exposure of the pups through postpartum day 42. Studies examining these effects following exposures for different durations and during specific life stages, including gestation and pre- and post-weaning, are also needed to more closely define the critical interval and lowest level of exposure at which this occurs. Decreased performance in neurobehavioral tests in female rats (increased locomotor activity, decreased click response, and decreased hindlimb grip strength) and male rats (increased handling reactivity, decreased click response, and approach stimulus) was seen following exposure to 5–150 mg/kg/day for intermediate durations *in utero*, during lactation, and/or postweaning (Chapin et al. 1997). However, there was no dose-response relationship for these effects. Methoxychlor caused a slight (but not significant) decrease in testes weight and sperm counts of male rats exposed during gestation and lactation (Gray et al. 1989).

There were several hypothesis-generating studies using extremely low doses that were not definitive enough to use for MRL derivation. Adult male mice exposed *in utero* to 0.02 mg/kg/day methoxychlor had increased prostate weights compared to vehicle controls (Welshons et al. 1999). Male mice exposed *in utero* to 0.02 mg/kg/day methoxychlor exhibited increased urine-marking behavior when placed in a new territory (vom Saal et al. 1995). A LOAEL of 1.8 mg/kg/day was observed for aggressive behavior (infanticide) in male mice exposed *in utero* toward an unrelated pup (Parmigiani et al. 1998), and a LOAEL of 0.02 mg/kg/day was observed for decreased aggression of young male mice exposed *in utero* toward male siblings (Palanza et al. 1999). Further oral studies that better define the threshold dose for developmental effects in rats, mice, and in other species, and examine the similarities and differences in response between species would be useful. Specifically, additional studies in mice using exposure levels encompassing the LOAEL of 0.02 mg/kg/day identified in the Welshons et al. (1999) study, and studies in species other than mice, may prove very useful. The Welshons et al. (1999) results need to be replicated using a larger number of exposed dams appropriate for a developmental toxicity study (Tyl 2000), testing all males in all litters for prostate size (Elswick et al. 2000a, 2000b), including estradiol and/or DES as simultaneous positive controls, and perhaps including more doses of methoxychlor above and below 0.02 mg/kg/day. A comparative study also needs to be done quantifying how much prostate weight in adult mice varies as a function of intrauterine position relative to female littermates and the estrogen that diffuses from them (Nonneman et al. 1992; Timms et al. 1999; vom Saal 1989); the results of such a study would aid in interpreting the biological significance of the Welshons et al. (1999) findings. Intrauterine position can be identified by caesarian delivery just prior to natural parturition. Additional studies are needed examining the effects of very low doses of methoxychlor on neurobehavioral parameters in mice and other species. The behavioral effects seen in male mice following *in utero* exposure need to verified and investigated further to determine their mechanism and their significance to human health.

New studies would be helpful to address the factors which make vom Saal et al. (1995), Parmigiani et al. (1998), and Palanza et al. (1999) difficult to interpret. Male mice exposed *in utero* to 0.02 mg/kg/day methoxychlor exhibited increased urine-marking behavior when placed in a new territory (vom Saal et al. 1995). This study was not used for MRL derivation because only two male pups/litter were tested and apparently each was only tested one time; the accuracy of the assessment of the test and how reproducible the results are were not determinable; and there was no indication of what, if any, statistical methods were used to evaluate the results. A LOAEL of 1.8 mg/kg/day for aggressive behavior (infanticide) in male mice exposed *in utero* toward an unrelated pup (Parmigiani et al. 1998) was not used for MRL derivation because only two male pups/litter were tested; there was no dose-response relationship, and no effect was

seen with DES, a positive estrogenic control. Similarly, a LOAEL of 0.02 mg/kg/day for decreased aggression of young male mice exposed *in utero* toward male siblings at postpartum day 39 but not at postpartum day 54 (Palanza et al. 1999) was not a suitable basis for an MRL because there was no dose-response relationship and the effect was transient.

There is puzzling, limited evidence that exposure of adult female animals may cause effects in subsequent, unexposed offspring. Female offspring (F1a) of mice dams exposed during gestation (gestation days 6–15) to 0, 50, or 100 mg/kg/day methoxychlor showed no change in day of vaginal opening and no change in ovarian weight (Swartz and Corkern 1992), although methoxychlor-exposed female pups had an increase in atretic follicles. Female mice exposed only via lactation and females exposed during gestation and lactation showed similar results. However, female offspring (F1b) from a second unexposed pregnancy showed a slight, but statistically significant, advance in the day of vaginal opening (25.0, 23.9, and 23.2 days for the control, 50–100-mg/kg/day groups, respectively). Since methoxychlor is eliminated rapidly from the body (Kapoor et al. 1970), the above data imply that methoxychlor might cause a long-term alteration in reproductive physiology that can affect subsequent offspring. More studies looking at effects on offspring of previous exposures to dams are needed in order to verify this effect. Upon confirmation, subsequent studies would be required to elucidate the mechanism(s) by which this effect on the offspring occurred.

Additional animal studies that identify reliable NOAELs and LOAELs for developmental effects for inhalation and dermal exposures would be helpful for extrapolating data to human exposures. Information on the offspring of female agricultural workers, pesticide workers, or residents who live at or near a hazardous waste site who may be exposed to methoxychlor may indicate whether the developmental effects observed in animals also occur in humans.

Immunotoxicity. No data were located regarding the immunological effects of methoxychlor in humans following inhalation, oral, or dermal exposure to methoxychlor, or in animals following inhalation or dermal exposure to methoxychlor. The immunotoxicity of methoxychlor has not been well investigated in animals following oral exposure to methoxychlor. A single study reported no histopathological changes in the thymus, spleen, and lymph nodes of mice and rats following chronic oral exposure to methoxychlor (NCI 1978). Male rats, but female rats, exposed to 5 or 50 mg/kg/day during gestation and maturation showed a decrease in plaque-forming cells/spleen, indicating a possible attenuation of primary immune responses (Chapin et al. 1997). Thymus weights were decreased in rats exposed to 50–150 mg/kg/day during prenatal and postnatal development (Chapin et al. 1997) and in

adult male rats exposed to 500 mg/kg/day (Okazaki et al. 2001). No histological data were provided. Spleen weight, splenic natural killer cell activity, splenic lymphoproliferative response, and splenic cell-surface phenotypes did not differ from controls (Chapin et al. 1997). These results provide only limited data that methoxychlor may influence immune responses. The lack of any immune effects in females or on other immune end points in males that are related to plaque-forming cell numbers led Chapin et al. (1997) to suggest that the observed effect may have been anomalous. Additional studies examining more sensitive functional immune end points following oral exposure would help to clarify the impact of methoxychlor on the immune system.

Neurotoxicity. Data regarding the neurological effects of methoxychlor in humans after inhalation, oral, or dermal exposure are limited to a single case report which described the development of deafness and peripheral neuropathies following inhalation exposure to a mixture of pesticides; no causality regarding methoxychlor could be determined (Harell et al. 1978). Based on its structural similarity to DDT, methoxychlor may be expected to have the potential to produce DDT-like neurological effects (tremors, convulsions) in animals and humans at very high acute doses. Effects of this nature have been reported following oral exposure to high doses in rats and dogs (Cannon Laboratories 1976; Tegeris et al. 1966) and in rats whose hepatic metabolic capacity was diminished by coadministration of carbon tetrachloride (Lehman 1952), but are not usually associated with lower exposures to methoxychlor. Neurological symptoms were also observed in rabbits following inhalation and dermal exposure, but since these effects were similar to those produced by a disease which is endemic to rabbits, these effects may not have been treatment related (Haag et al. 1950). Since these neurological effects of methoxychlor are probably due to the parent compound, additional studies which investigate species differences in the capacity to metabolize methoxychlor and differences in sensitivity to the neurotoxic effects of methoxychlor may help in evaluating neurotoxic risk to humans. Behavioral effects were noted in male and female rats and mice administered methoxychlor (Chapin et al. 1997; Gray et al. 1988; Parmigiani et al. 1998; vom Saal et al. 1995), which unlike the neurological effects discussed above, are most likely due to one or more metabolites of methoxychlor which exhibit estrogenic activity (Bulger et al. 1985). Additional studies that further characterize which neurotoxic effects are caused by methoxychlor and which are caused by its metabolites would be useful. Information obtained from humans accidentally exposed to large doses of methoxychlor may also serve to reveal whether the neurotoxic effects observed in animals also occur in humans.

Epidemiological and Human Dosimetry Studies. A single epidemiological study was located which identified a possible association between exposure to methoxychlor and leukemia in farmers (Brown et al. 1990). However, the exposed and control groups were small, and the increase only marginally significant. Moreover, exposure levels were not quantified and exposure to other chemicals probably occurred, so confidence in the study is low. Nevertheless, the possible association detected in this study suggests that further investigations should be conducted on agricultural workers, pesticide formulators, and pesticide applicators who are frequently exposed to methoxychlor. Other populations which could also be studied include residents at or near areas that undergo frequent aerial spraying of methoxychlor, areas at or near methoxychlor production facilities, and areas at or near hazardous waste sites that contain methoxychlor. Animal studies indicate that reproductive and developmental effects of methoxychlor are end points that are of primary concern. Therefore, epidemiological studies which focus on reproductive and developmental end points would also be valuable.

Biomarkers of Exposure and Effect

Exposure. The presence of methoxychlor and its phenolic metabolites in biological media may be used as specific biomarkers for exposure to methoxychlor. Methoxychlor has been detected in human breast milk samples from women living in rural and urban areas (Campoy et al. 2001a, 2001b). Methoxychlor and its metabolites have also been measured in the milk of lactating rats and were found to concentrate in the milk with increasing methoxychlor exposure level (Chapin et al. 1997). Plasma levels of methoxychlor and its metabolites (mono-hydroxy methoxychlor and di-hydroxy methoxychlor) in suckling rat pups have also been observed to increase with increasing dose of methoxychlor (Chapin et al. 1997). However, because of the relatively rapid clearance of these metabolites, measurements would probably only be useful in detecting recent exposures (within the past 24 hours). Measurements of methoxychlor and metabolite levels in relevant biological fluids in animal studies would be helpful in establishing a quantitative correlation between exposure level, body burden, and effect level. These correlations might prove useful in assessing risk in human exposures. The Chapin et al. (1997) study is the only study in which concentrations of methoxychlor and methoxychlor metabolites were measured in biological fluids at doses at which health effects were being observed in animals.

Effect. As discussed earlier, the primary effects of methoxychlor are on reproduction. In exposed female rodents, methoxychlor exposure may affect the estrus cycle (Gray et al. 1988; Harris et al. 1974; Martinez and Swartz 1992; Okazaki et al. 2001; Tegeris et al. 1966), serum hormone levels (Cummings and Gray 1987, 1989; Cummings and Laskey 1993; Gray et al. 1988), the uterus and ovaries (Bal 1984; Chapin et

al. 1997; Cummings and Gray 1987, 1989; Cummings and Laskey 1993; Cummings and Perreault 1990; Dikshith et al. 1990; Gray et al. 1988, 1989; Harris et al. 1974; Martinez and Swartz 1992; Mitsumori et al. 2000; Okazaki et al. 2001; Swartz et al. 1994; Tegeris et al. 1966; Tullner 1961), and may reduce fertility (Bal 1984; Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974; Haskell Laboratories 1966). In the female offspring of exposed female rodents, reproductive development, as indicated by age at vaginal opening and first estrus, may be accelerated (Chapin et al. 1997; Gray et al 1989; Harris et al. 1974). In male rodents, methoxychlor exposure may affect sexual maturity (age of preputial separation) (Gray et al. 1989, 1999), serum hormone levels (Cummings and Gray 1989; Goldman et al. 1986; Gray et al. 1989; Stoker et al. 1999), testes (Bal 1984; Chapin et al. 1997; Dikshith et al. 1990; Gray et al. 1989, 1999; Hodge et al. 1950; Tullner and Edgcomb 1962; Wenda-Rozewicka 1983), prostate (Okazaki et al. 2001; Shain et al. 1977; Stoker et al. 1999; Welshons et al. 1999), fertility (Chapin et al. 1997; Gray et al. 1989, 1999; Harris et al. 1974; Wenda-Rozewicka 1983), and behavior (Parmigiani et al. 1998; vom Saal et al. 1995). However, these effects are not specific to exposure to methoxychlor, but could occur following exposure to other chemicals with estrogenic activity as well. It is unknown whether any of these effects will occur in humans at environmental exposure levels. Some potential end points that might be examined in human studies include: increased cornification of vaginal epithelium as measured in a pap smear, changes in menstrual cycles or sperm counts, and changes in serum progesterone, prolactin, and testosterone levels. Additional studies which investigate the mechanism of action of methoxychlor at the molecular level, may be useful in developing a specific biomarker of effect for methoxychlor. Development of a specific and sensitive biomarker of effect would be useful to facilitate future medical surveillance that can lead to detection and possible treatment of exposed populations.

Absorption, Distribution, Metabolism, and Excretion. *In vivo* toxicokinetics data for methoxychlor in humans are absent. There are no data on inhalation exposure and only minimal information on dermal exposure. As discussed in Section 3.4, studies in which animals were given oral doses of radiolabeled methoxychlor indicate that methoxychlor is:

(1) rapidly and efficiently absorbed by the gastrointestinal tract in mice and goats (Davison et al. 1982, 1983; Kapoor et al. 1970);
(2) widely distributed to tissues and organs with some preferential distribution to fatty tissues in rats (Harris et al. 1974; Hodge et al. 1952; Kunze et al. 1950), sheep (Reynolds et al. 1976), dogs (Hodge et al. 1952), and goats (Davison et al. 1982); and
(3) very rapidly cleared, predominately as metabolites in feces (via biliary excretion) and urine, in mice (Kapoor et al. 1970), goats (Davison et al. 1982), and sheep (Reynolds et al. 1976).

Dermal absorption has been estimated to be 5–8% in goats (Davison et al. 1983) and 20% in cows (Skaare et al. 1982); however, because of differences in skin, dermal absorption by goats may not be a good model for dermal absorption by humans. Methoxychlor and methoxychlor metabolites have been detected in milk of lactating goats (Davison et al. 1982) and rats (Chapin et al. 1997) given oral doses of methoxychlor. Accumulation in milk and/or elimination through lactation do not appear to be predominant dispositional fates for methoxychlor, but transfer of methoxychlor and methoxychlor metabolites to suckling rat pups via milk has been demonstrated (Chapin et al 1997).

Studies examining placental transfer of methoxychlor or methoxychlor metabolites were not located, but subtle effects on the development of reproductive organs have been observed in male and female offspring of rodents exposed to oral doses of methoxychlor during gestation (Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974); however, it should be noted that exposure was not exclusively during gestation in any of these studies. Research examining the possibility of placental transport of methoxychlor and metabolites and quantifying possible distribution to developing fetuses may help to discern if these effects result directly from methoxychlor and metabolites passing through the placenta or indirectly from a disturbance of maternal physiological processes.

As discussed in Section 3.4, the metabolism of methoxychlor has been studied *in vivo* in orally exposed rats (Lehman 1952; Weikel and Laug 1958), mice (Kapoor et al. 1970), and goats (Davison et al. 1982, 1983). Demethylated, dechlorinated, and dehydrochlorinated metabolites of methoxychlor were identified in feces and urine, and minor ring hydroxylated metabolites were also found in urine. Most urinary metabolites were conjugated with glucuronic acid (Davison et al. 1982, 1983). I*n vitro* studies with human and rat liver microsomes, partially purified rat CYP isozymes, and/or human c-DNA-expressed CYP isozymes indicate that the predominant metabolic pathway involves initial demethylation of methoxychlor catalyzed by a number of CYP isozymes in rats (Kishimoto and Kurihara 1996; Kishimoto et al. 1995) and in humans (Stresser and Kupfer 1998). In humans, CYP2C19 and CYP1A2 may be the major demethylases for methoxychlor, but other forms may play significant roles in individuals deficient in these isozymes (Stresser and Kupfer (1998). In rats, CYP2C6 and another unidentified CYP isozyme may be the major catalysts for demethylation (Kishimoto and Kurihara 1996). Ring hydroxylation of methoxychlor or hydroxy-methoxychlor derivatives appears to be catalyzed by a different set of CYP isozymes than those catalyzing demethylation (Stresser and Kupfer 1997; Stresser et al. 1996). Studies with immature or mature rats given repeated intraperitoneal injections of methoxychlor indicate that methoxychlor can induce hepatic CYP enzymes involved in its metabolism (Li et al. 1995).

Animal data indicate that methoxychlor and its metabolites are excreted primarily in the bile with a lesser amount excreted in the urine (Davison et al. 1982, 1983; Kapoor et al. 1970). This is true regardless of the route of exposure. In bile-duct cannulated rats given a single intravenous dose of radiolabeled methoxychlor, 50% of the dose was excreted in the bile after 4 hours (Weikel 1957). The urinary excretion of label in bile-cannulated rats was only 0.1–0.2% compared to 5–10% in noncannulated rats, suggesting that the appearance of urinary metabolites was largely due to material that was reabsorbed in the gut (i.e., enterohepatic circulation). Because methoxychlor does not accumulate significantly in biological tissues and is rapidly eliminated, additional studies examining the excretion of methoxychlor and its metabolites are not warranted.

Comparative Toxicokinetics. The available data comparing rat and human liver microsomal metabolism of methoxychlor indicate qualitative similarities and some indications of quantitative differences. Rat liver microsomes were observed to have a higher capacity than human liver microsomes to metabolize methoxychlor to covalent binding intermediates (Bulger and Kupfer 1990). *In vitro* data also indicate that covalent binding of methoxychlor to human liver microsomes is similar across age and sex, whereas in rats, covalent binding in mature males is much higher than in mature females and immature males and females (Bulger and Kupfer 1989). Another comparison found that *in vitro* rates of demethylation of methoxychlor and mono-hydroxy methoxychlor with human liver microsomes were generally higher in rat liver microsomes than in human liver microsomes (Stresser and Kupfer 1998).

Further research comparing quantitative and qualitative aspects of metabolism with mouse, rat, and human systems may help in developing PBPK models for methoxychlor in these species. New studies comparing species-specific tissue/blood partition coefficients, cross-placental distribution in pregnant animals (mice and rats), distribution to milk in lactating animals (mice and rats), elimination kinetics for methoxychlor from blood (mice and rats), and/or kinetics of metabolite appearance in urine or feces (rats; data are available for mice, Kapoor et al. 1970) would facilitate development of relevant PBPK models predicting distribution of methoxychlor and methoxychlor metabolites to the developing fetus and suckling infants. Such models may be useful in extrapolating observations of reproductive effects in rodents following *in utero* and/or lactational methoxychlor exposure to relevant human exposure scenarios.

Methods for Reducing Toxic Effects. No data were located regarding the mechanism of absorption, distribution, or excretion of methoxychlor in humans or animals by any route. Based on the chemical and physical properties of methoxychlor, its absorption most likely occurs by passive diffusion. However, this has not been investigated. Studies which investigate the mechanism by which methoxychlor enters the body may be useful in developing methods for reducing its absorption. Once it enters the body, methoxychlor preferentially distributes to tissues with higher fat content; however, methoxychlor does not persist in tissues (Harris et al. 1974; Hodge et al. 1952; Kunze et al. 1950; Reynolds et al. 1976). Its rapid metabolism to more polar compounds facilitates its rapid clearance from these tissues (Davison et al. 1982, 1983; Lehman 1952; Weikel and Laug 1958). Since the majority of methoxychlor is cleared from the body within 24 hours (Kapoor et al. 1970), it seems unlikely that methods for reducing body burden of methoxychlor would be of much benefit. Therefore, studies examining the mechanism of methoxychlor distribution and excretion are not warranted.

Children's Susceptibility. There is no information on health effects observed in children after methoxychlor exposure via inhalation, oral, or dermal routes, and there is a data need for such information, particularly from the predominant oral route of exposure. Animal data indicate that the primary target of methoxychlor is the reproductive system, and methoxychlor can affect both adult animals and the developing reproductive system in males and females. Thus, there is concern about whether exposure to methoxychlor during any stage of development might potentially affect the developing reproductive system of fetuses, children, and adolescents. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection.

There are no studies of methoxychlor metabolism in children; such information would be useful. Numerous studies have shown that both adult and developing animals exhibit effects attributed to the estrogenic activity of methoxychlor or its metabolites. This suggests that either methoxychlor is metabolized by the mother first and the metabolites then cross the placenta, or that methoxychlor crosses the placenta and is metabolized by the fetus. However, there are no studies that specifically investigated metabolic enzyme activity in fetuses. Although there is no direct information regarding age-related differences in metabolism of methoxychlor in children, multiple CYP enzymes are involved in methoxychlor metabolism. It is likely that CYPs 2C19, 1A2, 2B6, 2C9, 2A6, and perhaps 2D6, 2E1, and 3A4 play a role in phase I human metabolism (Hong et al. 1987; Lacroix et al. 1997; Leeder and Kearns 1997; Mote et al. 1990; Ratenasavanh et al. 1991; Rich et al. 1990; Sonnier and Cresteil 1998; Treuler et al. 1997; Vieira et al. 1996; Yang et al. 1994). Many of these phase I enzymes are likely to have

overlapping roles. Although nothing is known about phase II metabolism in humans, Davison et al. (1982, 1983) found glucuronic acid conjugates of methoxychlor metabolites in goats; these conjugates are a product of glucuronosyltransferase and it is possible that this enzyme may be involved in human methoxychlor metabolism as well. Although it is unknown whether an overall age-related difference in methoxychlor metabolism would be observed *in vivo* in humans, there is some information that the following enzymes may be developmentally regulated: CYP2C19 (Leeder and Kearns 1997), CYP1A2 (Leeder and Kearns 1997; Rich et al. 1990), CYP2C9 (Ratenasavanh et al. 1991; Treuler et al. 1997), CYP2D6 (Leeder and Kearns 1997; Sonnier and Cresteil 1998), CYP2E1 (Hong et al. 1987; Vieira et al. 1996), CYP3A4 (Lacroix et al. 1997; Leeder and Kearns 1997; Mote et al. 1990; Yang et al. 1994), and glucuronosyltransferase (Leeder and Kearns 1997). In contrast, other researchers have found that CYPs 2A6, 2C9, 2D6, 2E1, and 3A have been found at the same levels in perinatal (37 weeks of gestation) to geriatric human livers (72 years) (Ratenasavanh et al. 1991; Tateishi et al. 1997).

There are no studies examining placental transfer of methoxychlor or methoxychlor metabolites, but subtle effects on the development of reproductive organs have been observed in male and female offspring of rodents exposed to oral doses of methoxychlor during gestation (Chapin et al. 1997; Gray et al. 1989, 1999; Harris et al. 1974; Swartz and Corkern 1992; Welshons et al. 1999). It is unclear whether these effects result directly from methoxychlor and metabolites passing through the placenta or indirectly from a disturbance of maternal physiological processes. Research examining the possibility of placental transport of methoxychlor and metabolites and quantifying possible distribution to developing fetuses may help in elucidating the mechanism by which offspring of an exposed dam are affected.

Methoxychlor and methoxychlor metabolites have been detected in milk of lactating goats (Davison et al. 1982) and rats (Chapin et al. 1997) given oral doses of methoxychlor. Accumulation in milk and/or elimination through lactation do not appear to be predominant dispositional fates for methoxychlor, but transfer of methoxychlor and methoxychlor metabolites to suckling rat pups via milk has been demonstrated (Chapin et al 1997). The Chapin et al. (1997) study measured plasma methoxychlor (and metabolite) levels 27–30 hours after the dam's last exposure to methoxychlor; it would be interesting to know what pup plasma levels would be shortly after the last exposure of the dams. Additional studies to quantitate the plasma levels of methoxychlor in pups exposed only via lactation and to investigate the resultant effects through puberty and reproduction would be helpful in understanding the risk to offspring of maternal exposure to methoxychlor, even after birth.

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

3.12.3 Ongoing Studies

Ongoing studies pertaining to methoxychlor have been identified and are shown in Table 3-5.

Table 3-5. Ongoing Studies on the Health Effects of Methoxychlor[a]

Investigator	Affiliation	Research description	Sponsor
Ahmed, SA et al.	Virginia Polytechnic Institute, College of Veterinary Medicine	In vivo effects of a pesticide on the immune system: Age and gender factors	CSREES VA
Chapin, RE	NIEHS, NIH	Prenatal/Juvenile exposure to pesticides on adult neural, immune function	NIEHS
Lubahn, DB	University of Missouri–Columbia	Environmental estrogen response proteins in ER-minus mice	NIEHS
Chambers, JE	Mississippi State University, College of Veterinary Medicine	Developmental and comparative toxicology of neurotoxic insecticides	U.S. Department of Agriculture, Cooperative Research Service
Gore, A	Mount Sinai School of Medicine	Neuroendocrine mechanisms of environmental toxicity during development	NIEHS
James, MO	University of Florida	Bioavailability of chlorinated compounds	NIEHS
Kupfer, D	Worcester Foundation of Experimental Biology	Effects of chlorinated hydrocarbons on mammalian systems	NIEHS
Poznanski, AA	Midwestern University	Methoxychlor effects on zebrafish forebrain development	NIEHS
Schiffenbauer, J	University of Florida	Autoimmune toxicity of chlorinated compounds	NIEHS
Wilson, EM	University of North Carolina–Chapel Hill	Mechanisms of action of environmental antiandrogens	NIEHS
Zacharewski, TR	Michigan State University	Comprehensive assessment of endocrine active mixtures	NIEHS

[a]Source: FEDRIP (2001)

NIEHS = National Institute of Environmental Health Sciences; NIH = National Institutes of Health