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 DDT, DDE, and DDD. 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. Information on the effects of DDT, DDE, and DDD in humans and in animal species traditionally used in laboratory experiments is presented in Section 3.2 Discussion of Health Effects by Route of Exposure, whereas information on the effects of these compounds in wildlife is presented in Appendix D, Health Effects in Wildlife Potentially Relevant to Human Health.

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

While this document is specifically focused on the primary forms or isomers of DDT, DDE, and DDD (namely *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD), other isomers of these compounds will be discussed when appropriate. In some cases, the term DDT will be used to refer to the collective forms of DDT, DDE, and DDD. Should this not be clear from the context, the term ΣDDT (Σ is used to mean sum of) will be used.

Typically, people are not exposed to DDT, DDE, or DDD individually, but rather to a mixture of all three compounds since DDE and DDD are degradation and metabolic products of DDT. In addition, DDT, DDE, and DDD each can exist in three isomeric forms based on the relative position of the chlorine substitutions on the two chlorophenyl rings (Chapter 4). The most prevalent isomer of DDT, DDE, or DDD in the environment is the *p,p'*-isomer. Technical-grade DDT contains 65–80% *p,p'*-DDT, 15–21% *o,p'*-DDT, and up to 4% of *p,p'*-DDD (Metcalf 1995). When the toxicity of the isomers of DDT, DDE, or DDD reported in the experimental data differ in an organ system, such as the reproductive or developmental systems, isomer-specific results are presented, when available. Therefore, the data presented in this document include some relevant toxicity information on DDE and DDD analogues and on the *o,p'*- and *p,p'*-isomers of DDT and technical-grade DDT. Levels of Significant Exposure (LSE) for the *o,p'*-isomers are presented in separate tables to emphasize the point that these isomers have a somewhat different spectrum of effects than the *p,p'*-isomers.

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.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of DDT, DDE, and DDD are indicated in Table 3-1 and Figure 3-1. Because cancer effects could occur at lower exposure levels, Figure 3-1 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for DDT, DDE, and DDD. 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 1990a), 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

Occupational exposure to DDT involves multiple routes of exposure. The primary routes of exposure were probably inhalation and dermal; however, absorption of DDT from the lungs may not have been significant, and ingestion due to the mucociliary apparatus of the respiratory tract is more likely. Therefore, with the exception of a report on lung cancer, epidemiological studies of occupational exposure will be discussed under oral exposure and the following section refers to nonoccupational inhalation exposure.

3.2.1.1 Death

No studies were located regarding death in humans or animals after inhalation exposure to DDT, DDE, or DDD.

3.2.1.2 Systemic Effects

No studies were located regarding cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, or dermal effects in humans or animals after inhalation exposure to DDT, DDE, or DDD.

Respiratory Effects. Volunteers were exposed by inhalation of aerosols containing DDT at concentrations that left a white deposit on the nasal hair (Neal et al. 1944). Except for moderate irritation of the nose, throat, and eyes, which may have been related to the vehicle to disperse DDT in an aerosol, no significant changes were reported. This study had several limitations. The study did not provide information concerning conditions of exposure, dose, or information on persons exposed.

No studies were located regarding the respiratory effects in animals after inhalation exposure to DDT, DDE, or DDD.

Ocular Effects. Reports of ocular effects in humans exposed to DDT in the air are limited to the study by Neal et al. (1944). In this study, moderate, nonspecific eye irritation was reported by volunteers exposed to an aerosol containing DDT. This effect is assumed to have been caused by direct contact of the aerosol with the eye and not by inhalation of the aerosol. No information on the length of exposure or the concentration of DDT in air was provided. Irritation may have been related to the vehicle in which DDT was dissolved.

No studies were located regarding the ocular effects in animals after inhalation exposure to DDT.

No studies were located regarding the following effects in humans or animals after inhalation exposure to DDT, DDE, or DDD:

3.2.1.3 Immunological and Lymphoreticular Effects

3.2.1.4 Neurological Effects

3.2.1.5 Reproductive Effects

3.2.1.6 Developmental Effects

3.2.1.7 Cancer

Occupational exposure to DDT was associated with increased lung cancer in a case control study of the Uruguayan work force (De Stefani et al. 1996). Elevated, but not statistically significant, odds ratios (OR) for any type of lung cancer were observed in 34 workers who had been exposed for 1–20 years (OR=1.6; 95% confidence interval [CI]=0.9–4.6), in 16 workers who had been exposed for greater than 20 years (OR=2.0; 95% CI=0.9–4.7), and in 50 workers who had ever been exposed to DDT (OR=1.7; 95% CI=1.0–2.8). Significantly elevated odds ratios were reported in a subset of 33 DDT-exposed lung cancer patients with small cell cancer (OR=3.6; 95% CI=1.5–8.9) or in 57 with adenocarcinoma (OR=2.3; 95% CI=1.2–4.7). Analyses were adjusted for age, residence, education, tobacco smoking, and alcohol consumption.

Several additional studies of workers occupationally exposed to DDT are discussed in Section 3.2.2.8 as exposure most probably occurred by ingestion of trapped particles rather than by inhalation.

No studies were located regarding cancer in animals after inhalation exposure to DDT, DDE, or DDD. EPA (IRIS 2001a, 2001b, 2001c) calculated an inhalation unit risk of 9.7×10^{-5} per $\mu g/m^3$ for DDT from oral data in animals (Section 3.2.2.8). The air concentrations corresponding to excess cancer risk levels of 1×10^{-4}, 1×10^{-5}, 1×10^{-6}, and 1×10^{-7} are 1×10^{-3}, 1×10^{-4}, 1×10^{-5}, and 1×10^{-6} mg/m^3, respectively.

3.2.2 Oral Exposure

Tables 3-1 and 3-2 and Figures 3-1 and 3-2 present the health effects observed in humans and animals associated with levels of significant oral exposure for each designated exposure duration. The levels of

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	ACUTE EXPOSURE						
	Death						
1	Rat (NS)	Once (NS)				300 (LD50)	Ben-Dyke et al. 1970 DDT, NS
2	Rat (NS)	Once (NS)				400 (LD50)	Ben-Dyke et al. 1970 DDD, NS
3	Rat (NS)	Once (G)				800 (LD50)	Cameron and Burgess 1945 DDT, NS
4	Rat (Sherman)	Once				113 (LD50)	Gaines 1969 DDT, p,p'
5	Rat (Sherman)	Once (G)				4000 (LD50)	Gaines 1969 DDD, Tech
6	Rat (Sherman)	Once (G)				880 (LD50)	Gaines 1969 DDE, Tech
7	Rat (NS)	4 d (G)				279.2 (4-day LD50, preweanling; cumulative dose)	Lu et al. 1965 DDT, Tech
8	Rat (NS)	4 d (G)				285.6 (4-day adult LD50; cumulative dose)	Lu et al. 1965 DDT, Tech
9	Rat (NS)	Once (G)				355.2 (LD50, weanling rats)	Lu et al. 1965 DDT, Tech
10	Rat (NS)	Once (G)				194.5 (LD50, adult rats)	Lu et al. 1965 DDT, Tech

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
11	Rat (NS)	Once (IG)				4000 (LD50, newborn rats)	Lu et al. 1965 DDT, Tech
12	Rat (NS)	Once (G)				437.8 (LD50, preweanling rats)	Lu et al. 1965 DDT, Tech
13	Mouse (Inbred Swiss)	Once (G)				300 M (LD50)	Kashyap et al. 1977 DDT, Tech
14	Mouse (C3H)	6 d (F)				85.7 F (50% of mice killed after a 6-day feeding period)	Okey and Page 1974 DDT, p,p'
15	Mouse (CF1)	Once (G)				1466 (LD50)	Tomatis et al. 1972 DDD, p,p'
16	Mouse (CF1)	Once (G)				237 (LD50)	Tomatis et al. 1972 DDT, Tech
17	Gn Pig (NS)	Once (G)				400 (LD50)	Cameron and Burgess 1945 DDT, NS
18	Rabbit (NS)	Once (G)				300 (LD50)	Cameron and Burgess 1945 DDT, NS
	Systemic						
19	Monkey (Rhesus)	Once (G)	Hepatic		150 (increased serum LDH, AP, and transaminases)		Agarwal et al. 1978 DDT, NS

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
20	Rat (Wistar)	12 d (G)	Hepatic		40 (18% increase in relative liver weight; increased liver GSH and AHH enzyme activities)		DeWaziers and Azais 1987 DDT, NS
21	Rat (Sprague Dawley)	Once (GO)	Endocr	25	50 (reduced capacity to concentrate iodine in thyroid)		Goldman 1981 DDT, Tech
22	Rat (Wistar)	14 d 1 x/d (GO)	Hepatic		12 M (increased relative liver weight; necrotic changes)		Kostka et al. 2000 DDT, Tech
23	Mouse (CF1)	1 wk (F)	Hepatic		42.9 (29% increase absolute liver weight; increase cytochrome-c reductase and P-450)		Pasha 1981 DDE, NS
24	Dog (Mongrels and Beagles)	Once (C)	Endocr		200 (reversible degenerative adrenal changes)		Powers et al. 1974 DDD, Tech
25	Dog (Mongrels and Beagles)	6 d or 15d (C)	Endocr			100 (necrosis, adrenal)	Powers et al. 1974 DDT, Tech
	Immunological/Lymphoreticular						
26	Rabbit (New Zealand)	10 d (G)		4.3			Shiplov et al. 1972 DDT, NS
	Neurological						
27	Human	Once (F)		10.3		16 (convulsions)	Hsieh 1954 DDT, NS

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
28	Monkey (Rhesus)	Once (G)			150 (decrease CNS total lipids, phospholipids and cholesterol)		Sanyal et al. 1986 DDT, Tech
29	Rat (Wistar)	Once (GO)				160 (tremors)	Hietanen and Vainio 1976 DDT, NS
30	Rat (Fischer)	Once (G)		25		50 (tremors)	Hong et al. 1986 DDT, p,p'
31	Rat (Albino Sprague Dawley)	Once (G)				100 (myoclonus)	Hwang and Van Woert 1978 DDT, p,p'
32	Mouse (albino)	once (GO)				160 M (tremors)	Hietanen and Vainio 1976 DDT, p,p'
33	Mouse (Albino)	Once (G)				200 (convulsions)	Matin et al. 1981 DDT, p,p'
34	Gn Pig (NS)	Once (G)				160 (paralysis of hind legs)	Hietanen and Vainio 1976 DDT, NS
35	Hamster (NS)	Once (G)		160			Hietanen and Vainio 1976 DDT, NS
	Reproductive						
36	Rat (Long-Evans)	1 x/d 4 d (GO)			200 M (reduced seminal vesicle and ventral prostate weight)		Kelce et al. 1995 DDE, p,p'

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
37	Rat (Sprague-Dawley)	1 x/d 5 d (GO)			200 M (reduced seminal vesicle and ventral prostate weight)		Kelce et al. 1997 DDE, p,p'
38	Rabbit (New Zealand)	Gd7-9 (GO)				50 (increased resorptions, 1.8% in controls, 25% in treated)	Hart et al. 1971 DDT, p,p'
39	Rabbit (New Zealand)	Gd7-9 (GO)				10 (increased resorptions, 1.3% in controls, 9.5% in treated)	Hart et al. 1972 DDT, p,p'
	Developmental						
40	Rat (Sprague-Dawley)	Gd15-19 (G)		28			Gellert and Heinrichs 1975 DDT, p,p'
41	Rat (Long-Evans)	5 d Gd 14-18 (GO)				100 M (significant decrease in ventral prostate weight)	Gray et al. 1999 DDE, p,p'
42	Rat (Sprague-Dawley)	5 d Gd 14-18 (GO)				100 M (decreased weight of glans penis; epididymis and ventral prostate; reduced anogenital distance)	Gray et al. 1999 DDE, p,p'
43	Rat (Long-Evans)	1 x/d Gd 14-18 (GO)				100 M (reduced anogenital distance at birth: ppd 13 retained thoracic nipples)	Kelce et al. 1995 DDE, p,p'
44	Rat (Holtzman)	1 x/d Gd 14-18 (GO)		10 M		50 M (reduced anogenital distance on ppd1 and relative ventral prostate weight on ppd21)	Loeffler and Peterson 1999 DDE, p,p'

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
45	Rat (Long-Evans)	1 x/d Gd 14-18 (GO)		10		100 M (reduced anogenital distance on ppd2; retained thoracic nipples on ppd13)	You et al. 1998 DDE, p,p'
46	Rat (Sprague-Dawley)	1 x/d Gd 14-18 (GO)			10 M (ppd13 males retained thoracic nipples)		You et al. 1998 DDE, p,p'
47	Mouse (NMRI)	Once ppd 10 (GO)			0.5[b] M (increased motor activity at 4 months)		Eriksson and Nordberg 1986; Eriksson et al. 1990a, 1990b DDT, Tech
48	Mouse (NMRI)	Once ppd 10 (GO)			0.5 M (decrease in cerebral cortex muscarinic acetylcholine receptors at 4 months)		Eriksson et al. 1992 DDT, Tech
49	Mouse (NMRI)	once ppd 10 (GO)			0.5 M (decreased muscarinic receptors in cerebral cortex; increased spontaneous activity at 5 months)		Johansson et al. 1995 DDT, Tech
50	Mouse (NMRI)	once ppd 10 (GO)			0.5 M (decreased muscarinic receptors in cerebral cortex; increased spontaneous activity at 5 and 7 months)		Johansson et al. 1996 DDT, Tech
51	Rabbit (New Zealand)	Gd4-7 (G)			1 (decreased fetal weight)		Fabro et al. 1984 DDT, NS

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
52	Rabbit (New Zealand)	Gd7-9 (GO)				50 (22% decreased offspring weight)	Hart et al. 1971 DDT, p,p'
53	Rabbit (New Zealand)	Gd7-9 (GO)			10 (11% decreased fetal weight on day 28)	50 (19% decreased fetal weight on day 28)	Hart et al. 1972 DDT, p,p'

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	INTERMEDIATE EXPOSURE						
	Death						
54	Monkey (Squirrel)	2,4 or 6 mo (G)				50 (death of 6/6 in 14 weeks)	Cranmer et al. 1972a DDT, p,p'
55	Mouse (B6C3F1)	6wk (F)				35 F (4 out 5 died)	NCI 1978 DDT, Tech
56	Mouse (B6C3F1)	6wk (F)				66 M (4 out of 5 died)	NCI 1978 DDE, p,p'
	Systemic						
57	Rat (albino)	120 d (GO)	Endocr			0.2 M (degeneration of adrenal cortex and medulla)	Chowdhury et al. 1990 DDT, Tech
			Bd Wt			0.2 M (30% reduced body weight gain)	
58	Rat (Wistar)	3 wk (GO)	Hepatic		15 (significant increase in liver weight and in cytochrome P-450 enzymes)		Gupta et al. 1989 DDT, p,p'
59	Rat (Sprague Dawley)	36 wk 7d/wk (F)	Hepatic		6.6 (focal necrosis/regeneration)		Jonsson et al. 1981 DDT, NS
60	Rat (Osborne-Mendel)	15-27 wk (F)	Hepatic	0.05[c]	0.25 (cellular hypertrophy, cytoplasmic eosinophilia)		Laug et al. 1950 DDT, Tech

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
61	Rat (Osborne-Mendel)	6 wk (F)	Bd Wt	28 M	50 M (16% reduced body weight gain)	97 F (45% reduced body weight gain)	NCI 1978 DDT, Tech
62	Rat (Osborne-Mendel)	6wk (F)	Bd Wt		88 M (11% decrease in body weight gain)	157 M (22% decrease in body weight gain)	NCI 1978 DDE, p,p'
63	Rat (Sherman)	2-18 mo (F)	Hepatic		5 M 20 F (minor liver vacuolation, hypertrophy and cell margination)		Ortega 1956 DDT, Tech
64	Mouse (B6C3F1)	6wk (F)	Bd Wt	310 F			NCI 1978 DDD, Tech
65	Mouse (B6C3F1)	6wk (F)	Bd Wt	101 F			NCI 1978 DDE, p,p'
66	Mouse (B6C3F1)	6wk (F)	Bd Wt	35			NCI 1978 DDT, Tech
67	Mouse (NMRI)	28 d (G)	Hepatic		6.25 (increased liver weight and P-450 induction)		Orberg and Lundberg 1974 DDT, p,p'
	Immunological/Lymphoreticular						
68	Rat (Wistar)	4 wk (F)		2.3 M	5.7 M (decreased IgG and IgM, increased albumin/globulin ratio)		Banerjee et al. 1995 DDT, p,p'
69	Rat (albino)	31 d 24hr/d (F)			1.9 (decreased mast cells)		Gabliks et al. 1975 DDT, NS

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
70	Mouse (Hissar)	3-12 wk (F)		10.5	21 M (decreased IgM antibody titer)		Banerjee 1987a DDT, NS
	Neurological						
71	Monkey (Squirrel)	2, 4, or 6 mo (G)		5		50 (staggering, weakness, loss equilibrium)	Cranmer et al. 1972a DDT, p,p'
72	Monkey (Rhesus)	100 d (G)			10 (15-20% decrease in brain lipids, CNS phospholipids, and cholesterol)		Sanyal et al. 1986 DDT, Tech
73	Rat (Osborne-Mendel)	26 wk (F)				16 F (body tremors)	NCI 1978 DDT, Tech
74	Rat (Wistar)	9 wk (F)				34.1 F (tremors in 80% of females after 9 weeks of treatment).	Rossi et al. 1977 DDT, Tech
	Reproductive						
75	Rat (Sprague Dawley)	36 wk (F)		6		12 (sterility)	Jonsson et al. 1976 DDT, Tech
76	Rat (Long-Evans)	1 x/d ppd 21-57 (GO)				100 M (delayed onset of puberty by 5 days)	Kelce et al. 1995 DDE, p,p'
77	Rat (Sprague Dawley)	9 wk (G)		10			Kornburst et al. 1986 DDE, p,p'

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
78	Rat (Wistar)	3 wk 3 x/wk (GO)			100 (marginal, but significant decrease in testosterone in the testis)		Krause 1977 DDT, NS
79	Rat (NS)	ppd 4-23 (G)			200 (decreased absolute testis weight)		Krause et al. 1975 DDT, NS
80	Mouse (C-57)	60-90 d (F)				51.4 (78% decreased fertility)	Bernard and Gaertner 1964 DDT, Tech
81	Mouse (B6C3F1)	86 d (F)		3.4			Ledoux et al. 1977 DDT, Tech
82	Mouse (NMRI)	7d/wk 12 wk (F)				1.67 (decreased implanted ova, increased-persistent- estrus)	Lundberg 1974 DDT, p,p'
83	Mouse (NMRI)	28 d (G)				1.67 (decreased corpora lutea and implants)	Lundberg 1974 DDT, p,p'
84	Mouse (NMRI)	28 d (G)		6.25			Orberg and Lundberg 1974 DDT, p,p'
85	Mouse (BALB/c)	120 d (F)		1.3			Ware and Good 1967 DDT, Tech
86	Rabbit (New Zealand)	3 x/wk 12 wk (GO)			3 F (reduced ovulation rate and slight decrease circulating progesterone post-insemination)		Lindenau et al. 1994 DDT, Tech

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Developmental						
87	Rat (Wistar)	42 d Gd 1-21 Ld 1-21 (F)		1.7	16.8 (decreased growth of nursing pups)	42.1 (pup death by 10 days)	Clement and Okey 1974 DDT, p,p'
88	Mouse (CF1)	Gd 1-21 Ld 1-21 (F)			34.3 (decreased maze performance learning at 1 and 2 months in survivors)		Craig and Ogilvie 1974 DDT, Tech
	Cancer						
89	Mouse (CF1)	15-30 wk (F)				42.8 (liver hepatomas)	Tomatis et al. 1974b DDT, p,p'

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - 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							
Death							
90	Monkey (Cynomolgus)	130 mo (F)				6.9 F (lowest dose associated with early death)	Takayama et al. 1999 DDT, p,p'
91	Rat (Osborne-Mendel)	78wk (F)				19 F (16% death rate compared to 0% in controls)	NCI 1978 DDE, p,p'
92	Mouse (B6C3F1)	78wk (F)				15.0 F (10% mortality compared to 0% in controls)	NCI 1978 DDT, Tech
93	Mouse (B6C3F1)	78wk (F)				49 F (40% death rate compared to 5% in controls)	NCI 1978 DDE, p,p'
Systemic							
94	Human	12-18 mo (F)	Cardio	0.5			Hayes et al. 1956 DDT, Tech
			Hemato	0.5			
			Hepatic	0.5			
			Bd Wt	0.5			
95	Monkey (Rhesus)	3.5-7 yr (F)	Hepatic	3.9			Durham et al. 1963 DDT, NS
96	Monkey (Cynomolgus)	130 mo (F)	Hepatic		6.4 F (lowest dose associated with fatty changes in the liver)		Takayama et al. 1999 DDT, p,p'

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
97	Rat (Osborne Mendel)	27 mo (F)	Resp	20			Deichmann et al. 1967 DDT, NS
			Hemato		20 (hemolysis in spleen)		
			Hepatic		20 (focal hepato-cellular necrosis)		
			Renal			20 (some tubular epithelial necrosis and polycystic degeneration; small hemorrhages)	
98	Rat (Osborne-Mendel)	2 yr (F)	Hepatic		7 (focal hepatocellular necrosis)		Fitzhugh and Nelson 1947 DDT, Tech
99	Rat (Osborne-Mendel)	78 wk (F)	Resp	45 M			NCI 1978 DDT, Tech
			Cardio	45 M			
			Gastro	45 M			
			Musc/skel	45 M			
			Hepatic		23 M (fatty metamorphosis)		
			Renal	45 M			
			Endocr	45 M			
			Dermal	45 M			
			Bd Wt		32 F (20% decrease in body weight gain)		

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - 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
100	Rat (Osborne-Mendel)	78wk (F)	Resp	231 M			NCI 1978 DDD, Tech
			Cardio	231 M			
			Gastro	231 M			
			Musc/skel	231 M			
			Hepatic	231 M			
			Renal		66 F (chronic inflammation of the kidney)		
			Endocr	231 M			
			Dermal	231 M			
			Bd Wt			66 F (26% decrease in body weight gain)	
101	Rat (Osborne-Mendel)	78wk (F)	Resp	59 M			NCI 1978 DDE, p,p'
			Cardio	59 M			
			Gastro	59 M			
			Musc/skel	59 M			
			Hepatic		31 M (fatty metamorphosis)		
			Renal	59 M			
			Endocr	59 M			
			Dermal	59 M			
			Bd Wt			19 F (21% decrease in body weight gain)	

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
102	Mouse (B6C3F1)	78wk (F)	Resp	30.2 F			NCI 1978 DDT, Tech
			Cardio	30.2 F			
			Gastro	30.2 F			
			Musc/skel	30.2 F			
			Hepatic		3.7 M (amyloidosis)		
			Renal	30.2 F			
			Endocr	30.2 F			
			Dermal	30.2 F			
			Bd Wt	30.2 F			
103	Mouse (B6C3F1)	78wk (F)	Resp	142 F			NCI 1978 DDD, Tech
			Cardio	142 F			
			Gastro	142 F			
			Musc/skel	142 F			
			Hepatic	142 F			
			Renal	142 F			
			Endocr	142 F			
			Dermal	142 F			
			Bd Wt		71 F (17% decrease in body weight gain)	142 F (28% decrease in body weight gain)	

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
104	Mouse (B6C3F1)	78wk (F)	Resp	49 F			NCI 1978 DDE, p,p'
			Cardio	49 F			
			Gastro	49 F			
			Musc/skel	49 F			
			Hepatic	49 F			
			Renal		27 M (chronic inflammation of the kidney)		
			Endocr	49 F			
			Dermal	49 F			
			Bd Wt			28 F (29% decrease in body weight gain)	
105	Hamster (Syrian)	life (F)	Hepatic	20	40 (focal necrosis)		Cabral et al. 1982a DDT, Tech
			Bd Wt	40			
106	Hamster (NS)	life (F)	Hepatic		67 (50% increase in relative liver weight)		Graillot et al. 1975 DDT, Tech
107	Hamster (Syrian)	128 wk (F)	Hepatic		47.5 (liver necrosis)		Rossi et al. 1983 DDE, p,p'
			Bd Wt		47.5 (unquantified reduction in body weight gain)		
108	Dog (NS)	39-40 mo (F)	Hepatic	16	80 (focal or diffuse liver alterations)	160 (severe liver damage)	Lehman 1965 DDT, Tech

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Neurological						
109	Human	12-18 mo (F)		0.61			Hayes et al. 1956 DDT, Tech
110	Monkey (Cynomolgus)	130 mo (F)				6.9 F (lowest dose associated with severe tremors)	Takayama et al. 1999 DDT, p,p'
111	Mouse (Swiss)	80 wk (F)				16.5 (tremors)	Kashyap et al. 1977 DDT, Tech
112	Mouse (CF1)	130-140 wk (F)		1.7		8.3 (tremors)	Turusov et al. 1973 DDT, Tech
	Reproductive						
113	Rat (Sprague-Dawley)	2 gen (F)		1.2			Duby et al. 1971 DDT, p,p'
114	Rat (Sprague-Dawley)	2 gen (F)		0.75			Duby et al. 1971 DDT, Tech
115	Rat (Sprague-Dawley)	2 gen (F)		10			Ottoboni 1969 DDT, Tech
116	Rat (Sprague Dawley)	7d/wk life (F)		1.6			Ottoboni 1972 DDT, Tech
117	Rat (Sprague-Dawley)	3 gen (F)		1.25			Treon et al. 1954 DDT, Tech

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
118	Mouse (Swiss-Webster)	3 gen (F)				20 (decreased fertility)	Keplinger et al. 1970 DDT, NS
119	Mouse (NS)	15 mo (F)		2.4			Wolfe et al. 1979 DDT, Tech
120	Dog (Beagle)	7d/wk-F2 gen (F)		10			Ottoboni et al. 1977 DDT, Tech
	Developmental						
121	Rat (Sprague Dawley)	2 gen (F)		1.9	18.6 (tail abnormalities, constriction rings)		Ottoboni 1969 DDT, Tech
122	Mouse (ICR)	70 wk (F)				16.5 (decreased neonatal survival)	Del Pup et al. 1978 DDT, Tech
123	Mouse (B6C3F1)	7d/wk-2gen (F)		10		50 (increased preweanling death)	Tomatis et al. 1972 DDT, p,p'
124	Mouse (CF1)	life (F)		8.3		41.3 (increased in preweanling death)	Turusov et al. 1973 DDT, Tech
	Cancer						
125	Rat (MRC Porton)	life (F)				19.7 F (liver tumors, NS)	Cabral et al. 1982b DDT, Tech
126	Rat (Osborne-Mendel)	78wk (F)				116 M (CEL: thyroid follicular cell adenoma and carcinoma)	NCI 1978 DDD, Tech

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
127	Rat (Wistar)	120 wk (F)				34.1 M (liver cell tumors; 33.3% incidence, 0% in controls)	Rossi et al. 1977 DDT, Tech
128	Mouse (C57BL/6N)	81 wk (F)				28 (liver tumors, NS)	Innes et al. 1969 DDT, Tech
129	Mouse (Swiss)	80 wk (F)				16.5 (lymphomas; lung and liver tumors NS)	Kashyap et al. 1977 DDT, Tech
130	Mouse (B6C3F1)	78wk (F)				27 M (CEL: hepatocellular carcinomas; 0/19, 7/41, 17/47)	NCI 1978 DDE, p,p'
131	Mouse (A strain)	5 gen (G)				1.3 (lung tumors, NS, lung adenomas)	Shabad et al. 1973 DDT, Tech
132	Mouse (BALB/c)	life 6 gen (F)				0.4 (lung adeno-carcinomas in F2, leukemia in F3)	Tarjan and Kemeny 1969 DDT, p,p'
133	Mouse (BALB/c)	life 2-gen (F)				50 F (liver tumors in F0 and F1)	Terracini et al. 1973 DDT, Tech
134	Mouse (CF1)	2 yr (F)				15.8 (liver tumors, NS)	Thorpe and Walker 1973 DDT, p,p'
135	Mouse (CF1)	life multi-gen (F)				0.38 M (liver tumors in F0 and F1)	Tomatis et al. 1972 DDT, Tech
						45.5 F (liver tumors in F0 and F1)	

Table 3-1. Levels of Significant Exposure to DDT, DDE, DDD (Except o,p'-Isomers) - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
136	Mouse (CF1)	130 wk (F)				42.6 M (significant increase in liver tumors)	Tomatis et al. 1974a DDE, p,p'
137	Mouse (CF1)	130 wk (F)				42.6 M (significant increase in lung and liver tumors)	Tomatis et al. 1974a DDD, p,p'
138	Mouse (CF1)	6 gen (F)				0.33 (liver tumors, NS)	Turusov et al. 1973 DDT, Tech
139	Hamster (Syrian)	128 wk (F)				95 (CEL: adrenal neoplasms; 14% in controls, 34% in treated)	Rossi et al. 1983 DDT, Tech
140	Hamster (Syrian)	128 wk (F)				47.5 (CEL: hepatocellular tumors; 0/73, 11/69, 14/78)	Rossi et al. 1983 DDE, p,p'

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

[b]Used to derive an acute oral minimal risk level (MRL) of 0.0005 mg/kg-day for DDT. The MRL was derived by dividing the LOAEL by an uncertainty factor of 1000 (10 to account for intraspecies variability, 10 for interspecies variability, and 10 for the use of a LOAEL).

[c] Used to derive an intermediate minimal risk level (MRL) of 0.0005 mg/kg/day for DDT. The MRL was derived by dividing the NOAEL by an uncertainty factor of 100 (10 to account for interspecies variability and 10 for intraspecies variability). See Appendix A, ATSDR Minimal Risk Level Worksheets, for explanation of how dietary concentrations were converted to doses.

Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; CNS = central nervous system; d = day(s); (F) = food; Endocr - endocrine; F = female; (G) = gavage; gastro = gastrointestinal; Gd = gestation day; gen = generation(s); (GO) = gavage in oil; Hemato = hematological; hr = hour(s); kg = killigram; ld = lactation day; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observable-adverse-effect level; M = male; mg = milligram; mo = month(s): Musc/skel = musculoskeletal; NOAEL = no-observable-adverse-effect level; NS = not specified; PND = postnatal day; ppd = postpartum day; Resp = respiratory; wk = week(s); x = times

Figure 3-1. Levels of Significant Exposure to DDT, DDE, DDD - Oral (Except o,p' isomers)

Acute (≤14 days)

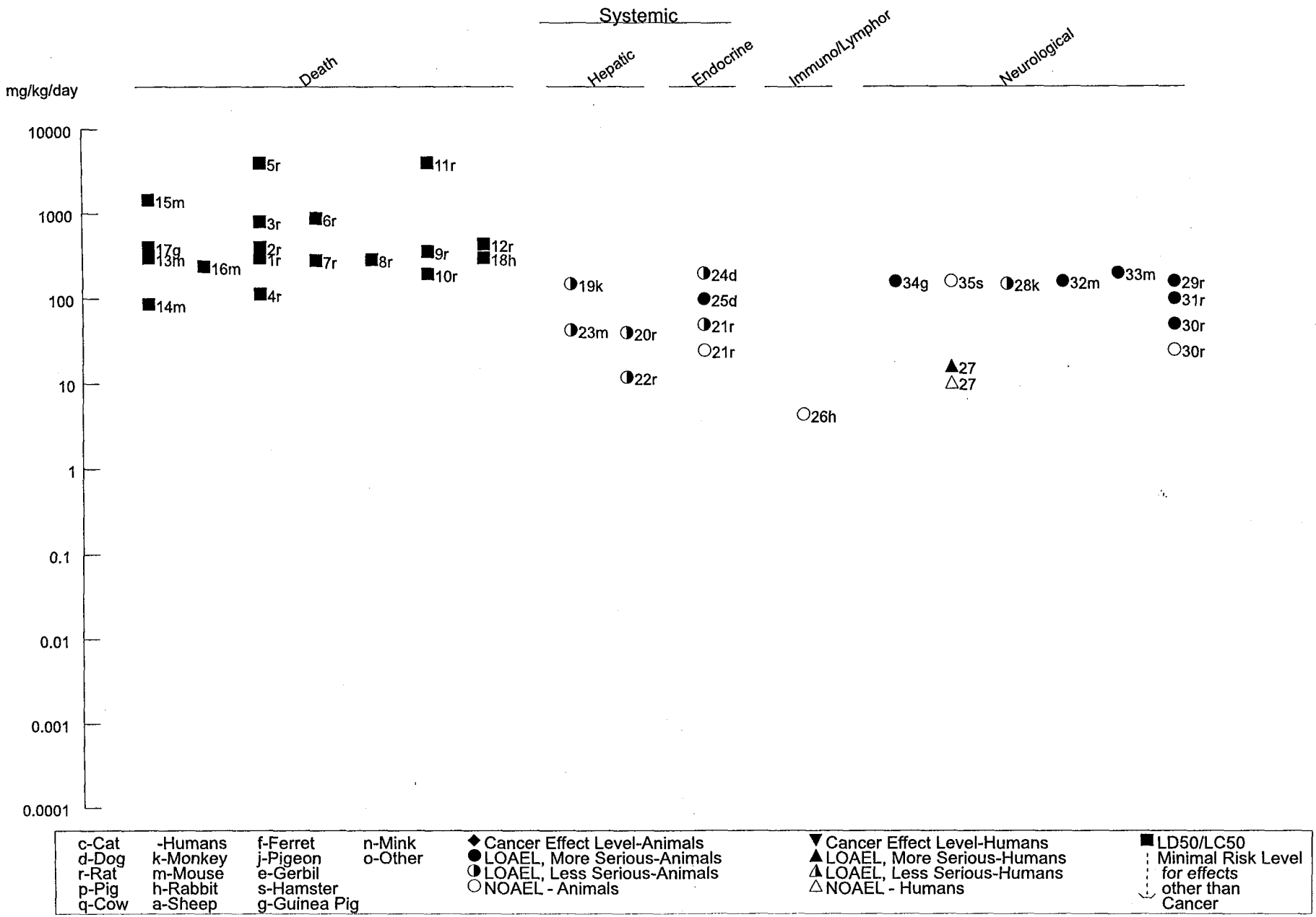


Figure 3-1. Levels of Significant Exposure to DDT, DDE, DDD - Oral (Except o.p' isomers) (*continued*)

Acute (≤14 days)

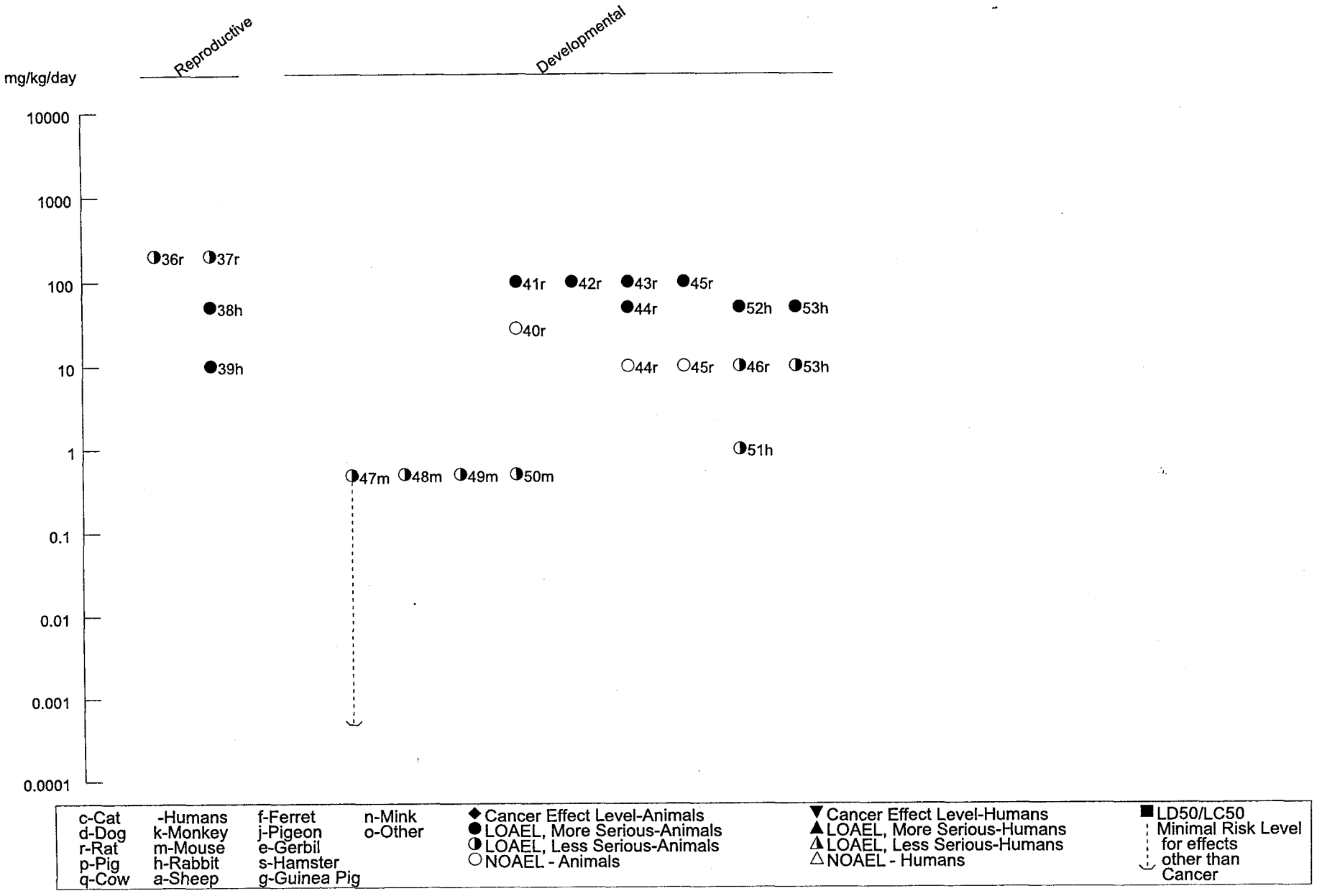


Figure 3-1. Levels of Significant Exposure to DDT, DDE, DDD - Oral (Except o,p' isomers) (*continued*)

Intermediate (15-364 days)

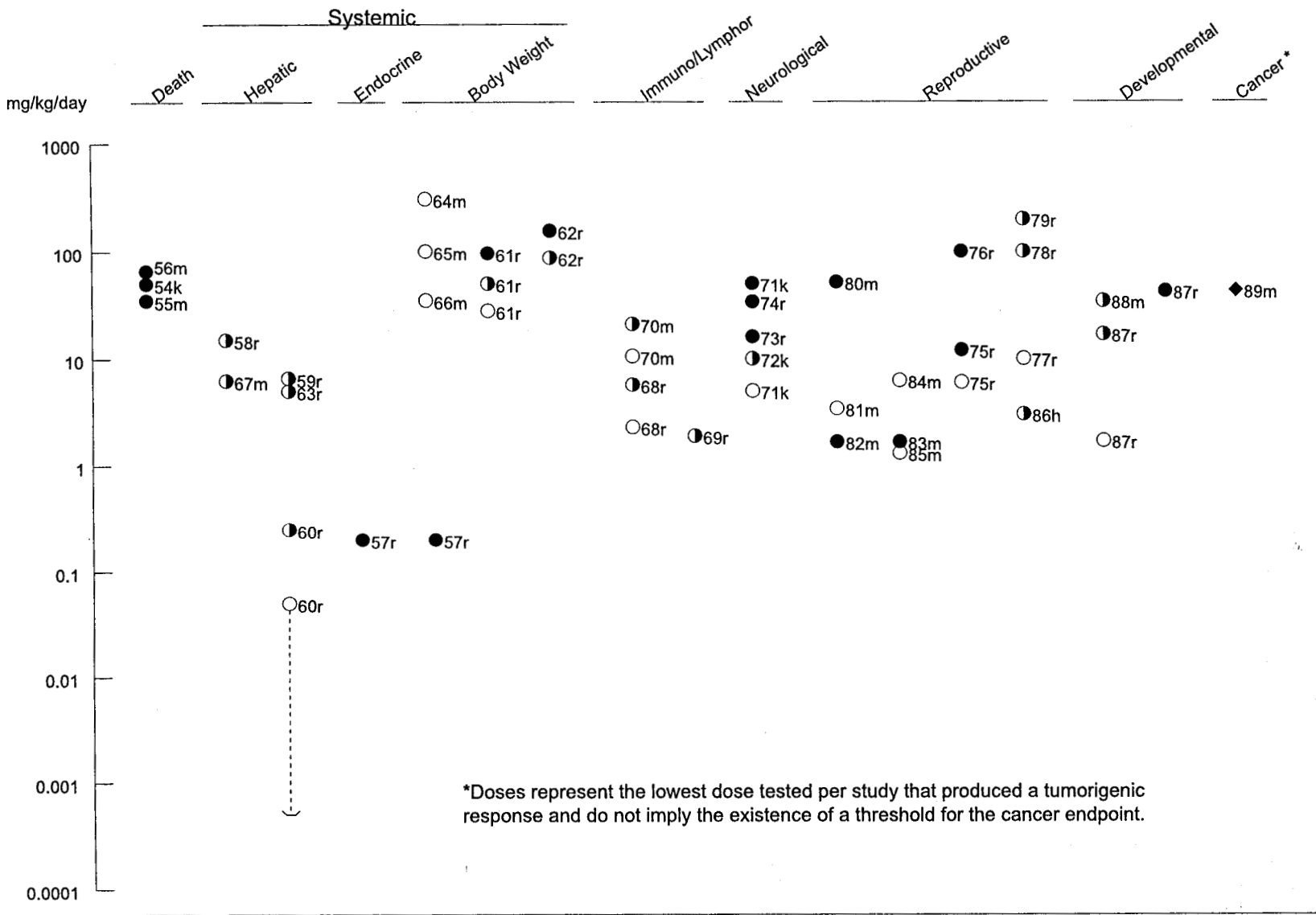


Figure 3-1. Levels of Significant Exposure to DDT, DDE, DDD - Oral (Except for o,p' isomers) (*continued*)

Chronic (≥365 days)

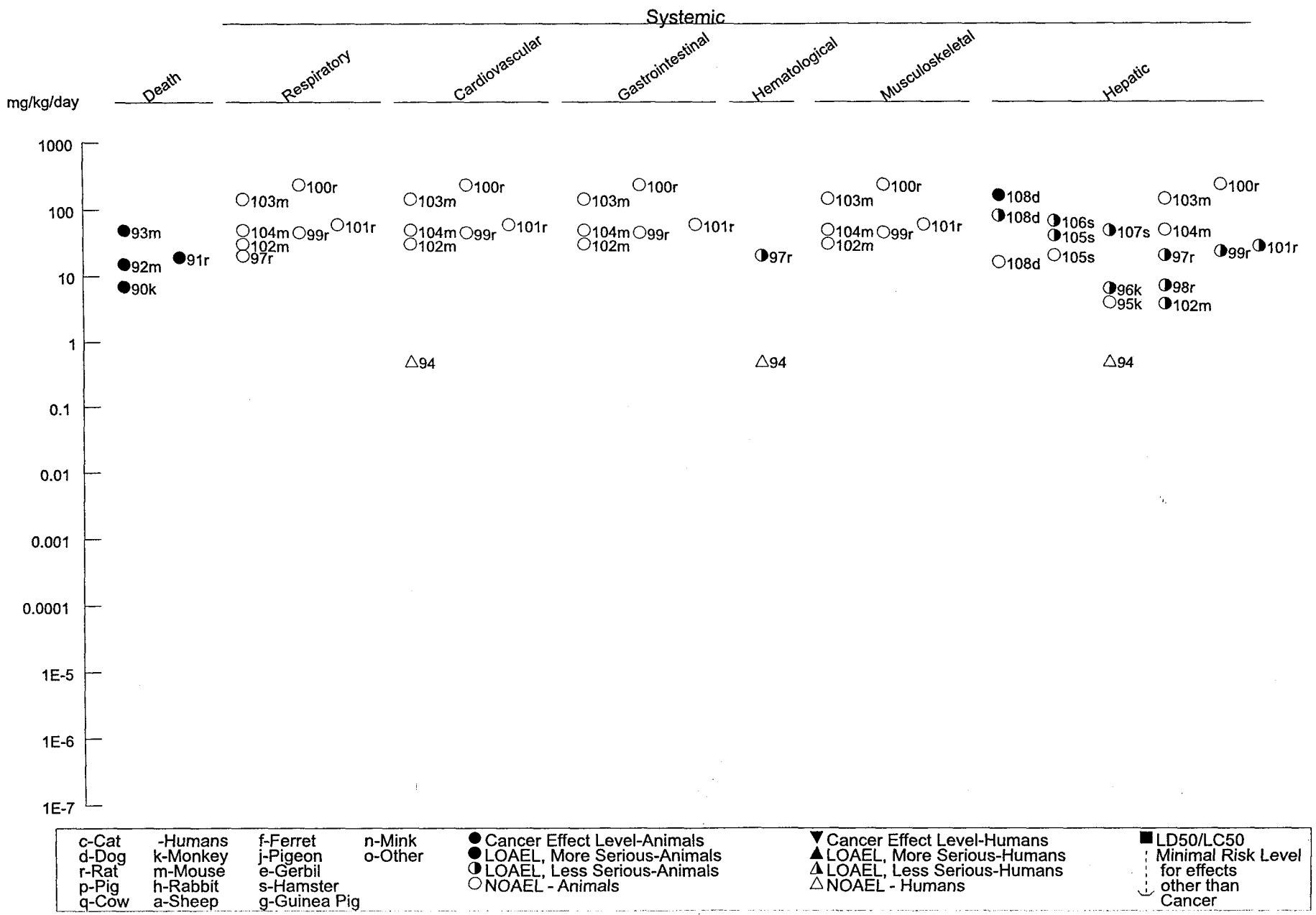


Figure 3-1. Levels of Significant Exposure to DDT, DDE, DDD - Oral (Except o,p' isomers) (*continued*)

Chronic (≥365 days)

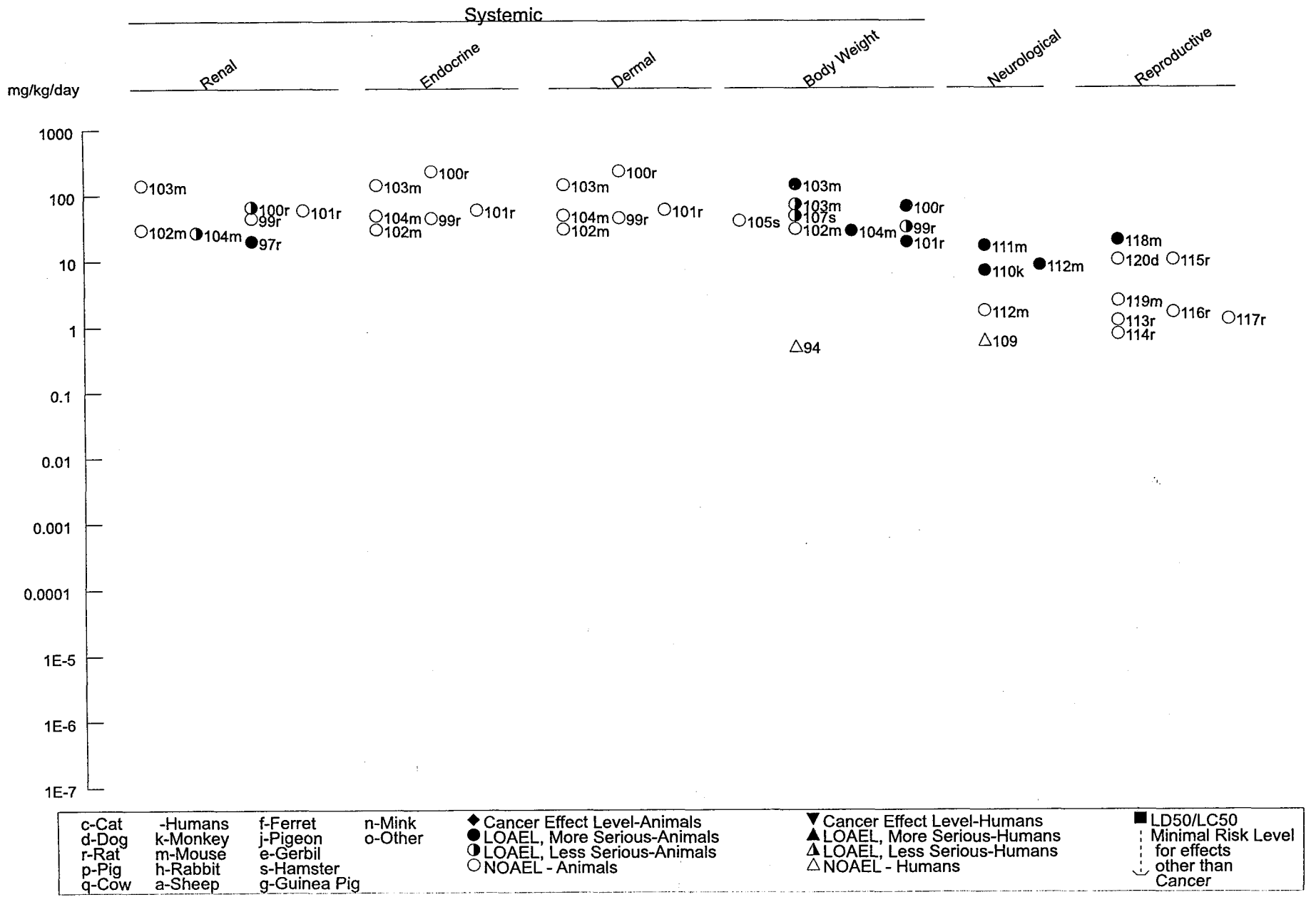


Figure 3-1. Levels of Significant Exposure to DDT, DDE, DDD - Oral (Except o,p' isomers) (*continued*)

Chronic (≥365 days)

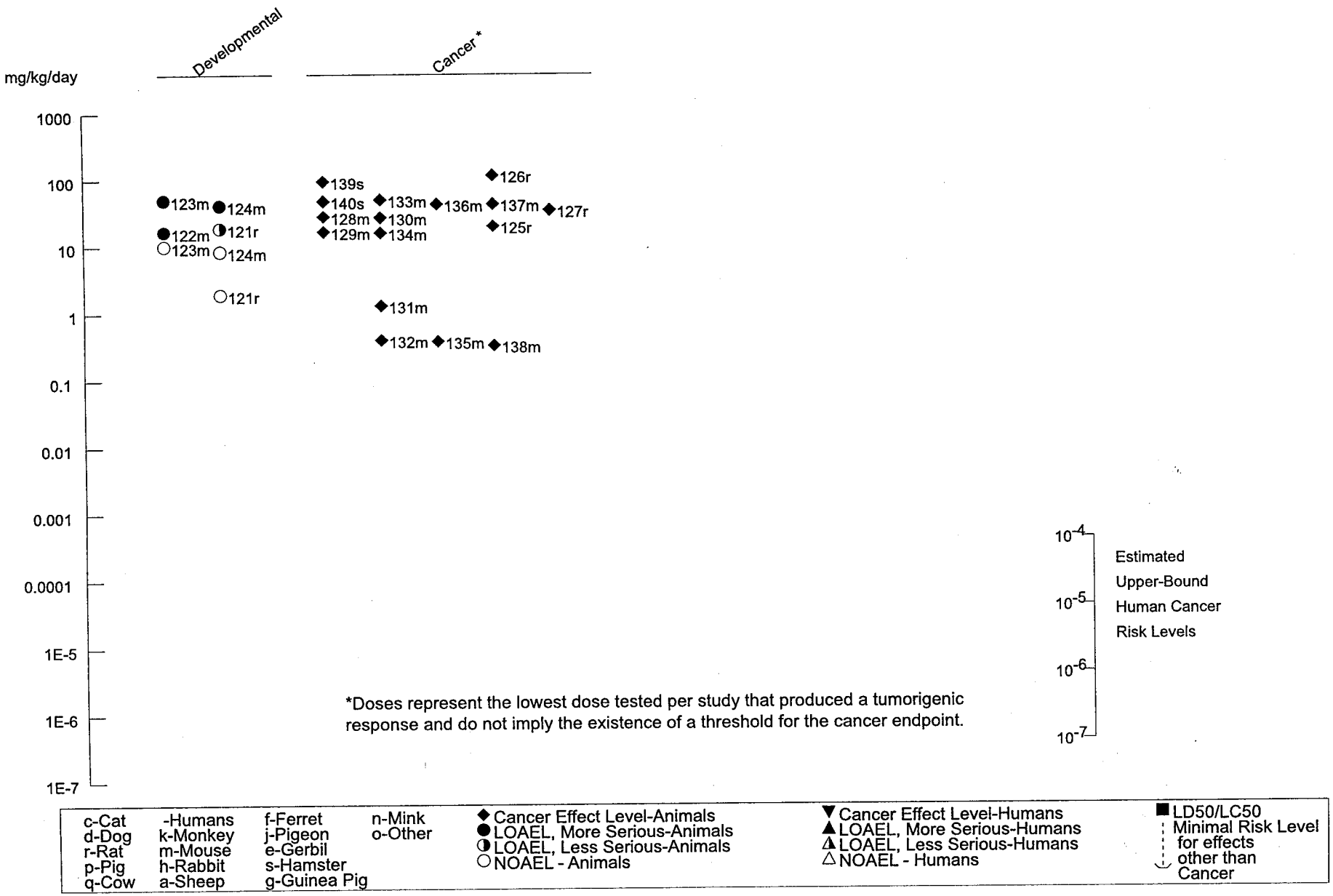


Table 3-2. Levels of Significant Exposure to o,p'-DDT, -DDE, -DDD - Oral

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
ACUTE EXPOSURE							
Death							
1	Mouse (CF1)	Once (G)				810 (LD50)	Tomatis et al. 1972 DDE, o,p'
Systemic							
2	Dog (NS)	14 d	Cardio			50 (decrease in contractile force)	Cueto 1970 DDD, o,p'
			Endocr		50 (decreased plasma glucocorticoids)		
3	Dog (NS)	10 d (C)	Endocr			138.5 (adrenal hemorrhage)	Kirk et al. 1974 DDD, o,p'
Reproductive							
4	Rat (Wistar)	7 d (F)			100 (increased uterus weight; premature vaginal opening)		Clement and Okey 1972 DDT, o,p'
5	Rat (DA/Han)	3 d 1 x/d (G)		10 F	100 F (significant increase in wet uterine weight)		Diel et al. 2000 DDT, o,p'
Developmental							
6	Rat (Sprague Dawley)	Gd15-19 (G)		28			Gellert and Heinrichs 1975 DDE, o,p'

Table 3-2. Levels of Significant Exposure to o,p'-DDT, -DDE, -DDD - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
7	Rat (Sprague Dawley)	Gd15-19 (G)		28			Gellert and Heinrichs 1975 DDT, o,p'
8	Rat (Sprague-Dawley)	Gd15-19 (G)			28 (delayed vaginal opening)		Gellert and Heinrichs 1975 DDD, o,p'
9	Mouse (CD-1)	7 d Gd 11-71 (GO)			0.018 M (decreased testes weight)		Palanza et al. 1999 DDT, o,p'
10	Mouse (CF1)	1 x/d Gd 11-17 (GO)		0.018 M	1.82 M (altered behavior; increase urine marking in a novel territory)		vom Saal et al. 1995 DDT, o,p'

Table 3-2. Levels of Significant Exposure to o,p'-DDT, -DDE, -DDD - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
INTERMEDIATE EXPOSURE							
Systemic							
11	Dog (NS)	36-150 d (C)	Endocr			50 (adrenocortical necrosis)	Kirk and Jenson 1975 DDD, o,p'
Immunological/Lymphoreticular							
12	Rat (Sprague-Dawley)	16 d (F)				121 (atrophy thymus)	Hamid et al. 1974 DDD, o,p'
Reproductive							
13	Rat (Wistar)	20 wk (F)		4.0			Wrenn et al. 1971 DDT, o,p'
Developmental							
14	Rat (Wistar)	pre-conception Gd 1-21 Ld 1-21 (F)		16.8	84 (17% less weight than controls at age 21 days)		Clement and Okey 1974 DDT, o,p'

Table 3-2. Levels of Significant Exposure to o,p'-DDT, -DDE, -DDD - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	CHRONIC EXPOSURE						
	Reproductive						
15	Rat (Sprague-Dawley)	2 gen (F)		0.3			Duby et al. 1971 DDT, o,p'

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

Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; CNS = central nervous system; d = day(s); (F) = food; Endocr - endocrine; F = female; (G) = gavage; gastro = gastrointestinal; Gd = gestation day; gen = generation(s); (GO) = gavage in oil; Hemato = hematological; hr = hour(s); kg = killigram; ld = lactation day; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observable-adverse-effect level; M = male; mg = milligram; mo = month(s): Musc/skel = musculoskeletal; NOAEL = no-observable-adverse-effect level; NS = not specified; PND = postnatal day; ppd = postpartum day; Resp = respiratory; wk = week(s); x = times

Figure 3-2. Levels of Significant Exposure to o,p'-DDT, -DDE, -DDD - Oral

Acute (≤14 days)

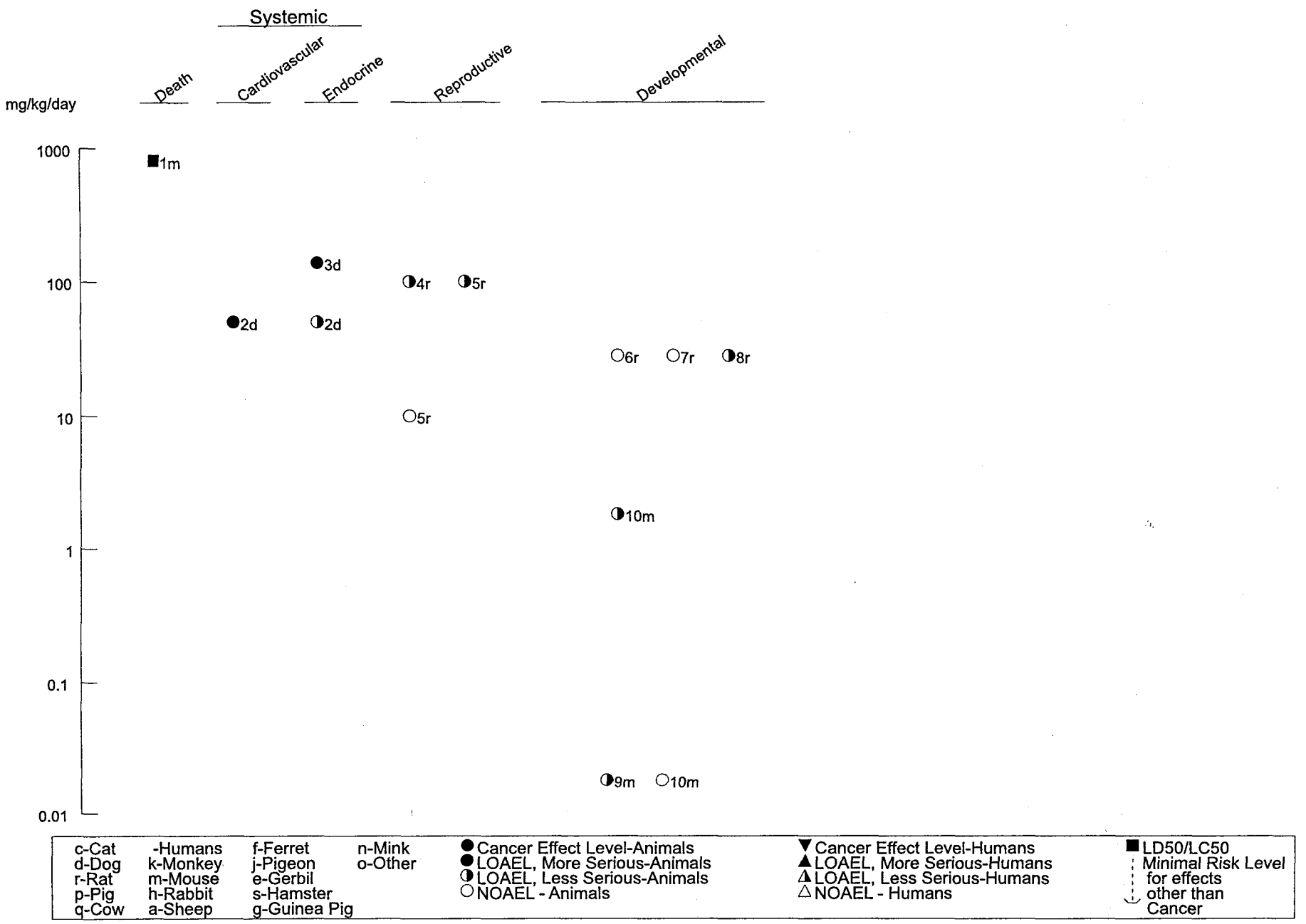


Figure 3-2. Levels of Significant Exposure to o,p'-DDT, -DDE, -DDD - Oral (*Continued*)

Intermediate (15-364 days)

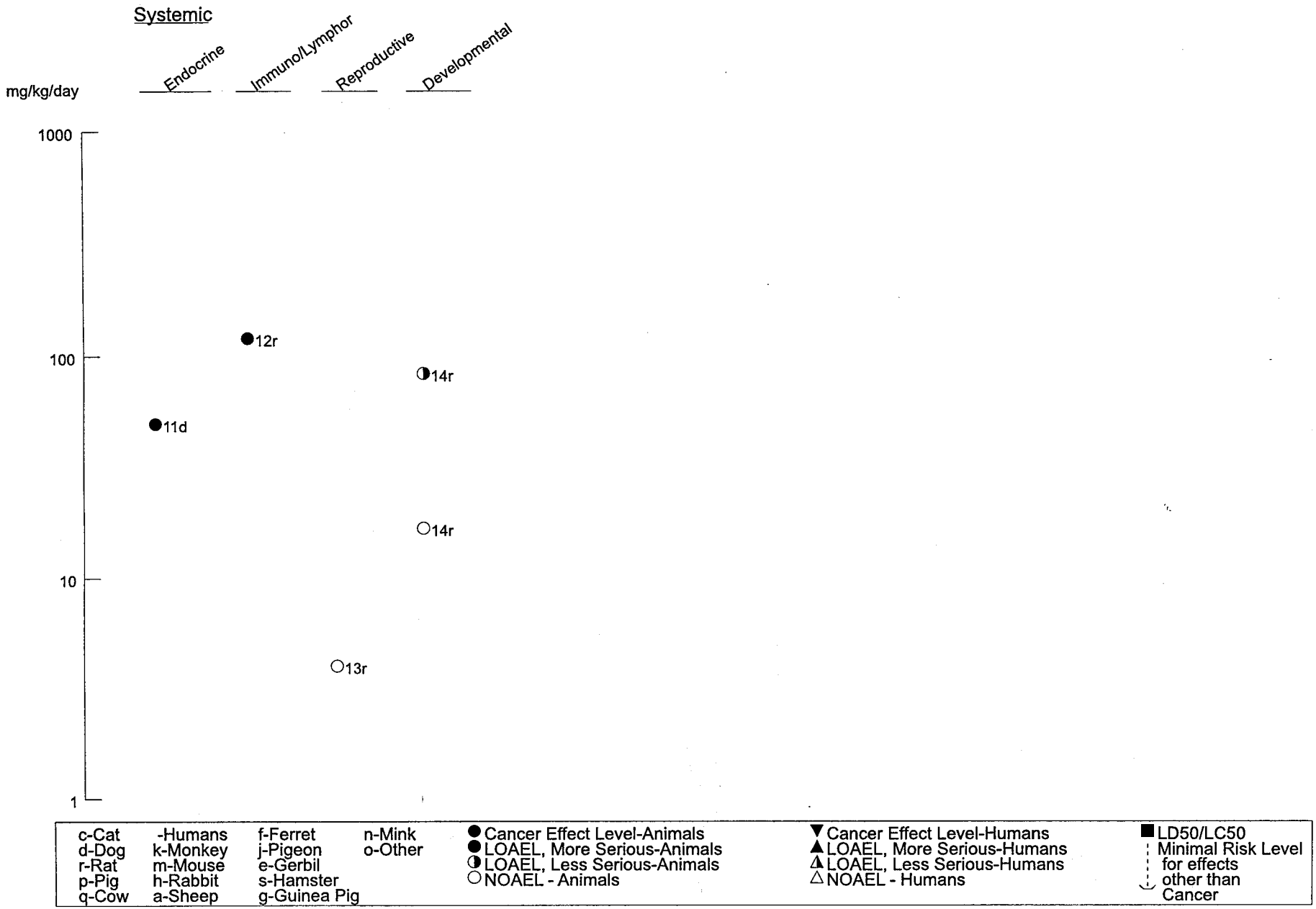
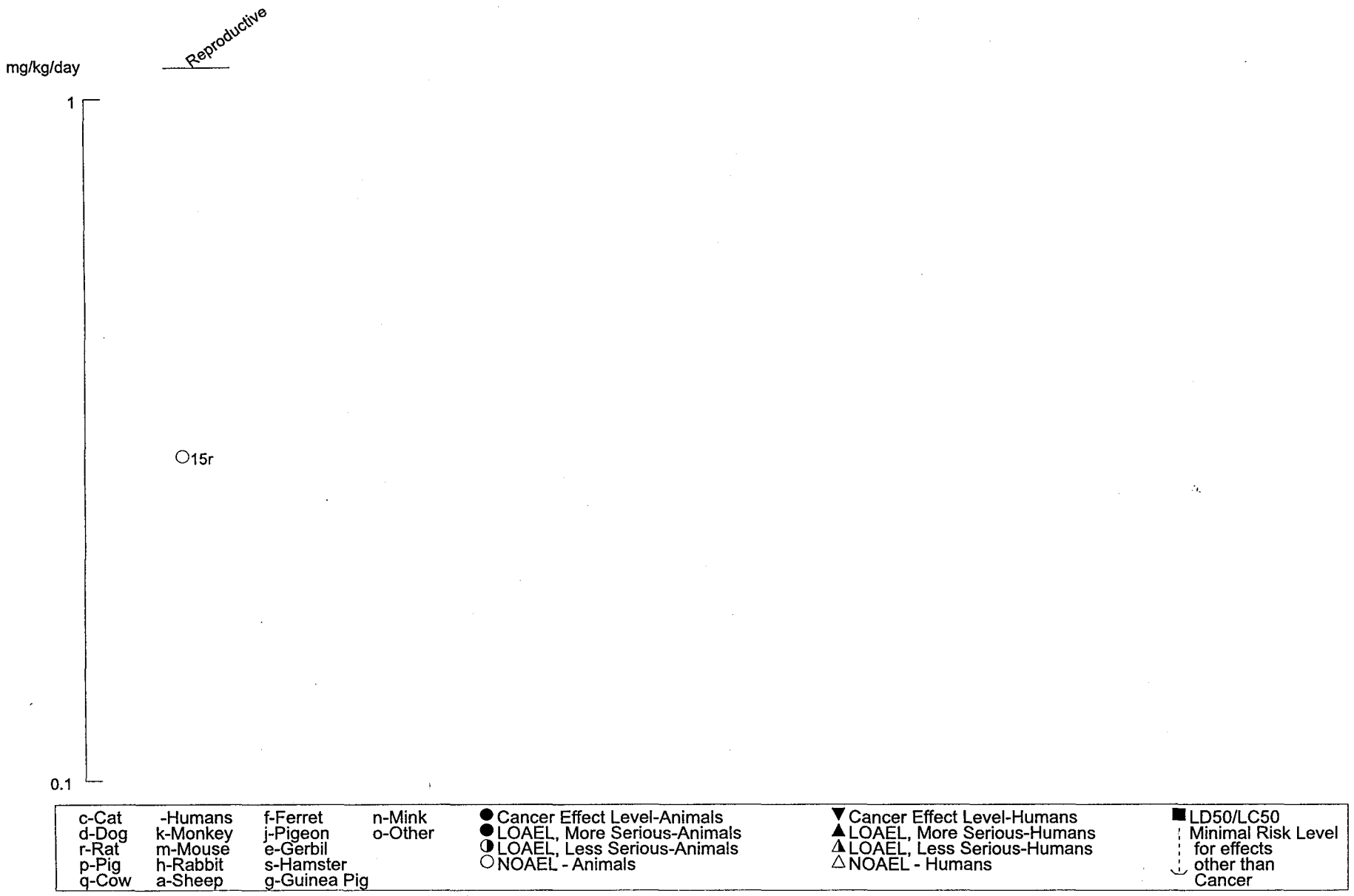


Figure 3-2. Levels of Significant Exposure to o,p'-DDT, -DDE, -DDD - Oral (*Continued*)

Chronic (≥365 days)



exposure to humans below which the risk of adverse effects (other than cancer) is presumed to be minimal are also presented.

Occupational exposure to DDT has involved multiple routes of exposure, the most significant of which involved ingestion of DDT particles via the mucociliary apparatus of the respiratory tract. Therefore, epidemiological studies of occupational exposure to DDT are discussed in this section.

3.2.2.1 Death

Only one case of fatal poisoning in humans after accidental oral exposure to DDT has been documented (Hill and Robinson 1945). One ounce of 5% DDT in kerosene was ingested by a 1-year-old child. Clinical signs included coughing and vomiting followed by tremors and convulsions. The child then became comatose and died; however, the contribution of the kerosene solvent to DDT toxicity was not clear. Doses as high as 285 mg DDT/kg body weight have been accidentally ingested by humans with no fatal results (Garrett 1947).

In a follow-up study to Morgan and Lin (1978) (see Section 3.2.2.2), Morgan et al. (1980) analyzed morbidity and mortality in an extensive cohort of male and female workers exposed (formulators, applicators, farmers) to organochlorine pesticides. The follow-up included 73% of an original cohort of 2,600 workers. The median duration of work in a pesticide-related occupation was 13 years. Disease incidence rates were studied in relation to job exposure categories and to serum levels of organochlorine pesticides measured in the original study. There were no significant differences in mortality patterns between pesticide-exposed workers and controls except for an excess of deaths by accidental trauma in workers engaged in structural pesticide application.

A historical prospective mortality study was conducted on 3,600 white male workers employed between 1935 and 1976 in occupations that involved exposures to various brominated compounds, organic and inorganic bromides, and DDT (Wong et al. 1984). Among individuals exposed to DDT, overall mortality, expressed as the standardized mortality ratio (SMR), was not elevated over expected values.

However, there was an excess in deaths from respiratory cancer. Several factors confound these results: those individuals exposed to DDT also were potentially exposed to other chemicals, and smoking history was not included in the analysis. Brown (1992) conducted an update of an historical prospective mortality study of workers in five pesticide manufacturing plants. In the plant where primary exposure

was DDT (320 persons and 90 deaths since 1964), there was a significant excess of deaths (11) from stroke. The SMR was 2.38. The study is limited by insufficient exposure data, possible confounding exposures, and by relatively small numbers of deaths from stroke.

The LD_{50} values reported in rats exposed to single oral doses of DDT ranged from 113 to 800 mg/kg (Ben-Dyke et al. 1970; Cameron and Burgess 1945; Gaines 1969). Results from a study by Lu et al. (1965) revealed age-dependent LD_{50} values in rats. The LD_{50} values in newborn, preweanling, weanling, and adult rats were >4,000, 438, 355, and 195 mg/kg, respectively. However, when preweanling and adult rats were administered one-quarter of the LD_{50} daily for 4 days, there was no significant difference in the 4-day LD_{50} between the two age groups. Lu et al. (1965) suggested that the elimination mechanism in the preweanling rats is less well developed, thus making them more susceptible to repeated doses than adults. The age-dependent susceptibility to single high oral doses of DDT in rats was confirmed by others who suggested that seizures and hyperthermia, observed in the adults but not in young rats, as well as less resistance to hypoxia, contribute to the apparent higher sensitivity of the adult rat (Henderson and Wooley 1969b, 1970).

In male and female mice, a single oral dose of >237 mg technical-grade DDT/kg caused death (Kashyap et al. 1977; Tomatis et al. 1972), and a daily dietary dose of about 85.7 mg *p,p*'-DDT/kg killed 50% of a group of mice after a 6-day feeding period (Okey and Page 1974). Exposure of pregnant mice to 34.3 mg technical DDT/kg on gestation days 1–21 followed by cross-fostering of the pups resulted in preweaning death in 39% of the neonates exposed *in utero* and through lactation and 10% of the pups exposed only through lactation (Craig and Ogilvie 1974). No deaths occurred in pups exposed *in utero* only. The LD_{50} values for guinea pigs and rabbits after oral exposure to DDT were 400 and 300 mg/kg, respectively (Cameron and Burgess 1945). Four out of five female B6C3F1 mice fed a diet that provided approximately 35 mg technical DDT/kg/day for 6 weeks died; the cause of death was not discussed (NCI 1978). Four out of six monkeys treated by gavage with 50 mg *p,p*'-DDT/kg/day died after 4 weeks of treatment; two additional monkeys died during the weeks nine and fourteen of treatment, the cause of death was not specified (Cranmer et al. 1972a). In a 130-month study that administered approximately 6.4–15.5 mg of *p,p*'-DDT/kg/day to 11 Rhesus and 14 Cynomolgus monkeys, there were 6 early deaths; the lowest dose associated with death was approximately 6.9 mg/kg/day (Takayama et al. 1999). A 10% mortality rate (relative to 0% in controls) was observed in the 78-week NCI (1978) bioassay for female B6C3F1 mice treated with approximately 15 mg technical DDT/kg/day; in the high-dose group (30.2 mg/kg/day), the mortality rate was 28%. There was no positive association between dose of DDT and mortality in male mice (NCI 1978).

DDE mortality studies also have been conducted. An LD_{50} of 880 mg/kg was reported for male Sherman rats (Gaines 1969). Death occurred in mice after single oral doses of *o,p'*-DDE ranging from 810 to 880 mg DDE/kg (Tomatis et al. 1974a).

An LD_{50} was reported for rats as a range of single oral doses (400–4,000 mg/kg) in which mortality was observed in 50% of rats exposed to DDD (Ben-Dyke et al. 1970). An LD_{50} for DDD in rats of >4,000 mg/kg was reported by Gaines (1969). Tomatis et al. (1974a) reported an LD_{50} in mice after a single oral dose ranging from 1,466 to 1,507 mg DDD/kg.

The LD_{50} values for the various isomers and technical-grades of DDT, DDE, and DDD are recorded in Tables 3-1 and 3-2 and plotted in Figures 3-1 and 3-2.

3.2.2.2 Systemic Effects

The highest NOAEL values and all LOAEL values from each reliable study for each systemic effect in each species and duration category are recorded in Tables 3-1 and 3-2 and plotted in Figures 3-1 and 3-2.

Respiratory Effects. No studies were located regarding respiratory effects in humans after oral exposure to DDT, DDE, or DDD. Rats fed a diet containing 20 mg DDT/kg/day for 27 months did not develop adverse respiratory effects with the exception of squamous bronchial metaplasia in one rat (Deichmann et al. 1967). In the 78-week chronic bioassay conducted by NCI (1978), no adverse effects on the respiratory system were observed in Osborne-Mendel rats treated in the diet with up to 45 mg technical DDT/kg/day, 59 mg *p,p*'-DDE/kg/day, or 231 mg technical DDD/kg/day. The same findings were reported for B6C3F1 mice treated with up to 30.2 mg technical DDT/kg/day, 49 mg *p,p*'-DDE/kg/day, or 142 mg technical DDD/kg/day (NCI 1978).

Cardiovascular Effects. Cardiovascular performance was one of the parameters evaluated in male volunteers orally administered 3.5 or 35 mg DDT/day by capsule for 12–18 months either as recrystallized or technical-grade DDT (Hayes et al. 1956). This dosing regimen resulted in administered doses of 0.05–0.063 or 0.36–0.5 mg/kg/day for 12–18 months. The background concentrations measured in the food of both controls and test subjects were 0.0021–0.0038 mg DDT/kg/day. Although some variations among individuals in heart rate (resting and with exercise), systolic blood pressure, and pulse pressure were noted, these variations did not correlate with increasing dosage of DDT or with duration of exposure. The authors concluded that DDT at these doses did not result in adverse cardiac effects.

Tachycardia (increased heart rate) was reported in 1 of 11 cases in humans after accidental ingestion of approximately 286–1,716 mg DDT in food, which when adjusted for individual body weights, was 5.1–120.5 mg/kg (Hsieh 1954). It is not known if the tachycardia was a direct result of damage to the heart or a result of a neurologically-mediated mechanism. A suggestive relationship between high serum levels of DDT and DDE and subsequent development of vascular disease, especially hypertension, was noted in pesticide-exposed individuals (Morgan et al. 1980). However, exposure to multiple pesticides is a significant confounding factor in this study.

In the 78-week chronic bioassay conducted by NCI (1978), no significant chemical-related adverse effects on the cardiovascular system were observed in Osborne-Mendel rats treated in the diet with up to 45 mg technical DDT/kg/day, 59 mg *p,p*'-DDE/kg/day, or 231 mg technical DDD/kg/day. The same findings were reported for B6C3F1 mice treated with up to 30.2 mg technical DDT/kg/day, 49 mg *p,p*'-DDE/kg/day, or 142 mg technical DDD/kg/day (NCI 1978).

Gastrointestinal Effects. No studies were located regarding the gastrointestinal effects in humans after oral exposure to DDT, DDE, or DDD. In the 78-week chronic bioassay conducted by NCI (1978), no significant chemical-related adverse effects on the gastrointestinal system were observed in Osborne-Mendel rats treated in the diet with up to 45 mg technical DDT/kg/day, 59 mg *p,p*'-DDE/kg/day, or 231 mg technical DDD/kg/day. The same findings were reported for B6C3F1 mice treated with up to 30.2 mg technical DDT/kg/day, 49 mg *p,p*'-DDE/kg/day, or 142 mg technical DDD/kg/day (NCI 1978).

Hematological Effects. Hematological effects of DDT (Hayes et al. 1956; Laws et al. 1967; Morgan and Lin 1978; Ortelee 1958) and DDE (Dunstan et al. 1996; Morgan and Lin 1978) have been assessed, but the statistical power in some of these studies is practically nil because of the small number of subjects. An extensive study measuring organochlorine pesticide concentrations in the blood of 2,600 pesticide-exposed individuals was conducted (Morgan and Lin 1978). One thousand controls with minimal exposure to pesticides were recruited and monitored. However, controls were not matched to pesticide-exposed individuals for age, sex, or race. Various clinical tests, including extensive hematologic analyses were performed during 1967–1973, and *p,p'*-DDT and *p,p'*-DDE levels in blood were determined (not adjusted for lipid content). None of the many hematological parameters measured had a correlation coefficient with DDT and/or DDE blood levels >0.17 (meaning that at best, 2.89% of the variance could be attributed to the pesticide levels) although a number of coefficients showed statistical significance. Morgan and Lin (1978) acknowledged that because the analysis was based on such large numbers of measurements, some statistically significant differences were likely to be found.

Clinical and laboratory examinations were performed on 40 workers exposed to DDT, some of whom had also been exposed to other pesticides (Ortelee 1958). Exposure was reported to have occurred primarily via dermal and inhalation routes, but absorption by these routes may not have been significant. No protective equipment was used and the workers were often coated with concentrated DDT dust. Examinations included a complete medical history, physical and neurological examination, and hematological and blood chemistry analyses. In addition, plasma and erythrocyte cholinesterase levels were determined as well as urinary excretion of bis(*p*-chlorophenyl) acetic acid (DDA), which is the predominant final metabolite of DDT. On the basis of DDA excretion, it was estimated that these workers received absorbed doses equivalent to oral doses of 14–42 mg/person/day (approximately 0.2–0.6 mg/kg/day). Despite this relatively high level of exposure, no correlation was found between DDT exposure and the frequency and distribution of abnormalities, including neurological effects, except for a few cases of minor skin and eye irritation.

A study was conducted on 35 workers who had been involved in the manufacture and formulation of DDT for an average of 15 years (Laws et al. 1967). Extensive medical examinations and blood, urine, and fat analyses were performed in an attempt to find any correlation between any health problems and exposure to DDT. The authors concluded that the clinical findings for this group were not significantly different from those expected in an appropriate control group with no occupational exposure to DDT. However, this study did not include a control group and comparisons of data were made by using information derived from "the general population".

Fifty-one male volunteers were exposed to 3.5 or 35 mg DDT/day, resulting in administered doses of 0.05–0.063 or 0.36–0.5 mg DDT/kg/day, respectively, for 12–18 months (Hayes et al. 1956). The background concentration measured in food of both controls and test subjects was 0.0021–0.0038 mg DDT/kg/day. Although some variation among individuals in hemoglobin levels, red and white blood cell count, and percentage of polymorphonuclear leukocytes was noted, these variations did not correlate with increased dosage of DDT or with duration of exposure.

In a case-control study of patients with chronic, debilitating fatigue lasting at least 6 months, the mean concentration of *p,p'*-DDE in blood serum was significantly higher in case subjects (11.9 ppb; n=14) than in controls (5.2 ppb; n=23) (Dunstan et al. 1996). When the 37 subjects were pooled and then redivided according to high serum DDE (>6 ppb) and low serum DDE (<6 ppb), the red blood cell distribution width (variation in erythrocyte cell width change; often a sign of anemia) was significantly greater in the

high DDE group than in the low DDE group; no differences were seen in other hematological parameters.

In a chronic study, exposure of rats to DDT at 20 mg/kg/day for 27 months resulted in alterations in the spleen, which consisted of congestion and hemolysis exceeding that observed in untreated control rats (Deichmann et al. 1967). In addition, squirrel monkeys exposed orally to doses of 0.05–50 mg DDT/kg/day for up to 6 months exhibited no hematological changes; however, all monkeys in the highest dose group (six animals) died by week 14 (Cranmer et al. 1972a); the cause of death was not determined, but before death, the monkeys had apparently recovered from severe neurotoxic symptoms.

Overall, the existing information does not suggest that hematological parameters are sensitive targets for DDT toxicity. The extent to which this is due to limitations in the available studies is uncertain.

Musculoskeletal Effects. The relationship between serum levels of DDE and bone mineral density was examined in a group of 103 (50 black, 53 white) women who had participated in the Mount Sinai Medical Center Longitudinal Normative Bone Density Study (1984–1987) (Bohannon et al. 2000). Because bone metabolism is both estrogen- and androgen-dependent, and DDT and related compounds have shown hormone-like properties, the authors hypothesized that high levels of DDE (the main stored metabolite of DDT) would be associated with lower bone density in peri- and postmenopausal women than in premenopausal women. Bone mineral density was measured at the lumbar spine and radius at 6 months intervals during a 2-year period. The results showed that black women had serum DDE levels significantly higher than white women (mean 13.9 vs. 8.4 ppb), but there was no correlation between either DDE and bone density or between DDE and the rate of bone loss. In a study of 68 sedentary Australian women (45–64 years of age), the authors state that an association was found between bone mineral density (lumbar spine) and serum DDE levels >2 ppb (Beard et al. 2000). However, the correlation coefficient was 0.269, meaning that only about 7.24% of the variance could be attributed to DDE; a stronger correlation was found for the relationship between bone mineral density and age.

An additional study examined associations between bone mineral density and serum levels of organochlorines (five DDT-related compounds among them) in 115 men (40–75 years of age) from the general Swedish population (Glynn et al. 2000). The most concentrated DDT-related compound was *p,p*'-DDE, with a mean value of 784 ppb (lipid basis). After adjusting for confounding factors in linear regression analyses, there was no significant association between serum concentrations of single organochlorines and bone mineral density.

Limited information exists from studies in animals. In the 78-week chronic bioassay conducted by NCI (1978), no significant chemical-related adverse musculoskeletal effects were observed in Osborne-Mendel rats treated in the diet with up to 45 mg technical DDT/kg/day, 59 mg *p,p*'-DDE/kg/day, or 231 mg technical DDD/kg/day. The same findings were reported for B6C3F1 mice treated with up to 30.2 mg technical DDT/kg/day, 49 mg *p,p*'-DDE/kg/day, or 142 mg technical DDD/kg/day (NCI 1978).

The existing information suggests that neither skeletal muscle nor bone are targets for DDT/DDE toxicity.

Hepatic Effects. Based on the data available, there is no conclusive evidence that DDT, DDD, and DDE cause adverse liver effects in humans, but in animals, the liver appears to be a sensitive target for DDT. Morgan and Lin (1978) measured organochlorine pesticide concentrations in the blood of 2,600 pesticide-exposed workers. Controls were not matched for age, sex, or race to pesticide-exposed individuals; data on alcohol consumption were not available. Weak correlations were reported between increasing serum DDT and DDE and elevated serum levels of alkaline phosphatase and lactic dehydrogenase. No significant correlation was found for serum glutamic oxaloacetic transaminase (SGOT; aspartate aminotransferase [AST]) and serum glutamic pyruvic transaminase (SGPT; alanine aminotransferase [ALT]). Morgan and Lin (1978) pointed out that the effects on serum enzyme activities were of such small magnitude that they were only detectable by exhaustive statistical analysis of data from thousands of subjects. In a follow-up study of Laws et al. (1967), Laws et al. (1973) reexamined 31 of the 35 workers who had been involved in the manufacture and formulation of DDT for an average of 15 years. These men completed detailed questionnaires concerning their daily contact with DDT. In addition, liver function tests and DDT serum sample tests were performed on 21 subjects. Despite the fact that these workers had an average exposure to DDT for 21 years at levels estimated to correspond to 3.6–18 mg daily (0.05–0.26 mg/kg/day), no evidence of hepatotoxicity, hepatic enlargement, or dysfunction (as measured by the Bromsulfthalein test, also known as sulfobromophthelein sodium) was found. Only one subject had an elevated Bromsulfthalein retention and slightly elevated serum levels of alkaline phosphatase and AST. Hayes et al. (1956) exposed 51 male volunteers to 3.5 or 35 mg DDT/day, resulting in administered doses of 0.05–0.063 or 0.36–0.5 mg DDT/kg/day for 12–18 months. The background concentration measured in food of both controls and test subjects was 0.0021–0.0038 mg DDT/kg/day. No signs of illness or adverse hepatic effects (as measured by liver function tests) were reported that were considered to be related to DDT exposure to humans.

Epidemiological studies of DDT-exposed workers often show increased activity of hepatic metabolic enzymes. Kolmodin et al. (1969) studied drug metabolism in 26 workers with occupational exposure to

several pesticides, primarily DDT, chlordane, and lindane. An appropriate control group of 33 subjects was included. Xenobiotic metabolism was assessed by administering antipyrine in a single dose of 10–15 mg/kg after which blood levels were monitored. Antipyrine was chosen because it is metabolized in the liver and not appreciably bound to plasma proteins. The half-life of antipyrine (both mean and median) was shorter in workers exposed to insecticides than in control subjects. In addition, the range of the half-life was much smaller in the exposed group. These results indicate that exposure to these insecticides induces hepatic enzyme activities, but the relative contribution of DDT to the observed enzyme induction is impossible to ascertain in this study.

The effect of high DDT exposure on phenylbutazone and endogenous cortisol metabolism was investigated in exposed workers by Poland et al. (1970). Both of these compounds are metabolized via hepatic microsomal enzymes. Nineteen workers with an average of 14 years of employment in a plant producing only DDT were selected for study, and matched controls were obtained. Both groups were screened for medical history and given a physical examination, and venous blood samples were drawn. After a single dose of 400 mg phenylbutazone, serum was obtained at regular intervals beginning at 24 and ending at 120 hours after drug administration. The serum half-life of phenylbutazone was significantly shorter in DDT-exposed workers than in the control population. However, the serum half-life of phenylbutazone did not correlate with the total concentration of DDT and related compounds in the serum of either group. The urinary excretion of the cortisol metabolite, 6β-hydroxycortisol, was increased by 57% in DDT-exposed individuals when compared to controls. However, there was no significant correlation found between the absolute serum concentration of total DDT-related compounds and the urinary excretion of 6β-hydroxycortisol. These results suggest that exposure to DDT can alter normal hepatic metabolic enzyme activity.

In animals, the liver appears to be one of the primary targets of DDT toxicity. Acute, subchronic, and chronic oral administration of DDT has been shown to cause dose-related mild-to-severe hepatic effects in numerous animal studies. In addition, DDT has been demonstrated to be an inducer of microsomal mixed function oxidases of the liver by its ability to promote the biotransformation of various chemicals (Pasha 1981; Street and Chadwick 1967).

Acute oral exposure to DDT is associated with a number of effects in animals including increased liver weights, increased serum levels of liver enzymes (suggestive of liver injury), and changes in the appearance of the liver. Both liver plasma membrane and serum gamma-glutamyl transpeptidase (GGTP) were increased 2-fold in rats treated with a single dose of 200 mg DDT/kg (Garcia and Mourelle 1984).

Plasma membrane enzyme activity returned to normal 48 hours after dosing, but enzyme activity in serum remained elevated for 48 hours. Relative liver weight increased 18% after 12 days exposure to 40 mg/kg/day DDT in rats previously deprived of vitamin A for several generations; DDT treatment began 1 week after vitamin A supplementation was started to increase the rats' vitamin A intake back to "normal" (de Waziers and Azais 1987). In both "normal" and vitamin A deficient rats, 12 days of DDT treatment increased liver CYP (P450) enzyme content per gram of liver by 2-fold, benzo(a)pyrene hydroxylase (a CYP enzyme) activity by 3-fold, and glutathione-S-transferase activity by 2-fold; all of these changes were statistically significant. A recent study showed that hepatic O-dealkylation activities associated with CYP2B were induced in a dose-related manner in rats treated orally with DDT for 14 days (Nims et al. 1998). The dose range tested was approximately 0.17–36 mg/kg/day. Maximal induction was more than 21 times greater than control values. There was no effect on *in vivo* CYP2B induction at <0.17 mg/kg/day. Limited induction of ethoxyresorufin O-dealkylation was observed, and no induction of immunoreactive hepatic microsomal CYP1A protein was seen. DDT also induced CYP3A1 and CYP3A2 proteins. Induction activity was also accompanied by an increase in relative liver weight with doses of approximately 4.6 mg/kg/day and higher. Mice administered 42.9 mg DDT/kg/day for 1 week by oral gavage had increased liver weights and increased microsomal enzyme activities (Pasha 1981) and Rhesus monkeys exposed to one oral dose of 150 mg DDT/kg had increased alkaline phosphatase (AP), LDH, AST, and ALT activities in serum (Agarwal et al. 1978). An increase in the levels of liver metabolic enzymes by itself is not considered adverse; however, continued microsomal enzyme induction may lead to hypertrophy and contribute to morphological changes in the liver. It should be noted also that microsomal enzyme induction may lead to the activation of some chemicals to toxic metabolites and the detoxification of other chemicals. In addition, increased serum levels of liver enzymes such as transaminases, LDH and AP can be predictive of more serious liver effects on prolonged exposure since this may indicate cell death and consequent lysis of membranes and leakage of enzymes.

Hepatic cell hypertrophy, histopathologic alterations (proliferation of the smooth endoplasmic reticulum and concentric membrane arrays), and increased microsomal enzyme activity have also been seen following intermediate exposure of rats to DDT. These effects were observed in rats following 3–27 weeks of dietary exposure at doses ranging from 0.25 to 20 mg DDT/kg/day (Gupta et al.1989; Laug et al. 1950; Ortega 1956). In general, as doses increased, hepatic effects in rats became more severe. Phase I metabolic enzymes were significantly increased in rats treated for 3 weeks with 15 mg *p,p*'-DDT/kg/day (Gupta et al. 1989). Minor hepatocyte vacuolation was seen in male rats receiving 2.5 mg technical DDT/kg/day in the diet for 3 months; females exhibited liver hypertrophy at 10 mg/kg/day (Ortega 1956). Rats administered approximately $(0.25–0.5) mg technical DDT/kg/day

(see Appendix A for dose calculation) in the diet for 12–27 weeks had liver changes consisting of hepatic cell enlargement, especially in the cental lobules, increased cytoplasmic oxyphilia with sometimes a semihyaline appearance, and more peripheral locations of the basophilic cytoplasmic granules (Laug et al. 1950). The severity of the effects was dose-related; a dose of approximately 0.05–0.09 mg/kg/day was a NOAEL. This study (Laug et al. 1950) was used as the basis for the derivation of an intermediate-duration oral MRL for DDT. Fitzhugh and Nelson (1947) fed female Osborne-Mendel rats a diet containing 1,000 ppm (96 mg/kg/day) technical-grade DDT for 12 weeks and observed reversible changes in the liver, including enlargement of centrolobular hepatocytes with accompanying histological changes. Rats exposed continuously to 6.6 mg DDT/kg/day in the diet for 36 weeks exhibited spotty cellular necrosis with moderate hepatocyte regeneration (Jonsson et al. 1981). Squirrel monkeys and mice had increased hepatic cytochrome P-450 enzyme activities and/or increased liver weights following short-term exposure by oral gavage to DDT. These effects were observed following doses of 1.67 or 6.25 mg DDT/kg/day for 28 days in mice (Lundberg 1974; Orberg and Lundberg 1974) and 5 mg DDT/kg/day for up to 6 months in squirrel monkeys (Cranmer et al. 1972a).

Hepatic effects ranging from increased liver weights to cellular necrosis have been reported in animals after chronic exposure to DDT in the diet. Necrosis, centrilobular hypertrophy, and hyperplasia have also been reported in rats exposed to 7–56 mg DDT/kg/day for 24–27 months (Deichmann et al. 1967; Fitzhugh and Nelson 1947). Increased incidence of fatty metamorphosis was seen in the liver of male rats treated with approximately 23 mg technical DDT/kg/day or more and of amyloidosis in the liver of male mice treated with about 3.7 mg DDT/kg/day for 78 weeks (NCI 1978). Increased relative liver weights, but no increase in serum ALT, LDH, AP, cholinesterase, or in liver ALT, AST, LDH, AP, or cholinesterase activities were reported in hamsters after exposure to 67–133 mg DDT/kg/day for life (Graillot et al. 1975). Cabral et al. (1982a) reported a significant increase in liver necrosis in hamsters exposed to approximately 71 mg DDT/kg/day in the diet for life, but not at lower doses. Both focal and diffuse liver alterations were observed in dogs exposed by diet to 80 mg DDT/kg/day for 39–40 months, but not at 16 mg/kg/day (Lehman 1965). In Rhesus monkeys given up to 3.9 mg DDT/kg/day for 3.5–7.5 years, periodic liver biopsies showed no “significant” observable alterations in liver histology and the Bromsulfthalein clearance test was normal, indicating no functional liver changes (Durham et al. 1963). However, in a 130-month dietary study in Rhesus and Cynomolgus monkeys, a dose of approximately 6.4 mg of *p,p*’-DDT/kg/day, the lowest dose tested, produced significant liver alterations including hepatocyte vacuolation, proliferation of bile duct cells, clear hepatocyte foci, and liver cell necrosis (Takayama et al. 1999).

Limited information exists on hepatic effects after oral exposure of animals to DDD or DDE. Rats exposed to two gavage doses of 350 mg DDE/kg/day exhibited an increase in enzyme levels (ornithine decarboxylase and cytochrome P-450) (Kitchin and Brown 1988), and mice showed increased liver weight, microsomal P450, cytochrome-C reductase, and serum total protein after daily gavage dosing with 42.9 mg/kg DDE/day for 7 days (Pasha 1981). A dose of 100 mg of *p,p'*-DDE/kg/day for 7 days caused elevated hepatic aromatase (enzyme involved in steroid metabolism) activity in adult rats, a potential mechanism by which DDE can affect reproduction (You et al. 2001). Chronic exposure of rats (78 weeks) to *p,p*,'-DDE resulted in increased incidence of fatty metamorphosis in the liver from males at a dose of approximately 31 mg/kg/day (NCI 1978). DDE produced significant induction of hepatic O-dealkylation activities associated with CYP2B in rats administered DDE in the diet at dose levels between approximately 0.15 and 36 mg/kg/day for 14 days (Nims et al. 1998). The NOEL for *in vivo* CYP2B induction was 0.17 mg/kg/day. No induction of immunoreactive hepatic microsomal CYP1A protein was observed, and induction of CYP1A associated activities was limited. DDE also induced CYP3A1 and CYP3A2 proteins. Induction activity was also accompanied by a significant increase in relative liver weight at a dose level of approximately 4.1 mg/kg/day and higher. No adverse liver effects were observed in mice chronically exposed to *p,p*'-DDE in the diet at up to 49 mg/kg/day for 78 weeks (NCI 1978). Chronic exposure to approximately 48 mg *p,p*'-DDE/kg/day resulted in focal necrosis of the liver in hamsters in a 128-week study (Rossi et al. 1983).

DDD induced CYP2B associated activities in the livers of rats treated with the test material in the diet at dose levels of approximately 1.4 mg/kg/day and higher for 14 days (Nims et al. 1998). The NOEL was between 0.5 and 1.4 mg/kg/day. No induction of immunoreactive hepatic microsomal CYP1A protein was observed, and limited induction of CYP1A associated enzyme activity was seen. DDD also slightly induced CYP3A2, but not CYP3A1. No significant treatment-related nonneoplastic alterations were seen in the livers from rats or mice exposed orally to up to 231 or 142 mg technical DDD/kg/day, respectively, for 78 weeks (NCI 1978). Mice exposed orally to 42.9 mg DDD/kg/day in the diet for 1 week had no change in levels of P-450 microsomal enzymes, but showed a decrease in cytosolic-enzyme-mediated hydroxylation of 2- and 4-biphenyls with no change in liver weight (Pasha 1981).

Renal Effects. No studies were located regarding renal effects in humans after oral exposure to DDT, DDE, or DDD. Male and female Osborne-Mendel rats exposed orally to 20 mg DDT/kg/day in the diet for 27 months had tubular polycystic degeneration, tubular epithelial necrosis, and hemorrhage of the kidney (Deichmann et al. 1967). In the 78-week chronic bioassay conducted by NCI (1978), no significant chemical-related adverse renal effects were observed in Osborne-Mendel rats treated in the

diet with up to 45 mg technical DDT/kg/day, or in mice treated with up to 30.2 mg DDT/kg/day. Similarly, no adverse renal effects were seen in rats treated with up to approximately 59 mg *p,p*'-DDE/kg/day for 78 weeks (NCI 1978). However, chronic inflammation of the kidneys was seen in male mice treated with approximately 27 mg *p,p*'-DDE/kg/day (NCI 1978); no such effect was seen in female mice treated with up to 49 mg/kg/day. Technical DDE, at a dose level of approximately 66 mg/kg/day, increased the incidence of chronic inflammation of the kidney in female rats in the 78-week NCI (1978) bioassay. No significant chemical-related increase in kidney lesions was seen in mice treated for 78 weeks treated with up to 231 mg technical DDD/kg/day (NCI 1978).

Endocrine Effects. Exposure to DDT and DDT-related compounds, particularly during development, can adversely affect the development and function of the reproductive system of both female and male animals. This is due primarily to the ability of some of these compounds to disrupt the action of natural steroids and bind to receptors for estrogens and androgens. Hormonal effects of DDT and residues that lead to altered reproduction and/or development are discussed in Sections 3.2.2.5 Reproductive Effects, 3.2.2.6 Developmental Effects, and 3.6.2 Mechanisms of Toxicity. Reported endocrine effects unrelated to reproduction or development are briefly mentioned below.

No studies were located regarding endocrine effects in humans after oral exposure to DDT or DDE. However, *o,p'*-DDD has been used to treat adrenocortical carcinoma for almost four decades (Bergenstal et al. 1960; Wooten and King 1993). The therapeutic action is based on the activation of the compound by local cytochrome P-450 (CYP11β) to a reactive metabolite that binds to macromolecules in the adrenal cortex (see Section 3.6.2, Mechanisms of Toxicity, for further details).

In rats administered 0.2 mg technical-grade DDT/kg/day for 120 days, atrophy of all zones of the adrenal gland, except the *zona glomerulosa*, was observed (Chowdhury et al. 1990). Hyalin degeneration of the medulla and cortex and a decrease in adrenal gland weight were also reported. Dogs given 1–15 oral doses of 100 mg/kg/day technical-grade DDD showed degenerative changes in mitochondria in both the *zona fasciculata* and *zona reticularis* of the adrenal cortex, which were reversible 56 weeks after dosing ceased (Powers et al. 1974). Similar degenerative adrenal changes were reported by Kirk et al. (1974) after dosing dogs (by capsule) with 138.5 mg/kg/day of *o,p'*-DDD for 10 days. Plasma levels of cortisol were decreased and a decreased response to ACTH stimulation was observed. In one animal, there was hemorrhage, invasion by lymphocytes, and necrosis of the adrenal cortex (Kirk et al. 1974). Adrenocortical necrosis was also reported in dogs treated for 36–150 days with 50 mg *o,p*'-DDD/kg/day in a capsule (Kirk and Jensen 1975). Studies in animals have also shown that the adrenal gland is affected

by sulfonyl metabolites of DDT. The metabolic pathways leading to these metabolites are discussed in Section 3.5.3, Metabolism, whereas information on their mechanism of toxic action can be found in Section 3.6.2, Mechanisms of Toxicity.

Reduced iodine concentrating capacity in the thyroid was reported in adult male Sprague-Dawley rats given a single oral gavage dose of 50 mg or higher of technical DDT/kg; the-no-observed-effect dose level was between 25 and 50 mg/kg (Goldman 1981).

In the 78-week chronic bioassay conducted by NCI (1978), no treatment-related adverse effects on the endocrine system (pituitary, adrenals, thyroid, parathyroid) were observed in Osborne-Mendel rats treated in the diet with up to 45 mg technical DDT/kg/day, 59 mg *p,p*'-DDE/kg/day, or 231 mg technical DDD/kg/day. The same findings were reported for B6C3F1 mice treated with up to 30.2 mg technical DDT/kg/day, 49 mg *p,p*'-DDE/kg/day, or 142 mg technical DDD/kg/day (NCI 1978).

Dermal Effects. Clinical examinations with laboratory workups were performed on 40 workers exposed to DDT, some of whom had also been exposed to other pesticides (Ortelee 1958). Exposure was reported to occur primarily via dermal and inhalation routes; however, no protective equipment was used, and the workers were often coated with concentrated DDT dust. Information collected on each worker included a complete medical history, physical and neurological examination results, and hematological and blood chemistry analyses results. Plasma and erythrocyte cholinesterase levels were determined as well as urinary excretion of DDA. On the basis of DDA excretion, it was estimated that these workers received absorbed doses equivalent to oral doses of 14–42 mg/day (approximately 0.2–0.6 mg/kg/day). Despite the relatively high estimated exposure, no correlation was found between DDT exposure and the frequency and distribution of skin abnormalities, except for a few cases of minor skin irritation.

No studies were located indicating adverse dermal effects in animals after oral exposure to DDT, DDE, or DDD.

Ocular Effects. The only information available is that from an earlier report by Ortelee (1958) regarding 40 workers exposed to DDT, some of whom had also been exposed to other pesticides. Based on information on DDA excretion, it was estimated that these workers received absorbed doses equivalent to oral doses of 14–42 mg/day (approximately 0.2–0.6 mg/kg/day). No correlation was found between DDT exposure and the frequency and distribution of abnormalities, except for a few cases of minor eye irritation, which were probably due to direct contact of the pesticides with the eye.

Body Weight Effects. No treatment-related effects on body weight were observed in a group of 51 male volunteers given daily doses of up to 0.5 mg technical DDT/kg for up to 18 months (Hayes et al. 1956).

Body weight gain was reduced by 30%, relative to controls, in male albino rats treated with 0.2 mg technical DDT/kg by gavage for 120 days; food consumption data were not provided (Chowdhury et al. 1990). Female and male Osborne-Mendel rats fed a diet that provided approximately 97 or 50 mg technical DDT/kg/day, respectively, for 6 weeks had a 45 and 16% reduction in body weight, respectively, at the end of the treatment period relative to controls (NCI 1978). Male and female B6C3F1 mice treated similarly with up to 35 mg technical DDT/kg/day showed no significant treatment-related alterations in body weight gain (NCI 1978). In a 78-week chronic study, female Osborne-Mendel rats showed a 20% decrease in body weight gain due to dietary administration of approximately 32 mg technical DDT/kg/day compared to controls; males exhibited a 16% decrease in weight gain with a dietary level of approximately 45 mg/kg/day (NCI 1978). B6C3F1 mice treated in the diet with up to approximately 30.2 mg technical DDT/kg/day for 78 weeks did not show treatment-related alterations in body weight (NCI 1978). Hamsters treated for life with up to 71 mg technical DDT/kg/day in the diet showed no significant treatment-related effects on body weight (Cabral et al. 1982a), but a dietary level of approximately 95 mg/kg/day of technical DDT for 128 weeks caused an unspecified reduction in body weight gain relative to controls (Rossi et al. 1983).

A 6-week treatment period with up to approximately 157 mg *p,p*'-DDE/kg/day in the diet induced a 22% decrease in body weight gain in male Osborne-Mendel rats, and a dose level of 88 mg/kg/day caused an 11% decrease in weight gain relative to controls (NCI 1978); a dose of 50 mg/kg/day was a NOAEL. No significant treatment-related effects on body weight were seen in female rats treated with up to 305 mg *p,p*'-DDE/kg/day or in B6C3F1 mice treated with up to 101 mg/kg/day (NCI 1978). Chronic administration of approximately 19 mg *p,p*'-DDE/kg/day in the diet to female Osborne-Mendel rats caused a 21% decrease in body weight gain; this was the lowest dose tested (NCI 1978). Female B6C3F1 mice administered approximately 28 mg *p,p*'-DDE/kg/day for 78 weeks had a 29% reduction in body weight gain (this was also the lowest dose tested); body weight gain in male mice was unaffected by doses of up to 47 mg *p,p*'-DDE/kg/day (NCI 1978). Hamsters fed a diet that provided approximately 47.5 mg *p,p*'-DDE/kg/day for 128 weeks showed an unspecified reduction in body weight gain compared to controls (Rossi et al. 1983).

Treatment of female Osborne-Mendel rats with approximately 97 mg technical DDD/kg/day for 6 weeks resulted in a 39% decrease in body weight gain relative to controls, but treatment with 172 mg/kg/day resulted in only a 4% reduction in weight gain (NCI 1978). In males, the highest dose tested, 279 mg/kg/day, caused a 10% reduction in weight gain at the end of the treatment period (NCI 1978). In the 78-week NCI (1978) bioassay, female rats treated with approximately 66 mg technical DDD/kg/day had a 26% decrease in weight gain compared to controls at the end of the study; this was the lowest dose level tested. In males, there was a 28% decrease in weight gain in a group dosed with approximately 116 mg/kg/day and a 39% decrease in a group receiving about 231 mg/kg/day of technical DDD.

3.2.2.3 Immunological and Lymphoreticular Effects

Limited information was located regarding immunological effects in humans after oral exposure to DDT, DDE, or DDD. In a study in which humans were challenged with an injection of *Salmonella typhimurium* vaccine, serum agglutinin titers were significantly higher in three volunteers given capsules containing 5 mg DDT/day (0.07 mg/kg) for 20 days when compared to volunteers who received only the bacterial antigen; immunoglobulin levels were unaffected by treatment with DDT (Shiplov et al. 1972). The volunteers exhibited no apparent symptoms of DDT exposure.

A different type of study assessed parameters of immunocompetence in a group of 23 men chronically exposed to DDT through consumption of fish from the Baltic Sea (Svensson et al. 1994). The levels of DDT in the fish were not provided. None of the subjects had symptoms of any infectious disease at the time of the study. Twenty men with almost no fish consumption served as controls. The parameters examined included white cell counts, lymphocyte levels, serum immunoglubulin levels, and lymphocyte subsets. Of all the parameters examined, only the level of natural killer (NK) cells was reduced in the fish eaters, but the difference between groups was not statistically significant. Weekly intake of fatty fish correlated significantly ($r=0.32$, $p<0.04$) with the reduction in NK cells. A correlation ($r=0.72$, $p=0.02$) was observed between NK cell level and plasma level of *p,p'*-DDT in 12 subjects. The toxicological significance of these findings is unknown.

Further information on the potential immunological effects of DDT is available in a study of 302 individuals residing near a waste site in North Carolina (Vine et al. 2001). Of 20 organochlorines tested, only DDE was detected in the blood of the participants; the median concentration in plasma was 2 ppb and the highest concentration was 32 ppb. End points evaluated included white blood cell counts, lymphocyte phenotypes, immunoglobulins, and mitogen-induced lymphoproliferative activity; a skin test

was also conducted to evaluate delayed-type hypersensitivity. When DDE levels were divided into five categories from #1 ppb or $7.6 ppb, subjects with higher blood DDE levels had lowered mitogen-induced lymphoproliferative activity (concanavalin A) and slightly increased total lymphocytes and immunoglobulin A levels. Results from the skin test showed no differences in response by blood DDE levels. Vine et al. (2001) concluded that relatively low DDE levels were associated with changes in immune markers, although the magnitude of the effects are of uncertain clinical importance.

Evidence of DDT-induced compromises in immune function has been obtained from studies conducted in animals. The effects of DDT on the humoral immune response were studied in mice (Banerjee 1987a; Banerjee et al. 1986, 1997b), in rats (Banerjee 1987b; Banerjee et al. 1995, 1996; Gabliks et al. 1975), and in rabbits (Shiplov et al. 1972). There was no evidence that DDT adversely affected the humoral response in rabbits; however, dosing was for only 10 days by oral gavage at a single dose level (4.3 mg/kg/day) (Shiplov et al. 1972). Following dietary dosing at levels to provide 13 mg DDT/kg/day for 3–12 weeks, mice showed an immunosuppression particularly of the secondary humoral immune response to immunization with sheep red blood cells (SRBC) or *Escherichia coli* lipopolysaccharide (LPS), which are T-cell dependent and T-cell-independent antigens, respectively. There was a significant reduction in splenic plaque-forming cells as well as decreased IgM titers to lipopolysaccharide compared to controls (Banerjee 1987a; Banerjee et al. 1986). Rats immunized with diphtheria toxoid were fed DDT in the diet for 31 days at an intake of 1.9 or 19 mg/kg/day. When challenged with diptheria toxoid, the severity of the anaphylactic response was decreased and the number of mast cells (producing histamine) in mesenteries was significantly decreased compared to controls; antibody titers to the toxoid were not decreased (Gabliks et al. 1975). Exposure of adult mice to DDT in the diet for 16 weeks at doses of 0.006 mg/kg/day had no effect on the humoral immune response but doses of 0.06 or 0.63 mg/kg/day for 16 weeks significantly stimulated the primary IgM response to SRBC and the lymphoproliferative response to LPS-coated SRBC (Rehana and Rao 1992). Dosing for longer periods (20 or 24 weeks) caused a sharp reduction in both responses. Dosing of dams during pregnancy and lactation caused suppression of the immune system in offspring as discussed in Section 3.2.2.6 (Rehana and Rao 1992); however, because appropriate controls were not used, the significance of this finding is not clear.

Effects in lymphoid organs were noted in rabbits (Street and Sharma 1975) and rats (Deichmann et al. 1967; Hamid et al. 1974). Rabbits administered an oral dose of 0.18 mg DDT/kg/day in the diet for 8 weeks exhibited a significant increase in gamma globulin levels and atrophy of the thymus whereas a much higher dose, 6.54 mg/kg/day, was found to significantly decrease the skin sensitivity to tuberculin (cell mediated immunity) (Street and Sharma 1975). Rats exposed to doses of 121 mg *o,p'*-DDD/kg/day

for 16 days had decreased (no statistical analysis) numbers of plaque-forming cells and rosette-forming cells in the spleen and thymus compared to controls and displayed atrophy of the thymus and adrenal gland (Hamid et al. 1974).

Dietary DDT increased the growth of the leprosy bacterium, *Mycobacterium leprae*, in mouse foot pads (Banerjee et al. 1997a). DDT was fed to male albino Rockfeller strain mice in the diet at 0, 20, 50, or 100 ppm (approximately 0, 4.3, 10.7, and 21.4 mg/kg/day) for 24 weeks following inoculation of the footpads of mice with the leprosy bacterium. At $50 ppm, bacillary growth was significantly increased.

Both humoral and cell-mediated responses were adversely affected in groups of 8–12 male Wistar rats fed diets containing 200 ppm DDT, DDE, or DDD (approximately 22.2 mg/kg/day) for 6 weeks (Banerjee et al. 1996). Compared to controls, effects on the humoral immune system were seen after dietary exposure to each of the compounds, including significantly increased serum albumin/globulin ratio, suppressed serum IgM, IgG after ovalbumin immunization, and decreased antibody titer. Likewise, cell-mediated effects were seen in rats fed DDT, DDE, and DDD, including increased inhibition of leucocyte and macrophage migration, and decreased footpad thickness. Mean relative liver weight was increased compared to controls in rats fed DDT and DDE, while relative spleen weight was decreased in DDD-fed rats; no other signs of toxicity were observed.

The influence of dietary protein on the humoral and cell-mediated immunotoxicity of DDT was evaluated in groups of male Wistar rats fed diets containing 0, 20, 50, or 100 ppm DDT (approximately 0, 2.3, 5.7, and 11.4 mg/kg/day, respectively) for 4 weeks (Banerjee et al. 1995). Dose groups were divided into subgroups that received either 3, 12, or 20% protein in the diet; half of each dietary subgroup, in turn, received a tetanus toxoid immunostimulant, resulting in allocation of 10–12 rats to each treatment group. The serum albumin/globulin ratio was significantly increased in mid- and high-DDT dose groups compared to treatment controls, but only in rats fed the low-protein diet and receiving the immunostimulant injection. Serum IgM and IgG were significantly reduced in mid- and high-DDT groups that were fed the 3% protein diet, regardless of whether they had been immunostimulated with tetanus toxoid injections. No effects were seen in any group fed diets containing 12 or 20% protein.

DDT enhanced stress-induced humoral immune response suppression in mice (Banerjee et al. 1997b). Groups of 80–90 albino male Hissar mice were fed 0, 20, 50, or 100 ppm *p,p'*-DDT in the diet (approximately 0, 4.1, 10.1, and 20.3 mg/kg/day, respectively). Equal subgroups were selected for SRBC or plaque-forming cell (PFC) assays. Assay subgroups were in turn divided into five equal groups,

resulting in treatment groups of 8–10 mice per group; one group received no stressor, and the other four groups received one of four different combinations of temperature (3 hours at 4 EC) and restraint stresses. Relative to controls, DDT alone caused no significant alterations in primary antibody titer to SRBC or in PFC response. DDT in combination with stress, and in a dose-related manner, significantly reduced both responses to a greater extent than did each stressor alone. The results of this study indicate the following: (1) certain types of stressors are sufficient to suppress the humoral immune response in mice, in the absence of known chemical stressors, and (2) dietary DDT at sufficient levels can enhance the immunosuppressant effect of other stressors that otherwise have no statistically discernable effect.

Sheep red blood cell (SRBC) antibody titers were reduced in groups of 16 or 20 male Wistar rats fed 100 or 200 ppm in the diet for 8 weeks (approximately 10.3 and 20.6 mg/kg/day, respectively); DDT dietary exposure groups were divided into two subgroups, one received ascorbic acid by gavage at 100 mg/kg/day and the other received no ascorbic acid (Koner et al. 1998). Dose-related decreases in SRBC antibody titers were seen in the DDT-treated rats compared to controls, with or without concurrent treatment with ascorbic acid; the decrease was statistically significant in the 200-ppm group. In the DDT-treated rats, the absolute decrease appeared to be attenuated in rats co-treated with ascorbic acid compared to those not receiving ascorbic acid, although statistical tests were not reported.

In the 78-week chronic bioassay conducted by NCI (1978), no treatment-related adverse effects on the thymus, spleen, or lymph nodes were observed in Osborne-Mendel rats treated in the diet with up to 45 mg technical DDT/kg/day, 59 mg *p,p*'-DDE/kg/day, or 231 mg technical DDD/kg/day. The same negative findings were reported for B6C3F1 mice treated with up to 30.2 mg technical DDT/kg/day, 49 mg *p,p*'-DDE/kg/day, or 142 mg technical DDD/kg/day (NCI 1978). Immunocompetence was not evaluated in this study.

The overall evidence suggests that DDT and related compounds can affect immunocompetence in animals, but there is no conclusive evidence regarding effects in humans.

The highest NOAEL values and all LOAEL values from each reliable study for immunological effects in each species and duration category are recorded in Tables 3-1 and 3-2 and plotted in Figures 3-1 and 3-2.

3.2.2.4 Neurological Effects

The nervous system appears to be one of the primary target systems for DDT toxicity in humans after acute, high exposures. A number of investigators conducted experimental studies on humans in the 1940s and 1950s at controlled doses that produced effects. Other data come from accidental poisonings where dose levels were crudely estimated. Persons exposed to 6 mg DDT/kg administered orally by capsule generally exhibited no illness, but perspiration, headache, and nausea have been reported (Hayes 1982). Convulsions in humans have been reported at doses of 16 mg DDT/kg or higher (Hsieh 1954). Velbinger (1947a, 1947b) exposed volunteers to oral doses of 250, 500, 750, 1,000, or 1,500 mg DDT (approximately up to 22 mg/kg) suspended in an oil solution. Variable sensitivity of the mouth (defined by the author as a prickle at the tip of the tongue, lower lip, and chin area) was reported in volunteers exposed to 250 and 500 mg DDT/person. Six hours after exposure to 750 or 1,000 mg DDT, disturbance of sensitivity of the lower part of the face, uncertain gait, malaise, cold moist skin, and hypersensitivity to contact were observed. Prickling of the tongue and around the mouth and nose, disturbance of equilibrium, dizziness, confusion, tremors, malaise, headache, fatigue, and severe vomiting were all observed in volunteers within 10 hours after oral exposure to 1,500 mg DDT. All volunteers exposed to DDT orally had achieved almost complete recovery from these acute effects within 24 hours after exposure. Similar symptoms were reported in persons after accidental or intentional ingestion of DDT (Francone et al. 1952; Garrett 1947; Hsieh 1954; Mulhens 1946).

Few studies have explicitly evaluated neurotoxicity in humans following chronic exposure. There was no correlation between DDT exposure and neurological effects in workers whose estimated exposure, based on DDA excretion data, was approximately 14–42 mg/person/day (Ortelee 1958). Occupational exposure involved all possible routes of exposure, but most of the intake is considered to be from the oral route. Inhaled dusts are poorly absorbed because of size, but they are cleared by the mucociliary mechanisms and a fair portion is then ingested. None of the subjects had any evidence of hyperexcitability, and the results of the neurological examinations were normal. No neurological effects related to DDT were noted in volunteers who ingested 3.5 or 35 mg DDT/day (0.05–0.05 or 0.36–0.5 mg/kg/day) for 12–18 months (Hayes et al. 1956). The subjects displayed no loss of coordination and there was no indication of tremors. Other tests (over 20) were negative and showed no peripheral neuropathy or central nervous system functional deficits. Background DDT levels in food of both controls and test subjects were 0.0021–0.0038 mg DDT/kg/day. Somewhat different findings were described in a recent study of retired malaria-control workers in Costa Rica (van Wendel de Joode et al. 2001). Neurological tests assessing cognitive, motor, and sensory functions were conducted on 27 former workers and 27 matched controls.

The men had been involved in DDT use between 1955 and 1986; the mean number of years of DDT application was 4.6 years. The exposed group had overall poorer performance; verbal attention and visuomotor speed and sequencing differed most between the groups. When years of DDT application was entered in the analysis as the explanatory variable, significant exposure-effect relations were seen for five tests in cognitive, motor, and sensory domains. Although the population sample was small, the study seems to have been well controlled. However, neither exposure levels nor past or current levels of DDT (or DDE) in blood or in adipose tissue were available.

As in humans, the nervous system appears to be one of the primary targets in animals after acute, subchronic, and chronic oral exposure to DDT. Acute oral exposure to high doses of DDT has been associated with DDT-induced tremors or myoclonus (abrupt, repeated involuntary contractions of skeletal muscles), hyperexcitability, and convulsions in several species. These effects have been observed in rats after single oral gavage doses of 50–600 mg DDT/kg/day (Henderson and Wooley 1969b; Herr and Tilson 1987; Herr et al. 1985; Hietanen and Vainio 1976; Hong et al. 1986; Hrdina and Singhal 1972; Hrdina et al. 1973; Hwang and Van Woert 1978; Philips and Gilman 1946; Pranzatelli and Tkach 1992; Pratt et al. 1986; Tilson et al. 1987). Mice receiving a single oral gavage dose of 160 mg DDT/kg had tremors (Hietanen and Vainio 1976), and single doses of 200–600 mg *p,p'*-DDT/kg/day induced convulsions (Matin et al. 1981). In guinea pigs and hamsters similarly dosed, no tremors were observed at 160 mg DDT/kg, but hind leg paralysis occurred in guinea pigs (Hietanen and Vainio 1976).

Acute oral exposure of animals to DDT has also been associated with increases in brain biogenic amine and neurotransmitter levels. Alterations in the metabolite 5-HIAA (5-hydroxy-indoleacetic acid), the degradation product of serotonin, have been reported to correlate with DDT-induced tremors; doses at 50 mg/kg/day or greater resulted in increases in the levels of 5-HIAA in the brain (Hong et al. 1986; Hrdina et al. 1973; Hudson et al. 1985; Hwang and Van Woert 1978; Tilson et al. 1986). Alterations in the levels of other neurotransmitters have been found. The neurotransmitter changes observed are consistent with one of the putative mechanisms for DDT toxicity; DDT is thought to influence membrane ion fluxes and consequently potentiate neurotransmitter release (see Section 3.6.2). Acetylcholine and norepinephrine decreased in rats after acute exposure to 400 mg/kg DDT (Hrdina et al. 1973). Also, aspartate and glutamine were increased in brain tissue of rats (Hong et al. 1986; Hudson et al. 1985; Tilson et al. 1986).

The effects of acute DDT exposures on adult learning and motor activity are discussed in Section 3.2.2.6 Developmental Effects, where they are compared to similar, but more pronounced, behavioral effects resulting from perinatal exposure.

Body tremors and hunched appearance were observed in female Osborne-Mendel rats after 26 weeks of treatment in the diet with approximately 16 mg technical DDT/kg/day in a 78-week study (NCI 1978). Similar findings were reported by Rossi et al. (1977) in female Wistar rats after 9 weeks of treatment with approximately 34 mg technical DDT/kg/day in the diet. Intermediate and acute exposure to DDT resulted in changes in brain lipid metabolism that affected total brain lipids and the relative brain lipid ratios in nonhuman primates. Rhesus monkeys exhibited a decrease in total brain lipids and the relative amount of cholesterol to phospholipid after oral exposure to 10 mg technical DDT/kg/day for 100 days or a single oral dose of 150 mg/kg/day (Sanyal et al. 1986). The results were more pronounced in the intermediate group than the acute group. Decreases in the levels of brain phospholipids and cholesterol may result in altered neuronal transmission (Sanyal et al. 1986). Lipids associated with the myelin sheath were not affected by DDT. Staggering, weakness, and loss of equilibrium were observed in monkeys treated for up to 14 weeks with 50 mg *p,p*'-DDT/kg/day, a dose level that was also lethal (Cranmer et al. 1972a). No such manifestations of toxicity were seen with a 5 mg/kg/day dose. Hunched appearance was reported in male rats after 8 weeks of treatment with approximately 59 mg of *p,p*'-DDE/kg/day and in male mice after 22 weeks of treatment with approximately 27 mg of *p,p*'-DDE/kg/day in 78-week duration studies (NCI 1978).

Some Rhesus and Cynomolgus monkeys exposed to approximately 6.9 mg of *p,p*'-DDT/kg/day or more in a 130-month dietary study experienced severe tremors (Takayama et al. 1999). Hyperactivity and tremors were also reported in chronic studies in mice at doses up to 8.3 mg DDT/kg/day (Kashyap et al. 1977; Turusov et al. 1973). Even after a change of diet or a decrease in dose, the tremors persisted for several weeks. No clinical signs of neurotoxicity were observed in hamsters fed diets to provide doses up to 95 mg technical DDT or *p,p*'-DDE/kg/day for life (Rossi et al. 1983).

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

3.2.2.5 Reproductive Effects

Effects discussed in this section are those that result from alterations affecting reproduction from the time of gametogenesis through implantation of the conceptus in the endometrium. In addition, this section includes descriptions of effects on male and female reproductive organs; these effects may be direct effects or triggered by hormonal changes. Developmental effects are distinguished from reproductive effects by evaluation of the conceptus after it is implanted and can be categorized as structural abnormalities, altered growth, functional deficiencies, and death of the developing organisms. Developmental effects are discussed in Section 3.2.2.6.

The potential association between DDT (and DDT metabolites) and reproductive end points has been examined in numerous studies. For example, a study of 240 women showed no difference in maternal DDT blood levels between 120 individuals who had a miscarriage and 120 controls; in both groups, the mean blood DDT levels were similar and below 6 ppb (Leoni et al. 1989). A more recent study of 89 women with a history of at least two miscarriages found that 15% of them had DDE blood levels above a reference value of 2.5 ppb; the mean was 1.2 ppb and the range was between 0.01 ppb (detection limit) and 8.6 ppb (Gerhard et al. 1998). Hexachlorobenzene, pentachlorophenol, and PCBs were also above reference values in 15–22% of the women. A study by Ron et al. (1988) reported no significant differences in DDT blood levels between 20 cases of premature rupture of fetal membranes and 15 controls; the mean concentrations of DDT in blood were comparable, approximately 24 ppb.

A difference in maternal DDE blood levels for women delivering full-term and premature infants was reported in a brief communication by O'Leary et al. (1970a). Twenty-three women who delivered early had infants with mean DDE blood levels of 19–22.1 ppb, while 44 women who delivered at full-term had infants with mean DDE blood levels of 4.9–6.1 ppb; DDE was the only organochlorine monitored.

Studies of women from India reported that total DDT residues (DDT plus DDE and DDD) in maternal blood from 25 full-term cases ranged from 7 to 73 ppb (mean 26 ppb) compared to 48–481 ppb (mean 66 ppb) in 15 pre-term and 90–1,280 ppb (mean 394 ppb) in 10 cases of spontaneous abortion (Saxena et al. 1980, 1981). Mean DDT residues in the respective placentas were 24, 66, and 162 ppb. Other organochlorine pesticides were also elevated in the pre-term and abortion cases relative to full-term cases. A study of 54 women from Brazil found no differences in maternal blood (at delivery) DDT residue levels between 30 cases of full-term delivery (31 ppb) and 24 cases of pre-term delivery (30 ppb) (Procianoy and Schvartsman 1981). However, analyses of umbilical cord blood showed significantly

elevated levels of DDT in pre-term newborns compared to full-term. No other chemical was monitored in this study. A similar study of Israeli women reported that maternal blood of 10 full-term cases had a concentration of 27 ppb of total DDT residues vs. 120 ppb in blood from 17 pre-term cases (Wassermann et al. 1982); other organochlorines such as PCBs, lindane, heptachlor epoxide, and dieldrin were also elevated in pre-term delivery cases. A more recent study of New York City women found no association between blood DDE levels and pre-term birth after comparing 20 cases with 20 matched controls (Berkowitz et al. 1996). The median DDE blood levels were 1.4 ppb in cases and 1.3 ppb in controls. Recently, Longnecker et al. (2001) presented the results of an analysis of 2,380 children, of whom 361 were born pre-term and 221 were small-for-gestational-age. The children were born between 1959 and 1966 and were part of the U.S. Collaborative Perinatal Project. Serum samples were taken from the mothers in the third trimester of pregnancy and were stored at -20 EC without recorded thaws. Analyses of DDE in serum were conducted between 1997 and 1999. DDE concentrations were evaluated in relation to odds of pre-term birth and small-for-gestational-age birth with logistic regression models. The median concentration of DDE in serum was 25 ppb and the range was 3–178 ppb. The authors found that the adjusted odds ratios for pre-term birth increased steadily with increasing DDE concentrations (trend $p<0.0001$). Quadratic spline models showed that the odds of pre-term birth began to increase at a DDE concentration of 10 ppb. Adjusted odds for small-for-gestational-age also increased, but less consistently (trend $p=0.04$).

A case-control study of women with endometriosis (n=86) and a matched control group of women without the condition (n=70) found no association between plasma concentration of DDT compounds and the occurrence of endometriosis (Lebel et al. 1998). No differences were observed between cases and controls in crude geometric mean total plasma DDT (DDT plus DDE) or in geometric mean total plasma DDT adjusted for age, body mass index, and symptoms indicating a need for laparoscopy. No differences were observed in crude geometric mean *p,p'*-DDE or *p,p'*-DDT concentrations in plasma between cases and controls.

An ecological study evaluated the relationship between several factors, including the concentration of *p,p*'-DDE in tree bark as a measure of DDT contamination in the environment, and birth rate among countries or pregnancy rate among states in the United States (Cocco 1997). In both multivariate regression analyses, no relationship between DDE exposure (as measured by the environmental concentration) and response was observed. A possible association between chlorinated hydrocarbons in blood and infertility was investigated in a group of 489 infertile women from Germany (Gerhard et al. 1999). Only eight of the women had total DDT blood levels higher than a reference value of 2.5 ppb; the

mean concentration was 1.0 ppb and the range span was 0–9.6 ppb. The authors reported that elevated *o,p'*-DDT and *p,p'*-DDT were found less often in women who conceived during the study than in women who did not. In addition, the conception rate was reduced with increasing total DDT levels from 18% for DDT levels below 0.5 ppb to 10% for DDT levels above 1.0 ppb.

Concentration of *p,p'*-DDE in human milk was inversely related to duration of lactation in women of Tlahualilo, Mexico, who had lactated previously, but not among women having their first lactation (Gladen and Rogan 1995). Median lactation duration declined from 7.5 months among women with lipid-adjusted DDE concentration of 0–2.5 ppm, to 3 months among women with $12.5 ppm in their milk; the difference was statistically significant. Significantly elevated crude hazard ratios (defined by the authors as estimated ratios of the hazard of weaning relative to the 0–2.5 ppm group; hazard was defined as the instantaneous probability of weaning) were observed in groups with milk DDE levels $7.5 ppm; hazard ratios adjusted for various determinant factors were elevated above unity in the group with milk DDE $12.5 ppm. A similar inverse relationship between milk DDE and lactation duration was seen in women in the United States (Rogan et al. 1987). In contrast, no correlation was seen between DDE concentration in maternal milk fat and birth weights, head circumference, or neonatal jaundice, but the authors indicated that higher levels of DDE (>4 ppm) in maternal milk fat were associated with hyporeflexia in infants (Rogan et al. 1986). The effects of DDE on lactation duration are likely due to the ability of DDE to disrupt the normal endocrine regulation of lactation, as estrogen is a potent inhibitor of milk secretion (Guyton and Hall 2000)

It has long been suspected that technical-grade DDT has estrogen-like properties based on findings in wildlife exposed to the pesticide (Bishop et al. 1991; Fry and Toone 1981; Fry et al. 1987; Guillette et al. 1994); results from studies in laboratory animals have left little doubt (Bitman and Cecil 1970; Clement and Okey 1972; Singhal et al. 1970; Welch et al. 1969). The estrogenic activity is largely due to the *o,p'*-isomer of DDT, which is present as a 15–21% contaminant of technical DDT (Metcalf 1995). For example, estrogenic effects on the uterus (uterotrophic: increased weight and glycogen content) and premature vaginal opening were observed in immature rats given diets that provided doses of 100 mg/kg *o,p'*-DDT from days 23–30 of life suggesting an agonistic action with estrogen; *p,p'*-DDT did not have such estrogenic activity (Clement and Okey 1972). Also, Singhal et al. (1970) showed that injection of a single dose of 100 mg *o,p'*-DDT/kg to ovariectomized rats mimicked estrogens in increasing uterine weight, glycogen content, and the activities of several enzymes involved in glycolysis and the hexose monophosphate shunt pathway. Recently, Diel et al. (2000) showed that oral administration of *o,p'*-DDT to juvenile ovariectomized rats produced the typical increase in uterine weight, although it induced a

pattern of gene expression different from ethinylestradiol. Much of the research in this area has been conducted in experimental animals administered the test substance parenterally or, more recently, in *in vitro* systems. Representative studies are discussed in Section 3.6.2, Mechanisms of Toxicity.

No spermatotoxic effects were observed in an acute screening test in rats given a single oral gavage dose of 100 mg *p,p'*-DDT/kg/day or five daily doses of 50 mg/kg/day (Linder et al. 1992). In juvenile male rats dosed by oral gavage on days 4 and 5 of life with 500 mg/kg/day or from day 4 to day 23 with 200 mg DDT/kg/day, a decrease in testis weight was observed (Krause et al. 1975). After two doses, significant decreases were seen at 34 days, and after repeated lower doses, decreases were significant at days 18, 26, and 34. Treated males were mated with healthy females on days 60 and 90. The number of fetuses and implantations was decreased 30% at the 60-day mating but not at the 90-day mating of rats dosed on days 4 and 5. For rats receiving multiple doses, the decreases were 95 and 35% after mating at days 60 and 90, respectively. In adult male rats dosed with 200 mg DDT/kg every other day (oral gavage) for 2 weeks, serum testosterone levels were decreased (statistically significant) compared to controls, but serum levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) were not significantly altered (Krause 1977). In the same study, testicular levels of testosterone were decreased, although not significantly, in adult male rats treated with 100 mg DDT/kg 3 times/week for 3 weeks, and no histologic effects on spermatogenesis were seen (Krause 1977). Oral gavage dosing of male mice at 6.25 mg/kg/day of *p,p'*-DDT for 28 days had no effect on testis weight (Orberg and Lundburg 1974). In a dominant lethal test in male rats with a single oral gavage dose of 100 mg/kg of *p,p'*-DDT, a clear genotoxic response was not reported, but the proportion of mated females with dead implants was somewhat increased.

The effect of DDT on fertility was examined in male and female rats fed diets providing an intake of 0.56 mg DDT/kg/day for 60 days before mating and continuing throughout gestation (Green 1969). A 75% depression of fertility was found, but there was no effect on litter size. The F_1 pups from these dams were completely infertile when mated. Jonsson et al. (1976) reported that dietary dosing of female rats with technical-grade DDT for 36 weeks caused sterility at 12 mg/kg/day, and this was accompanied by a decrease in serum progesterone; 6 mg/kg/day had no effect on fertility. When 21-day-old female rats were fed diets to provide *o,p'*-DDT levels of 0.1–4.0 mg/kg/day for up to 14 weeks, no effects on age of vaginal opening were seen, and after mating, there were no effects on litter size or pup weight at birth or weaning (Wrenn et al. 1971). No adverse reproductive effects were reported in rats treated with 10 mg *p,p*'-DDE/kg/day for 5 weeks before mating and during gestation and lactation (Kornbrust et al. 1986). Reproductive parameters examined included percent sperm positive, percent pregnant, gestation length,

litter size, sex ratio of pups, and milk production and composition. Significantly decreased fertility was observed in female mice fed technical-grade DDT for 60 days at a dose level of 51.4 mg/kg/day (Bernard and Gaertner 1964). Female mice exposed to 2.0 mg *p,p*'-DDT/kg/day for periods that included premating and gestation had decreased number of implanted ova, lengthening of the estrus cycle, decreased corpora lutea, and decreased implants (Lundberg 1973, 1974). Administration of up to approximately 3.4 mg technical DDT/kg/day in the diet to mice for 86 days, including mating, resulted in no adverse reproductive effects, but treated mice had larger litter sizes relative to controls (Ledoux et al. 1977). Treatment of male and female mice for 120 days with approximately 1.3 mg technical DDT/kg/day (30 days before mating plus 90 additional days) had no effect on fertility, fecundity, or litter size (Ware and Good 1967). Treatment of pregnant rabbits with $10 mg *p,p*'-DDT by gavage on days 7–9 of gestation resulted in premature deliveries and a significant increase in the number of resorptions (Hart et al. 1971, 1972); however, no such effects were seen after treatment on gestation days 21–23 (Hart et al. 1972). Neither fertility nor pre- or postimplantation embryonic losses were significantly affected in female rabbits administered 3 mg technical DDT/kg (only dose level tested) 3 days/week for 12–15 weeks before artificial insemination and throughout gestation (Seiler et al. 1994). However, this exposure regime significantly reduced ovulation rate, but did not cause treatment-related histopathological alterations in the ovarian cortex, corpora lutea, or uterus (Lindenau et al. 1994).

In recent years, evidence has been presented that the persistent DDT metabolite, *p,p'*-DDE, is an androgen receptor antagonist; this evidence is discussed in Section 3.6.2, Mechanisms of Toxicity. A number of experiments have investigated *in vivo* antiandrogenic effects. Kelce et al. (1995) conducted a series of experiments in male rats administered *p,p'*-DDE at different ages. Effects from prenatal plus juvenile exposure are discussed in Section 3.2.2.6, Developmental Effects. Treatment of adult male rats (120 days old) with 200 mg *p,p'*-DDE/kg/day for 4 days significantly reduced androgen-dependent seminal vesicle and ventral prostate weight relative to controls (Kelce et al. 1995). These rats had been castrated and implanted with testosterone-containing Silastic capsules to maintain a constant serum testosterone level. Prostates from treated rats had a 13-fold increase in androgen-repressed testosterone-repressed prostatic message 2 (TRPM-2) messenger RNA levels and a 35% decline in androgen-induced prostate binding subunit 3 (C3) mRNA levels relative to control rats (Kelce et al. 1995). These findings, coupled with results from *in vitro* studies (Section 3.6.2) on receptor (androgen and estrogen) binding, suggested that the antagonistic effects of DDT on the male reproductive system are mediated by *p,p'*-DDE through competitive inhibition of binding of androgens to the androgen receptor (AR) and subsequent inhibition of transcriptional activity. In a subsequent study, Kelce et al. (1997) observed a significant reduction in immunohistochemical staining of androgen receptor in epididymal nuclei of adult

rats given 200 mg *p,p'*-DDE/kg/day for 5 days as well as a significant increase in TRPM-2 and a decrease in testosterone-induced C3 (third subunit of prostatein or prostate specific binding protein mRNA). Kelce et al. (1997) also reported a significantly reduced seminal vesicle and ventral prostate weight in the rats.

Reproductive studies of chronic dietary DDT exposure or multigeneration studies have not generally indicated reproductive toxicity. However, the doses used were sufficiently low so that tremors, convulsions, and death would not be a confounding factor. Keplinger et al. (1970) conducted a six-generation dietary study in mice with two matings per generation; mice in all generations were mated at 4 months of age. No effects were observed at 5 mg/kg/day; 20 mg/kg/day caused decreased fertility evidenced by decreased viability and lactation indices, and the 50 mg technical-grade DDT/kg/day dose caused frank toxic effects and was discontinued after three generations. No adverse effects on litter size were reported after 15 months of exposure of field mice to technical-grade DDT at dietary levels providing up to 2.4 mg/kg/day (Wolfe et al. 1979). No adverse effects on reproduction (end points assessed limited mostly to fecundity and fertility) were observed in rats fed up to 18.6 mg technical-grade DDT/kg/day in the diet for 2 generations (Ottoboni 1969), 1.25 mg/kg/day for 3 generations (Treon et al. 1954), or 1.7 mg/kg/day for 11 breedings (Ottoboni 1972). Duby et al. (1971) found no reproductive effects in two successive generations of rats fed technical-grade DDT (0.5 mg/kg/day), *p,p'*-DDT (1.5 mg/kg/day) or *o,p'*-DDT (0.3 mg/kg/day). A three-generation study was conducted in Beagle dogs given daily oral doses of 0, 5, or 10 mg technical-grade DDT from weaning to termination (Ottoboni et al. 1977); all dogs were sacrificed by 28 months of age and all females were mated at the first estrus only. The parental generation consisted of 4 males and 7–10 females/group. In the F_1 and F_2 generations, 8–19 females/dose group were mated with males of the same generation. The only significant effect noted was a 2–3-month reduction in the age at first estrus in females of the F_2 generation, but the relationship of this effect to DDT administration is questionable. A total of 650 pups were produced. No effects were observed on length of gestation, fertility, litter size, viability, gestation, or lactation indices. In another study in dogs, Deichman et al. (1971) reported that daily administration of *p,p'*-DDT by capsule for 14 months at a level of 12 mg/kg/day caused subnormal reproduction in dogs. However, this was a one-generation study with several chlorinated pesticides, only one dose of DDT (without aldrin) was administered to four females, and these females were mated to males that had been fed DDT plus aldrin. In addition, the age of the dogs at initiation of study was not provided, and mating in some dogs took place up to 19 months after dosing was discontinued. No correlation between fertility and levels of DDT in adipose tissue at time of mating could be made, and no clear conclusions on the effect of DDT on reproduction can be determined.

In the 78-week chronic bioassay conducted by NCI (1978), no treatment-related adverse effects on the ovaries, uterus, mammary gland, or prostate were observed in Osborne-Mendel rats treated in the diet with up to 45 mg technical DDT/kg/day, 59 mg *p,p*'-DDE/kg/day, or 231 mg technical DDD/kg/day. The same findings were reported for B6C3F1 mice treated with up to 30.2 mg technical DDT/kg/day, 49 mg *p,p*'-DDE/kg/day, or 142 mg technical DDD/kg/day (NCI 1978). Reproductive function was not evaluated in this study.

In summary, studies in humans suggest that high DDT/DDE burdens may be associated with alterations in end points that are controlled by hormonal function such as duration of lactation, maintenance of pregnancy, and fertility. High blood levels of DDE during pregnancy have also been associated with increased odds of having pre-term infants and small-for-gestational-age infants. Perinatal exposure of animals to DDT/DDE has caused alterations in the reproductive organs and infertility.

The highest NOAEL values and all LOAEL values from reliable studies for reproductive effects in each species and duration category are recorded in Tables 3-1 and 3-2 and plotted in Figures 3-1 and 3-2.

3.2.2.6 Developmental Effects

The proper development of many systems and functions depends on the timely action of hormones, particularly sex steroids; therefore, interfering with such actions can lead to a wide array of effects that may include altered metabolic, sexual, immune, and neurobehavioral functions. Effects of this type, that occur following exposure during fetal life via the placenta or early in life caused by either direct exposure to chemicals or exposure via maternal milk, are discussed in this section.

A prospective longitudinal study of children from North Carolina examined whether prenatal or lactational exposure to background levels of DDE (or PCB) were associated with altered pubertal growth and development (Gladen et al. 2000). Exposure was estimated by measuring *p,p'*-DDE in maternal breast milk, maternal blood, cord blood, and placenta. The original cohort consisted of 856 children, and follow-up analysis at puberty was conducted on 594 of these individuals (316 girls and 278 boys). Most of the children were between 12 and 14 years old when they were first contacted and the duration of participation ranged from 1 to 5 years. The transplacental DDE index ranged from 0.3 to 23.8 ppm (median 2.4 ppm) and the lactational DDE index from 0.2 to 96.3 mg (median 6.2 mg). Height and weight adjusted for height of boys at puberty increased with transplacental exposure to DDE; those with the highest exposures were 6.3 cm taller and 6.9 kg larger than those with the lowest exposures; no such

effect was seen among girls. There was no effect on the age at which pubertal stages were attained. Lactational exposures to DDE had no apparent effects. Children from this cohort had normal birth weight and normal growth in the first year of life.

A study of German children examined the potential association between height and serum levels of DDE (Karmaus et al. 2002). The children were from three different regions from Germany; two regions were industrial and agricultural regions, but no information was given regarding the third region, or the distribution of children by region. Data on weight and height from birth to the age of 4 years (seven measurements) were obtained from medical records; measurements at the ages of 8, 9, and 10 years old were conducted by the investigators. Blood was collected only once, when the children were 8 years old, and was analyzed for DDE and PCBs. The number of children from whom weight and height were measured at each time point varied between 202 (at 43–48 months) and 323 (at birth). At all time points, boys outnumbered girls. Whole blood DDE concentrations were stratified in quartiles: 0.08–0.2, 0.21–0.29, 0.3–0.43, and 0.44–40.4 ppb. Analysis of the results controlling for relevant confounders showed that DDE was a significant predictor of height in girls starting at 1.3 months and remained significant through 8 years of age. On the average, girls in the highest DDE quartile were 1.8 cm shorter than girls in the lowest quartile. At 10 years old, no association was evident between height and DDE. The fact that only one DDE measurement was made, at 8 years of age, casts some uncertainty on the significance of the results.

Evaluation of Inuit infants exposed to *p,p*'-DDE (and other organochlorines) *in utero* revealed no association between immunological parameters (white blood cell counts, lymphocyte subsets, immunoglobulins) and prenatal exposure to organochlorines (Dewailly et al. 2000). Organochlorine concentration in breast milk fat was used as an index of exposure and evaluations were conducted at 3, 7, and 12 months of age. The authors also examined whether organochlorine exposure was associated with the incidence of infectious diseases and found that during the second evaluation period, that the risk of otitis media increased with prenatal exposure to *p,p*'-DDE, hexachlorobenzene, and dieldrin. They also found that the relative risk for 4- to 7-month-old infants in the highest tertile of *p,p*'-DDE exposure was 1.87 (95% CI, 1.07–3.26) compared to infants in the lowest tertile. In addition, the relative risk of otitis media over the entire first year of life also increased with prenatal exposure to hexachlorobenzene and *p,p*'-DDE. Dewailly et al. (2000) pointed out that "because all organochlorines originate from the same few food items and share a number of properties (persistence, liposolubility), their concentrations in breast milk are correlated with each other. Therefore, associations of health outcomes with *p,p*'-DDE or hexachlorobenzene may be due to other organochlorines found in the organochlorine mixture."

In animals, DDT can produce embryotoxicity, fetotoxicity, and abnormal development of the sex organs. Estrogen-like effects on the developing reproductive system have been reported. The *o,p'*-isomers of DDT and DDE have greater estrogen-like effects than other isomers and when administered to rats in the first few days of life can seriously affect the development and maturation of the reproductive system. Developmental effects have been observed in animals after acute oral exposure to DDT during gestation or in the early perinatal development period; the seriousness of these effects is dependent on the isomeric form, the dose, and the timing of exposure. Exposure during early gestation resulted in a 25% decrease in fetal body weights and in significant decreases in fetal brain and kidney weights in the offspring of pregnant rabbits given oral gavage doses of 1 mg DDT/kg/day on gestation days 4–7 (Fabro et al. 1984). Offspring from rabbit dams orally exposed to a dose level of $10 mg *p,p'*-DDT/kg/day by gavage on days 7–9 of gestation showed a significant reduction in weight on day 28 (Hart et al. 1971, 1972). However, treatment late in gestation (days 21, 22, and 23) did not induce such an effect (Hart et al. 1972). Gellert and Heinrichs (1975) exposed pregnant rats orally to 28 mg *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, or *o,p'*-DDD/kg/day on days 15–19 of gestation. No significant effects on body weight, weight of the ovaries and pituitary, estrous cycle, or vaginal opening in the offspring were noted with the exception of a small but significant delay (2 days) in vaginal opening with the *o,p*'-DDD isomer. The *p,p'*-isomer is most prevalent in the environment, accounting for approximately 85% of the total amount of DDT, DDE, or DDD found; also, technical-grade DDT contains between 65–80% *p,p'*-DDT, between 15–21% *o,p'*-isomer, and up to 4% *p,p*'-DDD (Metcalf 1995).

As previously mentioned in Section 3.2.2.5 Reproductive Effects, the DDT metabolite, *p,p'*-DDE, has been found to have antiandrogenic activity (Kelce et al. 1995, 1997). *In vivo* antiandrogenic effects on adult rats are discussed in that section. Kelce and co-workers showed that male pups from Long-Evans dams exposed during gestation days 14–18 to 100 mg *p,p'*-DDE/kg/day and then exposed indirectly to maternally stored *p,p'*-DDE via breast milk had significantly reduced anogenital distance at birth and retained thoracic nipples on postnatal day 13. Female rats normally have a shorter anogenital distance than males. Treatment of weanling male rats from either day 21 or 25 (specific day unclear in text) until day 57 of age with 100 mg *p,p'*-DDE/kg/day resulted in a statistically significant delayed onset of puberty (measured by the age of preputial separation) by 5 days. The fact that serum levels of testosterone were not reduced suggested that the antiandrogenic effects were not confounded by the reported ability of DDT-related chemicals to increase steroid metabolism. Other gestational exposure studies have confirmed these findings. For example, anogenital distance was not affected in male Sprague-Dawley rats on postnatal day 2 after treating the dams with up to 100 mg *p,p'*-DDE/kg on gestation days 14–18, but was significantly reduced in similarly exposed Long-Evans pups (You et al. 1998). A 10 mg/kg dose

to the dams was without effect in the Long-Evans pups. Anogenital distance was not affected in female pups from either strain. Treatment of the dams with 10 mg *p,p'*-DDE/kg resulted in retention of thoracic nipples in Sprague-Dawley pups, but only the higher dose (100 mg/kg) had this effect in Long-Evans pups. Treatment with *p,p'*-DDE also resulted in an apparent reduction of androgen receptor expression in male sex organs from mainly high-dose Sprague-Dawley pups, as shown by immunochemical staining; however, there were no changes in androgen receptor steady state mRNA levels in the high-dose Sprague-Dawley rats, but mRNA were increased 2-fold in the high-dose Long-Evans rats. Exposure of the pups to *p,p'*-DDE during gestation and lactation had no significant effect on the onset of puberty in either strain. Further studies by the same group showed that prenatal exposure to 100 mg/kg *p,p'*-DDE reduced prostate ventral weight in 85-day-old males, but did not alter the weight of the testes, epididymis, and seminal vesicles (You et al. 1999a); serum levels of testosterone and LH were not significantly altered by DDE, but treatment with DDE was associated with expression of TRPM-2, an androgen-repressed gene.

A similar study in Holtzman rats exposed during gestation days 14–18 to doses between 1 and 200 mg *p,p*'-DDE/kg (offspring were exposed to *p,p'*-DDE *in utero* and via breast milk) found reduced anogenital distance in males on postnatal day 1 and reduced relative ventral prostate weight on postnatal day 21 at 50 mg *p,p'*-DDE/kg and higher, but not at 10 mg *p,p'*-DDE/kg (Loeffler and Peterson 1999). On postnatal day 4, anogenital distance was reduced only at 200 mg/kg/day. Nipple retention on postnatal day 13 was significantly increased at 100 and 200 mg/kg/day. Doses up to 100 mg/kg/day to the dams had no effect on the onset of puberty, but 200 mg/kg/day did significantly delay puberty in males by less than 2 days. Androgen receptor staining in the ventral prostate was also reduced on postnatal day 21 at 100 mg/kg/day (the only dose tested). Serum levels of testosterone or 3α-diol androgens were not significantly altered at any time. mRNA levels of androgen regulated genes from both ventral and dorsolateral prostate were not significantly changed in treated animals on postnatal day 21. This study also reported that at the 100 mg/kg dose level, cauda epididymal sperm number was reduced by 17% on postnatal day 63 relative to controls. No measurements of DDE body burden were made in the 200 mg/kg/day offspring postnatally, so it is difficult to determine whether effects on puberty were due to the previous gestational plus lactational exposures or directly due to the effects of DDE present near the time of puberty. Gray et al. (1999) conducted similar studies and reported that gestational exposure (gestation day [Gd] 14–18) to *p,p'*-DDE (100 mg/kg/day) resulted in a significant decrease in ventral prostate weight at 15 months of age in Sprague-Dawley rats and also in decreases in weights of glans penis, ventral prostate, and epididymis in 10-month-old Long-Evans rats. Newborn Long-Evans rats also showed reduced anogenital distance and increased mean number of retained nipples. Exposure of pregnant mice to 0.18 mg/kg of the estrogenic *o,p'*-DDT during gestation days 11–17 had no significant

effect on preputial gland weight or testes weight at 6 months of age, but a lower dose of 0.018 mg kg/day reduced absolute testes weight by 13% (Palanza et al. 1999b). This was associated with a decreased intensity of aggressive behavior in the mice.

There are limited data suggesting that if mice exposed to DDT *in utero* and during lactation are further exposed to DDT postnatally, responses in both immunological plaque forming assays and lympho-proliferative assays are reduced (Rehana and Rao 1992). The results of this study are difficult to interpret because of experimental design issues, including the lack of a comparison unexposed group and ambiguity about whether statistical testing was done.

Administration of DDT *in utero* or to neonates during sensitive periods in nervous system development has caused behavioral and neurochemical changes in adult mice (Craig and Ogilvie 1974; Eriksson and Nordberg 1986; Eriksson et al. 1990a, 1990b, 1992, 1993; Johansson et al. 1995, 1996; Palanza et al. 1999b; vom Saal et al. 1995). Offspring of dams given 34.3 mg/kg/day DDT by oral gavage during gestation and lactation displayed impaired learning and decreased memory function in a maze when tested 1 and 2 months after weaning (Craig and Ogilvie 1974). However, the dose was sufficiently high in this study to cause 39% mortality in the pups before weaning.

Ten-day-old mice treated once with 0.5 mg technical DDT/kg/day demonstrated behavioral changes when tested at 4 months of age (Eriksson et al. 1990a, 1990b). The test entailed placing animals in a new cage with infrared motion detecting beams for 1 hour. Locomotion (horizontal movement), rearing (vertical movement), and total activity (vibration, tremors, grooming, movement) were scored and summed for each 20-minute period. Initially, motor activity was fairly comparable between DDT-treated mice and controls; locomotion and activity were similar, although rearing was increased. During the last 40 minutes in the box, the DDT-treated animals had significantly more locomotion, rearing, and activity than the controls. When control mice were placed in a new environment, such as the test cage, they initially increased their activity as they explored. Eventually, this exploratory activity decreased as they become familiar with their new environment; this phenomenon is known as habituation. Thus, the authors concluded that perinatal DDT exposure interferes with habituation, which is considered a simple, nonassociative learning process. The neurobehavioral effects in the 4-month-old mice only appeared after dosing at 10 days and not after similar dosing at day 3 or 19 of age. The induction period was limited to a peak in the rate of brain development and development of muscarinic cholinergic receptors (Ericksson et al. 1992). Since this behavioral assay used motor exploration as the end point, an alternate interpretation of the observed results is that the DDT directly affected neural regulation of activity rather than learning;

however, a problem with appropriate control of motor behavior would also be a functional deficit of concern. Hyperactivity has been observed in DDT-treated adult mice (Kashyap et al. 1977; Rossi 1977; Turusov et al. 1973).

One month after behavioral testing, the potassium-evoked release of acetylcholine from cortical slices was significantly increased in treated mice compared to controls. This is consistent with the well-characterized effect of DDT in slowing the closure of sodium channels and thus causing general central nervous system stimulation and neurotransmitter release. Apparently, behavioral effects seen in adult mice represented a persistent neurological change since no residual DDT from their neonatal exposure remained in the brain (Eriksson et al. 1990b). Radiolabeled DDT was found in the brain at 1 and 7 days but not at 30 days following a single oral dose of 0.5 mg DDT/kg to 10-day-old mice. The classification of this effect as developmental, rather than an immediate effect of current DDT body burden, hinges on the apparent clearance of DDT before the behavioral testing.

In parallel experiments, neurochemical changes were also observed shortly after 10-day-old mice were treated with DDT. However, all of these specific neurochemical changes did not persist into adulthood. A single low dose of DDT (0.5 mg/kg) administered orally to 10-day-old (preweaning) mice affected the muscarinic cholinergic receptors in the brain. A significant increase in the density of specific muscarinic receptors was observed in the cerebral cortex, but not the hippocampus, at 7 days postexposure, but was not evident as early as 24 hours postexposure (Eriksson and Nordberg 1986). Furthermore, a significant decrease in the percentage of muscarinic high-affinity binding sites and a corresponding increase in the percentage of muscarinic low-affinity binding sites were measured, indicating that most of the increased binding could be attributed to low affinity sites. According to the study authors, the low-affinity muscarinic binding sites are thought to correspond to the M_1 receptors in the cerebral cortex, which are postulated to be associated with neuronal excitation (McKinney and Richelson 1984). The persistence of these observed neurochemical changes in muscarinic receptors in the cerebral cortex was also evaluated (Eriksson et al. 1990b). The density of the muscarinic receptors in the cerebral cortex, hippocampus, and striatum of the 3-month-old NMRI mice that had been given a single dose of 0.5 mg DDT/kg at 10 days of age was determined. The study authors reported a tendency towards a decrease in the amount of specific binding in the cerebral cortex, but no significant changes in the hippocampus and striatum. No significant changes were noted in choline acetyltransferase activity in the cerebral cortex, hippocampus, or striatum. However, significant ($p<0.01$) decreases in cerebral cortex muscarinic receptors at 4, 5, and 7 months of age were seen in similar later studies from the same group of investigators (Eriksson et al. 1992, 1993; Johansson et al. 1995, 1996). Tests of spontaneous motor activity conducted at the ages of

5 and 7 months old revealed that mice treated perinatally at the age of 10 days with DDT still exhibited hyperactive behavior relative to controls (Johansson et al. 1995, 1996). Nicotinic cholinergic receptor density was not significantly altered in 5- or 7-month-old mice treated with DDT at the age of 10 days. In these more recent studies, Johansson et al. (1995, 1996) also compared the motor responses to bioallethrin (a type I pyrethroid insecticide) and paraoxon in mice treated with and without DDT perinatally. From the graphic presentation of the results, it appears that pretreatment with DDT altered (increase or decrease) some of the responses attributed to paraoxon alone, but the results with bioallethrin were much less clear. However, treatment with DDT followed by bioallethrin significantly increased the density of muscarinic receptors in the cerebral cortex relative to DDT alone. This increase was later attributed to increased expression of muscarinic receptor m4 mRNA (Talts et al. 1998).

Behavioral effects have also been observed in animals exposed as adults. Inhibition of nonassociative learning processes (habituation response) occurred in adult rats acutely exposed to DDT (Sobotka 1971). Open field activity was assessed in rats, and activity (crossing squares on a checkered board) was measured over a 5-minute period; the normal response is increased activity for the first 2 minutes and decreased activity in the last 2 minutes (e.g., habituation). This "habituation" response was significantly affected (continued activity) in adult rats when tested 24 hours after administration of a single dose of 25 mg/DDT/kg but not at the lower doses tested (1 and 10 mg DDT/kg). The open field test used by Sobotka was not as sensitive a test of habituation as the tests on the Eriksson studies reviewed above. No differences in problem solving, locomotion speed, or reaction to stress were found between untreated rats and rats given oral doses of DDT up to 30 mg/kg/day (Khairy 1959). However, the scores for the pattern of locomotion significantly increased with dose (Khairy 1959). These doses are approximately 50 times that administered to neonates in the Eriksson studies.

A different behavioral response, the rate of urine marking in a novel territory, was examined in a study by vom Saal et al. (1995). Pregnant mice were administered *o,p'*-DDT by gavage in doses ranging from approximately 0.018 to 91 mg/kg/day on gestation days 11–17. Behavioral testing of male mice was conducted at the age of 70 days. Exposure to $1.82 mg/kg/day resulted in a significant increase in the number of urine marks deposited by the mice during a 1-hour test in a novel environment. The authors (vom Saal et al. 1995) stated that since marking a territory is a central feature of the reproductive strategy of male mice, alteration of this behavior could markedly impact the social structure of the species. In a different study, the same group of investigators reported that exposure of mice to *o,p'*-DDT (0.018 or 0.18 mg/kg/day) during gestation days 11–17 had no significant effect on the behavior of the male progeny at 30 days of age towards mouse pups (Palanza et al. 1999b). However, the lowest dose tended

to increase the proportion of males exposed *in utero* that attacked a male intruder, although the intensity of the attacks was lower than observed in controls.

Intermediate-duration oral exposure to DDT in animals has been shown to produce developmental effects such as infertility, mortality, and slow development in offspring of exposed dams (Clement and Okey 1974; Craig and Ogilvie 1974; Deichmann and Keplinger 1966). Exposure throughout gestation and lactation is more fetotoxic in rats than exposure only in gestation. Clement and Okey (1974) exposed pregnant rats to 1.7, 16.8, or 42.1 mg *p,p'*-DDT/kg/day for an unspecified period before mating and during gestation and/or lactation. An increase in mortality of pups exposed perinatally via dams receiving 42.1 mg *p,p'*-DDT/kg/day was reported, along with a decrease in growth of the pups after exposure via nursing from dams receiving 16.8 or 42.1 mg *p,p'*-DDT/kg/day or 84 mg *o,p*'-DDT/kg/day (Clement and Okey 1974). In mice exposed from mothers receiving 34.3 mg/kg/day technical-grade DDT, preweaning mortality was observed in 10% of the neonates exposed to DDT prenatally and nursed on unexposed or "foster" mothers, while 39% of the neonates exposed perinatally (during gestation and through lactation) died before weaning (Craig and Ogilvie 1974). In addition, mice exposed perinatally subsequently showed learning impairment and decreased memory function in the maze test (Craig and Ogilvie 1974).

Developmental effects, including preweaning mortality and premature puberty, were reported in animals in multigeneration studies. An increase in preweaning mortality was observed in the offspring of mice chronically exposed to 41.3 mg technical or *p,p'*-DDT/kg/day (Tomatis et al. 1972; Turusov et al. 1973). Del Pup et al. (1978) found a decrease in 30-day survival of neonatal mice after exposing successive generations of dams to 16.5 mg DDT/kg/day in the diet for a total of 70 weeks. Increases in abortions, stillbirths, and pup mortality were reported in mice exposed to 1.3–6.5 mg DDT/kg/day in a multigeneration study; however, most of the females in the 6.5-mg/kg group died before delivery (Shabad et al. 1973). Green (1969) reported that there was an increase in the number of resorptions in rats exposed to 0.56 mg DDT/kg/day and that no litters were produced by the second-generation animals in a multigeneration study. However, it is unclear from the study whether females were mated to exposed males or to untreated males. Treon et al. (1954) conducted a multigeneration study in which dams were fed 0.125–1.25 mg DDT/kg/day throughout gestation and lactation. No reductions in litter size were noted. Changes in preweaning mortality were reported but were not considered to be dose related. Other developmental aspects evaluated were negative. No conclusions concerning the developmental effects of DDT could be drawn from the Treon et al. study.

In a multigeneration study, Ottoboni et al. (1977) reported an increase in the incidence of premature puberty that increased with dose and with each consecutive generation among female dogs (4–19 dogs per group) dosed with 1, 5, or 10 mg *p,p'*-DDT/kg/day. However, the increase was significant only in the two high-dose groups when all generations were combined. A significant increase in constricting rings of the tail was seen in the offspring of rats fed 18.6 mg DDT/kg/day through two generations (Ottoboni 1969).

In summary, there is no conclusive evidence that DDT/DDE at the levels found in the environment cause developmental effects in humans, although two studies suggested that there may be an association between prenatal exposure and/or exposure early in life to DDE and alterations in height in children. In animals, DDT/DDE can produce embryotoxicity, fetotoxicity, and abnormal development of the sex organs. *o,p'*-DDT has been shown to have estrogenic properties, whereas *p,p'*-DDE has shown anti-androgenic properties. In addition, adult mice administered DDT early in life showed neurobehavioral alterations when tested later in life.

The highest NOAEL values and all LOAEL values from reliable studies for developmental effects in each species and duration category are recorded in Tables 3-1 and 3-2 and plotted in Figures 3-1 and 3-2. Based on the studies by Eriksson and Nordberg (1986) and Eriksson et al. (1990a, 1990b), an acute oral MRL of 0.0005 mg/kg/day was calculated as described in the footnote in Table 3-1 and in Section 2.3.

3.2.2.7 Cancer

Numerous studies in the United States and abroad have examined the possible association between exposure to DDT and related compounds and cancer in humans. Studies have been conducted of members of the general population as well as of occupationally exposed individuals. Exposure has been traditionally assessed by measuring DDT residues (most often DDE because of its persistence in the body and in the environment) in blood or in adipose tissue. Current data indicate that DDT in either media is a valid biomarker of exposure to DDT residues and each media correlate well with one another providing that the concentration of residues is adjusted for lipid content. The drawback of trying to associate current residue levels determined at or near the time of diagnosis with the occurrence of cancer is that levels at diagnosis may be very different than those at the time when cancer began to develop, and this is particularly relevant for cancers that exhibit a long latency. These and other issues inherent to epidemiological studies make it difficult to draw definite conclusions about exposure to DDT/DDE/DDD and cancer. However, taking all factors into consideration, the existing information does not support the

hypothesis that exposure to DDT/DDE/DDD increase the risk of cancer in humans. The human studies below are presented in the following order: breast cancer, pancreatic cancer, Hodgkin's disease and non-Hodgkin's lymphoma, multiple myeloma, prostate and testicular cancer, endometrial cancer, and the occurrence of any cancer.

Human Studies

Breast Cancer. Many epidemiological studies have investigated the association between breast cancer and levels of DDT and DDT-derived compounds in blood or adipose tissue from humans. Some studies have suggested a positive association (Aronson et al. 2000; Dewailly et al. 1994; Falck et al. 1992; Güttes et al. 1998; Romieu et al. 2000; Wasserman et al. 1976; Wolff et al. 1993), while others do not support such an association (Demers et al. 2000; Dorgan et al. 1999; Helzlsouer et al. 1999; Laden et al. 2001a; Krieger et al. 1994; Liljegren et al. 1998; Lopez-Carrillo et al. 1997; Mendonca et al. 1999; Moysich et al. 1998; Schecter et al. 1997; Unger et al. 1984; van't Veer et al. 1997; Ward et al. 2000; Wolff et al. 2000a, 2000b; Zheng et al. 1999, 2000). Below, studies that found an association between DDT/DDE burdens and breast cancer are summarized first, followed by those that found no significant association. In each case, serum DDT/DDE as a biomarker of exposure is discussed before adipose tissue.

Evidence of a positive association between breast cancer and exposure to DDT and DDT-related compounds was found in a case-control analysis nested within a prospective study in which blood samples of New York City women attending a mammography clinic were collected between 1985 and 1991 (Wolff et al. 1993). Serum DDE (not lipid-corrected) was determined on the archived blood samples of women who were diagnosed with breast cancer within 6 months of entering the study (n=58) and in matched, cancer-free control women from the same cohort (n=171). Controls were matched to case patients with respect to menopausal status, age at entry into the study, number and dates of blood donations, and day of menstrual cycle (if premenopausal) at time of first blood drawing. Mean serum DDE was significantly higher in case patients (11.0 ppb) than in control subjects (7.7 ppb) (p=0.031). The adjusted odds ratio for breast cancer in the highest quintile of serum DDE was significantly elevated (OR=3.68; 95% CI=1.01–13.50), using the lowest quintile as the referent group. A significant positive trend was identified between the odds ratio for breast cancer and increasing quintile (p=0.035). A significant positive trend was also obtained for the relation between the adjusted odds ratio and serum DDE when serum DDE was evaluated as a continuous variable using conditional multiple logistic regression (p=0.0037). The odds ratios were adjusted for first-degree family history of breast cancer, lifetime months of lactation, and age at first full-term pregnancy; other potential confounders, including

age at menarche, history of benign breast disease, history of tobacco and alcohol use, and race were found not to affect the outcome in preliminary evaluations. This study supported a positive relation between serum DDE and breast cancer, in spite of a short follow-up period and a relatively small number of subjects. See below for follow-up data from this study (Wolff et al. 2000b).

The association between elevated serum DDE levels and risk of breast cancer was examined among a subsample of women from a larger breast cancer case-control study of women living in Mexico City (Romieu et al. 2000). The final analysis was restricted to 120 cases and 126 controls who had given birth to at least one child and had complete information on all key variables. Major predictors of DDE levels included DDT levels, age, duration of lactation, parity, and socioeconomic level. Mean lipid-adjusted serum DDE levels were higher among cases (3.84 ppm) than in controls (2.51 ppm) (p=0.02). This difference was more apparent for postmenopausal women; among premenopausal women, the difference was only marginally significant. Age-adjusted serum DDE levels were significantly related to breast cancer risk, but serum DDT levels were not. When DDE levels were examined as quartiles, after adjusting for age at menarche, duration of breast feeding, quetelet index, and menopausal status, there was a positive trend in the risk of breast cancer with increasing levels of serum DDE that was marginally significant (OR_{Q1-Q4}=2.16; CI 95%, 0.85–5.50; p for the trend=0.06). The association was found to be stronger among those women who had experienced menopause; no association was found among premenopausal women.

Several studies have reported positive associations between DDT/DDE/DDD body burden, measured in adipose tissue, and breast cancer. *p,p'*-DDE and *p,p'*-DDT levels in breast adipose tissue were significantly elevated in Hartford, Connecticut patients with breast cancer (n=20) in relation to age-matched controls (n=20) who had benign breast disease (Falck et al. 1992); samples were obtained from biopsy or mastectomy tissue, near the time of diagnosis in 1987. Women from Quebec City with estrogen receptor (ER)-positive breast cancer cells (n=9) had significantly higher *p,p*'-DDE levels in breast adipose tissue (p=0.01) and plasma (p=0.052) than in controls with benign breast disease (n=17) near the time of diagnosis during 1991–1992 (Dewailly et al. 1994); the difference was not statistically significant for women with ER-negative breast cancer cells. Wasserman et al. (1976) found that the concentrations of *p,p'*-DDT and *o,p'*-DDD in malignant breast tissue of hospitalized Brazilian women (n=9) collected after diagnosis (dates not reported) were significantly greater than those found in adjacent "normal" breast tissue in the same women (p<0.01 and p<0.1, respectively). Concentrations of *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT, and *o,p'*-DDE were also elevated in malignant breast tissue over adjacent normal tissue, but the differences were not statistically significant. A similar comparison was performed in a 1993–1994

case-control study of German women, in which the age-adjusted geometric mean concentration of *p,p'*-DDE in malignant breast tissue of recently mastectomized women (n=45) was found to be 62% higher (p=0.017) than the age-adjusted geometric mean concentration of *p,p'*-DDE in benign breast tissue in the control group (n=20); however, no statistically significant difference was found with respect to *p,p'*-DDT concentrations (Güttes et al. 1998). A hospital-base case-control study of Canadian women found a weak association between *p,p'*-DDE in breast adipose tissue and breast cancer (Aronson et al. 2000). Biopsy tissues from 217 cases and 213 benign controls were analyzed for *p,p'*-DDT, *p,p'*-DDE, and other organochlorines. The geometric mean concentrations of *p,p'*-DDE in cases and controls were 693 and 596 ppb, respectively; those of *p,p'*-DDT were 22.0 and 19.3 ppb, respectively. The only increased risk for *p,p'*-DDE was among a subgroup excluding current hormone replacement therapy users. Further evaluation of this cohort showed that the odds ratio of DDE was higher with risk of estrogen receptor-negative breast cancer than estrogen receptor-positive breast cancer (Woolcott et al. 2001). A possible explanation suggested by the investigators was that tumors that are receptor-negative when diagnosed may have a faster rate of progression than tumors that are receptor-positive when diagnosed.

Several studies found no association between serum DDT/DDE/DDD and breast cancer incidence in developed countries using blood samples collected prior to the diagnosis of breast cancer (Dorgan et al. 1999; Helzlsouer et al. 1999; Hunter et al. 1997; Krieger et al. 1994; Ward et al. 2000). Cholesterol-adjusted plasma DDE was determined in blood samples collected during 1989–1990 in a prospective study of the health of 121,700 married nurses in the United States (Hunter et al. 1997). Historical plasma DDE was no different between women who developed breast cancer before June 1992 (n=236) and pair-wise matched control women who did not subsequently develop breast cancer. The lack of an association was also observed within strata of menopausal status, age, age at menarche, age at birth of first child, number of children, and history of lactation. A follow-up report to the Hunter et al. (1997) study included the results of adding to the analysis 143 postmenopausal cases and controls and of adjusting plasma organochlorines for triglycerides in addition to cholesterol (Laden et al. 2001a). Median (lipid-adjusted) concentrations of DDE in cases and controls were 0.768 and 0.817 ppm, respectively. The study found no evidence of an association between DDE and breast cancer within strata of age, age at menarche, age at birth of first child, number of children, history of benign breast disease, or family history of breast cancer. For DDE, the multivariate relative risk of breast cancer for women in the highest quintile (1.5–6.0 ppm) of exposure as compared with women in the lowest quintile (0.007–0.43 ppm) was 0.82 (95% CI=0.49–1.37).

A prospective nested case-control study of blood samples collected between 1964 and 1971 compared DDE concentration in blood serum of northern California women who were later diagnosed with breast cancer (n=150; 50 each of white, black, and Asian women) with serum DDE of paired control women who did not develop breast cancer in the interval at least 6 months after the blood was drawn through the end of 1990 (Krieger et al. 1994). Serum DDE was not adjusted for serum lipid content. These blood serum samples were collected prior to the U.S. ban on DDT in 1972, and the serum DDE levels were much higher than those measured in the Wolff et al. (1993) study. Serum samples were collected an average of 14 years prior to cancer diagnosis. No significant difference in DDE serum level was observed in the combined analysis of 150 pairs of cases and controls (43.1 vs. 43.3 ppb). When the odds ratios were calculated for data stratified according to the amount of time between the serum sample collection and the breast cancer diagnosis, the results did not change. However, when ethnic groups were compared separately, high serum DDE concentrations were correlated with breast cancer incidence in Caucasian and African American women, but not Asian American women. For black women (50 pairs), serum DDE levels were higher in case patients than paired controls by an average of 5.7 ppb, although the difference was not statistically significantly higher (95% CI on the difference ranged from -3.3 to 14.8). The study also found that serum DDE was significantly higher in black and Asian women compared to white women.

Serum DDE (both lipid-adjusted and unadjusted) was measured in blood samples collected in 1974 or 1989 in a breast cancer case-control study nested within a prospective cohort study, consisting primarily of residents of Washington County, Maryland (Helzlsouer et al. 1999). Blood samples were collected from 20,305 residents of Washington County in 1974, and from 25,080 residents in 1989. A group of 346 women who were diagnosed with breast cancer by June 1994 after having donated blood (for a total follow-up period of up to 20 years), who were residents of Washington County at the time of donating blood, and who had no other invasive cancers comprised the case group; 346 cancer-free control women were matched for age, race, menopausal status, and date of blood donation. Mean and median lipid-adjusted DDE concentrations were nonsignificantly higher in controls than in women who subsequently developed breast cancer. Separate risk analyses conducted for quintiles of DDE concentration in samples from 1974 and for tertile samples from 1989 of lipid-adjusted serum DDE showed no association between DDE and breast cancer. Women who donated in both 1974 and 1989 and who were diagnosed with breast cancer after 1989 were included in both program-specific analyses.

A hospital-based case-control study investigated risk for breast cancer associated with organochlorine exposure among 175 cancer patients, 181 control patients with benign breast disease, and 175 women in a

second hospital control group (Wolff et al. 2000a). Fifty-seven percent of the subjects were Caucasian, 21% Hispanic, and 22% African-American. Chemicals evaluated in blood serum included *p,p'*-DDT, *p,p'*-DDE, *trans*-nonachlor, and higher and lower chlorinated biphenyls. Also, four tumor markers (estrogen, progesterone, p53, and erbB-2) were examined among cases. Most blood samples were collected prior to surgery, and none were taken more than 2 months after surgery. African-American women had significantly higher lipid-adjusted DDE levels (1.0 ppm, cases geometric mean) than Hispanic (0.71 ppm) and Caucasian (0.48 ppm) women, whereas DDT was highest among Hispanic women. However, organochlorine levels were not associated with risk of breast cancer, nor did organochlorines differ with respect to tumor stage or tumor markers. Higher DDE levels were associated with increasing body mass index (BMI), but with decreasing level of education, frequency of nulliparity, and frequency of family history of breast cancer. Wolff et al. (2000a) attributed their findings to historical patterns of exposure and to metabolic differences in organochlorines related to body mass index.

An additional study by Wolff et al. (2000b) provides prospective information on the cohort studied earlier (Wolff et al. 1993) by these investigators. Because the authors were interested in assessing the half-lives of DDE and PCBs, the study was restricted to only cases with at least three yearly blood donations, after the first year of serum analysis. Lipid measurements were available on 110 cases and 213 controls. Half-lives for DDE and PCBs were calculated for 84 cases and 196 controls who had contributed more than one blood donation. Odds ratios were calculated using conditional logistic regression analysis with DDE and PCB levels as both quartiles and continuous variables; some analyses, however, were conducted tertiles rather than quartiles. Adjustments were made for the following potential confounders: age at menarche, number of full-term pregnancies, age at first full-time pregnancy, first degree family history of breast cancer, months of lactation, height, BMI, and an interaction term for BMI and menopausal status. Breast cancer was significantly associated with nulliparity and family history of breast cancer. DDE levels were similar in cases and controls, whether lipid adjusted or not (geometric mean 977 ppb adjusted in cases vs. 1,097 ppb in controls). The lack of significance of these differences was maintained when estrogen receptor status of tumors was considered. Half-lives of DDE and PCBs were similar in cases and controls, and there was a strong correlation between the two chemicals. Half-life of DDE, but not serum level, was correlated with BMI (shorter among leaner women). The reason for the discrepancy between the earlier results (Wolff et al. 1993) and those from the present study was not apparent, and the authors could not rule out their earlier finding was a chance observation.

Demers et al. (2000) conducted a case-control study among women from the Quebec City area to assess not only cancer risk, but also disease aggressiveness in relation to plasma levels of several organochlorines, including *p,p'*-DDT and *p,p'*-DDE. The cohort consisted of 315 women newly diagnosed with breast cancer, 219 hospital-based controls, and 307 population controls. Mean concentrations of *p,p'*-DDT and *p,p'*-DDE in women with breast cancer were not significantly different from those of hospital or population controls. Moreover, high concentrations of *p,p'*-DDT and *p,p'*-DDE (concentrations divided in quintiles) were not related to breast cancer risk. Additional analyses restricted to cases showed that after adjusting for confounders, the relative risk of axillary lymph node involvement increased significantly with *p,p'*-DDE tertiles (and with other organochlorines as well). When tumor size and lymph node involvement were considered in the same model, odd ratios of having involved nodes after adjustment for tumor size and confounding factors increased with tertiles of *p,p'*-DDE. However, odd ratios of having a large tumor after adjustment for lymph node involvement and confounding factors did not increase with *p,p,'*-DDE plasma concentrations. There was no interaction between exposure to DDE/DDT and the hormonal status of the tumor (estrogen positive or negative) with regard to either tumor size or axillary lymph node involvement.

A case-control study of 240 breast cancer patients and 477 controls was nested within a prospective study initiated in 1976 to investigate the fate of 7,712 women in the Copenhagen City Heart Study (Høyer et al. 1998). Odds ratios for breast cancer were not increased in upper quartiles of lipid-adjusted serum total DDT, *p,p'*-DDT, or *p,p'*-DDE, compared to the lowest quartile of lipid-adjusted serum concentrations. The analysis was adjusted to account for the following potential confounders: weight, height, number of full-term pregnancies, alcohol consumption, smoking, physical activity, menopausal status, household income, marital status, and education. Blood samples were collected in 1976, and breast cancer diagnosis occurred up to 17 years following the sample. Exclusion of women who developed breast cancer within 5 years of serum sampling did not alter the results. Median serum levels of *p,p'*-DDE in samples taken 5 years after the first examination were not significantly changed (1,197 vs. 1,169 ppb), but *p,p'*-DDT levels were about 3 times lower in the more recent analysis (144 vs. 46 ppb) (Høyer et al. 2000). When the values for *p,p'*-DDT from the two sampling periods were averaged and the means were stratified in quartiles, a positive association between the average serum concentration of *p,p'*-DDT and breast cancer risk was observed (p for the trend was 0.02).

A study of 150 breast cancer cases and 150 controls from Norway found no association between *p,p'*-DDT or *p,p'*-DDE in serum levels and breast cancer (Ward et al. 2000). In this study, the interval between blood collection and diagnosis ranged from 2 to 18.2 years with a mean of 8.8 years. The mean

lipid-adjusted DDE concentrations in cases and controls were 1,230 and 1,260 ppb, respectively; the corresponding values for DDT were 120 and 138 ppb. Analyses of the data stratified by age at diagnosis, interval between blood collection and diagnosis, and estrogen and progesterone receptor status showed that DDE was higher in women cases age 50 years or older at diagnosis and in cancer cases with $10 years between blood sample and diagnosis. Neither one of these differences were statistically significant.

The association between breast cancer and DDT exposure was evaluated in 105 breast cancer cases relative to 208 matched controls in a case-control study nested within a prospective breast cancer study (Dorgan et al. 1999). Breast cancer was diagnosed up to 9.5 years after blood samples were obtained (between 1977 and 1987) from a cohort of women who participated in the Columbia, Missouri Breast Cancer Serum Bank study. DDT exposure was estimated using lipid-adjusted serum levels of *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, *p,p'*-DDE, *p,p'*-DDD, and total DDT. The percent of participants with serum DDT compound levels above the assay detection limit was not elevated in cases compared to controls. Risk ratios calculated by quartile of DDT compound level were evaluated for total DDT, *p,p'*-DDT, and *p,p'*-DDE, and found not to be elevated.

A case-control study of only postmenopausal women in western New York State (154 cases; 192 controls) found no association between risk of breast cancer and current age- and lipid-corrected serum *p,p'*-DDE concentrations, based on an odds ratio analysis (Moysich et al. 1998). Samples were collected between 1986 and 1991, within several months after diagnosis. The analysis controlled for a variety of breast cancer determinant factors, including age, education, familial breast cancer, parity, quetelet index (body mass index expressed as kg/m^2), age at first birth, duration of lactation, years since last pregnancy, fruit and vegetable intake, and serum lipids. A recent study by Zheng et al. (2000) examined the relationship between serum DDE (lipid-adjusted) and breast cancer risk by menopausal status, parity, and lactation, and by cases' estrogen receptor status among 475 cases and 502 control women in Connecticut. Age-adjusted mean serum DDE levels were comparable in cases and controls, 460 and 456 ppb, respectively. Stratification by disease stage showed that DDE levels in 20 patients with later stage disease were insignificantly lower (402 vs. 456 ppb) than in 389 patients with early stage disease. The authors also found no significant differences for mean serum levels of DDE between controls and the various types of treatment groups, estrogen receptor status, parity and lactation status, and menopausal status.

Two studies evaluated hospital populations of breast cancer patients in countries where DDT has been used in the recent past for malaria control programs, and found no relationship between risk of breast cancer and serum concentrations of *p,p'*-DDT or metabolites. Lopez-Carrillo et al. (1997) investigated the association between current serum levels of DDE and *p,p'*-DDT and the occurrence of breast cancer in hospital patients in Mexico City from 1994 to 1996. Neither arithmetic mean nor geometric mean serum DDE (either wet weight or lipid weight basis) were significantly different between Mexican women with breast cancer (n=141) and age-matched control women with no breast cancer. Arithmetic mean serum *p,p'*-DDT also was not different between the groups, either on a wet weight or lipid weight basis. Control women were recruited from various diagnostic areas of the hospitals, excluding oncology and gynecology; mean serum DDE was not statistically significantly different across diagnostic areas among control women. No significant effect of serum DDE on breast cancer risk was found for all subjects, or for subsets of subjects based on menopausal status, using an odds ratio analysis adjusted for age, quetelet index, breast feeding with first birth, parity, familial history of breast cancer, and time elapsed since first birth. Among northern Vietnamese women, no difference was found in 1994 plasma *p,p'*-DDE and *p,p'*-DDT concentrations between newly diagnosed breast cancer patients (n=21) and age- and residence-matched control patients who had fibrocystic breast disease (Schecter et al. 1997). Plasma levels of DDE and DDT were not adjusted for plasma lipid content. Relative risk of breast cancer was not significantly elevated for subjects in the higher tertiles of plasma DDE, DDT, and total DDT (DDT plus molar-adjusted DDE), compared to the lowest tertile. No differences were observed between controls and cases with respect to age, age at menarche, age at first pregnancy, parity, history of lactation, and maximum attained body weight. The authors concluded that exposure to *p,p'*-DDT is not an important factor in the etiology of breast cancer among northern Vietnamese women, although they acknowledge that the study was limited by small sample size.

Two studies of women from Brazil also found no association between blood DDT/DDE levels and breast cancer incidence. The first study investigated 177 cases of invasive breast cancer and 350 controls; cases and controls were residents of a large metropolitan region (Mendonca et al. 1999). The mean and median DDE concentrations in serum from patients were 5.1 and 3.1 ppb, respectively; the corresponding values in controls were 4.8 and 3.1 ppb. The age-adjusted odds ratio of breast cancer for women in the upper quintile compared with those in the lowest quintile was 0.90 (95% CI 0.47–1.73). The second study of 46 women with breast cancer and 152 controls found that mean total DDT serum levels was 13.6 ppb in cases and 15.9 in controls (Matuo et al. 2000). However, cases who never breast fed had significantly higher serum DDT than controls who never breast fed.

Studies that found a lack of association between DDT/DDE in adipose tissue and breast cancer include one by Liljegren et al. (1998) who found that the DDE concentration in breast tissue fat of women with malignant breast cancer (n=43) was no different from the DDE concentration in breast adipose tissue of women with benign breast disease (n=35), whether or not the study group was divided into pre- and postmenopause subgroups; samples were collected during surgical procedures in 1993–1995, after diagnosis. Odds ratio analysis of the entire study group, the postmenopausal subgroup, and the ER-positive subgroup revealed no association between breast cancer malignancy and tissue DDE concentration. Similarly, Unger et al. (1984) found that there was no difference in mean DDE levels in biopsied extractable breast fat tissue between newly diagnosed breast cancer patients and noncancer patients; the dates of biopsies were not provided. Zheng et al. (1999) also found no significant association between breast adipose tissue levels of DDT and DDE and breast cancer. The study included 304 cases of breast cancer and 186 benign breast disease controls in Connecticut. The age-adjusted geometric mean concentrations of DDE in breast adipose in cases and controls were 737 and 784 ppb, respectively; the corresponding values for DDT were 52 and 56 ppb. There was no increased risk of breast cancer associated with increasing breast adipose tissue levels of either DDT or DDE. An additional study compared breast adipose tissue levels of DDT/DDE/DDD between 73 breast cancer cases and 73 women undergoing breast reduction surgery in California (Bagga et al. 2000). Analysis of unadjusted mean concentrations revealed no significant differences for DDT and DDD between cases and controls, but DDE was significantly elevated in cases relative to controls. However, when adjusted for age (cases were significantly older than controls), there was no statistically significant relationship between either DDT, DDE, or DDD, or the sum of the three chemicals and breast cancer. Another case-control study evaluated postmenopausal women from several European countries, showing no significant difference in current *p,p'*-DDE concentration in buttocks adipose tissue between women with breast cancer (n=265, 1.35 ppm) and cancer-free women matched for age and study center (n=341, 1.51 ppm) (van't Veer et al. 1997). The dates of tissue sampling were not reported.

A combined analysis of the data from five studies (Helzlsouer et al. 1999; Laden et al. 2001a; Moysich et al. 1998; Wolff et al. 2000a; Zheng et al. 2000) including 1,400 case patients and 1,642 control subjects was recently published (Laden et al. 2001a). The authors calculated pooled odds ratios and 95% confidence intervals by use of the random-effects model. When women in the fifth quintile of lipid adjusted values for DDE were compared with those in the first quintile, the multivariate pooled odds ratio for breast cancer was 0.99 (95% CI, 0.77–1.27), suggesting that there was no association between breast cancer risk and circulating DDE concentrations.

Aschengrau et al. (1998) explored the relationship between occupational exposure to estrogenic chemicals and the occurrence of breast cancer. Investigators compared probable exposure to DDT (inferred from subjectively reported employment history and a job exposure analysis that related job type to probable xenobiotic exposure) in women from Cape Cod, Massachusetts who were diagnosed with breast cancer during 1983–1986 (n=261) with age- and race-matched controls (n=753) from Cape Cod. No relationship was found; the incidence of women who were occupationally exposed to DDT was regarded as "rare" and was not numerically reported in the study. No information was provided regarding the estimated timing of DDT exposure versus diagnosis of breast cancer.

Pancreatic Cancer. Pancreatic cancer was weakly associated with exposure to DDT in a nested case-control mortality study following up a cohort of 5,886 chemical manufacturing workers who were potentially exposed between 1948 and 1971 (Garabrant et al. 1992). Deaths due to pancreatic cancer occurred between 1953 and 1988. The mortality registry had been updated and periodically analyzed. An elevated mortality from pancreatic cancer was first seen in the cohort in 1987. The association between pancreatic cancer and exposure to 429 chemicals or groups of related chemicals was examined for 28 cases of pancreatic cancer and 76 matched controls. Only 16 of the 28 cases were medically verified; medical records were not available for 12 subjects whose death certificates indicated pancreatic cancer as the underlying cause of death. Only 11 of the cases had been exposed to DDT or related materials. The relative risk (RR) for exposure to DDT alone was 4.8 (6 cases, 7 controls; 95% CI=1.2–17.6) and for DDD was 4.3 (9 cases, 12 controls; 95% CI=1.5–12.4). The RR for DDT increased with greater-than-median exposure duration and more than 20 years of latency. Multivariate analyses to correct for confounding factors (e.g., cigarettes, decaffeinated coffee, antacid use) did not change the RR markedly. When exposures to other chemicals (e.g., ethylan, nitrofen, dinocap, carbon tetrachloride, dispersing agents) found associated with pancreatic cancer (i.e., RR>1) were added to the multivariate model the RR for DDT remained relatively stable, ranging from 3.1 to 5.4 (statistical significance was not reported). It was concluded that DDT was an independent risk factor for pancreatic cancer. The evidence was not strong enough to conclude that DDD was an independent risk factor. The study was limited because of the small number of pancreatic cancer cases in DDT-exposed persons and the large number of exposures. Although confounding factors and biases have been minimized, the evidence for association is weak.

The association between pancreatic cancer and self-reported exposure to organochlorine pesticides (DDT among them) was examined in group of 66 pancreas cancer cases and 131 controls in southeastern Michigan (Fryzek et al. 1997). Analysis of the results showed a nonsignificant increased odds ratio of

1.6 for pancreatic cancer (95% CI=0.8–3.1; cases=17, controls=23) for patients who were ever exposed to DDT, as compared to patients who had not been exposed.

A more recent study examined serum levels of *p,p'*-DDE and other organochlorine compounds in 108 pancreatic cancer cases and 82 controls (Hoppin et al. 2000). The study was part of an ongoing population-based case-control study of pancreatic cancer in the San Francisco Bay area. Controls were matched to cases by sex and age. After lipid adjustment, cases had higher concentrations of organochlorine than controls; the median *p,p'*-DDE concentration in cases was 1.3 ppm vs. 1.0 ppm in controls (p=0.05). When the *p,p'*-DDE concentration was measured by tertiles, the odds ratio for the highest tertile (2.1, 95% CI=0.9–4.7) was elevated compared to the lowest tertile, although it did not achieve statistical significance. The odds ratio of 2.1 decreased to 1.1 after controlling for PCB level. As part of the same ongoing study, Slebos et al. (2000) investigated the association between two molecular markers, K-*ras* oncogene and *p*53 tumor suppressor gene, and risk factors for pancreatic cancer in a group of 61 newly diagnosed patients. Activating point mutations of K-*ras* are common oncogene alterations in pancreatic carcinoma. Conversely, inactivation of the *p*53 tumor suppressor gene is common in almost all human cancers. The study found that patients with K-*ras* positive tumors tended to have lower serum levels of DDE than those with K-*ras* negative tumors and also that serum levels of DDE were not significantly different between the *p*53 positive and negative groups. In contrast to the findings of Slebos et al. (2000), Porta et al. (2000) found in a study of 51 subjects with pancreatic cancer that serum levels of *p,p'*-DDT and *p,p'*-DDE were significantly higher than among 26 controls. Among the 51 cases, 34 had mutated K-*ras* tumors and 17 had wild-type K-*ras*. A comparison of nonlipid adjusted values revealed that serum *p,p'*-DDE levels from wild-type cases did not differ from controls, but levels from mutated K-*ras* cases were about double those in controls; a similar comparison, but adjusted for lipid content, was not conducted.

Lymphoma. Hodgkin's disease and non-Hodgkin's lymphoma were investigated in a retrospective study of 31 cases of Hodgkin's disease, 93 cases of non-Hodgkin's lymphoma, and 204 referents. A crude ratio of 2.2 for subjectively reported DDT exposure was calculated for Hodgkin's disease, but no association was found when confounding factors were included into the logistic regression analysis (Persson et al. 1993). For non-Hodgkin's lymphoma, the logistic odds ratio was 2.0 (90% CI=0.3–13). Data were obtained by questionnaire from Swedish workers diagnosed between 1975 and 1984. The numbers of exposed cases and controls were too small to provide evidence of an association between DDT exposure and the diseases; DDT-exposed subjects included only one case of Hodgkin's disease, four cases of non-Hodgkin's lymphoma, and three referents. Exposure to phenoxy herbicides and fresh wood among

sawmill workers, lumberjacks, and paper pulp workers were found to be significant risk factors for Hodgkin's disease, whereas welding, working as a lumberjack, nursing, and past-smoking were significant increased risks for non-Hodgkin's lymphoma.

No significant difference in mean concentration of *p,p*'-DDE in abdominal adipose tissue was found between a group of 28 newly diagnosed patients with non-Hodgkin's lymphoma and 17 surgical controls in Sweden (Hardell et al. 1996). The mean *p,p*'-DDE concentrations in patients and controls were 1.4 ppm (lipid basis) and 1.1 ppm, respectively. However, almost all of 34 PCB congeners were significantly higher in 27 of the cases relative to the 17 controls.

Analysis of pooled data from three case-control studies in the United States revealed tenuous evidence of an association between DDT exposures and the occurrence of non-Hodgkin's lymphoma among male farmers (Baris et al. 1998). Exposure was reported subjectively, and was categorized into the following three groups: DDT use on crops and animals, DDT use on animals only, and DDT use on crops only. Odds ratios and confidence intervals were obtained by logistic regression. Using a reference group of nonfarmers and pooled data for farmers from four Midwestern states, the age- and state-adjusted odds ratio for the occurrence of non-Hodgkin's lymphoma were 1.5 (95% CI=1.1–2.1) for farmers who used DDT on crops (n=74) and 1.6 (95% CI=1.1–2.3) for farmers who personally handled DDT that was applied to crops (n=63). The odds ratios were lower and not statistically significantly elevated above unity for using or handling DDT applied to animals or applied to animals and crops combined. When adjusted for use of other individual pesticides or pesticide groups, when evaluated by type of non-Hodgkin's disease, or when stratified by co-exposure to 2,4-D and organophosphate pesticides, no significant odds ratios were observed. No association was observed between estimated duration of DDT use and occurrence of non-Hodgkin's lymphoma, adjusted for use of other pesticides.

Lipid-corrected total serum DDT (sum of *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, and *p,p'*-DDE) was not associated with risk for non-Hodgkin's lymphoma in patients diagnosed with the disease between 1975 and 1989 (n=74) when compared to matched, cancer-free controls (n=147) in a case-control study nested within a prospective cohort study of Maryland residents that was begun in 1974 (Rothman et al. 1997). A separate evaluation for each DDT compound was not reported. The median lipid-adjusted concentration of total DDT in archived blood of patients who subsequently developed non-Hodgkin's lymphoma (3.2 ppm) was not significantly higher than in patients who did not develop the disease (2.8 ppm) (Wilcoxon signed rank test; p=0.2). Odds ratios for non-Hodgkin's lymphoma in serum DDT

concentration quartiles, compared to the lowest quartile, were not statistically significant and ranged from 1.2 in the second quartile to 1.9 in the highest quartile.

A population case-control study of the association between subjectively reported DDT use and non-Hodgkin's lymphoma was conducted on farmers in Iowa and Minnesota with newly diagnosed or confirmed disease (n=622) and population-based controls (n=1,245) (Cantor et al. 1992). Significantly elevated maximum likelihood estimates of odds ratios were obtained for ever having handled DDT applied to crops (OR=1.7; CI=1.2–2.6; cases=57 and controls=75) and for having handled DDT applied to crops only prior to 1965 (OR=1.8; CI=1.1–2.7; cases=45 and controls=57). Odds ratios were not significant for exposure to DDT applied as an animal insecticide. In spite of the fact that the odds ratios were adjusted using logistic analysis for vital status, age, state, smoking, family history of lymphopoeitic cancer, high-risk occupation, and high-risk exposure, causality could not be established because of exposure to multiple pesticides.

Multiple Myeloma. Several studies evaluated a possible association between DDT exposure and risk of multiple myeloma. Eriksson and Karlson (1992) investigated the several environmental factors that are possibly related to the occurrence of multiple myeloma in Sweden. Using univariate statistical analysis, a significantly increased relative risk of multiple myeloma of 1.75 (90% CI=1.19–2.64) was obtained for any DDT exposure, but not for DDT exposures stratified by exposure duration. Relative risk decreased and was nonsignificant when multivariate analysis was used to correct for confounding risk factors. For a group of 20 Iowa farmers who reported that they mixed, handled, or applied DDT as a crop insecticide, a nonsignificant odds ratio for multiple myeloma of 1.7 (95% CI=0.9–3.1) was obtained using a control group of 52 nonfarmers (Brown et al. 1993). An odds ratio of 1.8 was obtained for farmers who failed to use protective equipment, although statistical significance cannot be determined since a confidence interval was not reported. A nonsignificant odds ratio of 1.1 (95% CI=0.6–1.9) for multiple myeloma was estimated for 20 farmers who mixed, handled, or applied DDT as an animal insecticide, using a control group of 84 unexposed individuals. The combined evaluation of crop DDT exposure plus animal DDT exposure was not provided.

Proportional mortality analysis was conducted on death certificates of a cohort of 590 persons who applied DDT or inspected DDT application areas, and 453 unexposed workers, in a malaria eradication campaign in Sardinia, Italy conducted during 1946–1950 (Cocco et al. 1997a, 1997b). Based on information on annual use of DDT, its concentration in the pesticide mix, and the concentration applied to surfaces, the investigators estimated that DDT exposure concentrations ranged from 170 to 600 mg/m^3 in

outdoor operations. They also estimated that on average, for a man working 6 hours/day in pesticide application, the minimum indoor exposure would have been 254 g/day (exactly how this was estimated is unclear). Proportional mortality ratios (PMR) were provided for numerous types of cancer, for both DDT-exposed and unexposed groups, and the general Italian male population was used as the reference group for estimating expected mortality. For most types of cancer, the PMR was either elevated in both exposed and unexposed groups, or was not elevated in either group. For deaths due to multiple myeloma, however, the PMR was elevated (statistically significant) in exposed workers (PMRx100=341) [where a value of 100 indicates no difference between observed and expected mortality from multiple myeloma, and values above 100 indicate that the observed mortality was greater than the expected]; 95% CI=110–795; n=5), but not in unexposed workers (PMRx100=94; 95% CI=1–522; n=1). These results provided only marginal support for a positive association between occupational DDT exposure and multiple myeloma, because of the small numbers of cases. No consistent pattern of association was observed when data were categorized by estimated duration of exposure in days.

In a case-control study of workers in Italy who worked in an agricultural profession at some time, the odds ratio for multiple myeloma was significantly elevated above unity for self-reported exposure to chlorinated pesticides, including DDT (OR=1.6; 95% CI=1.1–2.4) (Nanni et al. 1998). Very similar, albeit marginally significant results were obtained for exposure specifically to DDT (OR=1.6; 95% CI=1.0–2.5). The referent group was comprised of age- and gender-matched individuals who had never worked in agriculture. A higher, but not statistically significant, odds ratio was obtained for exposure to DDT among workers whose primary occupation was agricultural (OR=2.6; 95% CI=0.9–7.8).

Prostate and Testicular Cancer. An ecologic study evaluated the relationships between *p,p'*-DDE concentration in subcutaneous fat, or *p,p'*-DDE in tree bark, and mortality from prostate and testicular cancers using multivariate statistical techniques (Cocco and Benichou 1998). Adipose DDE was obtained from samples collected in the EPA Human Monitoring Program in 1968 for people in 22 states, tree bark DDE data were available for 18 states representing the years 1992–1995, and age-adjusted mortality rates from prostate and testicular cancers during 1971–1994 were available by state from the National Center for Health Statistics. Numerous demographic factors were considered as possible confounders, and data were obtained from public sources. Separate analyses were conducted for whites and African Americans since mean adipose DDE in African Americans was 74% higher than in whites ($p<0.001$). The authors concluded that study results do not support an association between prostate and testicular cancer mortality and DDE exposure.

Endometrial Cancer. No association was found between newly-diagnosed endometrial cancer and lipid-corrected blood serum concentrations of *p*,p'-DDE in a multicenter case-control study of 180 women in the United States (Sturgeon et al. 1998). The median blood lipid-adjusted concentrations of *p,p'*-DDE were very similar between cases and controls (about 1.4 ppm). The adjusted relative risk of endometrial cancer in the highest quartile of exposure compared with women in the lowest quartile was 0.7 (95% CI=0.2–2.0). The blood concentration of *o,p'*-DDT was slightly higher among controls relative to cancer cases, but there was no significant association between increased cancer risk and this isomer. In contrast, cancer cases had higher blood levels of *p,p'*-DDT and a slightly higher risk of endometrial cancer. Similar overall results were obtained when the analysis was restricted to women over 50 years of age, as the authors sought to evaluate the influence of menopausal status.

The mean concentration of ' DDT (sum of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD) was significantly higher ($p<0.001$) in uterine leiomyomatous tissue (0.845 ppm) obtained from 25 recent hysterectomies of 36- to 55-year-old women than in normal uterine tissue (0.103 ppm) obtained from 25 recent autopsies of women from the same age range (Saxena et al. 1987). The mean concentration of each of the individual forms was nonsignificantly elevated above control levels in the leiomyomatous tissue. Dates that tissue samples were obtained were not reported.

The occurrence of endometriosis, a benign proliferation of endometrial tissue outside the endometrial cavity, was not associated with DDT exposure as measured by lipid-adjusted plasma *p,p'*-DDT, *p,p'*-DDE, and ' DDT in a study of 86 cases and 70 controls confirmed by laparoscopy in 1994 (Lebel et al. 1998). Geometric mean plasma concentrations of DDT compounds were not elevated in all cases compared to all controls, nor in subgroups of cases compared to controls matched by symptomatology (e.g., pelvic pain, infertility, and tubal fulguration).

All Cancer. In a prospective cancer mortality study of 919 adults from Charleston, South Carolina (Austin et al. 1989), serum levels of total DDT (DDT plus DDT molar equivalents of DDE) were estimated in 1974–1975, and 10 years later, the individuals were followed up and the cause of death was determined for the deceased. The cohort was divided into exposure tertiles based on total serum DDT; the lowest tertile was used as the reference group for calculating relative mortality rates. Adjustments were made for age, race, gender, years of education, and smoking habits. Relative risk of death, and specifically of death due to any cancer, was not significantly elevated in the high serum DDT tertile groups. No consistent positive trend in risk of cancer mortality relative to serum DDT was observed.

However, there was some evidence of a dose-response relation between serum DDT and respiratory cancer, but the point estimates were unstable and the trend was not statistically significant.

A historical prospective mortality study was conducted on 740 white male workers employed between 1935–1976 in occupations that involved exposures to DDT (Wong et al. 1984). Follow-up was conducted in 1976, and expected age- and cause-specific mortality rates were calculated from U.S. rates for white males for 5-year periods during 1935–1975. Among individuals exposed to DDT, overall mortality (all cancers), expressed as the SMR, was not elevated over expected values. Standardized mortality ratios were also not significantly elevated for individual cancers of the digestive, respiratory, urogenital, and lymphohaematopoietic systems. Several factors confound these results: individuals exposed to DDT also were potentially exposed to other chemicals, and smoking history was not included in the analysis.

Mortality and health impairments in a cohort of 2,620 pesticide workers and 1,049 controls were tracked during 1971–1977 (Morgan et al. 1980), and cancer rates were found to be significantly elevated in at least one category of exposed workers for internal cancers, leukemias, and lymphomas (evaluated together), as well as for skin cancers and all cancers. However, geometric mean total serum DDT (DDT plus DDE) measured in 1971–1973 was not significantly different between subjects who developed cancer of leukemia by the end of 1977 (n=43) and a group of subjects without the disease (n=45). Exposure to multiple pesticides was a significant confounding factor in this study.

A recent study examined the association of the 1968 adipose DDE levels of population samples from 22 U.S. states with age-adjusted mortality rates between 1975 and 1994 for multiple myeloma, non-Hodgkin's lymphoma, and breast, uterus, liver, and pancreas cancer (Cocco et al. 2000). Past body burdens were estimated by using half-lives for DDT and DDE of 7 years and several decades, respectively. Separate analyses were conducted for gender and race. The authors found no association for pancreatic cancer and multiple myeloma. Breast cancer mortality was inversely correlated with adipose DDE levels among both white and African American women. Significant inverse correlations were also observed for uterine cancer among white women, while no association was observed for African Americans and for non-Hodgkin's lymphoma among white (men and women) and African American women. Liver cancer mortality significantly increased with adipose DDE levels in white males and females, but not among African Americans. A previous study of Italian workers exposed to DDT in a malaria eradication campaign in Sardinia, Italy also found a significant increase in the PMR for liver and

biliary tract cancer; however, liver cancer was also elevated among unexposed workers and it did not show a trend with duration of exposure (Cocco et al. 1997a).

Overall, in spite of some positive associations for some cancers within certain subgroups of people, there is no clear evidence that exposure to DDT/DDE causes cancer in humans.

Animal Studies

DDT is one of the most widely studied pesticides in animals, and data are available on a number of carcinogenicity studies in several species. Intermediate exposures, in which animals were exposed to DDT in food, caused cancer increases in mice but not in rats or hamsters. Mice that were observed for 50–105 weeks after cessation of treatment developed liver hepatomas following dietary exposure to 42.8 mg *p,p'*-DDT/kg/day for 15–30 weeks (Tomatis et al. 1974b). DDT did not produce increases in the tumor incidence in rats exposed to 10–20 mg/kg/day in the food for up to 45 weeks (Kimbrough et al. 1964; Laug et al. 1950; Numoto et al. 1985) or in hamsters fed 50 mg DDT/kg/day for 30 weeks (Tanaka et al. 1987).

Chronic exposure (>1 year) to DDT caused cancer in multiple strains of mice but not in dogs; most studies in nonhuman primates have also been negative. Chronic exposure to DDT produced liver neoplasms in mice strains ([C57BL/6 x C3H/Anf]F_1, [C57BL/6 x AKR]F_1, BALB/c, and CF1) fed DDT at doses as low as 0.38 mg DDT/kg/day for a minimum of 78 weeks (Innes et al. 1969; Kashyap et al. 1977; Terracini et al. 1973; Thorpe and Walker 1973; Tomatis et al. 1972, 1974a; Turusov et al. 1973). An increased incidence of pulmonary adenomas was observed in mice after chronic gavage administration (Shabad et al. 1973). Malignant lymphomas and lung and liver tumors were also observed in mice treated with DDT in the food (Kashyap et al. 1977).

Some evidence exists to indicate that DDT may be carcinogenic in the rat. Rats maintained on diets containing DDT for more than 2 years or at doses higher than 25 mg DDT/kg/day developed liver tumors, primarily in female rats (Cabral et al. 1982b; Fitzhugh and Nelson 1947; Rossi et al. 1977). Liver tumors occurred in rats at doses of 19.7 mg DDT/kg/day for 2 years (Cabral 1982b). In contrast, no evidence of carcinogenicity was seen in rats receiving up to 45 mg technical DDT/kg/day for 78 weeks in the NCI (1978) bioassay. No significant increases in tumor incidence were observed in mice administered DDT at doses of 3–30 mg/kg/day (Del Pup et al. 1978; NCI 1978). Long-term exposure failed to induce significant increases in tumors in monkeys at doses of 3.9–20 mg/kg/day for up to 5 years (Adamson and

Sieber 1979, 1983; Durham et al. 1963); or in dogs at 80 mg/kg/day for 49 months (Lehman 1965). A more recent study that involved 11 Rhesus and 13 Cynomolgus monkeys administered approximately 6.4–15.5 *p,p'*-DDT/kg/day in the diet for up to 130 months reported that 2 out of 13 Cynomolgus monkeys (15%) developed malignant tumors, one hepatocellular carcinoma and one adenocarcinoma of the prostate (Takayama et al. 1999). No neoplasms were found in a group of nine Cynomolgus and eight Rhesus untreated control monkeys.

Evidence of carcinogenicity of DDT in hamsters is equivocal. Rossi et al. (1983) reported an increased incidence (14% in controls, 34% in treated hamsters) of adrenal neoplasms in hamsters administered approximately 95 mg DDT/kg/day via the diet for 30 months. At lower doses, Cabral et al. (1982a) did not observe a statistically significant increase in adrenal gland tumors; however, the incidence in males was increased compared to controls in animals receiving 71 mg DDT/kg/day via the diet for 28 months. Other studies in hamsters did not indicate any carcinogenic effects of DDT; however, early deaths occurred in one study (Agthe et al. 1970) and the duration of exposure was shorter in another (Graillot et al. 1975).

Several multigeneration studies have been conducted in mice. In these studies, exposure of the F_1 and subsequent generations to DDT was initially perinatal (i.e., *in utero* and through lactation) and was followed postweaning by oral exposure to DDT in the diet. In a study by Tarjan and Kemeny (1969), exposure to 0.4 mg *p,p'*-DDT/kg/day resulted in significant increases in leukemia and pulmonary carcinomas in the F_2 generation and occurred with increasing frequency with each subsequent generation of mice. Liver tumors (0.3–0.4 mg/kg/day) (Tomatis et al. 1972; Turusov et al. 1973) and pulmonary tumors (1.3 mg/kg/day) (Shabad et al. 1973) in the F_1 generation had a shorter latency period than in the parental generation, but the tumor incidence was comparable and did not increase with consecutive generations.

There are several studies of the potential carcinogenicity of DDE and DDD in rats, mice, and hamsters. DDE administered chronically in the diet produced liver tumors in male and female mice at doses of 27–43 mg/kg/day for 30–78 weeks (NCI 1978; Tomatis et al. 1974a) and in hamsters dosed with approximately 48 mg *p,p*'-DDE/kg/day for 128 weeks (Rossi et al. 1983). DDE did not induce significant increases in tumor incidence in rats exposed to DDE in the diet at doses ranging from 12 to 42 mg/kg/day for 78 weeks (NCI 1978), but doses of approximately 43 mg/kg/day for 130 weeks significantly increased the incidence of liver tumors in mice (Tomatis et al. 1974a). DDD induced liver tumors and lung adenomas in CF-1 mice at doses of approximately 43 mg/kg/day (Tomatis et al. 1974a),

but it was not tumorigenic in $B6C3F_1$ mice in a 78-week study at doses of approximately 142 mg/kg/day (NCI 1978). In the NCI (1978) bioassay, the combined incidences of thyroid follicular cell adenoma and follicular cell carcinomas were 1/19, 16/49, and 11/49 in controls, low-dose (116 mg/kg/day), and high-dose (231 mg/kg/day) male rats, respectively. The difference between the control and low-dose group was significant according to the Fisher Exact test. However, NCI (1978) pointed out that the variation of these tumors in control male rats in the study did not permit a more conclusive interpretation of the lesion.

EPA has estimated an oral cancer potency factor (q_1^*) for DDT of $3.4x10^{-1}$ $(mg/kg/day)^{-1}$, which was derived using the linearized multistage model (IRIS 2001a). This potency factor is derived from the geometric mean of potency factors based on the incidence of liver tumors in mice and rats as reported by Cabral et al. (1982b), Rossi et al. (1977), Terracini et al. (1973), Thorpe and Walker (1973), Tomatis and Turusov (1975), and Turusov et al. (1973). At this potency, $3.4x10^{-1}$ $(mg/kg/day)^{-1}$, the lifetime average daily doses that would result in risks of $1x10^{-4}$, $1x10^{-5}$, $1x10^{-6}$, and $1x10^{-7}$ are $2.9x10^{-4}$, $2.9x10^{-5}$, $2.9x10^{-6}$, and $2.9x10^{-7}$ mg/kg/day, respectively.

The oral cancer potency factor (q_1*) for DDD is $2.4x10^{-1}$ $(mg/kg/day)^{-1}$ based on the incidence of liver tumors in CF-1 mice (Tomatis et al. 1974a) and the oral q_1* for DDE is $3.4x10^{-1}$ based on the geometric mean of six slope factors for liver tumors in both sexes of $B6C3F_1$ mice (NCI 1978), CF-1 mice (Tomatis et al. 1974a), and Syrian hamsters (Rossi et al. 1983). At these potencies, the lifetime average doses that would result in risk of $1x10^{-4}$, $1x10^{-5}$, $1x10^{-6}$, and $1x10^{-7}$ are $4.2x10^{-4}$, $4.2x10^{-5}$, $4.2x10^{-6}$, and $4.2x10^{-7}$ mg/kg/day, respectively, for DDD and $2.9x10^{-4}$, $2.9x10^{-5}$, $2.9x10^{-6}$, and $2.9x10^{-7}$ mg/kg/day, respectively, for DDE.

Cancer Effect Levels (CELS) are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.3 Dermal Exposure

Occupational exposure to DDT involved multiple routes of exposure. The primary contact was probably inhalation and dermal; however, absorption of DDT from the lungs may not have been significant, and ingestion via the mucociliary apparatus of the respiratory tract is more likely. Therefore, epidemiological studies of occupational exposure are discussed under oral exposure.

3.2.3.1 Death

The dermal LD_{50} of DDT in rats was reported by Ben-Dyke et al. (1970), Cameron and Burgess (1945), and Gaines (1969) to range from 2,500 to 3,000 mg DDT/kg. In guinea pigs, a single dose of 1,000 mg DDT/kg resulted in death of 50% of the animals (Cameron and Burgess 1945). The LD_{50} in rabbits was 300 mg DDT/kg (Cameron and Burgess 1945) and 4,000–5,000 mg DDD/kg (Ben-Dyke et al. 1970). In the study by Cameron and Burgess (1945), the animals were dermally exposed to various doses of DDT in solvents including kerosene (1 or 10%), ethyl alcohol, acetone, or ether. It is uncertain what contribution these solvents made to the toxic effects observed; the authors stated that kerosene itself may have caused some deaths.

3.2.3.2 Systemic Effects

No studies were located regarding gastrointestinal, musculoskeletal, or ocular effects in humans or animals after dermal exposure to DDT, DDE, or DDD.

All of the information on the systemic and neurological effects of dermal exposure to DDT in animals is derived from a study by Cameron and Burgess (1945). In this study, rabbits, guinea pigs, and rats were dermally exposed to various doses of DDT in solvents including kerosene (1 or 10%), ethyl alcohol, acetone, or ether. It is uncertain what contribution these solvents made to the toxic effects observed; the authors stated that kerosene itself may have caused some deaths. The only information reported on the method of application stated that the skin area was shaved 24 hours before application of DDT impregnated in cloth and that precautions were taken to prevent animals from licking contaminated skin. The duration of exposure was not clearly reported. In addition, the species and the number of animals exhibiting specific toxic symptoms were not clearly reported and no statistical analysis was conducted.

Respiratory Effects. No studies were located regarding respiratory effects in humans after dermal exposure to DDT, DDE, or DDD. In rats, guinea pigs, and rabbits exposed to acute dermal doses ranging from 50 to 200 mg DDT/kg, pulmonary edema and respiratory failure were reported (Cameron and Burgess 1945).

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after dermal exposure to DDT, DDE, or DDD. Cameron and Burgess (1945) exposed rats, guinea pigs, and rabbits to acute dermal doses ranging from 50 to 200 mg DDT/kg and reported fat in the fibers of the heart.

Hematological Effects. No studies were located regarding hematological effects in humans after dermal exposure to DDT, DDE, or DDD. Cameron and Burgess (1945) exposed rats, guinea pigs, and rabbits to acute dermal doses ranging from 50 to 200 mg DDT/kg. A decrease in hemoglobin and leukocytosis was reported.

Hepatic Effects. No studies were located regarding hepatic effects in humans after dermal exposure to DDT, DDE, or DDD. Cameron and Burgess (1945) exposed rats, rabbits, and guinea pigs to acute dermal doses of 10, 50, or 100 mg DDT/kg and reported fatty degeneration, calcification, and necrosis in the liver.

Renal Effects. No studies were located regarding renal effects in humans after dermal exposure to DDT, DDE, or DDD. Cameron and Burgess (1945) exposed rats, rabbits, and guinea pigs to acute dermal doses ranging from 50 to 100 mg DDT/kg and reported fat deposits, tubular changes, calcification, and necrosis of the kidneys.

Dermal Effects. Cameron (1945) conducted a series of experiments on volunteers wearing undergarments impregnated with 1% DDT in order to determine whether this treatment would protect soldiers against body lice. Several individuals had transient dermatitis, but no other symptoms were observed. The length of exposure via this route was not specified. Cameron and Burgess (1945) exposed rats, rabbits, and guinea pigs to 10, 50, or 100 mg DDT/kg and reported inflammation, edema, and destruction of the epidermis. Guinea pigs were dosed 5 days a week for 3 weeks with 322–400 mg DDT/kg (Kar and Dikshith 1970). A decrease in skin amino acids, disruption and degeneration of the basal cell layer, and morphologic changes in the cells were reported.

3.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans or animals after dermal exposure to DDT, DDE, or DDD.

3.2.3.4 Neurological Effects

No studies were located regarding neurological effects in humans after dermal exposure to DDT, DDE, or DDD. Cameron and Burgess (1945) exposed rats, rabbits, and guinea pigs to acute, dermal doses ranging from 50 to 200 mg/kg DDT and reported tremors and nervousness.

No studies were located regarding the following effects in humans or animals after dermal exposure to DDT, DDE, or DDD:

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

No case studies or epidemiological investigations concerning the carcinogenic effects in humans after dermal exposure exclusively to DDT, DDE, or DDD were located. Occupational studies that probably involved both dermal and inhalation routes of exposure are discussed in Section 3.2.2.8.

Dermal exposure (skin painting) of mice to DDT did not result in a significant increase in tumor incidence when applied in a 5% solution in kerosene once weekly for 52 weeks (Bennison and Mostofi 1950) or at 8 mg/kg twice weekly for 80 weeks (Kashyap et al. 1977). No information on dermal exposure of rats or hamsters to DDT or dermal exposure to DDE or DDD was located.

3.3 GENOTOXICITY

DDT, DDE, and DDD have been tested in several genotoxicity studies in animals and in bacterial systems, but studies in humans are limited. Tables 3-3 and 3-4 report the results of *in vivo* and *in vitro* studies, respectively. Chromosomal aberrations have been reported in human studies, but simultaneous exposure to other chemicals and/or lack of control of potential covariates (such as smoking) make the findings inconclusive. For example, blood cultures of men occupationally exposed to several pesticides, including DDT, exhibited an increase in chromatid lesions (Yoder et al. 1973). Rabello et al. (1975) reported chromosomal aberrations in workers occupationally exposed to DDT. When all workers were considered, regardless of direct or indirect exposure, a significant increase in the incidence of

Table 3-3. Genotoxicity of DDT, DDE, and DDD *In Vivo*

Species (test system)	End point	Results	Reference
Mammalian cells:			
Human (plasma)	Chromosomal aberrations	+	Rabello et al. 1975
Human (plasma)	Chromosomal aberrations	+	Yoder et al. 1973
Human (lymphocytes)	Chromosomal aberrations	+	Rupa et al. 1991
Human (lymphocytes)	Micronuclei	–	Vine et al. 2001
Mouse	Chromosomal aberrations	+	Clark 1974
Rat	Chromosomal aberrations	–	Legator et al. 1973; Palmer et al. 1973
Rabbit (fetus' liver)	Chromosomal aberrations	–	Hart et al. 1972
Mouse (bone marrow)	Chromosomal aberrations	+	Johnson and Jalal 1973; Larsen and Jalal 1974
Mouse	Dominant lethal	+	Clark 1974
Rat	Dominant lethal	(+)	Palmer et al. 1973
Mouse (inhibition of testicular synthesis)	DNA synthesis	– (DDE)	Seiler 1977
Rat (liver)	DNA lesions	+	Hilpert et al. 1983
Host-mediated assays:			
Serratia marcescens (Mouse hosted-mediated)	Gene mutation	– (DDT, DDE) + (DDD)	Buselmaier et al. 1973
Neurospora crassa	Gene mutation	–	Clark 1974
Invertebrate systems:			
Drosophila melanogaster	Dominant lethal	+	Clark 1974

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

Table 3-4. Genotoxicity of DDT, DDE, and DDD *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium (TA1535, TA1537, TA98, TA100)	Gene mutation	–	–	McCann et al. 1975
Escherichia coli (Pol-A)	Gene mutation	–	–	Fluck et al. 1976
E. coli (Back mutation)	Gene mutation	–	No data	Fahrig 1974
Escherichia marcescens (glucose prototrophy)	Gene mutation	–	No data	Fahrig 1974
Bacillus subtilis (rec-assay)	DNA damage	–	No data	Shirasu et al. 1976
E. coli (col E1 plasmid DNA)	DNA damage	–	No data	Griffin and Hill 1978
E. coli (DNA cell binding assay)	DNA damage	–	No data	Kubinski et al. 1981
Fungal and plant cells:				
Neurospora crassa	Recessive lethal	–	No data	Clark 1974
Saccharomyces cerevisiae	Mitotic gene conversion	–	No data	Fahrig 1974
Mammalian cells:				
Chinese hamster V79 cells	Chromosomal aberrations	+ (DDE) – (DDT)	No data No data	Kelly-Garvert and Legator 1973
Chinese hamster (B14F28 cells [chromosomal damage])	Chromosomal aberrations	+	No data	Mahr and Miltenburger 1976
Kangaroo rat (cells)	Chromosomal aberrations	+	No data	Palmer et al. 1972
Human (hepatocyte-mediated cell)	Gene mutation	–	–	Tong et al. 1981
Chinese hamster (V79 cells [6-thioguanine resistent mutation])	Gene mutation	–	No data	Tsushimoto et al. 1983
Mouse (L51784 lymphoma cells)	Gene mutation	+	No data	Amacher and Zelljadt 1984
Rat (liver epithelial cell)	Gene mutation	–	No data	Telang et al. 1981
Human SV-40 (unscheduled DNA synthesis)	DNA damage	–	–	Ahmed et al. 1977
Mouse (hepatocytes-UDS)	DNA damage	–	–	Probst et al. 1981
Rat (hepatocytes-UDS)	DNA damage	No data	–	Probst and Hill 1980
Hamster (hepatocytes-UDS)	DNA damage	No data	–	Maslansky and Williams 1981

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

chromosomal damage was reported; no information was provided regarding control for relevant covariates. In pesticide sprayers who were exposed to DDT as well as seven other pesticides, increased frequencies of sister chromatid exchanges and chromosomal aberrations in peripheral lymphocytes were reported, compared to controls (Rupa et al. 1988). Increases in sister chromatid exchanges, the proliferation rate index, and the mitotic index were also reported in pesticide sprayers exposed to DDT along with several other pesticides (Rupa et al. 1989). A recent study of 302 individuals residing near a waste site in North Carolina found that plasma DDE levels were not associated with a higher frequency of micronuclei (Vine et al. 2001).

Studies in animals have provided mixed results (Table 3-3). In a dominant lethal assay, treatment of male rats with a single dose of 100 mg *p,p'*-DDT/kg resulted in a statistically significant increase in the proportion of females with one or more dead implantations only in animals mated during the postmeiotic stage of spermatogenesis (Palmer et al. 1973). In a dominant lethal assay in mice, DDT was administered orally to male mice at 150 mg/kg/day for 2 days (acute), or 100 mg DDT/kg twice weekly for 10 weeks (intermediate); the final dose was given 24 hours before sequential mating began (Clark 1974). Significant increases occurred in the number of dead implants per female. Acute doses resulted in maximum sensitivity in the induction of dominant lethal effects in week 5 and chronic doses in week 2, with continued increases above control through week 6. Repeated dosing caused significant reductions in testes weight, sperm viability, and a reduction of cell numbers in all stages of spermatogenesis. With acute treatment, the meiotic stage of spermatogenesis appeared to be the most sensitive. Acute treatment produced a significantly increased frequency of chromosome breakage, univalents, and stickiness in spermatocytes. BALB/C mice exposed *in vivo* to DDT exhibited chromosomal aberrations of the bone marrow (Johnson and Jalal 1973; Larsen and Jalal 1974).

Rats treated orally (by gavage) with DDT in single doses of 50–100 mg/kg or daily doses of 20–80 mg/kg/day for 5 days did not show a dose-related increase in percent of chromosomal aberrations over the solvent control (Legator et al. 1973). DDE, when administered in a single oral dose to male mice at the rate of 50 mg/kg, did not inhibit testicular DNA synthesis (Seiler 1977).

Administration of up to 50 mg *p,p'*-DDT/kg by gavage to rabbits on gestation days 7–9 did not affect chromosomal number distribution or the percentage of aberrations compared with controls (Hart et al. 1972). In addition, the distribution of chromosomes in liver samples from fetuses of DDT-treated rabbits and the percentage of chromosomal aberrations in these fetuses did not differ from controls (Hart et al. 1972).

As shown in Table 3-4, DDT and related compounds were, for the most part, nonmutagenic in prokaryotic organisms and did not induce DNA damage under the conditions tested. However, a few studies with mammalian cells *in vitro* found positive results. For example, Mahr and Miltenburger (1976) reported chromosomal damage in the B14F28 Chinese hamster cell line after exposure to DDT, DDE, or DDD. Palmer et al. (1972) also observed these same results in kangaroo rat cells (*Potorus tridactylis*) *in vitro* after exposure to DDT, DDE, or DDD. Kelly-Garvert and Legator (1973) reported a significant increase in chromosomal aberrations in Chinese hamster V79 cells after exposure to DDE, but not DDT.

Collectively, the data do not suggest that DDT and related compounds present a genotoxic hazard at environmentally relevant concentrations.

3.4 HEALTH EFFECTS IN WILDLIFE POTENTIALLY RELEVANT TO HUMAN HEALTH

The 1972 EPA decision to ban DDT for most uses in the United States was significantly influenced by a large body of scientific information documenting adverse effects to wildlife (EPA 1975). These observed effects were severe, including the lethality of DDT to birds and fish and the DDE-induced reproductive effects in birds, particularly eggshell thinning (EPA 1975). Although it is difficult to draw firm conclusions about adverse effects to human health based on those observed in wildlife, it is impossible to ignore that the documented effects to wildlife have motivated the investigation of human health effects. It is reasonable to assume that the adverse effects observed in wildlife may also be a concern to humans and that wildlife are possible "sentinels" for human health (NRC 1991). In order to completely address potential concerns for human health, it is necessary to review and evaluate the observed effects of DDT/DDE/DDD to terrestrial wildlife. Exposures for wildlife to DDT and its metabolites in the natural environment are primarily associated with the accumulation and persistence of these contaminants in both aquatic and terrestrial food chains. Ingestion of contaminated food results in the deposition of DDT/DDE/DDD in tissues with subsequent reproductive, developmental, and neurological effects. The most important reproductive effect observed in wildlife concerns eggshell thinning in birds. These and other effects on terrestrial wildlife are discussed in greater detail in Appendix D with the purpose of providing a qualitative synopsis of effects in terrestrial wildlife to address potential concerns that these effects from DDT/DDE/DDD exposure may also occur in humans.

Eggshell Thinning in Birds. Eggshell thinning in birds reached widespread public awareness in the 1960s and 1970s largely because of field observations in wild raptor populations including the bald eagle, peregrine falcon, and osprey, and the association of these effects with abrupt population declines. Experimental studies established a scientific link between DDT/DDE/DDD exposure, particularly DDE, and avian eggshell thinning, which weighed significantly in the decision to ban most domestic crop uses of DDT in the 1970s (EPA 1975). In general, raptors, waterfowl, passerines, and nonpasserine ground birds were more susceptible to eggshell thinning than domestic fowl and other gallinaceous birds, and DDE appears to have been a more potent inducer of eggshell thinning than DDT (Cooke 1973b; EPA 1975; Lundholm 1997; WHO 1989). Further, reproductive disturbances associated with DDT/DDE/DDD exposure continue to be reported in North American populations of predatory birds and/or birds that migrate to regions such as South America where DDT is still used (Lundholm 1997).

Numerous experimental studies have shown that dietary exposures to DDT/DDE/DDD are associated with eggshell thinning and breakage in wild birds including the barn owl (*Tyto alba*) (Mendenhall et al. 1983), the American kestrel (Porter and Wiemeyer 1969), the mallard duck (*Anas platyrhynchus*) (Heath et al. 1969; Risebrough and Anderson 1975; Vangilder and Peterle 1980), the black duck (*Anas rubripes*) (Longcore et al. 1971), the Japanese quail (*Coturnix coturnix japonica*) (Kenney et al. 1972), the bobwhite quail (*Colinus virginianus*) (Wilson et al. 1973) and the the Ringed turtle doves (*Streptopelia risoria*) (Haegele and Hudson 1973; Peakall 1970; Peakall et al. 1975). These experimental results have verified that the field observations of eggshell thinning and reductions in wild raptor populations are associated with releases of DDT. Possible mechanisms of eggshell thinning in birds have been extensively studied and reviewed (Cooke 1973b; EPA 1975; Lundholm 1997; Peakall et al. 1975; WHO 1989). The leading hypothesis for DDE-induced thinning involves an inhibition by *p,p'*-DDE (but not by *o,p'*-DDE or DDT or DDD isomers) of prostaglandin synthesis in the shell gland mucosa (Lundholm 1997). Overall, there is still some question as to the primary mechanism and reviewers have suggested that these may differ between bird species or differ with environmental conditions or physiological state for a given species. There is some question, however, as to the relevance of avian eggshell thinning to human health. There is no anatomical or physiological counterpart of the shell gland, a specialized segment of the oviduct, in humans. The shell gland lays down calcite ($CaCO_3$, calcium carbonate) onto the developing avian egg to form the eggshell (EPA 1975). Mechanisms of action that involve a direct action of DDT/DDE/DDD on the shell gland itself probably have no human relevance, but mechanisms of action that involve intermediate effects, such as reduced blood calcium, may have relevance to human health.

Reproductive Effects. Exposure to DDT/DDD/DDE is associated with reproductive toxicity in avian wildlife including embryolethality (Heath et al. 1969; Longcore et al. 1971; Porter and Wiemeyer 1969), decreased egg size and weight (Jefferies 1969; Peakall 1970; Wilson et al. 1973), delayed oviposition after mating (Cecil et al. 1971; Jefferies 1967, 1969; Peakall 1970; Richie and Peterle 1979; Vangilder and Peterle 1980), ovarian effects (Bitman et al. 1968; Gish and Chura 1970; Keith and Mitchell 1993) and testicular effects (Burlington and Lindeman 1950; George and Sunararaj 1995; Gish and Chura 1970; Locke et al. 1966). Several authors have speculated that these effects are associated with DDT-induced hormonal imbalances (Jefferies 1967) such as DDT induced estrogen-like inhibition of FSH and LH secretion by the pituitary inhibiting ovary development.

In most studies, egg production is not affected by DDT/DDD/DDE exposure (Azevedo et al. 1965; Chen et al. 1994; Davison et al. 1976; Davison and Sell 1972; Heath et al. 1969; Jefferies 1969; Longcore et al. 1971; Mendenhall et al. 1983; Porter and Wiemeyer 1969; Risebrough and Anderson 1975; Scott et al. 1975; Shellenberger 1978; Vangilder and Peterle 1980; Wilson et al. 1973). There are, however, a few reported cases of decreased egg production especially in birds with restricted diets (Cecil et al. 1971; Gish and Chura 1970; Haegele and Hudson 1973; Kenney et al. 1972). Egg fertility and hatchability are not consistently affected by DDT/DDD/DDE exposure. Some studies report significantly decreased fertility and hatchability (Porter and Wiemeyer 1969; Vangilder and Peterle 1980; Wilson et al. 1973), while others do not document significant effects (Azevedo et al. 1965; Haegele and Hudson 1973; Jones and Summers 1968; Scott et al. 1975; Shellenberger 1978). When considered collectively, egg production, fertility, and hatchibility were largely unaffected in numerous studies in a variety of bird species. This may be inconsequential to the overall reproductive success of birds since DDT/DDD/DDE exposure is clearly associated with decreased embryonic survival or fledgling success (Keith and Mitchell 1993).

DDT exposure has been shown to be associated with reduced post-hatch survival in avian wildlife. This effect has been observed in laboratory testing with mallards, pheasant, black duck, chicks; Japanese quail and ringed turtle doves (Azevedo et al.1965; Genelly and Rudd 1956; Haegele and Hudson 1973; Heath et al. 1969; Jones and Summers 1968; Keith and Mitchell 1993; Longcore et al. 1971; Porter and Wiemeyer 1969; Shellenberger 1978). The mechanism of DDT-induced reduced survival after oral exposures to DDT or DDE in maternal birds is hypothesized to be associated with increased body burdens of DDT/DDD/DDE in chicks as either a result of direct toxicity to the chick, or a reduction in parental care-giving among treated birds resulting in chick malnutrition and poor survival.

The implications of these observed effects in wildlife to human health is uncertain as the mechanisms of toxicity are not thoroughly understood. The consistency of the observed reproductive effects to avian wildlife and the field observations of effects to birds and reptiles have stimulated the investigation of reproductive effects in mammalian models that are more directly relevant to humans. *In vitro* mechanism of action studies have resulted in the identification of some DDT isomers and metabolites as androgen antagonists and estrogen agonists. There have been a number of intriguing mechanistic studies of DDT isomers and metabolites in fish that relate to reproductive and developmental effects (Das and Thomas 1999; Faulk et al. 1999; Khan and Thomas 1998; Loomis and Thomas 1999; Sperry and Thomas 1999; Thomas 1999). There are some interesting parallels between mammalian wildlife and human health studies. Similar to the associations made between DDT and preterm deliveries in humans (Saxena et al. 1980, 1981; Wassermann et al. 1982), premature births in California sea lions (*Zalophus californianus californianus*) are associated with elevated DDT concentrations (DeLong et al. 1973). However, the effect could not be solely, causally isolated to DDT due to the presence of PCBs.

Developmental Effects. The developmental effects of DDT/DDD/DDE on reptiles and avian wildlife have received considerable attention. Studies of alligator populations at Lake Apopka in Florida, where a pesticide spill occurred in 1980, have reported various reproductive effects including reduced clutch viability (Woodward et al. 1993), altered steroidogenesis (Crain et al. 1997; Guillette et al. 1995), abnormal ovarian morphology and plasma 17β-estradiol levels (Guillette et al. 1994), and reductions of phallus size and serum testosterone (Guillette et al. 1994, 1995, 1996, 1997, 1999). The authors hypothesized that the estrogenicity of DDT and other contaminants induced hormonal imbalance in the alligators, causing the observed effects (Guillette and Crain 1996). The contribution of DDT/DDE/DDD (only one component of the mixture of pesticides present) to the observed effects is uncertain. However, other experimental findings support the hypothesis that certain DDT-related compounds induce estrogenic effects in reptiles which could potentially adversely affect reproduction in a population (*in ovo* DDE exposures in alligators by Matter et al. 1998). In general, reptiles may be particularly susceptible to the endocrine-altering effects of DDT/DDE/DDD, as sex in many species are determined by environmental factors (temperature, etc.,) compared to the genetic sex determining mechanisms in birds and mammals (Crain and Guillette 1998). Organochlorine contaminants in general and *p,p*'-DDE, specifically, are thought to influence sexual dimorphism in the common snapping turtle (*Chelydra serpentina*)(de Solla et al. 1998). Snapping turtles in Ontario, Canada, lacked the normal sexual dimorphism in the distance between cloaca and plastron which was attributed to the antiandrogenic effects of *p,p*'-DDE.

DDT exposure is also associated with developmental abnormalities in amphibians and avian wildlife. DDT exposures are associated with delayed tadpole metamorphosis in the frog (*Rana temporaria*) (Cooke 1972, 1973a) and altered facial features (Cooke 1970a). Developmental effects in avian wildlife associated with exposure to DDT/DDD/DDE include a reduced growth (Seidensticker 1968), decreased ability to thermoregulate (Vangilder and Peterle 1980), behavioral alterations (Heinz 1976), reduced testicular development (Burlington and Lindeman 1952), and development of ovarian tissue and oviducts in genetic males (Fry and Toone 1981). Wildlife species may be appropriate sentinels of developmental effects in humans because certain effects, particularly reduced early survival in young, occurred consistently across several species under various exposure conditions.

Neurological and Behavioral Effects. Neurological effects (tremors, convulsions, hyperactivity, and behavioral changes) were observed in mammalian wildlife, amphibians, and avian wildlife experimentally exposed to DDT or DDE, particularly after administration of lethal doses or after administration of lower doses when food intake was restricted. Tremors were the most commonly reported neurological effect and have been reported in the brown bat (the short-tailed shrew (*Blarina brevicauda*) (Blus 1978), the free-tailed bat (*Tadarida brasiliensis*) (Clark and Kroll 1977) the big brown bat (*Eptesicus fuscus*) (Luckens and Davis 1964) and Pipistrelle bats (Jefferies 1972). Studies generally did not offer explanations as to the possible mechanisms that caused tremors, although it is reasonable to assume a mechanism similar to that seen in laboratory animals. Diets were experimentally restricted in several studies to simulate the health effects of DDT/DDE/DDD mobilized from fat during periods of energetic stress in the wild such as may occur, for example, during periods of nesting, migration, or thermal or other stress. Reviews (EPA 1975; WHO 1989) have postulated that during periods of energy stress, DDT mobilized from fat is redistributed to the brain (presumably because of the high lipid content in brain tissue) where it induces neurological effects and death. A study in bats (Clark and Kroll 1977) demonstrated that DDT residues in the brain increase substantially when the diet was restricted. Although a direct action on the central nervous system in wildlife has not been confirmed by observations of brain lesions, one study documented significant decreases in brain neurotransmitter levels associated with increased brain DDE residue levels after sublethal dietary exposures (Heinz et al. 1980). Alterations in neurotransmitter levels may explain changes in bird behavior that were observed in several species. Neurological effects observed in amphibians exposed to DDT/DDE/DDD in water include uncoordinated movement (Cooke 1970b) and hyperactivity (Cooke 1972, 1973a), tremors, lack of muscular coordination and weakness (Harri et al. 1979). Most available data suggest that wildlife species exhibit neurological effects similar to those observed in humans. These neurological effects, however were observed in wildlife at lethal exposure levels or in energy-stressed animals at lower exposure levels.

In avian wildlife DDT/DDD/DDE exposures are associated with decreased brain dopamine levels with a significant negative correlation observed between neurotransmitter levels and DDE residues in the brain (Heinz et al. 1980). Tremors have also been observed in bald eagles (*Haliaetus leucocephalus*) (Chura and Stewart 1967; Locke et al. 1966), kestrels (*Falco sparverius*) (Porter and Wiemeyer 1972), double-crested cormorants (*Phalacrocorax auritus*) (Greichus and Hannon 1973), pheasants (Azevedo et al. 1965), bobwhite quail (*Colinus virginianus*) (Hill et al. 1971), Japanese quail (Davison et al. 1976; Gish and Chura 1970), house sparrows *(Passer domesticus)* (Boykins 1967), cardinals (*Richmondena cardinalis*), and blue jays (*Cyanocitta cristata*) (Hill et al. 1971), homing pigeons (*Columba livia)* (Jefferies and French 1971) (Jefferies and French 1972), and domestic chickens (Glick 1974). Balance disturbances have also been observed (in some cases prior to death) in pheasants that (Azevedo et al. 1965), Bobwhite quail (*Colinus virginianus*) (Hill et al. 1971), cardinals (*Richmondena cardinalis*), and blue jays (*Cyanocitta cristata*) (Hill et al. 1971). Neurological effects in avian wildlife are also manifested as behavioral effects. These include the delayed onset of nocturnal restlessness indicative of normal migratory behavior (Mahoney 1975), significantly decreased courting behavior (Haegele and Hudson 1977), and decreased nest attendance by parental birds (Keith and Mitchell 1993).

Other adverse effects observed in wildlife species are described in detail in Appendix D. This section only provides a summary of reproductive, developmental and neurological effects that are the primary adverse effects to terrestrial wildlife associated with DDT/DDD/DDE exposure.

3.5 TOXICOKINETICS

Overview. Although oral exposure is considered the most significant route of entry in humans, DDT, DDE, and DDD are absorbed following inhalation, oral, and dermal exposures. Oral exposure to DDT, DDE, and DDD results in preferential absorption by the intestinal lymphatic system. Some absorption into the portal blood also occurs. Limited data exist regarding the rate and extent of DDT absorption in humans. DDT, DDE, and DDD are readily distributed in the lymph and blood to all body tissues and ultimately stored in proportion to the lipid content of the tissue, regardless of the route of exposure. Metabolism of DDT in humans appears similar to that seen in rats, mice, and hamsters, except that not all intermediate metabolites detected in animals have been identified in humans. Excretion of DDT in the form of its metabolites (e.g., DDA and its conjugates) is largely via the urine, regardless of route of exposure, but DDT excretion may occur via feces, semen, and breast milk. Some experiments have

suggested that fecal excretion may be the major route of elimination at high doses; however, this has not been confirmed by later investigations.

3.5.1 Absorption

3.5.1.1 Inhalation Exposure

Absorption of DDT by the lung is considered to be a minor route of entry. It is assumed that the large particle size of DDT (crystalline) prevents it from entering the deeper, smaller spaces of the lung, and that it is deposited on the upper respiratory tract mucosa and then eventually swallowed because of the action of the mucociliary apparatus (Hayes 1982). Some crystalline DDT, however, could be small enough to pass through the tracheal-bronchial passages. In occupational settings, human exposure has occurred by a mixture of routes, including inhalation with subsequent oral ingestion, and dermal absorption. Evidence of DDT absorption was indicated by the appearance of DDA (a DDT metabolite) in the urine (Laws et al. 1967; Ortelee 1958) and the presence of DDT in adipose tissue (Laws et al. 1967) and plasma or serum (Morgan et al. 1980; Rabello et al. 1975). However, no studies were located that quantify the rate or extent of absorption of DDT, DDE, or DDD in humans after inhalation exposure. No studies were located regarding the absorption of DDT, DDE, or DDD after inhalation exposure in animals.

3.5.1.2 Oral Exposure

Absorption following ingestion of DDT, DDE, and DDD is evident in humans both from measurements of serum and adipose tissue concentrations of these chemicals and from measurements of DDA in the urine (Hayes et al. 1971; Morgan and Roan 1971, 1974). Indirect evidence of absorption is provided in the development of toxicity following accidental or intentional (suicidal) ingestion of DDT (Hsieh 1954). In subjects chronically exposed to oral doses of DDT up to 20 mg/day (approximately 0.3 mg/kg/day), DDT appeared in the serum and reached peak serum concentrations 3 hours after ingestion (Morgan and Roan 1971). Serum levels remained elevated but returned to near pre-dose values 24 hours after each dose.

Gastrointestinal absorption can be inferred in animals. The presence of urinary metabolites in mice, rats, and hamsters (Fawcett et al. 1987; Gold and Brunk 1982, 1983, 1984), the presence of DDT and its metabolites in bile collections (Jensen et al. 1957), and the induction of tumors and other toxic effects in animals after oral administration of DDT, DDE, or DDD provide evidence of gastrointestinal absorption.

In animals, absorption of orally administered DDT is enhanced when it is dissolved in digestible oils (Keller and Yeary 1980). Approximately 70–90% of the administered dose is absorbed by rats after oral exposure to DDT in vegetable oils (Keller and Yeary 1980; Rothe et al. 1957). DDT is absorbed 1.5–10 times more effectively in laboratory animals when given in digestible oils than when dissolved in nonabsorbable solvents (Hayes 1982).

Gastrointestinal absorption by way of the intestinal lymphatic system plays a major role in the uptake of DDT in animals (Noguchi et al. 1985; Pocock and Vost 1974; Sieber 1976; Turner and Shanks 1980). For example, Sieber (1976) showed that 12–24% of the administered dose was recovered in the 24-hour lymph after intraduodenal administration of ^{14}C-isomers to thoracic duct-cannulated rats, and most of the radioactivity was attributed to parent compounds. Other studies indicate that little DDT is absorbed from the gastrointestinal tract directly into the blood (Palin et al. 1982; Rothe et al. 1957).

3.5.1.3 Dermal Exposure

Dermal absorption of DDT in humans and animals is considered to be limited, but can be inferred by observation of toxicity after dermal application of DDT. Acute toxicity studies in several species demonstrate that toxicity, expressed as an LD_{50}, is less when DDT is applied dermally than when given by gavage or by injection, which reflects the difference in the amount of DDT absorbed by the dermal route. The data indicate that DDT is 4 times more toxic when given by intraperitoneal injection than when administered orally and 40 times more potent when given by intraperitoneal injection than when administered by the dermal route (Hayes 1982). Absorption of DDT from soil applied to the abdomen of monkeys, as extrapolated from urinary excretion data, was 3.3% of the applied dose in 24 hours (Wester et al. 1990).

3.5.2 Distribution

The distribution and storage of DDT in humans and animals has been extensively studied. DDT and its metabolites, DDE and DDD, are lipid-soluble compounds. Once absorbed, they are readily distributed via the lymph and blood to all body tissues and are stored in these tissues generally in proportion to organ tissue lipid content (Morgan and Roan 1971).

Hayes et al. (1971) and Morgan and Roan (1971, 1974) evaluated the distribution of orally administered DDT, DDE, or DDD in volunteers. Morgan and Roan (1971, 1974) and Roan et al. (1971) measured the

concentration of DDT, DDE, DDD, and DDA in blood, fat, and urine after oral dosing. The administered doses ranged from 5 to 20 mg DDT/kg/day for up to 6 months; the ratio of concentration of DDT stored in adipose tissue to that present in blood was estimated to be 280:1.

DDT uptake into tissues is a function of the blood flow, lipid content of that tissue, and the partition coefficient for DDT between the blood and lipids in specific organs. The ratio of DDT concentrations in adipose tissue to blood may remain relatively constant; however, the amount of DDT from past exposure cannot be determined from present blood levels only. DDT, DDE, and DDD have been reported to be distributed to and retained in the adipose tissue of humans (Morgan and Roan 1971). The affinity for storage in adipose tissue is related to each chemical's lipophilicity and increases in the order *p,p'*-DDD # *o,p,'*-DDT < *p,p'*-DDT < *p,p'*-DDE (Morgan and Roan 1971).

DDT and DDE selectively partition into fatty tissue and into human breast milk, which has a higher fat content than cow's milk. Takei et al. (1983) reported concentrations from the 1969–1970 U.S. national human milk study. The *p,p'*-isomer of DDT and DDE was found in 100% of the samples tested, with mean concentrations of 0.19 and 1.9 ppm (lipid-basis), respectively. However, variance in levels of DDT and its metabolites may be influenced by such factors as number of parity, children nursed, diet, and cigarette smoking (Bouwman et al. 1990; Bradt and Herrenkohl 1976; Mes et al. 1993; Rogan et al. 1986). A steady decrease in the levels of DDT and its metabolites in human milk has been reported as a result of decreased intake of DDT. In Finland, samples taken between 1973 and 1982 indicate a reduction of more than 50% in total DDT concentration in human milk (Wickstrom et al. 1983). Using data from the United States and Canada, Smith (1999) estimated that since 1975, there has been an 11–21% decline in average DDT in breast milk. Table 6-4 shows levels of DDT and related compounds in breast milk from some recent studies.

LaKind et al. (2000) formulated a model to estimate doses of DDE to nursing infants. Included in the model were nursing variables (duration of breast-feeding, volume of breast milk consumed, percent lipid in breast milk, and milk density), infant variables (percent absorption of chemical, half-life of chemical, and body weight), and maternal depuration rate of the chemical via breast-feeding (time-dependent concentration of the chemical in the mother's milk). Several exposure scenarios were evaluated: 3, 6, 9, and 12 months, and a randomly simulated duration. The concentration of DDE in milk at time zero used in the model was 222.3 ppb (lipid basis), which was the mean value obtained in a Canadian breast milk evaluation of 497 donors from across Canada conducted in 1992 (Newsome et al. 1995). The results showed that, regardless of the exposure scenario simulated, infant body burdens of DDE increase rapidly

at the start of lactation, but decrease after approximately 5–6 months, even if nursing continues. The maximum mean body burden of DDE was about 70 µg/kg, and by 24 months postpartum, it was <10 µg/kg, regardless of the duration of breast-feeding.

DDT and DDT metabolites have been shown to cross the placenta. A study of 90 mother/infant pairs from Mexico found that all 90 cord blood samples had detectable levels of *p,p'*-DDE, 9 had detectable levels of *o,p'*-DDT, and 44 had measurable levels of *p,p'*-DDT (Waliszewki et al. 2000). The mothers were volunteers from the general population with no known occupational exposure to DDT. The concentrations of the pesticide residues in maternal blood were very similar to those in cord blood. *p,p'*-DDE was the most concentrated (lipid-adjusted means, 4.4 ppm maternal; 4.7 ppm cord blood), followed by *p,p'*-DDT (1.8 ppm maternal, 2.8 ppm cord blood), and *o,p'*-DDT (0.30 ppm maternal, 0.35 ppm cord blood). A study of Spanish women reported a geometric mean of 2.2 ppb (not lipid-adjusted) for *p,p'*-DDE in maternal blood at delivery (n=72) and 0.83 ppb in cord blood (n=69) (Sala et al. 2001). The women in the study lived in the vicinity of an organochlorine-compound factory.

The distribution of *p,p'*-DDT in newborn rats from dams administered *p,p'*-DDT in the diet before mating and throughout gestation was evaluated before and after suckling had occurred (Woolley and Talens 1971). In newborn rats sacrificed 0–1 hours after birth, before suckling had occurred, levels of *p,p'*-DDT were noted in the brain, liver, kidneys, and stomach. These results demonstrate that DDT readily passes through the placental barrier to enter tissues of the developing fetus. In newborn rats sacrificed after suckling, the tissue levels were relatively higher than levels in newborns before suckling. This could be attributed in part to the higher levels of DDT in the maternal milk, compared to DDT levels in maternal plasma (Woolley and Talens 1971). Recently, You et al. (1999b) studied the transplacental and lactational transfer of *p,p*'-DDE in rats. Pregnant rats were administered 10 or 100 mg *p,p*'-DDE/kg/day on gestation days (Gd) 14–18. In dams killed on day 15, the concentration of *p,p*'-DDE in the placenta was about 3-fold higher than in fetal tissues; similar observations were made in rats killed on Gd 17. Using a cross-fostering scheme, the authors found that lactational transfer provided the pups with a *p,p*'-DDE tissue burden far greater than that provided through placental transfer. For example, 10-day-old pups exposed only *in utero* to the low-dose had no detectable *p,p*'-DDE in blood, liver, or brain (only samples collected); *p,p*'-DDE could be detected only in the liver of high-dose pups. In contrast, in pups exposed only via dam's milk or through combined gestational and lactational exposure, *p,p*'-DDE was detected at much higher concentrations in the samples in the order of liver > brain > blood, and no significant differences were seen between the two groups. In 78-day-old rats, *p,p*'-DDE was detected only in fat in the three exposure groups, and rats exposed only *in utero* had at least two orders of

magnitude less *p,p*'-DDE in fat than the other two groups. In dams, at the end of nursing, *p,p*'-DDE levels in tissues and plasma were approximately 1/3 those at the end of gestation, suggesting that a large portion of the *p,p*'-DDE in storage sites was mobilized during lactation. See Section 3.5.5 for a description of physiologically based pharmacokinetic (PBPK) models developed by You et al. (1999b) to describe transplacental and lactational transfer of *p,p*'-DDE.

The time course of distribution of DDT (evaluated as *o,p'*- and *p,p'*-isomers) in reproductive tissues of female rabbits was examined by Seiler et al. (1994). The rabbits were administered the test material by gavage 3 days/week for 12–15 weeks before artificial insemination and throughout gestation. The authors examined DDT residues in oviductal and uterine luminal fluid, cleavage stage embryos (day 1 postcoitum), blastocytes (day 6 postcoitum), and in fetuses, exocoelic fluid and placenta (day 11 postcoitum). No demonstrable residues were detected in cleavage stage embryos and tubal flushings. Relative to controls, DDT residues were significantly increased in blastocytes (14-fold), uterine fluid (7-fold), fetuses (40-fold), and exocoelic fluid (700-fold). The higher concentration of DDT residues found in fetuses relative to blastocytes suggested that transplacental passage may be more easily accomplished than passage into blastocytes via uterine secretions.

Mühlebach et al. (1991) examined the kinetics of distribution of *p,p*'-DDE in rats after a single intravenous dose of 5 mg/kg of the radiolabeled material. Peak concentrations of DDE were observed before 1 hour in the liver and muscle, at 3 hours in the skin, and between 1 and 4 days in adipose tissue. Between 4 and 14 days after exposure, the tissue/blood concentration ratio was about 6 for liver and muscle, 35 for skin, and 400 for adipose tissue. The results also showed that the distribution kinetics of DDE was characterized by a redistribution from blood to liver and muscle, to skin, and ultimately to adipose tissue, and this process appeared to take about 1 day.

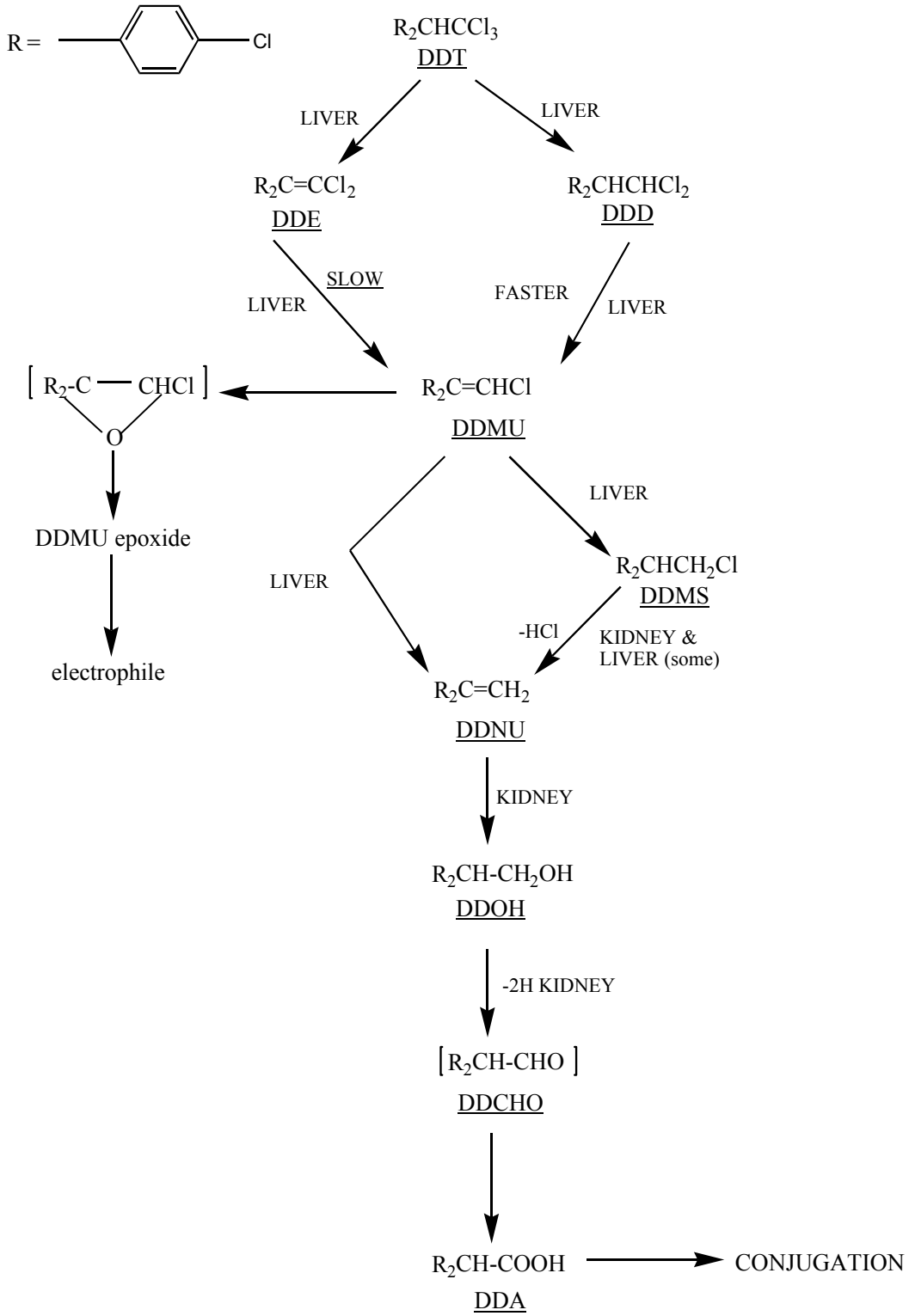
3.5.3 Metabolism

The metabolism of DDT, DDE, and DDD has been studied in humans and a variety of other mammalian species. The metabolism in rats, mice, and hamsters is similar to that in humans; however, not all of the intermediary metabolites identified in animals have been identified in humans. It has been proposed by a number of investigators that in mammals, the major urinary metabolite of DDT, 2,2-*bis*(*p*-chlorophenyl) acetic acid (DDA), is produced by a sequence involving reductive dechlorination, dehydrochlorination, reduction, hydroxylation, and oxidation of the aliphatic portion of the molecule (Gingell 1976; Peterson and Robison 1964). In this proposed pathway (Model I: see Figure 3-3a), DDT is initially metabolized in

the liver to two intermediary metabolites, DDE (Mattson et al. 1953; Pearce et al. 1952) and DDD (Klein et al. 1964). In rats, DDE is slowly converted in the liver to 1-chloro-2,2-*bis*(*p*-chlorophenyl)ethene (DDMU), and then to DDA in the kidney by way of 1,1-*bis*(*p*-chlorophenyl)ethene (DDNU) (Datta 1970; Datta and Nelson 1970). DDD is rapidly detoxified by way of DDMU to 1-chloro-2,2-*bis*(*p*-chlorophenyl)ethane (DDMS) and then to DDNU (Datta 1970). Metabolism of DDMS to DDNU occurs in both the liver and kidney, but the kidney is the primary site (Datta 1970). DDNU is then further metabolized, primarily in the kidney, to 2,2-*bis*(*p*-chlorophenyl)ethanol (DDOH) then to 2,2-*bis*(*p*-chlorophenyl)ethanal (DDCHO) (Suggs et al. 1970), which is further oxidized to DDA (Peterson and Robison 1964).

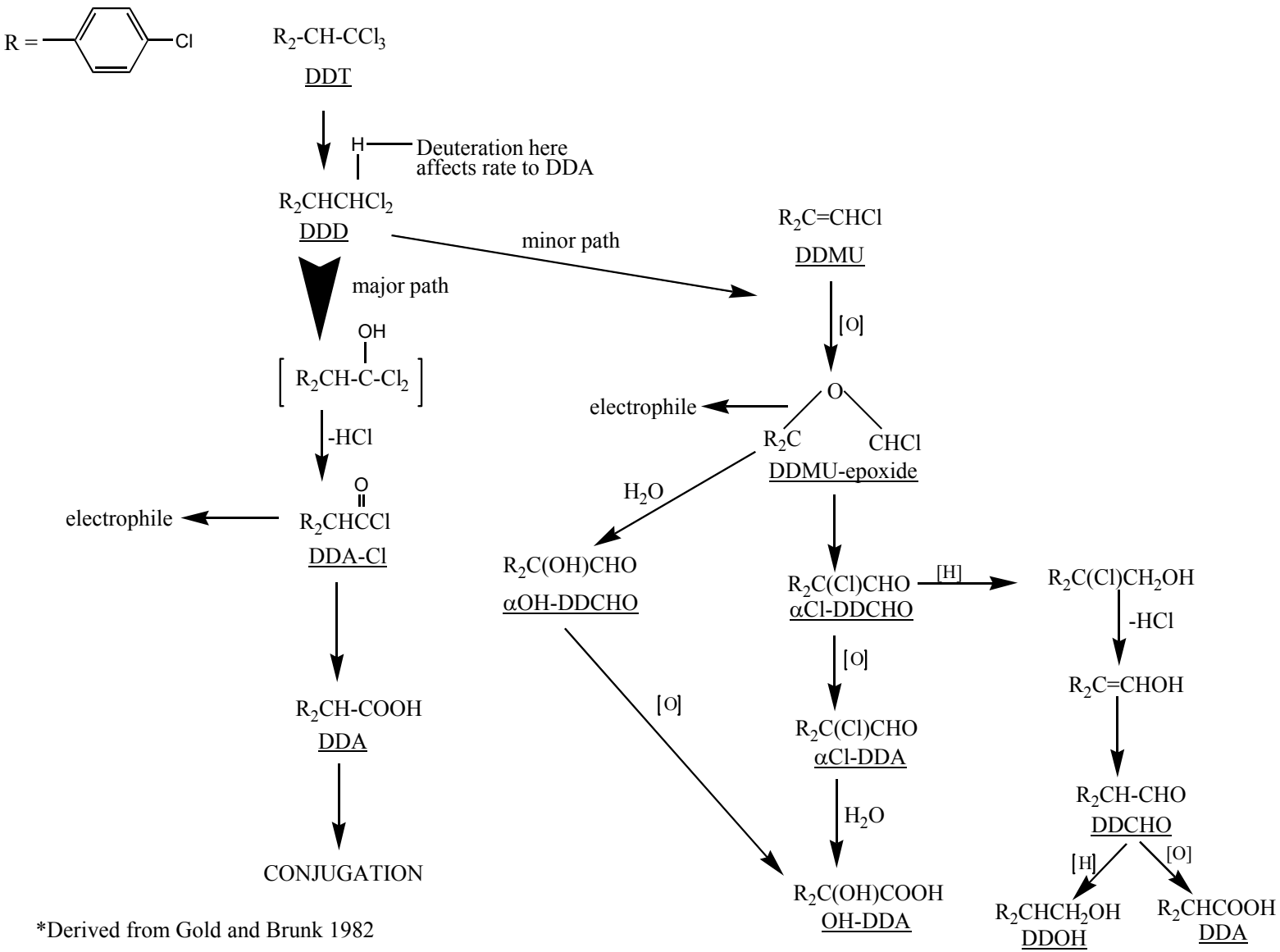
Other evidence in mice and hamsters suggests an alternative metabolic scheme (Gold and Brunk 1982, 1983) (Model II: see Figure 3-3b). In these studies, one dose of radioactively-labeled DDT or DDD was administered by gavage, and urine was collected for 72 hours for analysis. The principal urinary

Figure 3-3a. Model I Metabolic Scheme for DDT*



*Adapted from Peterson and Robinson 1964

Figure 3-3b. Model II Metabolic Scheme for DDT*



*Derived from Gold and Brunk 1982

metabolites were DDA and its conjugates. Few metabolite intermediates on the Model I pathway were isolated in the urine of DDT- or DDD-treated animals; however, if DDMU, a Model I pathway intermediate, was administered, downstream Model I metabolites were found in greater quantities. Based on this and the fact that deuterium labeling studies (Gold and Brunk 1984) indicate that removal of the alpha hydrogen from DDD is a rate limiting step in DDA formation, Gold et al. (1981) hypothesized that DDD is hydroxylated at the chlorinated 1-ethane side-chain carbon to yield 2,2-*bis*(*p*-chlorophenyl)acetyl chloride (DDA-Cl), which in turn can be hydrolyzed to the major urinary metabolite, DDA. Epoxidation of DDMU, a minor metabolite of DDD in Model II (note that in Model I it is not minor), yields DDMU-epoxide (Gold et al. 1981). The major (Model II) urinary metabolite DDA-Cl, which is an electrophile capable of acylating nucleophilic cellular molecules, and the minor DDMU-epoxide metabolite may contribute to the known tumorigenicity of DDT and DDD via the formation of covalent DDA adducts in the mouse. Urinary metabolites were similar in both female Swiss mice and female Golden Syrian hamsters (Gold and Brunk 1982, 1983, 1984). The metabolic disposition of DDT, DDD, and DDMU in the hamster is similar to that of the mouse and proceeds by the same oxidative metabolic pathways in both species (Gold and Brunk 1983). Therefore, it is unlikely that the observed differences in species sensitivity to the DDT-induced tumorigenicity in the mouse and the resistance to tumor production from DDT exposure in the hamster are due to differences in the production of the DDMU-epoxide or DDA-Cl. However, there was a species difference in the metabolic conversion of DDT to DDE. DDE was detected at much higher levels in the urine of mice after both acute and chronic studies than in hamsters (Gingell 1976; Gold and Brunk 1983). The data indicate that the hamster was less efficient than the mouse in the conversion of DDT to DDE. Fawcett et al. (1987) evaluated the metabolism of radiolabeled DDT, DDE, DDD, and DDMU in male Wistar rats. These results suggested that the metabolism of DDT to DDA proceeding via the acid chloride (DDA-Cl), as had also been found in the mouse and hamster (Gold and Brunk 1982, 1983, 1984).

Several investigators have isolated DDT metabolites from human urine, serum, and adipose tissue. The DDT metabolites in humans are the same as some of those produced in animals and it can be inferred that the metabolic pathways in humans and animals are similar. In humans, ingested DDT undergoes reductive dechlorination to DDD, which is further degraded and readily excreted as DDA (Roan et al. 1971). DDT is also converted by dehydrodechlorination to DDE, although at a much slower rate than the DDT-to-DDD pathway (Morgan and Roan 1971). Morgan and Roan (1971) concluded that the conversion of DDT to DDE occurs with considerable latency and that the extent of the conversion was estimated to be less than 20% over the course of the 3-year study. Further metabolism of DDE is apparently slow, and DDE is retained in adipose tissue (Hayes et al. 1971; Morgan and Roan 1971).

According to Roan et al. (1971) and Morgan and Roan (1971), oral administration of DDT or DDD to volunteers resulted in an increased urinary excretion of DDA, but no increase in excretion of DDA above predose values was noted after oral ingestion of DDE. According to the study authors, the data indicate that DDD, not DDE, is the precursor for DDA in humans and that little if any DDE is further converted to DDA.

After Phase I metabolism (reactions involving oxidation, reduction, and hydrolysis), many of the DDT metabolites ultimately are excreted in the conjugated form. Conjugates have been reported to include glycine, bile acid conjugates, serine, aspartic acid, and glucuronic acid (Gingell 1975; Pinto et al. 1965; Reif and Sinsheimer 1975).

DDT induces microsomal mixed function oxidases that are involved in the catabolism of both xenobiotics and many endogenous hormones. DDT has also been shown to induce its own metabolism in rats (Morello 1965) and hamsters (Gingell and Wallcave 1974). DDE and DDD have also caused the induction of hepatic cytochrome P-450 microsomal enzymes (Pasha 1981). DDT, DDE, and DDD were each found to be phenobarbital-type cytochrome P-450 inducers in male F344 rats, causing induction of hepatic CYP2B and less of CYP3A, but not CYP1A proteins (Nims et al. 1998). Limited induction of CYP1A1-associated enzyme activity was observed. DDT and DDE appeared to have similar inducing potencies, whereas the potency of DDD was within one order of magnitude lower than the other two compounds. Nims et al. (1998) further demonstrated that CYP2B induction resulting from DDT administration was due to the combined inductive effects of DDT, DDE, and DDD, and not exclusively to the DDE generated metabolically.

The metabolism of DDT can also produce methylsulfonyl metabolites, which are potent toxicants, particularly in the adrenal gland, after metabolic activation. Methylsulfonyl metabolites of DDT (specifically 3- and 2-methylsulfonyl-DDE) were first identified in seal blubber from the Baltic Sea (Jensen and Jansson 1976); they have later been found in several species of animals (Bergman et al. 1994) and in humans (Weistrand and Norén 1997). Methylsulfonyl-DDE is formed as follows: products of the reaction between arene oxides, formed in phase I metabolism, and glutathione are degraded and excreted in the bile into the large intestine where they undergo cleavage by a microbial C-S lyase (Bakke et al. 1982; Preston et al. 1984). The thiols formed are methylated, reabsorbed, and the sulfur is further oxidized to the corresponding methylsulfones, which are distributed by the blood (Haraguchi et al. 1989). Figure 3-4 shows the proposed pathway for sulfonyl metabolites. Further information regarding the

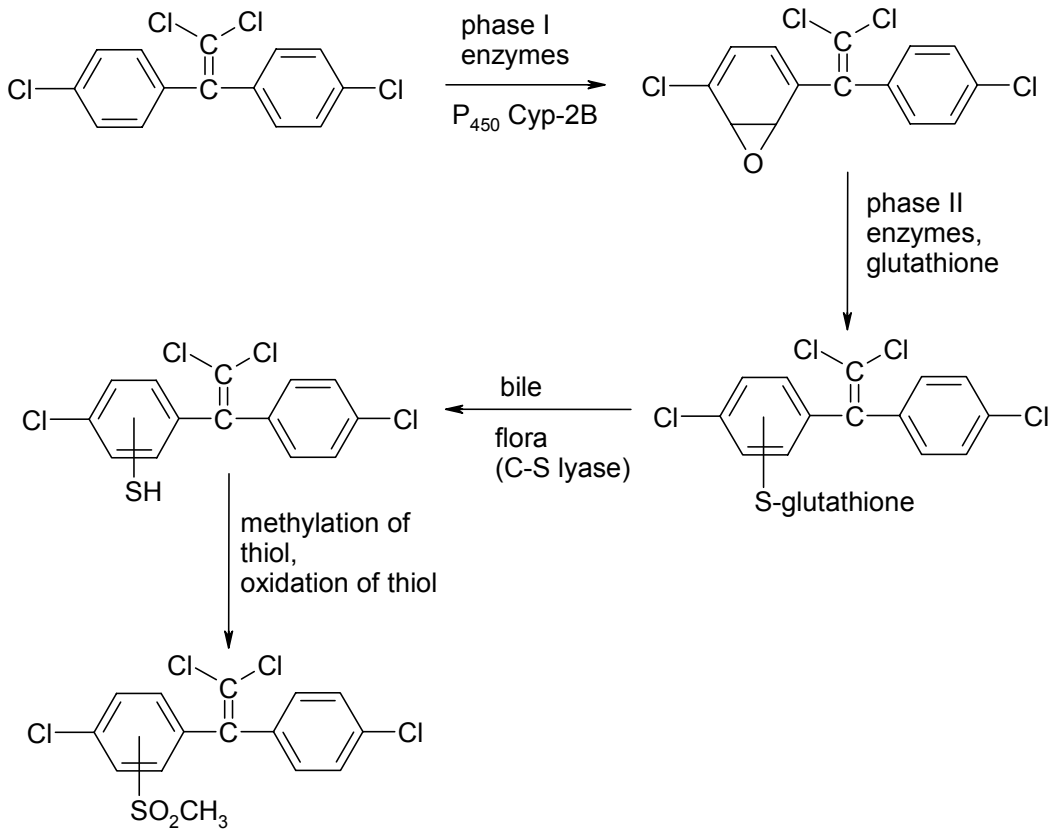
toxicity of this metabolite is presented in Section 3.7, Toxicities Mediated Through the Neuroendocrine Axis.

3.5.4 Elimination and Excretion

Excretion of DDT has been studied in humans and a variety of animals. The major route of excretion of absorbed DDT in humans appears to be in the urine, but some excretion also occurs by way of feces (via biliary excretion) (Jensen et al. 1957) and breast milk (Takei et al. 1983). Results of studies with mice, rats, and hamsters indicate that the metabolites of DDT and small amounts of unmetabolized DDT are excreted primarily in the urine and feces (Gold and Brunk 1982, 1983, 1984).

The biological half-lives for the elimination of these compounds are ranked as follows: DDE>DDT>DDD. This relationship is based on the chemical stability of each compound in the body, efficiency of excretory mechanisms, and possibly transport in and out of fat depots (Morgan and Roan 1971). The excretion of DDT was investigated in volunteers who ingested DDT. Hayes et al. (1971) reported that, in subjects receiving 35 mg/day (approximately 0.5 mg/kg) for up to 18 months, urinary excretion of DDA increased rapidly for the first few days and a steady state level of approximately 13–16% of the daily dose was reached and remained stable for 56 weeks. However, although the rate of excretion of DDA was relatively constant in each individual, there were marked differences observed between men receiving the same dose. Urinary excretion of DDA fell rapidly after cessation of dosing. It appears that a steady state for storage was reached within 12–18 months of daily dosing, after which humans were apparently able to eliminate the entire daily dose of 35 mg/day. Although no excretion of DDT metabolites was detected in the feces, the authors stated that there were probably DDT metabolites in the feces which were organic unextractable polar conjugates (Hayes et al. 1971). Since only 5.7 mg/day of all DDT isomers were found in the urine at steady state, it was postulated that other routes of excretion, such as biliary transport, may be involved. This was shown by Paschal et al. (1974). Roan et al. (1971) reported that increased urinary excretion of DDA is detectable within 24 hours of ingestion of DDT (5, 10, or 20 mg/day), DDD (5 mg/day), or DDA (5 mg/day). DDA excretion returns to predose levels within 2–3 days of dose termination for DDA, but continues significantly above predose levels for over 4 months after termination of DDD or DDT doses. Ingestion of DDE (5 mg/day) failed to produce any increase in DDA excretion.

Figure 3-4. Proposed Metabolic Pathway for the Conversion of *p,p*'-DDE to its Methylsulfone Derivative



Source: Bergman et al. 1994; Letcher et al. 1998; Weistrand and Norén 1997

DDT excretion in the feces may be a major route of excretion at high doses of DDT. DDT and DDT-related metabolites have been identified in the feces of humans receiving 35 mg/day (Hayes et al. 1956); however, this result has not been confirmed by later investigations (Hayes et al. 1971). According to Jensen et al. (1957), biliary excretion is the major source of DDT metabolites found in the feces of rats, as demonstrated in bile-cannulated rats given an intravenous injection of DDT. Following intravenous administration of DDT in rats, bile cannulation results indicated that enterohepatic circulation was occurring for conjugated DDA, a DDT metabolite (Gingell 1975; Pinto 1965).

Analysis of urine from humans occupationally exposed to DDT showed the presence of DDA (Laws et al. 1967; Ortelee 1958; Ramachandran et al. 1984). By comparing the urinary excretion of DDA with that of volunteers given known doses of DDT, the average occupational exposure can be estimated (WHO 1979). The observations by Laws et al. (1967) and Ortelee (1958) indicate that the urinary excretion of DDA is correlated with the level of exposure to DDT. The concentration of DDA in the urine in occupationally exposed workers was reported to be greater than that observed in the general population, while DDE excretion was reported to be only slightly higher than in the general population. It is of interest that monkeys fed DDT stored DDE in their fat (not DDT) and when feeding ceased, it was rapidly lost, probably by urinary excretion (Durham et al. 1963).

Parameters of elimination of DDE and DDE-derived radioactivity in rats were estimated by Mühlebach et al. (1991) after administration of a single intravenous dose. Over a 14-day period following dosing, 34% of the administered dose was excreted in the feces and 1% in the urine. In the feces, 10% of the excreted radioactivity represented unchanged DDE, whereas no unchanged DDE could be detected in the urine. No hexane-extractable lipophilic metabolites were found in the feces. The average total DDE recovered in tissues and excreta was 90%. The total body burden half-life was 120 days.

3.5.5 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 3-5 shows a conceptualized representation of a PBPK model.

Models of the pharmacokinetics of *p,p′*-DDE, a principle metabolite of DDT, have been proposed by You et al. (1999b). The You et al. (1999b) models are PBPK models of *p,p′*-DDE uptake and disposition in pregnant and lactating/nursing rats. The models are based on experimental studies in which pregnant Sprague-Dawley rats were administered gavage doses of *p,p′*-DDE, and the kinetics of *p,p′*-DDE tissue levels in the dams, fetuses, and pups were measured. The models provide an approach to estimating tissue doses in fetuses and pups associated with maternal exposures to *p,p′*-DDE and can be used to explore dose-response relationships for the developmental effects of *p,p′*-DDE in the Sprague-Dawley rat.

Description of the model. Figures 3-6 and 3-7 show conceptualized representations of the gestation model and the lactation/nursing models, respectively. Parameters used in the models are shown in Tables 3-5 and 3-6. The gestation model simulates the absorption of an oral dose of *p,p'*-DDE from the of *p,p'*-DDE from the dam to the nursing pup from mammary milk, followed by exchanges with pup fat, kidney, and other richly-perfused and poorly-perfused tissues in the pups.

All exchanges with blood plasma, in both models, are simulated as flow-limited processes, with the exception of the following. Exchanges between maternal fat and a *deep fat* compartment are assumed to be diffusion-limited and are represented with first order rate constants. Exchanges between the embryo/fetus and placenta are modeled as diffusion-limited processes and are represented with diffusion coefficients (L/day). Parameters used in the model were either taken from the literature, estimated by using the SIMUSOLV simulation program, or set by visually inspecting the fit of the data.

Elimination pathways in the maternal model include metabolism, transfer from mammary tissue to maternal milk (in the lactation model), and fecal excretion from the gastrointestinal tract, including transfer from bile to the gastrointestinal tract. A fecal pathway from liver (presumably through bile and the gastrointestinal tract) is included in the pup model.

Table 3-5. Tissue:Blood Partition Coefficients and Pharmacokinetic Constants for Modeling DDE Disposition in the Pregnant Rat[a]

Tissue:blood partition coefficients	
Liver	7
Fat	450
Poorly-perfused tissues	12
Well-perfused tissues	6
Kidney	6
Uterus	6
Placenta	2
Mammary gland	12
Pharmacokinetic constants	
K_{AS} (L/day) Portal absorption rate constant	24
K_{LY} (L/day) Lymphatic absorption rate constant	74
K_{FX} (L/day) Fecal excretion rate constant	230
K_B (L/day) Biliary excretion rate constant	1.2
PA_F (L/day) Fat diffusion coefficient	5
PA1 (L/day) Placenta-to-embryo/fetus diffusion coefficient	1.6
PA2 (L/day) Embryo/fetus-to-placenta diffusion coefficient	1.9
K_{12}/K_{21} Diffusion to deep fat	1.0/0.1
T_{del} (day) Delay in time	0.1

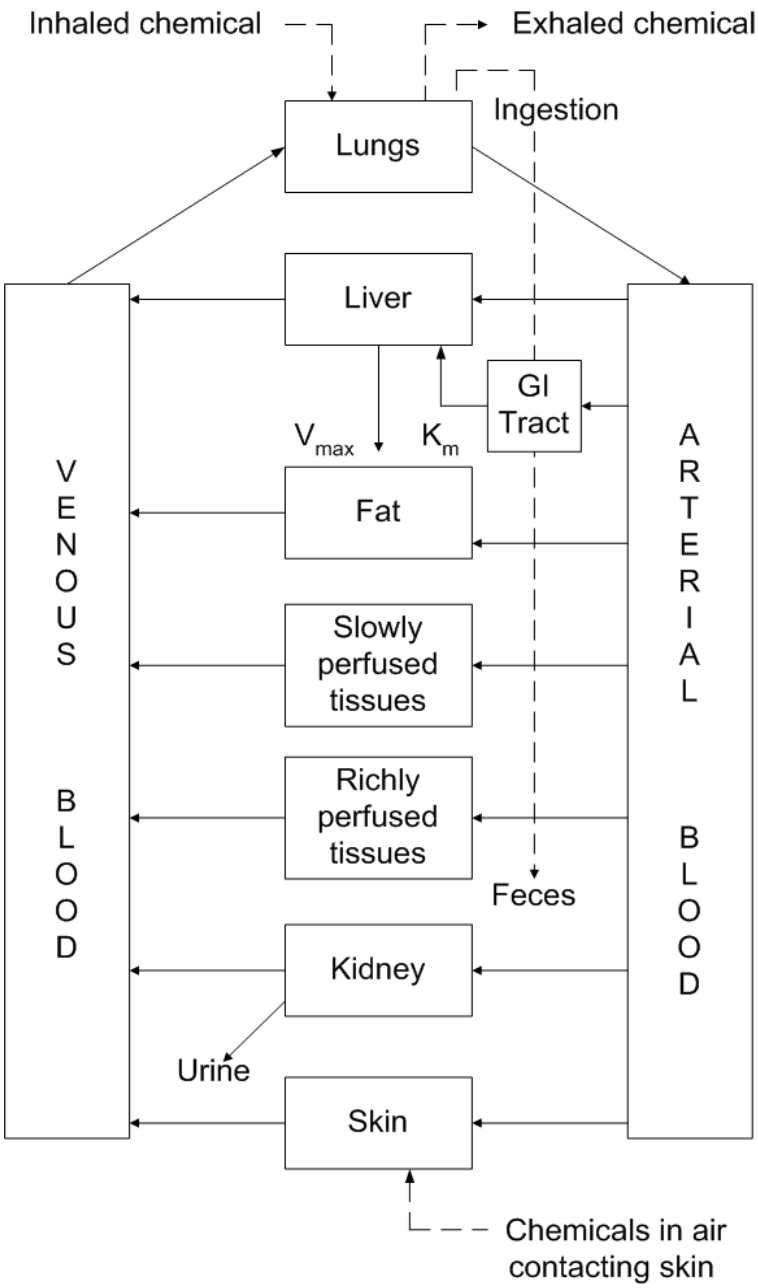
[a]Adapted from You et al. 1999b

Table 3-6. Physiological Constants Used in the PBPK Model for the Lactating Dam and the Nursing Pup[a]

	Dam	Pup
Body weight (kg) (BW)	0.290–0.340	0.0061–0.58
Tissue volumes (% of body weight)		
Liver, V_L	4	4
Well-perfused tissues, V_{WP}	8	8
Poorly-perfused tissues, V_{PP}	76-V_{MT}	76
Fat, V_F	7	0.0199*pBW+1.664
Mammary tissue, V_{MT}	4.4–9.6	
Milk, V_{milk}	0.002L	
Cardiac output (L/h)	$14*pBW^{0.75}$	$18*pBW^{0.74}$
Blood flows (% of cardiac output)		
Liver, Q_L	25	25
Well-perfused tissues, Q_{WP}	41-Q_{MT}	49
Poorly perfused tissues, Q_{PP}	25	25
Fat, Q_F	7	1
Mammary tissue, Q_{MT}	9–15	

[a]Adapted from You et al. 1999b

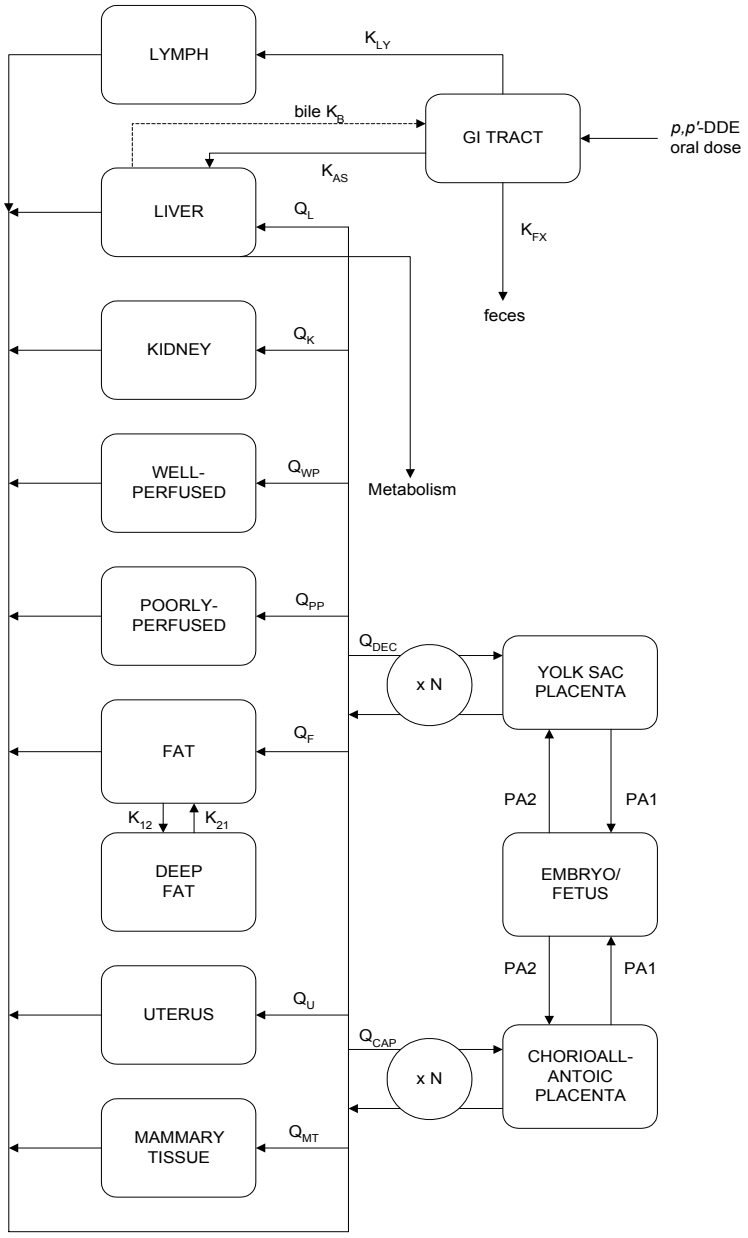
Figure 3-5. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



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.

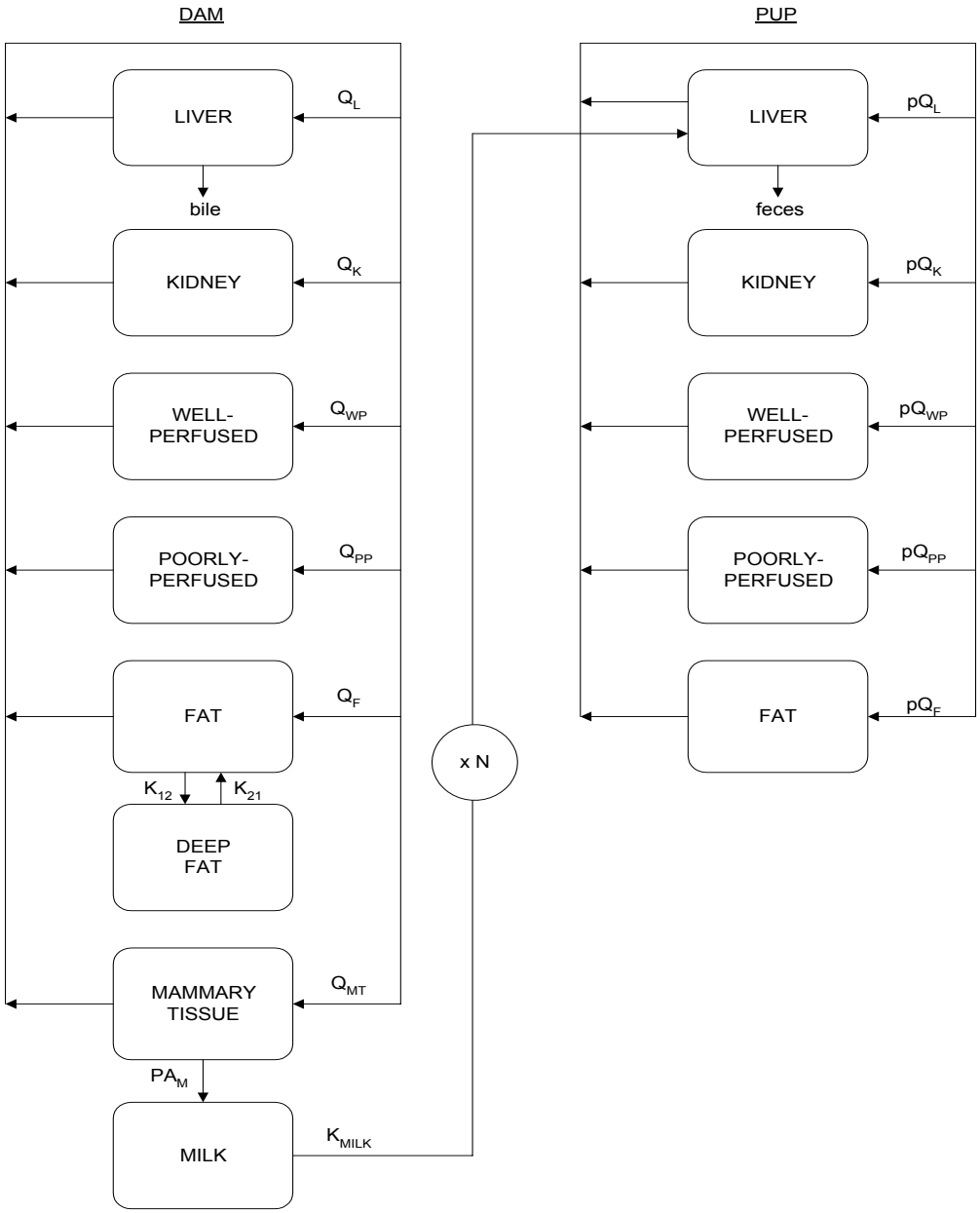
Figure 3-6. Diagrammatic Representation of the PBPK Model for Gestation*.



N = number of concepti
Terms are Defined in Table 3-6 and Table 3-7.

*Adapted from You et al. 1999b

Figure 3-7. Diagrammatic Representation of the PBPK Model for the Lactating Dam and Nursing Pup*.



N = number of pups

Portal and lymphatic absorption routes for dams are not shown (see Figure 3-6).

Terms are Defined in Table 3-5 and Table 3-6.

*Adapted from You et al. 1999b

Validation of the model. The models have been calibrated with data from experimental studies. Pregnant Sprague-Dawley rats were administered gavage doses of 0, 10, or 100 mg *p,p′*-DDE on Gd 14–18. A subset of the dams were killed 4 hours after each dosing, and tissue levels of *p,p′*-DDE were measured in the dams, placenta, and fetuses. A subset of pups in each dose group was cross-fostered to assess *p,p′*-DDE transfer to tissues from maternal milk. The gestational model was calibrated by adjusting variables to achieve agreement between estimated and observed concentrations of *p,p′*-DDE in maternal and pup plasma, liver, and fat, and in placenta and fetal tissue. The lactation/nursing model was calibrated with data on *p,p′*-DDE concentrations in pup blood, liver, and fat. Reasonable agreement was achieved between model output and observations.

Risk assessment. The models provide an approach to estimating tissue doses in fetuses and pups associated with maternal gavage doses of *p,p′*-DDE to pregnant Sprague-Dawley rats. This information can be used to interpret the results of developmental toxicity bioassays of *p,p′*-DDE in terms of maternal, fetal, and pup tissue dose-response relationships.

Target tissues. Output from the You models that are described in You et al. (1999b) are estimates of the *p,p′*-DDE concentrations in maternal and pup blood and plasma, liver, and fat, and in placenta and embryo/fetus. Estimates of *p,p′*-DDE concentrations in other tissues may also be feasible, although they are not described in model evaluations thus far reported (You et al. 1999b)

Species extrapolation. The models have been calibrated to predict *p,p′*-DDE pharmacokinetics in pregnant and lactating/nursing Sprague-Dawley rats. Extrapolation to other physiological states (e.g., immature rats, nonpregnant adults, senescent rats), other rat strains, or other species would require modification to the models to account for different tissue masses, blood flows, and possibly other kinetic variables.

Interroute extrapolation. The models are calibrated to simulate the pharmacokinetics of *p,p′*-DDE when it is administered by gavage doses in corn oil or in a similar lipid vehicle. The gestation model includes a lymphatic absorption pathway from the gastrointestinal tract that is intended to simulate the absorption kinetics of *p,p′*-DDE dissolved in a lipophilic vehicle. The kinetics would be expected to be different for aqueous vehicles. Therefore, the output of the models cannot be extrapolated to other types of vehicles or to other exposure pathways (e.g., dietary, drinking water) or routes (e.g., dermal, inhalation) without re-evaluation and possibly recalibration and modification of the models.

3.6 MECHANISMS OF ACTION

3.6.1 Pharmacokinetic Mechanisms

DDT, DDE, and DDD are efficiently absorbed, distributed, and stored because of their high lipophilicity. There are not thought to be specific transport or distribution mechanisms for DDT, DDE, or DDD other than their affinity for other hydrophobic fats. These chemicals are absorbed in the stomach and, to a larger extent, in the intestine. Sieber (1976) studied lymphatic absorption of DDT and related compounds in rats and found these compounds to be preferentially absorbed via the intestinal lymphatic system with some absorption into blood. Pocock and Vost (1974) and Sieber et al. (1974) reported that most DDT absorbed into lymph is carried in the lipid core of chylomicrons. Once absorbed into the lymphatic system, DDT is carried throughout the body and incorporated into fatty tissues. In addition to facilitating the absorption of these compounds from the gastrointestinal tract, the lipophilicity of DDT, DDE, and DDD enables them to cross the blood-brain barrier readily without a specific transporter. Uptake into tissues is a function of the blood flow, lipid content of the specific tissue, and the partition coefficient for DDT between the blood and lipids in specific tissues.

Studies in humans suggest that DDT and related compounds are primarily transported in the blood bound to protein. In occupationally exposed workers, Morgan et al. (1972) found that less than 18% of *p,p'*-DDT and *p,p'*-DDE in human blood is carried in erythrocytes. Less than 1% of all DDT-related compounds is carried by the chylomicrons in plasma of normal fat content. Instead, DDT-related compounds are carried by proteins and are undetectable in plasma from which protein has been precipitated. Following ultracentrifugation, *p,p'*-DDT and *p,p'*-DDE are found in relation to lipoproteins of various densities, but mainly in the triglyceride-rich, low density (LDL), and very low density lipoproteins (VLDL). Results from electrophoresis experiments showed that plasma albumin, and secondarily the smaller globulins are the principal plasma constituents associated with blood-borne *p,p*'-DDT and *p,p*'-DDE. In agreement with the findings of Morgan et al. (1972), Norén et al. (1999) found that in blood from humans, *p,p*'-DDE and its methylsulfonyl metabolite, 3-$MeSO_2$-DDE, were associated to a great extent (almost 80%) with a fraction containing primarily albumin. In this case, the subjects (three men and two women) had no known occupational exposure to organochlorine compounds. Of the remaining DDE, 12% was recovered associated with LDL, 9% with VLDL, and 6% with high density lipoprotein (HDL). The corresponding figures for 3-$MeSO_2$-DDE were 8, 4, and 11%. Results from studies *in vitro* are consistent with findings *in vivo*. Gómez-Catalán et al. (1991) showed that 82.3% of *p,p'*-DDE in human blood was associated with plasma and only 17.7% with cells. In plasma,

approximately 60% was associated with protein and 9–15% with the various lipid fractions. Similar results were reported by Mohammed et al. (1990) in an *in vitro* study with radiolabeled DDT.

Extensive information regarding the metabolism of DDT and DDT-related compounds was presented in Section 3.5.3. DDT and DDT-related compounds are metabolized primarily in the liver and kidney, but important activation reactions also occur in other tissues such as the lung and adrenal glands. The metabolism of DDT can be considered both a detoxification reaction as well as an activation reaction. For effects associated with the parent compound, such as neurotoxicity, metabolism means detoxification. For other toxicities, such as antiandrogenicity and adrenal effects, biotransformation of DDT may be an activation process. In general, the metabolism of DDT in animals is similar to that in humans; however, there are also differences between species, and within species, there are differences between tissues. DDT, DDE, and DDD induce CYP2B-associated activities in rat liver, which makes them phenobarbital-type cytochrome P-450 inducers. Studies in humans have shown that the major route of excretion of absorbed DDT is the urine and that DDA is the main urinary metabolite.

3.6.2 Mechanisms of Toxicity

Mechanisms for the major effects of DDT are discussed in this section. In animals, these adverse effects include neurotoxicity, hepatotoxicity, metabolic effects, reproductive effects, and cancer.

Neurological Effects. DDT acts on the central nervous system by interfering with the movement of ions through neuronal membranes. There appear to be at least four mechanisms by which DDT affects ion movement, all possibly functioning simultaneously. DDT both delays the closing of the sodium ion channel and prevents the full opening of the potassium gates (Ecobichon 1995; Narahashi and Haas 1967). DDT has been shown to target a specific neuronal adenosine triphosphatase (ATPase) thought to be involved in the control of the rate of sodium, potassium, and calcium fluxes through the nerve membrane (Matsumura and Patil 1969). This ATPase plays a vital role in neuronal repolarization. In addition, it has been suggested that DDT inhibits the ability to transport calcium ions in nerves possibly by binding with a hydrophobic site on calmodulin and secondarily effecting a Ca/Mg ATPase; however, this mechanism has not been confirmed (Matsumura 1985). Calcium ions are essential to the release of neurotransmitters. These actions combine to effectively maintain the depolarization of the nerve membrane, potentiating the release of transmitters and leading to central nervous system excitation manifested as hyperexcitability, tremors, and convulsions, along with secondary effects from convulsions, such as tachycardia, metabolic acidosis, and hyperthermia.

The action of DDT as a central nervous system stimulant may also be related to a deficiency of brain serotonin (Hwang and Van Woert 1978). Much research has focused on changes in levels of biogenic amines and amino acids resulting from DDT exposure. It is possible that some of these changes could be a result of the effects of DDT on membrane ion transport and consequent neurotransmitter release. Several researchers have reported increased levels of 5-HIAA, 3-methoxy-4-hydroxyphenylglycol (MHPG), aspartate, and glutamate in the brains of rats exposed to DDT (Hong et al. 1986; Hudson et al. 1985; Pratt et al. 1985). 5-HIAA and MHPG are metabolites of the biogenic amines serotonin and norepinephrine, respectively, which act as inhibitors in the central nervous system. The increases in levels of 5-HIAA and MHPG indicate increased breakdown of these inhibitory neurotransmitters. Both aspartate and glutamate function as excitatory amino acids. The overall effect of increased levels of aspartate and glutamate is the induction of a state of increased excitability in the neurons of the central nervous system. This leads to hyperexcitability in the exposed animal and contributes to tremors and convulsions, which have been observed following DDT exposure. Serotonin, in addition to being active in the inhibition of neurons, plays an important role in the regulation of body temperature. It has been suggested that the increased turnover of serotonin in DDT-exposed organisms may be responsible for DDT-induced hyperthermia (Hudson et al. 1985). Other data suggest that DDT-induced hyperthermia may be due to heat generated by muscular movement during tremors or convulsions (Herr et al. 1986).

Reproductive and Developmental Effects. DDT intake, particularly during sexual differentiation, can adversely affect the reproductive system of male animals. Such effects have been attributed to DDT and related compounds acting in any of the following manners or in any combination of them: (1) mimicking endogenous hormones, (2) antagonizing endogenous androgenic hormones, (3) altering the pattern of synthesis or metabolism of hormones, and (4) modifying hormone receptor levels. DDT is primarily suspected of influencing reproduction and development through its interaction with steroid hormones receptors for estrogens and androgens.

Androgens and estrogens are very lipid soluble and thus, diffuse easily through the cell membrane into the cytosol and nucleus. Unoccupied steroid receptors bound to a complex of molecular chaperones are in dynamic equilibrium between the cytosol and the 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. This RNA is spliced to form mRNA, which directs the synthesis of proteins that cause the characteristic responses to the steroid hormone. 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). 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). None of the studies examining the difference in estrogen receptor binding affinity for DDT isomers and 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.

Results from numerous studies using a wide range of experimental approaches suggest that binding to the estrogen receptor and subsequent events are the predominant mechanism by which estrogenic effects are expressed. Tests for estrogenicity fall into two general categories, *in vivo* and *in vitro* (Zacharewski 1998). Examples of the former include the uterotrophic (increase in uterine wet weight) and vaginal cell cornification assays. *In vitro* assays include (i) measuring the activities of enzymes involved in steroid synthesis, (ii) competitive ligand binding assays using binding globulins and receptors, (iii) cell proliferation assays, and (iv) gene expression assays in mammalian cells and yeast. If possible, both *in vivo* and *in vitro* tests should be conducted since, for instance, *in vitro* assays can give false positives if the chemical is not absorbed or distributed to the target tissue or is rapidly metabolized. Conversely, false negatives may arise from the lack of an activation system in the *in vitro* system; other factors may play a role too. Also, both *in vivo* and *in vitro* tests are available for assessing androgenic effects such as measurement of anogenital distance in new born male rodents and androgen receptor binding assays in prostate cytosol, respectively. Some representative studies that examined mechanistic aspects of estrogenicity and antiandrogenicity of DDT and related compounds are briefly summarized below. Bulger and Kupfer (1983) reviewed the literature and references to most of the earlier studies on estrogenic effects can be found therein.

p,p'-DDT (200 mg/kg twice) and *o,p'*-DDD (200 mg/kg up to 5 times) administered intraperitoneally to rats had almost no estrogenic activity for initiating implantation, in contrast with responses observed after administration of *o,p'*-DDE (200 mg/kg once) and *o,p'*-DDT (200 mg/kg once) (Johnson et al. 1992). The latter two chemicals not only initiated implantation but also maintained pregnancy when administered repeatedly. *p,p'*-DDT was as efficient as *o,p'*-DDT in a uterine response assay in rats *in vitro*, but exhibited low estrogenic activity *in vivo* (Galand et al. 1987). Welch et al. (1969) reported an estrogenic activity ranking of *o,p'*-DDT > technical DDT > *p,p'*-DDT in immature female rats treated intraperitoneally. Singhal et al. (1970) showed that *o,p*'-DDT (100 mg/kg) was much more effective in increasing uterine weight in young rats than *p,p*'-DDT after a single intramuscular injection. Shelby et al. (1996) examined the estrogenicity of *o,p'*-DDT in three different assays; (1) competitive binding with the mouse uterine receptor, (2) transcriptional activation in HeLa cells transfected with plasmids containing an estrogen receptor and an estrogen response element linked to a reporter gene, and (3) the uterotropic assay in mouse. The first two assays are *in vitro* assays, whereas the third one was carried out by injecting immature mice subcutaneously with *o,p'*-DDT on three consecutive days and determining uterine weight on the fourth day. *o,p'*-DDT gave positive estrogenic responses in the three assays, but with a potency that was several orders of magnitude weaker than 17β-estradiol and diethylstilbestrol (DES). *o,p'*-DDT, *o,p'*-DDD, and *p,p'*-DDT were full estrogenic agonists in the *in vitro* E-screen test, *p,p'*-DDE and *p,p'*-DDD were partial agonists and technical DDT was a full agonist (Soto et al. 1997). The E-screen test uses breast cancer estrogen-sensitive MCF-7 cells, and the rationale of the assay is that a human serum-borne molecule specifically inhibits the proliferation of human estrogen-sensitive cells, and estrogens induce proliferation by neutralizing this inhibitory effect. 10^7 times more *o,p'*-DDT, *o,p'*-DDD, *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, and technical DDT was needed to produce maximal cell yields than 17β-estradiol. *p,p'*-DDE also showed partial antagonistic effects on MCF-7 cell proliferation (Soto et al. 1998).

Gaido et al. (1997) used a yeast system to express the estrogen receptor, a reporter gene regulated by two estrogen receptor response elements, and a yeast gene that is supposed to enhance steroid receptor mediated gene transcription. *o,p'*-DDT and *o,p'*-DDD appeared to stimulate estrogen receptor responsive genes at very high doses, but a complete curve of expression versus dose was not done for these two chemicals. The authors extrapolated EC_{50} (effective concentration for 50% maximal response) values for *o,p'*-DDT and *o,p'*-DDD assuming that these chemicals would have been as effective as estradiol in inducing reporter gene expression if a complete dose response experiment had been done. The calculated EC_{50} values were 1.8 mM for *o,p'*-DDT ($8x10^6$ less potent than estradiol) and 3.32 mM for *o,p'*-DDD ($15x10^7$ less potent than estradiol) compared to an actual estradiol EC_{50} of 2.25 nM. *o,p'*-DDE produced

little apparent response. Sohoni and Sumpter (1998) investigated the ability of *o,p'*-DDT and *p,p'*-DDE to induce estrogen receptor regulated gene expression in yeast expressing the human estrogen receptor and a secreted reporter gene controlled by an estrogen response element. A complete dose-response curve was generated for *o,p'*-DDT and it was found to be about 10^5 less potent than 17β-estradiol in inducing estrogen regulated gene transcription. There was little response to *p,p'*-DDE even at concentrations as high as 0.5 mM. In a similar study using recombinant receptor-reporter gene assays in stably transfected MCF-7 and HeLa cells, both *o,p'*-DDE and *p,p'*-DDE showed weak estrogenic activity (Balaguer et al. 1999). *o,p'*-DDE was slightly more potent than *p,p'*-DDE , but it was still approximately 10^6 times less potent than 17β-estradiol on a molar basis. In an additional study using HeLa cells cotransfected with an expression vector containing ER and an RE-responsive chloramphenicol acetyltransferase reporter, *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD showed no appreciable transcriptional induction (Tully et al. 2000). The highest concentration tested was 10 μM; for comparison, 0.01 nM 17β-estradiol produced approximately a 3-fold induction over the vehicle. Moreover, when the chemicals were tested in binary combinations, the mixtures showed no additional estrogenicity (Tully et al. 2000).

Several investigators have demonstrated that *o,p'*-DDT can compete with estradiol for binding to the estrogen receptor. An IC_{50} is the concentration at which 50% of the maximal inhibition of binding to the labeled standard (in this case, estradiol) is achieved. Danzo (1997) showed that *o,p'*-DDE (IC_{50}=40 μM) competed with 17β-estradiol (IC_{50}=2.7 nM) for binding to the estrogen receptor in rabbit uterine extracts and *p,p'*-DDT and *p,p'*-DDE were much less effective competitors. Kelce et al. (1995) showed in uterine extracts from immature rats that *o,p'*-DDE (IC_{50}=5 μM) competed with 17β-estradiol (IC_{50}=0.8 nM) for estrogen receptor binding and that *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD were relatively ineffective competitors (IC_{50}>1,000 μM). The observations of the lack estrogenicity of the *p,p'*-isomers were consistent with results from earlier *in vivo* studies (Bitman and Cecil 1970; Gellert et al. 1972; Nelson 1974).

Kelce et al. (1995) characterized the binding of *p,p'*-DDT, *p,p'*-DDE, *o,p'*- DDT, and *p,p'*-DDD to the androgen and estrogen receptors *in vitro* in uterine cytosolic extracts from immature rats and in rat ventral prostate cytosol, respectively. In competitive androgen receptor binding assays using a radiolabeled synthetic androgen (R1881), the four chemicals showed dose-dependent competitive inhibition. *p,p'*-DDE was the greatest competitor with an inhibition constant (K_i) of 3.5 μM, which was similar to that of DES and about 30 times weaker than 17β-estradiol. The other three isomers were 12–20-fold less effective than *p,p'*-DDE. Based on IC_{50} values for displacement of the synthetic androgen from the androgen receptor or 17β-estradiol from the estrogen receptor, *o,p'*-DDT was 20-fold more effective as a

competitor of estrogen binding to the estrogen receptor than androgen binding to the androgen receptor. *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE bound the androgen receptor 14, 11, and 200 times more effectively than the estrogen receptor, respectively. A control experiment was done to demonstrate that the antiandrogenic effects of the DDT isomers were not due to their ability to inhibit the conversion of testosterone by 5α-reductase to 5α-dihydrotestosterone and 5α-androstan-3α-17β-diol. In microsomes isolated from the adult rat caput and corpus epididymis, the four DDT isomers were all 20-fold less effective in inhibiting this reaction than DES or 17β-estradiol. Danzo (1997) also confirmed that *p,p'*-DDE (IC_{50}=6.8 μM) could compete with dihydrotestosterone (IC_{50}=1.1 nM) for binding to androgen receptors in rat prostate cytosol. In contrast to Kelce, Danzo found that *o,p'*-DDT and *p,p'*-DDT were only slightly less effective than *p,p'*-DDE in competing with dihydrotestosterone for androgen receptor binding.

The binding experiments described above cannot distinguish between an agonist and an antagonist as both might bind equally well to the androgen receptor. *p,p'*-DDE's action as an androgen receptor antagonist has been demonstrated by its ability to inhibit the androgen receptor from either appropriately inducing or repressing transcription from androgen responsive genes containing androgen receptor binding sites in their regulatory sequences. Kelce et al. (1995) transiently transvected monkey kidney cells with an androgen receptor expression vector and a reporter gene containing the MMTV promoter, which contains binding sites for the androgen receptor. In a 5-hour expression assay, both 0.2 μM *p,p'*-DDE and 1 μM hydroxyflutamide were able to inhibit 5α-dihydrotestosterone (0.1 nM) induced transcription by about 50%. In contrast, in lysates of rat ventral prostate, the androgen antagonist hydroxyflutamide was 10 times more effective than *p,p'*-DDE in inhibiting binding to the androgen receptor. In the transient tranvection assay, levels of 5α-dihydrotestosterone were not measured in the wells, so there was no control for whether the DDE could have affected its metabolism. *p,p'*-DDT and *o,p'*-DDT also showed some ability to inhibit the transcription of androgen responsive genes in this assay. Kelce et al. (1997) performed an *in vivo* experiment on castrated adult male rats, which were maintained on a constant dose of testosterone from implanted Silastic capsules; this experiment was designed to eliminate possible variations in testosterone levels from feedback at the hypothalamus or pituitary. After 5 days gavage with 200 mg/kg/day of *p,p'*-DDE, northern blots of mRNA isolated from the prostate showed significant increases in steady state levels for mRNA for testosterone repressed prostatic message and decreases in testosterone induced C3 (third subunit of prostatic specific binding protein). The *p,p'*-DDE did not affect testosterone metabolism in these animals. Kelce et al. (1995) suggested that their findings raised the possibility that the androgen receptor, rather than the estrogen receptor, is the site of hormonal blockade by persistent environmental pollutants such as *p,p'*-DDE.

Other investigators have confirmed Kelce's results showing the antiandrogenic effects of *p,p'*-DDE on androgen receptor regulated genes. Maness et al. (1998) investigated how isomers of DDT, DDE, and DDD inhibited androgen receptor regulated gene expression *in vitro* in HEPG2 human hepatoma cells transiently transvected with the human androgen receptor and a reporter gene linked to an androgen responsive promoter. These cell were exposed for 24 hours to a maximally inducing dose (0.1 μM) of dihydrotestosterone and various doses of either *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT, *o,p'*-DDE, or *o,p'*-DDD. Details of the data were not presented, but from the graphical presentation of the data, both isomers of DDT, DDE, and DDD appear to be equipotent (IC_{50} . 1–10 μM) in inhibiting dihydrotestosterone induced gene transcription. The authors state that *p,p'*-DDE was the most potent inhibitor (IC_{50}=1.86 μM), but no evidence of statistical significance is discussed. No sampling of the cells or media was done to determine whether DDT isomers might have been affecting the metabolism of dihydrotestosterone in these experiments on liver cells.

Sohoni and Sumpter (1998) investigated antiandrogenic effects in yeast expressing the human androgen receptor plus a secreted reporter gene controlled by androgen response elements. Both *o,p'*-DDT and *p,p'*-DDE were approximately equipotent in inhibiting dihydrotestosterone (DHT) responsive gene expression. Both were about as effective as the antiandrogen flutamide. From graphs, they appeared to have IC_{50} values on the order of 10 μM from 4-day incubations with a submaximally inducing dose of DHT (1.25 nM) and DDT or DDE; however, the authors felt that explicit calculations of IC_{50} values were inappropriate, because if incubation times were extended to 5 or 6 days, DHT seemed to partially overcome the inhibition by *p,p'*-DDE (*o,p'*-DDT was not tested in these tests). Again, no sampling of yeast media was done to determine if the DDT or DDE was affecting metabolism of DHT.

Collectively, the results of these studies suggest that DDT-related compounds might have estrogenic or antiandrogenic activities if sufficient doses are used. The androgen antagonist mechanism demonstrated in these studies would explain a number of reproductive and developmental effects seen in male rats of various ages exposed to *p,p*'-DDE. These include reduced anogenital distance and retention of thoracic nipples in pups exposed during gestation and lactation (Kelce et al. 1995; Loeffler and Peterson 1999; You et al. 1998;); delayed puberty in rats exposed either during juvenile development (Kelce et al. 1995) or gestation and lactation (Loeffler and Peterson 1999), and reduced accessory sex organ weights in exposed adult males (Kelce et al. 1995, 1997). The experimental details of these studies are discussed extensively in Section 3.2.2.5 Reproductive Effects and Section 3.2.2.6 Developmental Effects.

Alterations in development and reproduction can also be induced by altering the metabolism of sex hormones. For example, in perinatally exposed rats, *p,p'*-DDE was shown to increase liver CYP enzymes systems that hydroxylate testosterone (You et al. 1999c). *p,p'*-DDE was also shown to increase liver aromatase activity in adult male rats (You et al. 2001); aromatase plays a critical role in steroidogenesis by catalyzing the conversion of C19 steroids to estrogens.

DDT and metabolites apparently also exhibit additional estrogen or androgen receptor-independent effects as shown for example in a study by Juberg and Loch-Caruso (1992) in which the estrogenic *o,p'*-DDT increased contractions of rat uterus strips *ex vivo*, but 17β-estradiol did not. The estrogen antagonist, tamoxifen, failed to block the stimulatory effect of *o,p'*-DDT. Furthermore, the nonestrogenic DDT analogue, *p,p'*-DDD, significantly stimulated uterine contraction. The authors also demonstrated that the increase in contractility was not due to release of prostaglandin E2 (PGE_2) from the uterine strips since PGE_2 levels in the muscle bath showed no significant differences between control and DDT-treated strips. A more recent study from the same group directly showed that *p,p'*-DDD causes a dramatic increase in intracellular free calcium in rat myometrial smooth muscle cells (Juberg et al. 1995). This increase resulted primarily from an increase in calcium influx from the extracellular medium and release from intracellular calcium stores. The elevation in intracellular calcium is consistent with an increase in uterine contractility.

Mechanistic Studies of DDT in Fish. There have been a number of intriguing mechanistic studies of DDT isomers and metabolites in fish that relate to reproductive and developmental effects. Two subtypes of nuclear androgen receptors have been identified in both kelp bass and Atlantic croaker; one subtype is expressed in the brain and the other in ovarian tissue (Sperry and Thomas 1999). The brain receptor subtype seems more similar to mammalian androgen receptors in binding characteristics; the ovarian receptor is unique to teleost fish. Both *p,p'* and *o,p'* isomers of DDT, DDD, and DDE can displace dihydrotestosterone from the ovarian androgen receptor subtype. Analogous to ERα and ERβ in humans, there are also different estrogen receptor subtypes in Atlantic croaker fish; two slightly different estrogen receptors have been identified in the testis and liver of these fish (Loomis and Thomas 1999). *o,p'*-DDT, as well as *o,p'*-DDE and *o,p'*-DDD, can bind to both of these receptors with low affinity (50,000–500,000 times lower) relative to DES; binding of these isomers was somewhat better to the testicular receptor than to the hepatic receptor. There has been one report in Atlantic croaker that *o,p'*-DDD can competitively inhibit binding of maturation-inducing steroid to its plasma membrane receptor (Das and Thomas 1999; Thomas 1999). Similar to estrogen, *o,p'*-DDT can stimulate

gonadotropin release in Atlantic croaker (Khan and Thomas 1998). Another report reveals that *o,p'*-DDT impairs survival related behavior in larval offspring of exposed Atlantic croaker (Faulk et al. 1999).

Metabolic Effects. DDT and DDT-analogues were found to be phenobarbital-type (CYP2B and to a lesser extent CYP3B) inducers in rat liver (Nims et al. 1998). Since some of these isozymes metabolize endogenous steroids and other hormones, it is possible DDT could increase the turnover or change serum levels of endogenous macromolecules metabolized by these P-450 isozymes. This would be consistent with the increases in microsomal catabolism of cortisol observed in DDT-exposed humans. These subjects excreted significantly increased amounts of the cortisol metabolite 17-hydroxycortisone in their urine (Nhachi and Loewenson 1989; Poland et al. 1970). Thus, hormonal homeostasis could be indirectly affected and effects would be seen on several systems including the reproductive system. Evidence that the latter may happen was provided by You et al. (1999c) who examined the potential of *in utero* DDE exposure to affect the developmental expression of hepatic CYPs enzymes responsible for testosterone hydroxylations. They treated pregnant Sprague-Dawley rats on gestation days 14–18 and also adult male rats with *p,p'*-DDE and found that the responses of CYP2C11 and CYP2A1 were development-regulated. DDE induced 2A1 in males on postnatal day (PND) 10 but not PND 21; pronounced induction of 2B1 was seen in males and females on both PND 10 and 21 and to a lesser extent of 3A1; there was no induction of 2C11. In adult rats, DDE induced 2B1, 3A1, and 2C11, but not 2A1. Similar results, also in adult Sprague-Dawley rats, but with technical DDT were published by Sierra-Santoyo et al. (2000). Another enzyme involved in steroid metabolism that was affected by *p,p'*-DDE was aromatase (catalyzes the conversion of C19 steroids to estrogens), whose activity was significantly increased in hepatic microsomes from treated adult male rats, as were the levels of aromatase protein in the liver (You et al. 2001).

Hepatic Effects. Hepatic effects in animals associated with DDT, DDE, and DDD exposure include increased liver weights, hypertrophy, hyperplasia, microsomal enzyme and cytochrome P-450 induction (CYP2B, phenobarbital-type induction), cell necrosis, and increased levels of SGPT (ALT) and SGOT (AST) enzymes released from damaged liver cells. DDT and its metabolites have been reported to disrupt the ultrastructure of mitochondrial membranes (Byczkowski 1977). This disruption may result in cell damage and some cell death. Consequently, the liver could begin cell regeneration to compensate for this loss of cells. This regenerative process often leads to hyperplasia and hypertrophy, which combine to increase the weight of the liver, and may contribute to the promotion of liver tumors (Fitzhugh and Nelson 1947; Schulte-Hermann 1974).

Adrenal Gland Effects. The adrenal gland consists of the adrenal medulla, which secretes epinephrine and norepinephrine, and the adrenal cortex, which is composed of three zones. These zones are: (1) the *zona glomerulosa*, producing mineralocorticoids involved in controlling electrolyte homeostasis; (2) the *zona fasciculate*, the primary source of glucocorticoids involved in controlling carbohydrate metabolism and stress responses, and downregulating some immune responses; and (3) the *zona reticularis*, the primary adrenal source of very small quantities of sex steroids (Ross and Reith 1985). *o,p'*-DDD has been used to treat adrenocortical carcinoma and Cushing's disease in humans for almost 4 decades (Bergenstal et al. 1960; Wooten and King 1993). The therapeutic action is based on the induction of selective necrosis of the *zona fasciculata* and *zona reticularis* of the adrenal cortex and inhibition of cortisol synthesis. The latter is accomplished by inhibiting the intramitochondrial conversion of pregnenolone and the conversion of 11-deoxycortisol to cortisol (Hart and Straw 1971; Hart et al. 1971). Damage to the adrenal gland was first observed in dogs given DDD orally in earlier studies by Nelson and Woodard (1949). Many additional studies in dogs have confirmed the original observations after acute and long-term administration of *o,p'*-DDD to the animals (Kirk and Jensen 1975; Kirk et al. 1974; Powers et al. 1974). Studies *in vitro* have shown a high correlation between adrenocorticolytic activity and metabolic activation by adrenocortical mitochondria. Dog adrenal mitochondria had significantly greater metabolism of *o,p'*-DDD and covalent binding activities than did adrenal mitochondria from rabbits, rats, or guinea pigs (Martz and Straw 1980). Levels of metabolism and covalent binding measured in human adrenal mitochondria were intermediate between levels measured in dogs and rabbits and those measured in rats and guinea pigs (Martz and Straw 1980). A more recent study with dog adrenal cortical homogenates showed that the majority of the ^{14}C-*o,p'*-DDD-derived radioactivity was covalently bound to proteins, and that no radioactivity was associated with DNA (Cai et al. 1995). The rank order of species regarding metabolism and protein binding was cow > dog > rat adrenal homogenates > human normal adrenal or tumor homogenates. According to Cai et al. (1995), their results are consistent with an acyl chloride being the reactive intermediate. This reactive intermediate is thought to be formed by hydroxylation of *o,p'*-DDD at the β-carbon and quick transformation by dehydrochlorination into the acyl chloride. The latter either binds covalently to nucleophiles, or by losing water, is transformed to DDA for renal excretion (Schteingart 2000).

As previously mentioned in Section 3.5.3 Metabolism, methylsulfonyl metabolites of DDT (3-$MeSO_2$-DDE and 2-$MeSO_2$-DDE; all studies have been conducted with the *p,p'*-isomers, and therefore, the chlorine position is not specified in the abbreviation) have been identified in humans and animals (Haraguchi et al. 1989; Norén et al. 1996; Weistrand and Norén 1997). 3-$MeSO_2$-DDE is a potent toxicant in the adrenal cortex, particularly of mice, and possibly in humans after local activation by

cytochrome P-450. For example, whole body autoradiography of ^{14}C-labeled 3-$MeSO_2$-DDE in female mice injected with the test material intravenously showed heavy accumulation of radioactivity confined to the *zona fasciculata* of the adrenal cortex (Lund et al. 1988). Morphological evaluation of the adrenals showed extensive vacuolation and necrosis of the *zona fasciculata* 1–12 days after a single dose of 25 mg/kg; degenerative changes were seen at 12.5 mg/kg. Studies *in vitro* showed a dose- and time-dependent covalent irreversible binding to protein and formation of water soluble metabolites (Lund et al. 1988). This was blocked by the cytochrome P-450 inhibitor, metyrapone, and the addition of reduced glutathione decreased binding. A subsequent study in mice administered 3-$MeSO_2$-DDE intraperitoneally identified mitochondria in the *zona fasciculata* as the primary targets for 3-$MeSO_2$-DDE-induced toxicity (Jönsson et al. 1991). Disorganization and disappearance of central cristae were observed as soon as 6 hours after a dose of 3 mg/kg. Higher doses showed mitochondrial vacuolization, followed by disappearance of mitochondria or cellular necrosis. Jönsson et al. (1991) further suggested that of the two cytochrome P-450s expressed predominantly in the adrenal cortex, P-45011β and P-450SCC, the former (which converts 11-deoxycorticosterone to cortisone) is involved in the metabolic activation of 3-$MeSO_2$-DDE in mice. P-450SCC is also expressed in the ovary and testes, but no binding or toxicity was seen in these organs. Lund and Lund (1995) later provided direct evidence for an involvement of adrenocortical mitochondrial P-450s in the bioactivation of 3-$MeSO_2$-DDE. Intraperitoneal administration of radioactive 3-$MeSO_2$-DDE to pregnant or lactating mice resulted in specific accumulation and binding of 3-$MeSO_2$-DDE-derived radioactivity in the *zona fasciculata* of adrenals from 16- to 18-day-old fetuses or suckling pups, showing ready transplacental passage of the metabolite and transfer via maternal milk (Jönsson et al. 1992). The results also suggested that cytochrome P-45011β was involved in the activation of 3-$MeSO_2$-DDE in the fetal adrenal cortex. Quantitative measurements showed that 7 days after dosing, the amount of radioactive label of the pups adrenals was 2 and 3.6 times higher than maternal adrenals at the 1.5 and 25 mg/kg dose levels, respectively, suggesting that mother's milk may be an important route of exposure of 3-$MeSO_2$-DDE in DDT-exposed animals. A later study showed that P-45011β seems to be expressed during gestation days 10–12 in the adrenal cortex in the mouse fetus (Jönsson et al. 1995). 3-$MeSO_2$-DDE was also found to reduce the capacity of pups and maternal adrenals to secrete corticosterone (Jönsson 1994) by a mechanism possibly involving competitive inhibition of adrenocortical CYP11B1 (Johansson et al. 1998). Human adrenal glands *in vitro* were found also to bioactivate 3-$MeSO_2$-DDE to a metabolite that bound irreversibly to mitochondria (Jönsson and Lund 1994). Binding was inhibited by metyrapone indicating the involvement of cytochrome P-450, but the specific isozyme was not elucidated. The bioactivation and mitochondrial toxicity of 3-$MeSO_2$-DDE observed *in vivo* has recently been reproduced in an *ex vivo* tissue-slice culture preparation (Lindhe et al. 2001).

Comparative studies by Brandt et al. (1992) showed that adrenal cortical cytochrome P-450 from mink and otter metabolically activate and bind both *p,p'*-DDD and *o,p'*-DDD, whereas 3-$MeSO_2$-DDE is not activated by these species *in vitro*. Both isomers induced necrosis and focal bleeding in the *zona fascicularis/zona reticularis in vivo* in mink. Adrenals from chickens also activated and covalently bound 3-$MeSO_2$-DDE and *o,p'*-DDD *in vitro*, and both compounds were adrenotoxic *in vivo*.

Cancer. Although the evidence regarding the carcinogenicity of DDT in humans is inconclusive, DDT and related compounds have been shown to be carcinogenic in some laboratory animals. Several mechanisms have been proposed: direct mutagenicity through formation of covalent DNA adducts; promotion of pre-existing abnormal cells; or cytotoxicity leading to hyperplasia and promotional tumor development. These mechanisms may not be mutually exclusive. DDMU epoxide and DDA-Cl are two electrophiles that potentially can be produced during DDT metabolism. These could form covalent adducts with DNA, and thereby contribute to the cytotoxicity as well as the carcinogenicity of DDT. DDMU epoxide is a minor side-product by both of the predominant models (I and II) for DDT biotransformation. DDA-Cl is a major DDT metabolite in the Model II catabolism scheme (Gold and Brunk 1983). Electrophiles, as a class, are frequently mutagenic. Consistent with this hypothesis, DDMU-epoxide has been found to be mutagenic in the *S. typhimurium* Ames assay (Gold and Brunk 1983). DDA-Cl is not known to have been similarly assayed, so there is no direct biological evidence of its mutagenicity. There are apparently species differences in carcinogenicity of DDT. In the hamster and mouse, metabolism of DDT, DDD, and DDMU is similar (Gold and Brunk 1983). Therefore, it is unlikely that the observed differences in response between the mouse and hamster are due to relative differences in the production of DDMU-epoxide or DDA-Cl. There is a species difference in the conversion of DDT to DDE in that the hamster was less efficient than the mouse in the conversion of DDT to DDE (Gingell 1976; Gold and Brunk 1983).

A possible mechanism of carcinogenicity may be a phenobarbital-like promotion of pre-existing abnormal cells that develop into types of tumors commonly seen in aging rats (Williams and Weisburger 1991). DDT has demonstrated promoting activity in several initiation-promotion assays (see Section 3.10). Mechanisms by which DDT promotes tumors may include direct liver injury leading to cell death and cell regeneration that could subsequently result in excess cell proliferation, hyperplasia, and tumor development (Schulte-Herman 1974); inhibition of apoptosis, which is programmed cell death characterized by DNA fragmentation (Wright et al. 1994); and reduction of gap junctional intercellular communication (GJIC) (Ren et al. 1998). DDT reduced DNA fragmentation in seven different cell types with chemical- or UV-induced apoptosis (Wright et al. 1994), supporting the hypothesis that DDT

promotes tumors in part by inhibiting the death of initiated cells. Another possible mechanism of carcinogenicity may be hormonal promotion of initiated cells via estrogenic action. The estrogenic activity of DDT isomers is discussed above under the subheading Reproductive and Developmental Effects.

A tumor promoter may reduce GJIC by reducing gap junction channel permeability, gap junction number, or connexin expression (Ren et al. 1998), by increasing Ca^{+2} concentration in the cell (Adenuga et al. 1992), or by disrupting gap junction plaque stability by producing reactive oxygen radicals that affect plasma membrane fluidity (Jansen and Jongen 1996). The effects of a specific tumor promoter are frequently cell- or connexin-specific. DDT has induced GJIC inhibition *in vitro* in rat liver epithelial cells (Guan and Ruch 1996; Ruch et al. 1994), primary hepatocytes (Budunova et al. 1993), Syrian hamster embryo cells (Rivedal et al. 1994, 2000; Roseng et al. 1994), rat precision-cut liver slices (de Graaf et al. 2000), mouse skin cells (Jansen and Jongen 1996), bovine oviductal cells (Tiemann and Pöhland 1999), human breast cells (Kang et al. 1996), and human urothelial cells (Morimoto 1996), and *in vivo* in rat liver cells (Tateno et al. 1994). Mechanisms and determinant factors of DDT-induced GJIC inhibition have been investigated extensively.

Using several combinations of cell lines and connexin types, Ren et al. (1998) showed that DDT-induced GJIC inhibition was more consistent with a cell type-dependent mechanism of GJIC inhibition than a connexin-dependent mechanism.

In “normal” human breast epithelial cells that did not express the estrogen receptor, GJIC inhibition was strongly DDT dose-dependent after a 90-minute *in vitro* exposure to $25 μM (Kang et al. 1996). Levels of phosphorylated connexin43 were significantly reduced in cells with DDT-induced GJIC inhibition, suggesting that intercellular communication in human breast tissue may be modulated by dephosphorylation of the connexin proteins. The same levels of DDT did not alter steady-state levels of connexin43 mRNA, suggesting that DDT alters connexin43 proteins after translation, rather than during transcription (Kang et al. 1996). Human urothelial cell lines were susceptible to DDT-induced GJIC inhibition at DDT concentrations that were not cytotoxic for up to 7 days (Morimoto 1996). For 48-hour exposures, the degree of inhibition was not dose-dependent, but dose dependence was evident in 96-hour exposures.

Jansen and Jongen (1996) investigated whether tumor stage affected the degree of DDT-induced GJIC inhibition in mouse skin cells by conducting parallel fluorescent dye transfer assays in primary mouse

keratinocytes, DMBA-initiated mouse epidermal 3PC cells, and mouse skin carcinoma-derived CA3/7 cells. The investigators found that the order of GJIC percent inhibition in cell types was primary keratinocytes > initiated 3PC cells > carcinoma-derived cells, where percent inhibition of GJIC was measured by comparison to cell type-specific controls. DDT-induced GJIC inhibition in the 3PC initiated cell line was both dose- and time-dependent within a 90-minute exposure. GJIC inhibition by DDT was also dose-dependent in liver cells of rats orally administered DDT *in vivo* for 2 weeks (Tateno et al. 1994). The number and size of immunostained connexin32 spots were reduced in liver cells of rats administered $25 mg/kg/day, while the area of dye spread was significantly reduced only at 50 mg/kg/day. The time course of GJIC inhibition showed significantly reduced area of dye spread after 1, 2, and 4 weeks of exposure, with recovery to control levels at 6 weeks.

As mentioned in Section 3.2.2.8, numerous studies have examined the role of environmental estrogens, DDT and related compounds among them, as possible contributors to the increased incidence of breast cancer. Several mechanisms for DDT-induced increased breast cancer risk have been proposed including: (1) by binding to the ER, which induces a series of biochemical reactions ultimately resulting in protein synthesis and cell proliferation in estrogen-sensitive tissues including the breast; (2) by shifting the balance of endogenous 17β-estradiol metabolites in favor of the genotoxic 16α-hydroxyestrone, at the expense of the nongenotoxic 2-hydroxyestrone; and (3) by mimicking epidermal growth factor in human mammary epithelial cells, thus stimulating cell proliferation.

In addition to the studies that characterized the binding of DDT to the estrogen receptor already mentioned in the discussion of reproductive effects, many additional similar studies have specifically used breast cancer cells to demonstrate the involvement of the estrogen receptor in breast cancer cell proliferation (Dees et al. 1996, 1997a, 1997b; Shekhar et al. 1997; Zava et al. 1997). These and other studies clearly demonstrated that DDT analogues increase cell proliferation in human breast cancer cells by an ER-mediated mechanism that can be blocked by antiestrogens, and that their estrogenic potencies are several orders of magnitude lower than that of 17β-estradiol.

Another mechanism by which DDT compounds may increase cancer risk is by increasing the production of genotoxic metabolites from the breakdown of 17β-estradiol, at the expense of producing nongenotoxic metabolites. Two 17β-estradiol metabolites that have been studied extensively are 16α-hydroxyestrone (16α-OHE1) and 2-hydroxysterone (2-OHE1), which are formed via two mutually exclusive metabolic pathways. 16α-OHE1 binds covalently to the estrogen receptor in human breast cancer cells (Swaneck and Fishman 1988), and has induced genetic and proliferative changes in mouse mammary epithelial cells

(Telang et al. 1992). 2-OHE1, in contrast, inhibited both basal and estradiol-induced growth of MCF-7 human breast cancer cells, acting as an anticarcinogen (Schneider et al. 1984). Several studies addressed the hypothesis that the balance of the two metabolites, expressed as the ratio 16α-OHE1/2-OHE1, may be predictive of xenoestrogen-induced mammary cancer in humans; as the ratio of 16α-OHE1/2-OHE1 increases, so does the risk of breast cancer.

The ratio of estradiol metabolites 16α-OHE1/2-OHE1 was increased over controls in cultures of ER-positive MCF-7 human breast cancer cells by *o,p'*-DDT, *o,p'*-DDE, and *p,p'*-DDE (Bradlow et al. 1995). McDougal and Safe (1998) also found that the 16α-OHE1/2-OHE1 ratio in a MCF-7 human cancer cell assay was elevated when the cells were exposed to *o,p'*-DDE, and E_2 2-hydroxylation was reduced compared to controls in cells exposed to *o,p'*-DDE and *o,p'*-DDT; however, the ratio did not consistently predict known mammary carcinogens in the same assay. In ER-negative MCF-10 and MDA-MB-231 cell lines, on the other hand, neither *o,p'*-DDE nor *o,p'*-DDT induced significant changes in either C2- or 16α-hydroxylation of estradiol (Bradlow et al. 1997), suggesting an estrogenic mechanistic pathway. In the MCF-7 cell line, C2-hydroxylation was reduced to 67% of the control level by *o,p'*-DDT, but addition of the antiestrogen indole-3-carbinol blocked the effect, again suggesting that the effect is ER-mediated.

The 16α-OHE1/2-OHE1 ratio hypothesis is controversial, however. Epidemiology studies of the association of 16α-OHE1 with breast cancer have provided mixed results (Safe 1998). In addition, although 16α-OHE1 induced cell proliferation, a closely related compound, 16α-OHE2, did not (Schneider et al. 1984, as cited in Safe 1998). Also, while 2-OHE1 inhibited cell growth, the isomer 2-OHE2 enhanced cell proliferation in MCF-7 human breast cancer cells (Schneider et al. 1984, as cited in Safe 1998). Safe (1998) did not elaborate on the relative metabolic production of the different isomers.

A third mechanism by which DDT compounds may increase the risk of breast cancer is by producing proliferative changes in ER-negative breast cells via a nonestrogenic pathway by mimicking epithelial growth factor (EGF). *p,p'*-DDT stimulated two growth factor receptors associated with malignant breast lesions and increased STATS-mediated gene expression, ultimately increasing proliferation of untransformed ER-negative MCF-10A human breast epithelial cells (Shen and Novak 1997a). The study results indicated that *p,p'*-DDT may modulate breast cell proliferation by multiple mechanisms not regulated by the ER, while the *o,p'*-isomer was inactive in this test system.

When endogenous growth factors bind to growth factor receptors that are receptor tyrosine kinases, a chain of biochemical reactions is initiated that leads to transcription of genes involved in cellular proliferation and differentiation (Shen and Novak 1997b). *p,p'*-DDT mimicked EGF in human mammary epithelial cells by increasing EGF receptor tyrosine kinase activity at 0.01 and 0.1 μM concentrations, but appeared to impede the activity at 1 and 10 μM concentrations (Shen and Novak 1997b).

3.6.3 Animal-to-Human Extrapolations

In humans and in animals, acute high doses of DDT primarily affect the nervous system. However, it is uncertain whether effects on other systems and/or organs seen in animals exposed to lower doses for prolonged periods of time would also manifest in humans exposed under similar exposure conditions. In general, the metabolism of DDT in animals is similar to that in humans; however, there are also differences between species, and within species, differences between tissues. Comparisons of elimination rates of DDT from fat showed that the process is faster in rats followed by dogs and monkeys and slowest in humans (Morgan and Roan 1974). Rats eliminate DDT 10 to 100 times faster than humans. Morgan and Roan (1974) suggested that the differences in elimination rats could be due to differences in liver metabolism, gut bacterial metabolism, enterohepatic recirculation, or factors related to the accessibility of plasma-transported pesticide to the excretory cells of the liver. For specific effects, such as adrenal gland toxicity, *in vivo* and *in vitro* susceptibility to *o,p'*-DDD and 3-methylsulfonyl-*p,p*'-DDE varied among animal species (Brandt et al. 1992), and human adrenals *in vitro* showed potentially different susceptibility than animal glands *in vitro*. This is most likely due to differences in metabolism and covalent binding activities. Thus far, the endocrine-disrupting activity of DDT and analogues observed in experimental animals and in wildlife has not been observed in humans exposed to DDT.

3.7 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, reproductive, growth, immune, and neurobehavioral functions. 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).

Numerous reviews have been written regarding the endocrine disrupting capabilities of DDT and related compounds (Bulger and Kupfer 1983; Chapin et al. 1996; Colborn et al. 1993; Crisp et al.1998; Gillesby and Zacharewski 1998; Golden et al. 1998; Gray et al. 1997; and many others). Much of the historical information summarized below, particularly the early data, has been extracted from these reviews. The focus of this section is on effects in humans and in animal species traditionally used in laboratory experiments; information on endocrine effects, as well as other health effects, on wildlife is presented in Appendix D, Health Effects in Wildlife Potentially Relevant to Human Health.

It has been known for several decades that DDT and related compounds have weak estrogenic action in experimental animals and wildlife, but there is insufficient information to show that these chemicals have estrogenic action in humans. Estrogen influences the growth, differentiation, and functioning of many target tissues, including male and female reproductive systems such as mammary gland, uterus, vagina, ovary, testes, epididymis, and prostate. Several studies in humans have examined possible associations between body burdens of DDT and analogues and the incidence of alterations in these systems and tissues

and in other relevant end points; the results have been mixed. For instance, no association could be shown between the incidence of endometriosis and plasma concentrations of DDT (DDT plus DDE) in a case-control study by Lebel et al. (1998). Other studies reported that levels of DDT, DDE, and DDD were higher in maternal blood and in placental tissue in mothers who gave birth to premature infants or who spontaneously aborted fetuses compared to mothers who gave birth to full-term infants (Saxena et al. 1980, 1981; Wassermann et al. 1982). However, other potentially endocrine-disrupting chemicals, such as PCBs and other chlorinated pesticides, were also increased in the maternal blood of these subjects, and the specific contribution of DDT, DDE, or DDD could not be determined. In contrast, a similar study did not show the same association between serum DDE levels and pre-term delivery (Berkowitz et al. 1996). An earlier study by Procianoy and Schvartsman (1981) found no differences in DDT levels in maternal blood at delivery full-term babies and cases of pre-term delivery; however, they found that umbilical cord blood from pre-term newborns had higher DDT than full-term newborns. A recent study of 361 pre-term infants and 221 small-for-gestational-age cases found that the odds for both outcomes increased steadily with increasing maternal blood concentrations of DDE in samples taken in the third trimester of pregnancy (Longnecker et al. 2001). The association was evident at DDE concentration $10 ppb, but there was essentially no relation at lower DDE concentrations. With regard to cancers of the reproductive tissues, no association was found between risk of endometrial cancer and lipid-corrected blood serum concentrations *p,p'*-DDE and *o,p'*-DDT in a multicenter case-control study of women in the United States, but there was a slight increased risk associated with *p,p'*-DDT (Sturgeon et al. 1998). Also, there was no association found between *p,p'*-DDE concentration in subcutaneous fat and incidence of prostate and testicular cancer mortality (Cocco and Benichou 1998) or between DDE and death from uterine cancer in a study of women in the United States (Cocco et al. 2000).

Numerous studies have been conducted in the United States and elsewhere that evaluate the association between DDT and related compounds and breast cancer. Although a few studies have found positive associations among some subgroups of women (Aronson et al. 2000; Dewailly et al. 1994; Falck et al. 1992; Güttes et al. 1998; Romieu et al. 2000; Wasserman et al. 1976; Wolff et al. 1993), others have found no association between these chemicals and breast cancer (Demers et al. 2000; Dorgan et al. 1999; Helzlsouer et al. 1999; Laden et al. 2001a; Krieger et al. 1994; Liljegren et al. 1998; Lopez-Carrillo et al. 1997; Mendonca et al. 1999; Moysich et al. 1998; Schecter et al. 1997; Unger et al. 1984; van't Veer et al. 1997; Ward et al. 2000; Wolff et al. 2000a, 2000b; Zheng et al. 1999, 2000). A recently published combined analysis of five U.S. studies that included 1,400 cases and 1,642 controls found no evidence linking breast cancer and DDE body burdens (Laden et al. 2001a). Details of the individual studies about breast, endometrial, testicular, and prostate cancer are presented in Section 3.2.2.8 Cancer.

Early studies in experimental animals administered the chemicals orally or by parenteral routes, whereas in recent years, much research has focused on elucidating the mechanisms of action involved using *in vitro* test systems. Such tests can be used to assay for estrogenic, antiestrogenic, androgenic, and antiandrogenic activity. In general, results from *in vivo* and *in vitro* studies indicate that DDT and analogues have much lower estrogenic potency than the endogenous hormone, 17β-estradiol. Experiments conducted by Gellert et al. (1972) showed that young rats administered 5 mg *o,p'*-DDT/kg/day for 27 days had delayed vaginal opening and increased uterine and ovarian weights. In another study, using the uterine glycogen response assay, Bitman and Cecil (1970) observed that *o,p'*-DDT was the most potent among several isomers; *o,p'*-DDE and *p,p'*-DDT were approximately 16 times less active than *o,p'*-DDT and o,p'-DDD was inactive. Clement and Okey (1972) showed that *o,p'*-DDT (200 ppm in the diet) induced premature vaginal opening in Wistar rats. An *in vivo* assay with rats and mink showed that *o,p'*-DDT had uterotropic activity, whereas *p,p'*-DDT had only slight activity, and the activity of technical DDT was dependent on the level of *o,p'*-DDT that it contained (Duby et al. 1971). However, no reproductive effects were observed in two successive generations of rats fed technical DDT, *p,p'*-DDT, or *o,p'*-DDT (Duby et al. 1971). Many other *in vivo* studies have shown the estrogenic potential of DDT and related analogues (Gellert and Heinrichs 1975; Gellert et al. 1974; Heinrichs et al. 1971; Welch et al. 1969).

More recent *in vitro* experiments have examined binding of DDT, DDE, and DDD isomers to estrogen and androgen receptors and subsequent steroid regulated gene transcription. The experimental details of these studies are discussed in Section 3.6.2, Mechanisms of Toxicity: Reproductive and Developmental Effects. Although the results have varied somewhat between different investigators, in general, *o,p*'-DDT and *o,p*'-DDE appear to act as weak estrogen agonists, while *p,p*'-DDE can function as an androgen antagonist. In particular, *p,p*'-DDE functions as an antagonist after it has bound to the androgen receptor (Kelce et al. 1995). This androgen antagonism or antiandrogenic activity can explain a number of reproductive and developmental effects seen in male rats of various ages exposed to *p,p*'-DDE. These effects include reduced anogenital distance and retention of thoracic nipples in pups exposed during gestation and lactation (Gray et al. 1999; Kelce et al. 1995; Loeffler and Peterson 1999; You et al. 1998); delayed puberty in rats exposed either during juvenile development (Kelce et al. 1995) or at very high doses during gestation and lactation (Loeffler and Peterson 1999); and reduced accessory sex organ weights in exposed adult males (Gray et al. 1999; Kelce et al. 1995, 1997; You et al. 1999a). The experimental details of those studies are discussed extensively in Section 3.2.2.5, Reproductive Effects and Section 3.2.2.6, Developmental Effects.

The results of these and other studies suggest that DDT analogues can produce both agonistic and antagonistic responses by interfering with the binding of endogenous androgens and estrogens to their receptors. The type and magnitude of the response varied between assays and was dependent on the concentration of test material, as well as the temporal aspects of the exposure. Reviews of published data suggest that the amount of naturally occurring estrogens ingested daily through a normal diet may be far greater than the daily intake of estrogenic pesticides (Safe 1995). In addition, many experiments (Balaguer et al. 1999; Danzo 1997; Gaido et al. 1997; Kelce et al. 1995; Shelby et al. 1996; Soto et al. 1997) show that DDT isomers and metabolites have orders of magnitude less estrogenic activity than 17β-estradiol. This does not imply a lack of related risk, since estrogenic pesticides such as DDT and analogues bioconcentrate in the food chain and accumulate in the body and many have additive effects. Moreover, key endocrine processes can be profoundly affected by exposure to extremely small amounts of active chemicals during critical windows of embryonic, fetal, and neonatal development. As for antiandrogenic effects, Kelce et al. (1995) stated that the concentration of *p,p'*-DDE required to inhibit androgen receptor transcriptional activity in cell culture (64 ppb) is less than levels that accumulate from the environment such as in eggs of demasculinized male alligators in Florida's Lake Apopka (5,800 ppb) and in humans in areas where DDT remains in use or is present in contaminated ecosystems.

The adrenal gland may also be the target of some DDT isomers and metabolites. The metabolism of DDT can produce methylsulfonyl metabolites, such as methylsulfonyl-DDE, which are potent adrenal toxicants (Bakke et al. 1982; Brandt et al. 1992; Preston et al. 1984). *o,p'*-DDD is used therapeutically to treat adrenocortical carcinomas in humans. Furthermore, in some wild birds, but not others, adrenal weights or cortico-medullary ratios were affected by *p,p'*-DDT, *p,p'*-DDE, or technical-grade DDT (Hurst et al. 1974; Jefferies and French 1972; Jefferies et al. 1971; Lehman et al. 1974; Peterleef et al. 1973). Adrenal toxicity is discussed more extensively in Section 3.6.2, Mechanisms of Toxicity.

3.8 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 are particularly susceptible to hazardous chemicals due to the fact that many physiological systems, such as the nervous system, are being organized during the childhood years.

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 while 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).

No studies were located that specifically addressed effects of exposure to DDT in children, with the exception of a report by Hill and Robinson (1945) that described the case of a 1-year-old child who ingested 1 ounce of 5% DDT in kerosene that led to coughing, vomiting, tremors, convulsions, and eventually, death. The contribution of the kerosene solvent to the DDT toxicity was not clear. Data in adults are derived mostly from cases of accidental and/or intentional acute exposure (ingestion) of large amounts of DDT and from controlled studies in volunteers. Information from these studies indicate that the primary target of DDT toxicity is the nervous system. The effects are manifested as hyperexcitability, tremors, and convulsions (Francone et al. 1952; Garrett 1947; Hayes 1982; Hsieh 1954). It is reasonable to assume that the same effects would be seen in children similarly exposed. Additional information is derived from early occupational studies with limitations including lack of precise exposure data and presence of other compounds. Some of the long-term exposure reports provided suggestive evidence of adverse liver effects (Hayes 1956; Morgan and Lin 1978). None of these exposure scenarios appear likely for children in the United States at the present time.

Results from a few animal studies suggest that young and older animals exhibit different susceptibility to DDT toxicity, at least regarding neurotoxicity in response to relatively high doses of DDT. For example, the LD_{50} values for DDT in newborn, preweanling, weanling, and adult rats were $4,000, 438, 355, and 195 mg/kg, respectively (Lu et al. 1965). However, when one-quarter of the daily LD_{50} dose was administered daily for 4 days to preweanling and adult rats, both groups had similar 4-day LD_{50} values. Lu et al. (1965) suggested that the elimination mechanisms in the preweaning rats is less developed than in the adult rats, thus making them more susceptible to repeated small doses. In another study, 10-day-old rats were more resistant to the acute lethal toxicity of purified *p,p'*-DDT than 60-day-old rats (Henderson and Woolley 1970). In both groups, respiratory failure was the cause of death; however, the time course of DDT poisoning in the young rats was prolonged considerably as compared to the adults. Furthermore, the immature rats did not exhibit seizures nor the hyperthermia that preceded death in the older animals. The decreased sensitivity of the younger rats was attributed to an incomplete development of the neural pathways involved in seizure activity and in thermoregulation. The relevance of these findings to human health is unknown.

A decrease in testis weight was observed in juvenile male rats dosed by oral gavage on days 4 and 5 of life with 500 mg/kg/day or from day 4 to day 23 with 200 mg DDT/kg/day (Krause et al. 1975). After two doses, significant decreases were seen at 34 days, and after repeated lower doses, decreases were significant at days 18, 26, and 34. Treated males were mated with healthy females on days 60 and 90. The number of fetuses and implantations was decreased 30% at the 60-day mating but not at the 90-day mating of rats dosed on days 4 and 5. For rats receiving multiple doses, the decreases were 95% and 35% after mating at days 60 and 90, respectively. Treatment of weanling rats from either day 21 or 25 until day 57 of age with 100 mg *p,p*'-DDE/kg/day did not significantly alter serum levels of testosterone, but this treatment significantly delayed onset of puberty (see below) and was likely due to the antiandrogenic effects of DDE (Kelce et al. 1995).

Limited information exists regarding developmental effects of DDT on humans (Reproductive effects are discussed in Section 3.2.2.5). A study of 594 children from North Carolina found that height and weight adjusted for height of boys at puberty increased with transplacental exposure to DDE, but there was no association between any measure of DDE exposure (DDE in breast milk, maternal blood, cord blood, placenta) and age at which pubertal stages were attained (Gladen et al. 2000). Children from this cohort has normal birth weight and normal growth in the first year of life. A study of German children found an inverse association between serum DDE levels and height in girls (Karmaus et al. 2002). A study of Inuit infants exposed to *p,p*'-DDE (and other organochlorines) *in utero* revealed no association between immunological parameters (white blood cell counts, lymphocyte subsets, immunoglobulins) and prenatal exposure to organochlorines (Dewailly et al. 2000). However, the authors found that organochlorine exposure was associated with the incidence of infectious diseases and that the risk of contracting otitis media at 7 months of age increased with prenatal exposure to *p,p*'-DDE, hexachlorobenzene, and dieldrin. They also found that the relative risk for 4- to 7-month-old infants in the highest tertile of *p,p*'-DDE exposure was 1.87 (95% CI, 1.07–3.26) compared to infants in the lowest tertile. In addition, the relative risk of otitis media over the entire first year of life also increased with prenatal exposure to *p,p*'-DDE and hexachlorobenzene.

In animals, DDT can cause abnormal development o sex organs, embryotoxicity and fetotoxicity in the absence of maternal toxicity (Clement and Okey 1974; Fabro et al. 1984; Hart et al. 1971, 1972). Developmental effects, including preweanling mortality and premature puberty, have been reported in animals in multigeneration studies (Del Pup et al. 1978; Green 1969; Ottoboni et al. 1969, 1977; Tomatis et al. 1972, Turusov et al. 1973). DDT has shown estrogenic properties in animals administered the pesticide orally or parenterally (Bitman and Cecil 1970; Clement and Okey 1972; Fabro et al. 1984;

Gellert et al. 1972, 1974; Singhal et al. 1970). In female neonates injected subcutaneously with *o,p'*-DDT or *o,p'*-DDD, there were significant alterations in the estrous cycle, decreases in ovary weight, and decreases in corpora lutea when the animals were evaluated as adults (Gellert et al. 1972, 1974). In general, the estrogenic potency of DDT is orders of magnitude lower than that of estradiol.

p,p'-DDE, a persistent metabolite of DDT, was an androgen receptor antagonist in male rats exposed *in utero*, and also as juveniles (Gray et al. 1999; Kelce et al. 1995, 1997; Loeffler and Peterson 1999; You et al. 1998, 1999a). Kelce and co-workers showed that pups from dams exposed during gestation days 14–18 to 100 mg *p,p'*-DDE/kg/day and then exposed indirectly to maternally stored *p,p'*-DDE via breast milk had significantly reduced anogenital distance at birth and retained thoracic nipples on postnatal day 13. Treatment of weanling male rats from either day 21 or 25 (specific day unclear in text) until day 57 of age with 100 mg *p,p'*-DDE/kg/day resulted in a statistically significant delayed onset of puberty (measured by the age of preputial separation) by 5 days. Gray et al. (1999) and You et al. (1998) reported that anogenital distance was not affected in male Sprague-Dawley rats on postnatal day 2 after treating the dams with up to 100 mg *p,p'*-DDE/kg on gestation days 14–18, but was significantly reduced in similarly exposed Long-Evans pups. A 10 mg/kg dose to the dams was without effect in the Long-Evans pups. Anogenital distance was not affected in female pups from either strain. Treatment of the dams with 10 mg *p,p'*-DDE/kg resulted in retention of thoracic nipples in Sprague-Dawley pups, but only the higher dose (100 mg/kg) had this effect in Long-Evans pups. Treatment with *p,p'*-DDE also resulted in an apparent reduction of androgen receptor expression in male sex organs from mainly high-dose Sprague-Dawley pups, as shown by immunochemical staining; however, there were no changes in androgen receptor steady state mRNA levels in the high-dose Sprague-Dawley rats, but androgen receptor mRNA were increased 2-fold in the high-dose Long-Evans rats. Exposure of the pups to *p,p'*-DDE during gestation and lactation had no significant effect on the onset of puberty. An additional study from the same group showed that prenatal exposure to *p,p'*-DDE was associated with expression of TRPM-2, an androgen-repressed gene (You et al. 1999a). A similar study in Holtzman rats exposed during gestation days 14–18 to doses between 1 and 200 mg *p,p'*-DDE/kg (offspring were exposed to *p,p'*-DDE *in utero* and via breast milk) found reduced anogenital distance in males on postnatal day 1 and reduced relative ventral prostate weight on postnatal day 21 at 50 mg *p,p'*-DDE/kg, but not at 10 mg *p,p'*-DDE/kg (Loeffler and Peterson 1999). Doses up to 100 mg/kg/day to the dams had no effect on onset of puberty, but 200 mg/kg/day did significantly delay puberty in males by less than 2 days. Androgen receptor staining in the ventral prostate was also reduced on postnatal 21. Serum levels of testosterone or 3α-diol androgens were not significantly altered at any time. This study also reported that at the 100 mg/kg dose level, cauda epididymal sperm number was reduced by 17% on postnatal day 63 relative to controls. No

measurement of DDE body burden were made in the 200 mg/kg/day offspring postnatally, so it is difficult to determine whether effects on puberty were due to the previous gestational plus lactational exposures or directly due to the effects of DDE present near the time of puberty. More details about these studies can be found in Section 3.2.2.6, Developmental Effects. Kelce et al. (1995) also showed that treatment of adult male rats with 200 mg *p,p'*-DDE/kg for 4 days significantly reduced androgen-dependent seminal vesicle and ventral prostate weight relative to controls.

There are limited data suggesting that if mice exposed to DDT *in utero* and during lactation are further exposed to DDT postnatally, responses in both immunological plaque forming assays and lympho-proliferative assays are reduced (Rehana and Rao 1992). The results of this study are difficult to interpret because of experimental design issues, including the lack of a comparison unexposed group and ambiguity about whether statistical testing was done.

Alterations in learning processes and in other behavioral patterns have also been described in adult mice exposed to DDT perinatally (Craig and Ogilvie 1974; Palanza et al. 1999b; vom Saal et al. 1995) or as neonates (Eriksson et al. 1990a, 1990b, 1992, 1993; Johansson et al. 1995, 1996); this end point is the basis of an acute oral MRL, which is discussed in detail in Section 2.3, Minimal Risk Levels. These studies suggest that exposure of the developing fetus or newborn to DDT during critical stages in nervous system development can cause developmental toxicity manifested later in life. Eriksson et al. (1990a, 1990b) pointed out that the dose levels that caused behavioral alterations in mice are comparable to those levels to which human neonates might be exposed in areas where DDT is still being used. Behavioral neurotoxicity has been described in rats treated with DDT as adults (Sobotka 1971), but only at doses at least 50 times those that produced learning deficits in neonates.

There is no information regarding transgenerational effects associated with DDT exposure in humans, despite some limited evidence of genotoxicity in humans. Chromosomal aberrations have been reported in blood cells from subjects occupationally exposed to DDT (Rabello et al. 1975; Rupa et al. 1988; Yoder et al. 1973) and in human lymphocytes exposed *in vitro* to DDT (Lessa et al. 1976). These occupational exposures, however, also included exposure to many other pesticides. Two studies, one in rats (Palmer et al. 1973) and one in mice (Clark 1974), reported an increase in dominant lethality after exposure to high doses of DDT (Clark 1974). A study in rabbits administered 50 mg *p,p*'-DDT/kg by gavage on Gd 7–9 found no significant alterations in the distribution of chromosomes in liver samples from fetuses or in the percentage of chromosomal aberrations relative to controls (Hart et al. 1972).

There is no information regarding the pharmacokinetics of DDT in children or regarding the nutritional factors that may influence the absorption of DDT. Analysis of urine samples from humans exposed to DDT suggest the involvement of both phase I and phase II metabolic enzymes in the biotransformation and elimination of DDT and metabolites. The specific P-450 isoenzymes involved in phase I metabolism and the particular phase II conjugating enzymes are not known with certainty, so no conclusions can be drawn based on general differences in isozyme activities between adults and children. It is not known whether the metabolism of DDT in children might be different than in adults. Recent studies have suggested that enzymes involved in the metabolism of steroids are development-regulated and can be affected by DDE. For example, You et al. (1999c) examined the effects of *p,p'*-DDE on CYPs enzymes responsible for testosterone hydroxylations. They found that exposure of pregnant Sprague-Dawley rats to *p,p'*-DDE during gestation days 14–18 resulted in induction of CYP2A1 in males on PND 10 but not PND 21; pronounced induction of CYP2B1 was seen in males and females on both PND 10 and 21 and to a lesser extent of CYP3A1; there was no induction of CYP2C11. In contrast, in adult rats, DDE induced 2B1, 3A1, and 2C11, but not 2A1.

DDT and DDT-related compounds, particularly DDE, accumulate in fatty tissues and have been found in human milk (Dewailly et al. 1996; Scheele et al. 1995; Smith 1999; Torres-Arreola et al. 1999), amniotic fluid (Foster et al. 2000), placenta (Gladen and Rogan 1995; Procianoy and Schvartsman 1981; Wassermann et al. 1982), and in most organs from stillborn infants (Curley et al. 1969). Although, there is no direct evidence of adverse health outcomes in human infants exposed in such a manner, mobilization of adipose fat for lactation might provide an increased amount of DDT to a breast-feeding infant. Model estimates of increasing body burden of DDE in infants from breast-feeding milk with a DDE concentration of 222 ppb (lipid basis) predict that maximum body burden (approximately 70 μg/kg) is achieved after approximately 5–6 months of nursing, even if nursing continues, and then gradually decreases to <10 μg/kg by 2 years postpartum (LaKind et al. 2000). Levels of DDT and related compounds in human tissues from recent studies are presented in Table 6-4. Studies in animals have demonstrated placental transfer of DDT and DDE to fetuses and also to newborns via mother's milk (Fang et al. 1977; Seiler et al. 1994; Wooley and Talens 1971; You et al. 1999b). The results of these studies indicate that the amounts of chemical transferred via mother's milk are much greater than the amounts that reach the fetus through the placenta. PBPK models for the transplacental and lactational transfer of *p,p*'-DDE in rats were proposed by You et al. (1999b). The models provide an approach to estimating tissue doses in fetuses and pups associated with maternal exposures to *p,p*'-DDE, and can be used to explore dose-response relationships for the developmental effects of *p,p*'-DDE in the rat. In a study in rabbits administered DDT by gavage before artificial insemination and throughout gestation,

Seiler et al. (1994) found higher concentrations of DDT residues in fetuses (day 11 postcoitum) than in blastocytes (day 6 postcoitum), suggesting that transplacental passage may be more easily accomplished than passage into blastocytes via uterine secretions.

Intraperitoneal administration of the DDT metabolite, 3-$MeSO_2$-DDE, to pregnant or lactating mice resulted in specific accumulation and binding of 3-$MeSO_2$-DDE-derived radioactivity in the *zona fasciculata* of adrenals from 16- to 18-day-old fetuses or suckling pups, showing ready transplacental passage of the metabolite and transfer via maternal milk (Jönsson et al. 1992). The results also suggested that cytochrome P-45011β was involved in the activation of 3-$MeSO_2$-DDE in the fetal adrenal cortex. Quantitative measurements showed that 7 days after dosing, the labeling of the pups adrenals was 2 and 3.6 times higher than maternal adrenals at 1.5 and 25 mg/kg dose levels, respectively, suggesting that mother's milk may be an important route of exposure of 3-$MeSO_2$-DDE in DDT-exposed animals. A later study showed that P-45011β seems to be expressed during Gd 10–12 in the adrenal cortex in the mouse fetus (Jönsson et al. 1995). 3-$MeSO_2$-DDE was also found to reduce the capacity of pups and maternal adrenals to secrete corticosterone (Jönsson 1994) by a mechanism possibly involving competitive inhibition of adrenocortical CYP11B1 (Johansson et al. 1998).

There are no biomarkers of exposure or effect for DDT or DDT-related compounds that have been validated in children or in adults exposed as children. There are no biomarkers in adults that identify previous childhood exposure. No studies were located regarding interactions of DDT with other chemicals in children or adults. No information was located regarding pediatric-specific methods for reducing peak absorption following exposure to DDT or DDT-related compounds, reducing body burden, or interfering with the mechanism of action for toxic effects. In addition, no data were located regarding whether methods for reducing toxic effects in adults might be contraindicated in children.

3.9 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 DDT, DDE, and DDD are discussed in Section 3.9.1.

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 DDT, DDE, and DDD are discussed in Section 3.9.2.

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 3.11, Populations That Are Unusually Susceptible.

3.9.1 Biomarkers Used to Identify or Quantify Exposure to DDT, DDE, and DDD

In general, biomarkers of exposure to DDT can be classified as specific, such as DDT itself and its metabolites, and nonspecific, such as changes in endogenous chemicals that might indicate exposure to DDT, but also to other unrelated chemicals as well. DDT, DDE, and DDD have been detected and measured in adipose tissue, blood, serum, urine, feces, semen, and breast milk using several analytical techniques (see Chapter 7). Metabolites of DDT have also been measured in body fluids. The major

urinary metabolite identified in humans is DDA (Gingell 1976), while the *p,p'*-isomers of DDT and DDE as well as *o,p'*-DDT have been detected in breast milk (Takei et al. 1983; Torres-Arreola et al. 1999; see also Table 6-4). Nair and Pillai (1992) detected *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, and *p,p'*-DDD in human adipose tissue and breast milk, and *o,p'*-DDE, *p,p'*-DDE, and *p,p'*-DDT in whole blood. An earlier study had identified *p,p'*-DDE, *p,p'*-DDD, and *o,p'*-DDT in whole blood (Agarwal et al. 1976). *p,p'*-DDE has also been detected in amniotic fluid (Foster et al. 2000). Smith (1999) compiled values for DDT in human milk from 130 published studies since 1951 and observed that population means have declined in much of the world from 5,000–10,000 ppb (lipid-based) to around 1,000 ppb currently in many areas. Using a set of 13 studies from the United States and Canada, Smith (1999) estimated that since 1975, there has been an 11–21% decline in average DDT concentrations in breast milk. He also estimated a half-life of 4.2–5.6 years for DDT on a population basis. Levels of DDT in breast milk from some recent studies are presented in Table 6-4. Because DDT/DDE/DDD are fat soluble, they tend to concentrate in the fat portion of the specific biological medium (i.e., milk, serum, etc.) and therefore, their levels in the biological medium may vary with the fat content of that medium. This variability in the amount of DDT/DDE/DDD in the various media, is often accounted for by using lipid-adjusted measurements.

However, there are no quantitative data available that allow correlation of DDT/DDD/DDE levels in human tissue or fluids and exposure to particular levels of environmental contamination. Studies of pesticide production workers reported that blood levels of these compounds are generally higher in persons exposed in the workplace. Since the biological half-lives for elimination of these compounds are ranked as follows: DDE > DDT > DDD, detection of higher ratios of DDD or DDT to DDE is believed to indicate more recent exposure while lower ratios are believed to correlate with long-term exposure and storage capacity (Morgan and Roan 1971). There is a direct correlation between DDT and DDE levels in blood and adipose tissue when concentrations are expressed on a lipid basis (Hayes et al. 1971; Morgan and Roan 1971; Mussalo-Rauhamaa 1991). On a wet tissue basis, concentrations of DDT in adipose tissue are approximately 280 times higher than those of blood (Anderson 1985). However, because DDT and DDE are extensively stored in fatty tissue and slowly released from storage sites, there is no correlation between levels in tissues and the time course of exposure in short time spans. Analysis of residue levels of *p,p'*-DDT in skin lipids collected by wiping the skin with cotton and purifying lipids by gel permeation chromatography has been investigated by Sasaki et al. (1991b) as a noninvasive method for monitoring DDT burdens in pesticide-exposed persons; a correlation was found between skin levels and adipose tissue levels; however, these results reflect both body burden and dermal exposure. Levels of DDT (total DDT) in adipose tissue from the U.S. population have been continuously declining over the

last decades from about 8 ppm (lipid adjusted) in 1970 to about 2 ppm in 1983 (Kutz et al. 1991). A review by Kutz et al. (1991) also lists levels of DDT, DDE, and DDD in samples of adipose tissue collected during the 1960s through the 1980s from populations around the world. Table 6-4 presents additional information on DDT levels in blood and adipose tissue from some recent studies.

Changes in plasma concentrations of endogenous chemicals that might be consistent with DDT exposure include increased vitamin A plasma levels, which have been shown to increase with increasing plasma levels of DDE in humans (Nhachi and Kasilo 1990). In rats, DDT administration (oral gavage of 40 mg/kg) decreased hepatic vitamin A storage (deWaziers and Azais 1987). Increased levels of urinary 17-hydroxycortisone have also been reported as indicators of DDT exposure in humans (Nhachi and Loewenson 1989; Poland et al. 1970). This is consistent with DDT induction of hepatic P-450 enzymes increasing the catabolism of cortisol to 17-hydroxycortisol (see Section 3.5.3). A potential biomarker of exposure, which has been identified from studies in laboratory animals, is an increase in GGTP. In rats, after acute oral exposure to DDT, serum levels of GGTP doubled and remained elevated for 48 hours (Garcia and Mourelle 1984). However, none of these potential biomarkers are specific to DDT, DDE, or DDD exposure, and not all the body compartments in which these changes occur are accessible for sampling in living humans.

3.9.2 Biomarkers Used to Characterize Effects Caused by DDT, DDE, and DDD

The primary target organs for DDT, DDE, and DDD toxicity include the nervous system, the reproductive system, and the liver. No biomarkers of effect specific for DDT, DDE, or DDD exposure alone were identified in the literature. Tremors and convulsions have been observed in both humans and laboratory animals after DDT exposure (Hsieh 1954; Hwang and Van Woert 1978; Matin et al. 1981). Exposure to DDT has been shown to induce hepatic microsomal enzymes in both humans and laboratory animals (Kolmodin et al. 1969; Morgan and Lin 1978; Pasha 1981; Street and Chadwick 1967). However, these biomarkers of effect are not specific for DDT, DDE, or DDD exposure, and not all the body compartments in which these changes occur are accessible for sampling in living humans.

For more general information on biomarkers of effect for the immune, renal, and hepatic systems, see CDC/ATSDR Subcommittee Report on Biological Indicators of Organ Damage (CDC/ATSDR 1990), and for biomarkers of effect for the neurological system, see the Office of Technology Assessment report (OTA 1990). For more information on the health effects after exposure to DDT, DDE, and DDD see Section 3.2.

3.10 INTERACTIONS WITH OTHER CHEMICALS

This section discusses the potential for DDT to act synergistically and/or antagonistically with other chemicals to cause physiological harm. DDT may have broad effects by changing the metabolism of other chemicals, both xenobiotics and endogenous macromolecules. DDT induces microsomal mixed function oxidases that are involved in the catabolism of both xenobiotics and many endogenous hormones, such as cortisol. DDE has also caused the induction of hepatic enzymes (Conney 1971), including cytochrome P-450 microsomal enzymes (Pasha 1981). Its effects on the latter are discussed in Section 3.5.3. In most cases, this biotransformation results in compounds that are less toxic than the parent compound and more readily excreted from the body. For some chemicals, this metabolism results in the production of metabolites that are more toxic than the parent compound and that may be carcinogenic. One interaction of concern is the enhanced conversion of other chemicals to active, carcinogenic forms mediated by microsomal enzymes induced by DDT. Several investigations indicate that DDT administered to animals along with a known carcinogen may result in either increase or decrease in tumor production relative to the carcinogen tested without DDT. A study by Walker et al. (1972) suggested that the liver enlargement was greater and the time to palpability of liver masses was earlier in mice fed dieldrin and DDT than those fed either pesticide separately. A potentiation of carcinogenic activity of dieldrin was suggested but not conclusively shown. DDT alone is thought to produce hepatic tumors both through the formation of DNA adducts and through promotional mechanisms involving cytotoxicity and compensatory cell proliferation (see Section 3.5.2). It is possible that DDT could also promote the formation of hepatic tumors initiated by other carcinogens.

DDT is reported to promote the tumorigenic effects of several known carcinogens, such as 3-methyl-(4-dimethylamine)-azobenzene (Kitagawa et al. 1984), 2-acetylaminofluorene (2-AAF) (Peraino et al. 1975), diethyl-nitrosamine (DEN) (Diwan et al. 1994; Nishizumi 1979), and carbon tetrachloride (CCl_4) (Preat et al. 1986) when given after the putative carcinogen. The promoting effect of DDT in rats is reported to act in a dose-dependent fashion, with DDT decreasing the latency period of tumor development and increasing the incidence and yield of hepatic tumors, mainly hepatocellular carcinomas.

DDT acted as a hepatocellular tumor promoter in D2B6F1 mice when administered in the diet at 300 ppm (53 mg/kg/day) for 53 weeks, beginning 2 weeks following initiation with an intraperitoneal injection of N-nitrosodiethylamine (Diwan et al. 1994). The incidence of mice with tumors was 22/22 in the DDT-treated group, compared to 12/30 in the group receiving only N-nitrosodiethylamine; the difference was statistically significant.

Pretreatment of animals with DDT was also reported to decrease the tumorigenic effects of some previously determined carcinogens. For example, pretreatment of rats with DDT significantly lowered the incidence of mammary tumors per rat after treatment with 7,12-dimethylbenz[a]anthracene (DMBA), versus DMBA-treated controls (Silinskas and Okey 1975). The authors suggested that DDT may inhibit DMBA-induced mammary tumors by stimulating hepatic metabolism and accelerating the excretion of DMBA, so that less carcinogen is available to peripheral tissues. Other studies also have reported the DDT induction of hepatic microsomal enzymes, which reduced the carcinogenicity of azo dyes and similar carcinogens (Williams and Weisburger 1991).

Similarly, the hepatocarcinogenicity of aflatoxic B_1 in mice was inhibited by pretreatment with DDT and by co-treatment with DDT when given throughout aflatoxin B_1 dosing (Rojanapo et al. 1988, 1993). However, DDT acted as a hepatocarcinogenic promoter to aflatoxin B_1 initiation when a 14-week DDT administration followed an 8-week aflatoxic B_1 treatment, or when the DDT administration began halfway through aflatoxin B_1 treatment (Rojanapo et al. 1988, 1993). Also, in groups receiving both aflatoxic B_1 and DDT, in any order, absolute and relative liver weights were significantly increased over both the vehicle control and the group receiving just aflatoxin B_1; treatment with aflatoxin B_1 alone increased liver weights, while treatment with DDT alone did not (Rojanapo et al. 1993). The proposed mechanisms of tumor promotion are discussed in Section 3.5.2.

The effects of DDT on the nervous system are altered when DDT is given in combination with certain neurologically-active pharmacological agents. Some pharmacological agents (hydantoin, phenobarbital), prevent some or all of the neurological effects seen in animals treated with DDT (see Section 3.2.2.4), while other agents (trihexyphenidyl, haloperidol, propranolol) enhance DDT-induced neurotoxicity (Herr et al. 1985; Hong et al. 1986; Matin et al. 1981). One of the effects of DDT is to hold sodium channels open, which probably contributes to DDT-induced neurological effects (tremors and hyperexcitability). Studies by Rubin et al. (1993) have shown that DDT analogues and metabolites as well as several pyrethroids modify radioligand binding of batrachotoxinin to sodium channels in mouse brain synaptosomes. DDT and pyrethroids do not by themselves stimulate Na^+ uptake, but they enhance activator-dependent uptake. DDT is more efficacious than the pyrethroids tested. Eriksson et al. (1993) have shown that the pyrethroid bioallethrin and DDT can interact *in vivo* in rats.

As previously mentioned, in the United States, exposure of the general population to DDT/DDE/DDD occurs mainly through consumption of contaminated food (i.e., meat, fish, dairy products). Because organochlorines, in general, bioaccumulate in the food chain, it is very likely that there will be also

simultaneous exposure to several other persistent organochlorine chemicals, many also with hormonal activity. Therefore, interactions at the level of the estrogen and/or androgen receptor may occur, and the final outcome probably represents the summation of effects (positive or negative; linear or non-linear) of chemicals with similar modes of action.

3.11 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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

No data are available on human differences in susceptibility to DDT, DDE, or DDD. Groups who might be particularly susceptible to the toxic effects of DDT are individuals with diseases of the nervous system or liver. Persons with nervous system disorders in which normal function is altered due to physiological changes, such as changes in neurotransmitter balance or impaired neuronal conduction, might be more susceptible to DDT neurotoxicity. Persons with diseases of the liver might be more sensitive to the hepatotoxic effects of DDT since normal repair function may already be compromised.

The susceptibility of children is discussed in detail in Section 3.8, Children's Susceptibility.

3.12 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to DDT, DDE, and DDD. 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 DDT, DDE, and DDD. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

Most of the strategies discussed in the following sections apply to high-dose exposures. The balance between the benefits and detriments of mitigation for low-dose chronic exposures might differ from those

for high-dose exposures. Methods to reduce toxic effects should not be applied indiscriminately to all individuals exposed to DDT, DDE, or DDD; good clinical judgement should be used.

3.12.1 Reducing Peak Absorption Following Exposure

A number of strategies have been suggested to minimize absorption from the gastrointestinal tract. Ipecac-induced emesis has been suggested unless there is a risk of lung aspiration due to unconsciousness or convulsions (HSDB 2002a, 2002b, 2002c, 2002d). Gastric lavage with mannitol may also be useful for limiting absorption, particularly when emesis is contraindicated (Dreisbach 1983). Since activated charcoal can absorb DDT, it has also been commonly used as a method for reducing intestinal uptake (Dreisbach 1983). Another method of reducing absorption is the use of a cathartic, and in practice, activated charcoal is frequently given in a slurry of one of the saline cathartics (Dreisbach 1983; HSDB 2002a, 2002b, 2002c, 2002d). Vegetable oils should not be used as cathartics because they have been shown to promote the absorption of DDT, DDE, and DDD in animals (Keller and Yeary 1980). At this point, it is unknown whether there are any specific binding or reactive agents that might prevent absorption, but this might be a strategy for future research.

Dermal absorption of DDT, DDE, and DDD is less efficient than absorption by the oral route. After dermal or ocular exposure, absorption may be reduced by decontaminating the exposed area; the generally used method is washing (HSDB 2002a, 2002b, 2002c, 2002d).

3.12.2 Reducing Body Burden

In humans, DDT and its metabolites distribute in the general circulation, but are eventually selectively concentrated in adipose tissue where they are retained for long periods of time. After the pesticide is sequestered in adipose tissue, common methods to reduce body burden such as dialysis, exchange transfusion, and hemoperfusion are probably ineffective because only small amounts are present in the blood. However, the interval immediately after absorption may be a window of opportunity for removing the xenobiotic from the circulation before it partitions into adipose tissue. Potential strategies that might be investigated include hemodialysis and hemoperfusion (Klaassen 1990).

Absorbed DDT is primarily excreted in the urine (mostly as conjugated DDA), with minor amounts excreted in feces (via biliary excretion), semen, and breast milk. There are several potential strategies for enhancing fecal DDT excretion that might be worth investigation. Studies of DDT excretion in

laboratory rats indicated that oral administration of sodium muconate resulted in fecal excretion of injected DDT that was 22 times greater during the first day than in animals that had not received sodium muconate. The *trans, trans* muconate isomer was given by oral gavage (75 mg/kg) 2 hours after intraperitoneal injection of ^{14}C-*p,p'*-DDT (6.7 mg/kg). Over the first 10 days following sodium muconate treatment, fecal excretion was 2.34 times greater in rats receiving sodium muconate compared to rats that did not receive this treatment (Boileau et al. 1985). Ten days posttreatment, the experimental rats had significantly lower DDT in abdominal fat, liver, and brain showing effective reduction of body burden (Boileau 1985). The mechanism of *trans, trans* muconate in enhancing fecal excretion is unknown. Therefore, it is possible that administration of sodium muconate to humans could result in greater fecal excretion. Muconate may only be effective in acute poisonings, and it has not been shown if a similar effect in rats occurs after acute oral exposure to DDT.

Fecal metabolites have been measured, and at least in one case compared with biliary excretion straight from the bile duct (see Section 3.4.4). If significant enterohepatic circulation could be demonstrated, then methods to interfere might be effective in accelerating the excretion of DDT metabolites. There are several possible strategies for reducing intestinal resorption of metabolites excreted in the bile; the simplest is repeat doses of activated charcoal (Levy 1982). Another strategy that has been effective with other lipophilic xenobiotics has been the oral administration of the anion exchange resin, cholestryamine (Boylan 1978). Daily administration of mineral oil, a cathartic, in the diet of monkeys beginning 7 days after a single oral dose of DDT resulted in a reduction of DDT in adipose tissue and greater elimination in feces (Rozman et al. 1983).

3.12.3 Interfering with the Mechanism of Action for Toxic Effects

DDT, DDE, and DDD are stimulants that can cause tremors and convulsions by several postulated mechanisms (Section 3.5.2). The most well accepted mechanism is interference with membrane ion fluxes, which leads to prolongation of the action potential and repetitive firing (Narahashi and Haas 1967). Other contributory mechanisms that may be secondary to inferences with ion fluxes may include decreases in brain serotonin and increases in levels of aspartate and glutamate. A number of drugs can alleviate this type of central nervous system excitation on experimental animals. These include the anticonvulsant barbiturate phenobarbital, the sedative-antianxiety drug diazepam, and the anticonvulsant phenytoin (Herr et al. 1985; HSDB 2002a, 2002b, 2002c, 2002d; Matin et al. 1981; Tilson et al. 1987). A reduction in the serotonergic activity in the brain has also been postulated to be responsible for the neurotoxic syndrome of myoclonus associated with DDT. Agents that enhance the action of serotonin,

such as L-5-hydroxytryptophan, a serotonin precursor; chlorimipramine, a serotonin uptake blocker; and phenoxybenzomine or trazodone, two α-receptor blockers, can reduce the neurotoxic effects (myoclonus) of DDT in mice (Hwang and Van Woert 1978). Caution should be used in extrapolating from animal therapeutics to human applicability. Myoclonus in the rat induced by *p,p'*-DDT has been studied by Pratt et al. (1986), and from electrophysiological and pharmacological analysis it was concluded that the rat was not a good model for studying 5-hydroxytryptamine-sensitive myoclonus in humans.

The reproductive system in animals is another sensitive target organ for DDT toxicity. There are no medically proven-methods for reducing DDT, DDD, and DDE reproductive toxicity by interfering with the mechanism of action in this organ.

Mitigation strategies developed in the future for other lipophilic pesticides should be investigated for their applicability to DDT, DDE, and DDD.

3.13 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 DDT, DDE, and DDD 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 DDT, DDE, and DDD.

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.

3.13.1 Existing Information on Health Effects of DDT, DDE, and DDD

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to DDT are summarized in Figure 3-8. The purpose of this figure is to illustrate the existing information concerning the health effects of DDT, DDE, and DDD. Each dot in the figure indicates that one or more

studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Figure 3-8. Existing Information of Health Effects of DDT, DDE, and DDD

	Death	Acute	Intermediate	Chronic	Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
	Systemic									
Inhalation										●
Oral	●	●	●	●	●	●	●	●	●	●
Dermal										

Human

	Death	Acute	Intermediate	Chronic	Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
	Systemic									
Inhalation										
Oral	●	●	●	●	●	●	●	●	●	●
Dermal	●	●				●				●

Animal

● Existing Studies

3.13.2 Identification of Data Needs

Acute-Duration Exposure. With acute oral exposure to high doses, the nervous system appears to be the major target in both humans and animals. Acute oral exposure has been associated with tremors or convulsions in humans (Hsieh 1954; Velbinger 1947a, 1947b) and animals (Hong et al. 1986; Matin et al. 1981). An acute MRL has been derived based on neurobehavioral effects observed in adult mice following acute perinatal exposure to DDT (Eriksson et al. 1990a, 1990b, 1992, 1993; Johansson et al. 1995, 1996; Talts et al. 1998). Studies of single oral or injection exposures of rats, guinea pigs, and mice have provided information on lethal and nonlethal levels of DDT. Further acute oral exposure studies during critical windows of embryonic, fetal, or neonatal development may be very informative.

Information on health effects following acute inhalation of DDT, DDE, or DDD in humans (Neal et al. 1944) or dermal exposure in animals (Cameron and Burgess 1945) was limited. Because of the lack of inhalation data in animals, an acute inhalation MRL could not be derived. Exposure via inhalation at the ambient levels in air (Whitmore et al. 1994) is thought to be insignificant compared with dietary uptake (see Section 6.5, General Population and Occupational Exposure). Also, in the atmosphere, about 50% of DDT is adsorbed to particulate matter and 50% exists in the vapor phase (Bidleman 1988); it is likely that particulate-absorbed DDT will be deposited in the upper respiratory tract and swallowed after mucociliary transport upward (Hayes 1982).

Intermediate-Duration. Intermediate-duration exposures in humans and animals have been reported. In most human studies, the exact duration and level of exposure cannot be quantified because the information is derived from case reports or epidemiological studies that do not adequately characterize exposure. Studies on volunteers have been performed in the past that provide useful information (Hayes et al. 1956). The animal studies describe predominantly neurological (Cranmer et al. 1972a; Sanyal et al. 1986), hepatic (Gupta et al. 1989; Laug et al. 1950; Ortega 1956), immunological (Banerjee et al. 1987a, 1995, 1996, 1997a, 1997b), and reproductive/developmental (Clement and Okey 1974; Jonsson et al. 1976; Kelce et al. 1995; Krause 1977; Krause et al. 1977; Lundberg 1974; Wrenn et al. 1971) end points. Little or no information on respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, or dermal/ocular effects in animals exists. An intermediate oral MRL was calculated for DDT, based on cytoplasmic eosinophilia and cellular hypertrophy of hepatocytes in rats described in the Laug et al. (1950) study. Because this study was conducted several decades ago, it would be desirable to replicate the findings using modern histopathological techniques and current views on the interpretation of specific liver changes. The immunological studies on DDT are not considered to be adequate for MRL

development. The 1992 study reported by Rehana and Rao has several deficiencies in reporting that don't allow adequate data interpretation. The animal data indicate that the liver is the main target organ following intermediate or chronic oral exposure, but there is little evidence that liver function has been impaired in humans occupationally exposed, although the data are limited. The importance of subtle biochemical changes in humans, such as the induction of microsomal enzymes in the liver and decreases in biogenic amines in the nervous system, is not known with certainty. This information would be helpful in evaluating the toxic effects of DDT, DDE, and DDD following intermediate exposure. Levels in environmental media are not expected to be high enough to result in high inhalation or dermal exposures.

Chronic-Duration Exposure and Cancer. Studies have been conducted in animals in which oral exposure was for a chronic duration. Reproductive, neurological, and hepatic effects have been observed in animals following chronic oral exposure (Deichmann et al. 1967, 1971; Durham et al. 1963; Fitzhugh 1948; NCI 1978; Rossi et al. 1977; Takayama et al. 1999). No chronic-duration toxicity studies in which animals were exposed dermally or by inhalation were located. However, the inhalation and dermal routes are considered minor routes of entry with regard to absorption. Inhaled DDT is largely deposited in the upper respiratory tract and then swallowed (Hayes 1982). Pharmacokinetic data using lung dosimetry models would provide useful information as to the contribution of the inhalation route to total intake; however, direct absorption in the lung is probably minimal. An oral MRL for chronic-duration exposure was not derived since the most sensitive noncancer (hepatic) effects were observed at doses higher than the doses at which the most sensitive acute- and intermediate-duration effects occurred. This dietary level was the lowest level tested in the study (Fitzhugh 1948). Because few experimental details were given in the Fitzhugh (1948) report, it may be desirable to replicate the findings using modern histopathological techniques and current views on the physiological meaning of specific liver changes.

The possible association between exposure to DDT and various types of cancers in humans has been studied extensively (Aronson et al. 2000; Cocco and Benichou 1998; Cocco et al. 1997a, 1997b, 2000; Demers et al. 2000; Dewailly et al. 1994; Dorgan et al. 1999; Garabrant et al. 1992; Hardell et al. 1996; Helzlsouer et al. 1999; Hoppin et al. 2000; Krieger et al. 1994; Laden et al. 2001a; Liljegren et al. 1998; Lopez-Carrillo et al. 1997; Moysich et al. 1998; Romieu et al. 2000; Schecter et al. 1997; Sturgeon et al. 1998; van't Veer et al. 1997; Ward et al. 2000; Wasserman et al. 1976; Wolff et al. 1993, 2000a; Zheng et al. 1999, 2000). Nearly all of the human studies evaluated the carcinogenicity of either *p,p'*-DDT or *p,p'*-DDE. Cancer end points that have been investigated in humans include respiratory system, pancreatic, endometrial, breast, prostate, liver, and testicular cancers, Hodgkin's and non-Hodgkin's lymphomas, and multiple myeloma. Thus far, there is no conclusive evidence linking DDT and related

compounds to cancer in humans. It is expected that some of these cohorts will continue to be monitored to ensure that latency is appropriately accounted for. Other areas where data gaps exist include exposure assessment in epidemiological studies; studies on validity and reliability of recall of pesticide use for occupationally and nonoccupationally exposed populations; and information on inert ingredients of pesticide formulations, where specific pesticides are still used, since inert components are not necessarily biologically inert. The finding of Demers et al. (2000) of a potential association between blood DDE levels in breast cancer patients and aggressiveness of the disease requires replication in additional studies to rule it out as artifactual. In addition, further attention should be paid to physiological changes that may occur in cancer patients that could alter the pharmacokinetics of the biomarkers of exposure. This issue was brought up by Hoppin et al. (2000) in their study of patients with pancreatic cancer, a condition in which wasting may greatly diminish the lipid pool and thus give artificially-elevated values of body burden for lipid-soluble chemicals.

Animal data provide sufficient evidence of carcinogenicity via oral exposure. DDT has been shown to be carcinogenic in a number of studies in rodents (Cabral et al. 1982b; Innes et al. 1969; Kashyap et al. 1977; Rossi et al. 1977; Thorpe and Walker 1973). DDT and related compounds caused primarily liver cancer. Information on the mechanism of action for cancer induction by DDT in these susceptible species and on whether or not species-specific biomarkers exist would be helpful. For example, mice and hamsters metabolize DDT by similar metabolic pathways. Therefore, it is unlikely that the species difference is due to differences in the production of DDMU-epoxide or DDA-Cl (Gold and Brunk 1983). However, the hamster is less effective in the conversion of DDT to DDE (Gingell 1976; Gold and Brunk 1983). DDE has been shown to cause liver tumors in hamsters (Rossi et al. 1983). DDT has been shown to promote the carcinogenicity of other substances (Diwan et al. 1994; Kitagawa et al. 1984; Nishizumi 1979; Peraino et al. 1975; Preat et al. 1986; Rojanapo et al. 1993). On the other hand, some studies have observed an inhibitory action of DDT on the carcinogenicity of other chemicals (Rojanapo et al. 1993; Silinskas and Okey 1975). Virtually no information was located regarding the carcinogenicity of DDT by the inhalation and dermal routes of exposure, but additional bioassays do not seem necessary at this time.

Genotoxicity. There is no conclusive evidence that DDT and related compounds are genotoxic in humans. While some studies of humans exposed occupationally have reported chromosomal aberrations (Rabello et al. 1975; Rupa et al. 1988, 1989; Yoder et al. 1975), the subjects were also exposed to other pesticides and it is unclear whether additional potential confounders were adequately controlled. A recent study of subjects residing near a waste site found no association between serum levels of DDE and the incidence of micronuclei in peripheral lymphocytes (Vine et al. 2001). Two high-dose oral studies in

rodents, one in rats (Palmer et al. 1973) and the other in mice (Clark 1974), reported increases in dominant lethality. In general, the results from standard gene mutation tests in bacteria did not show mutagenicity (Fahrig 1974; Fluck et al. 1976; McCann et al. 1975; Shirasu et al. 1976) and conflicting results have been obtained in tests for chromosomal aberrations in cultured rodent and human cells (Kelly-Garvert and Legator 1973; Palmer et al. 1972; Probst et al. 1981; Tong et al. 1981; Tsuchimoto et al. 1983). It is unlikely that further studies will provide new key information.

Reproductive Toxicity. Several studies in humans have examined possible associations between body burdens of DDT and analogues and alterations of some aspects of reproduction. Duration of lactation was found to be inversely related to the concentration of *p,p'*-DDE in breast milk in two studies (Gladen and Rogan 1995; Rogan et al. 1987). Some studies also found weak associations between blood DDT/DDE levels and miscarriages (Gerhardt et al. 1998) and infertility (Gerhardt et al. 1999), but these findings need to be confirmed. Additional studies have examined associations between body burdens of DDT, DDE, and DDD and premature infants and abortions (Saxena et al. 1980, 1981; Wassermann et al. 1982), but the possible role of other chemicals, such as PCBs and other chlorinated pesticides, could not be ruled out. A recent study in the United States found increased odds of having pre-term infants and small-for-gestational-age infants in women having blood concentrations of DDE $10 ppb in samples taken in the third trimester of pregnancy (Longnecker et al. 2001). There was essentially no relation at lower DDE concentrations. Overall, there is no conclusive evidence that body burdens of DDT/DDE/DDD currently found in the general U.S. population represent a reproductive hazard, but adverse reproductive outcomes may be a concern for women in countries where these chemicals are still used.

Acute exposure to DDT by the oral route in animals has been associated with reproductive effects, including a decrease in fertility in male rats (Krause et al. 1975). Reproductive effects observed in animals following DDT exposures of intermediate duration are similar to those effects observed with excess estrogen, including infertility (Green 1969; Jonsson et al. 1976), and decreases in implanted ova (Lundberg 1973). Adult male rats showed a decrease in seminal vesicle and ventral prostate weight after short-term treatment with *p,p'*-DDE, which is thought to be due to the antiandrogenic effects of *p,p*'-DDE (Kelce et al. 1995, 1997). One study *in vitro* showed that all DDT/DDE/DDD isomers can exhibit some degree of antiandrogenicity (Maness et al. 1998), but it is not known whether isomers other than *p,p'*-DDE have antiandrogenic properties *in vivo*. Also, it would be useful to determine whether the persistent DDT metabolite, 3-MeSO_2-DDE, functions as an androgen antagonist in both *in vitro* binding assays and gene expression assays, and how its potency compares to that of DDE itself.

Multigeneration studies have been conducted in rats (Duby et al. 1971; Green 1969; Ottoboni 1969, 1972; Treon et al. 1954), mice (Keplinger 1970), and dogs (Deichman et al. 1971; Ottoboni et al. 1977). The infertility observed in the Green (1969) study at the very low dose (0.56 mg/kg/day) raises potential concerns about human reproductive effects. However, Keplinger (1970) saw no effect at 3.2 mg/kg/day, although there was a decrease in fertility (as defined by decreased viability through the lactation period) at 32 mg/kg/day. No effects on fertility were seen in 2 or 3 generations of rats exposed to 1.25 mg/kg/day (Treon et al. 1954; Ottoboni 1969) or 1.5 mg/kg/day of technical-grade DDT (Duby et al. 1971), nor in 3 generations of beagles exposed to 10 mg/kg/day (Ottoboni et al. 1977). The Deichman et al. (1971) was a mixed exposure to aldrin and DDT from which no conclusions can be drawn. Unfortunately, none of the multigeneration studies mentioned above was conducted according to current scientific standards; flaws exist in the areas of adequate sample sizes, statistical testing (an lack thereof), and reporting of sufficient experimental detail. Thus, there is a critical data need for a well designed multigeneration animal study to reduce the scientific uncertainties about the effects of DDT on fertility.

Well-designed experiments to identify sensitive time periods of exposure and to clarify dose-response relationships for these effects would be useful when deriving an MRL or assessing the potential hazard resulting from environmental exposures. Because exposure to DDT from hazardous waste sites is of concern, additional studies are needed to assess the reproductive toxicity following exposure to doses similar to those estimated at hazardous waste sites. However, DDT-induced effects on fertility may be difficult to detect since the baseline human infertility rate is so high. Levels in environmental media are not expected to be high enough to result in high inhalation or dermal exposures, and therefore, additional inhalation or dermal exposure reproductive studies do not seem to be warranted at this time.

Developmental Toxicity. There are limited data on developmental effects of DDT and analogues in humans. A study of children from North Carolina found that transplacental exposure of DDE was associated with increased height and weight adjusted for height in boys at puberty (Gladen et al. 2000). A study of German children found an inverse association between serum DDE levels and height in girls (Karmaus et al. 2002). Also, a study of Inuit infants found that prenatal exposure to DDE and other chemicals may have been associated with increased risk of otitis media during the first year of life (Dewailly et al. 2000).

While the limited data in humans do not allow for definite conclusions to be drawn, data in animals indicate that perinatal exposure to DDT/DDE/DDD (Craig and Ogilvie 1974; Fabro et al. 1984; Gellert and Heinricks 1975; Gray et al. 1999; Hart et al. 1971, 1972; Kelce et al. 1995; Loeffler and Peterson

1999; You et al. 1998, 1999a) or neonatal exposure to DDT (Eriksson et al. 1990a, 1990b, 1992, 1993; Johansson et al. 1995, 1996; Talts et al. 1998) can cause adverse developmental effects in the offspring. These effects depend on the dose administered, the timing of exposure during or after gestation, and the specific isomer administered. The *o,p'*-isomers of DDT, DDE, and DDD have been associated with estrogen-like effects in the reproductive system and *p,p'*-DDE has been associated with antiandrogenic effects. Further information concerning these factors and their impact on the developmental toxicity of DDT, DDE, and DDD would be helpful. For example, cross-fostering studies could help in determining the relative impact of gestational versus lactational exposure to *p,p'*-DDE in relation to delays in the onset of puberty. Standardization of both *in vivo* (pre and postnatal exposures) and *in vitro* tests for estrogenicity and antiandrogenicity of DDT and related compounds would be helpful to compare results between research groups. There are limited data suggesting that if mice exposed to DDT *in utero* and during lactation are further exposed to DDT postnatally, responses in both immunological plaque forming assays and lymphoproliferative assays are reduced (Rehana and Rao 1992). The results of this study are difficult to interpret because of experimental design issues, including the lack of a comparison unexposed group and ambiguity about whether statistical testing was done. It would be useful to try to replicate these results using a better study design. The results from the series of studies by Eriksson et al. (1990a, 1990b, 1992, 1993) and Johansson et al. (1995, 1996) on mice exposed perinatally were used as the basis for deriving an acute oral MRL. Duplication of these results by other laboratories would greatly increase the confidence in these findings. Developmental effects have also been observed in animals following intermediate- and chronic-duration oral exposures to DDT. These effects included slowed development and premature puberty (Clement and Okey 1974; Craig and Ogilvie 1974; Naishtein and Leibovich 1971; Ottoboni et al. 1977; Tomatis et al. 1972; Turusov et al. 1973). Additional studies to assess the mechanism of the developmental toxicity, the critical stages in perinatal development affected by DDT, and the dose-response relationships would be helpful. More data on prenatal and postnatal exposures and postnatal developmental effects might also be useful. No developmental studies by the inhalation and dermal routes of exposure were located. However, since levels in environmental media are not expected to be high enough to result in high inhalation or dermal exposures, additional inhalation or dermal exposure studies assessing developmental effects do not seem to be warranted at this time.

Immunotoxicity. Evidence of immunotoxicity in humans is inconclusive and is limited to a study with only 3 volunteers (Shiplov et al. 1972), a study of 12 fish-consumers in Sweden (Svensson et al. 1994) and a more recent study of subjects who resided near a waste site (Vine et al. 2001). Acute-, intermediate-, and chronic-duration oral studies in animals provide evidence that DDT may cause immunological effects. Effects reported included decreases in antibody titers and plaque-forming cells

(Banerjee 1987a; Banerjee et al. 1986), increases in gamma globulin and serum immunoglobulin and a decreased tuberculin skin reaction (Street and Sharma 1975), alterations in the spleen (Deichmann et al. 1967), and increased growth of the leprosy bacterium (Banerjee et al. 1997a). The immunotoxicity of DDT compounds is enhanced by a low-protein diet (Banerjee et al. 1995) and physical/emotional stress (Banerjee et al. 1997b), while concurrent treatment with ascorbic acid appears to attenuate DDT-induced immunotoxicity (Koner et al. 1998). No clear pattern of relative humoral or cell-mediated immunotoxicity was seen from either DDT, DDE, or DDD (Banerjee et al. 1996). In view of the complexity of the immune system, a multiple assay battery would be helpful in order to evaluate the effects of DDT on major components of the immune system. No immunological studies by the inhalation and dermal routes of exposure were located. However, since levels in environmental media are not expected to be high enough to result in high inhalation or dermal exposures, additional inhalation or dermal exposure studies assessing immunological effects do not seem to be warranted at this time.

Neurotoxicity. While there are several studies that indicate overt clinical signs of neurotoxicity to humans acutely exposed to relatively high oral doses (Hayes 1982; Hsieh 1954; Velbinger 1947a, 1947b), there is limited information on effects of chronic exposure. Two studies, one in workers (Ortelee 1958) and one in volunteers (Hayes et al. 1956), found no evidence of adverse neurological effects among the subjects studied. However, a recent small study of retired malaria-control workers in Costa Rica found mildly impaired neurological functions among the workers relative to a control group (van Wendel de Joode et al. 2001). Evaluation of further cohorts from countries where DDT is still used may provide relevant information.

Studies in animals indicate that DDT may affect the level of neurotransmitters and the amount of lipids in the brain (Eriksson and Nordberg 1986; Herr et al. 1986; Hong et al. 1986; Hudson et al. 1985; Sanyal et al. 1986). Clinical observations of overt neurotoxicity such as tremors or convulsions have been reported (Herr and Tilson 1987; Hong et al. 1986; Matin et al. 1981). Neurotoxicity can be caused in adult animals and can be manifested as tremors or convulsions. Developmental neurotoxicity has also been reported in a number of studies (Eriksson and Nordberg 1986; Eriksson et al. 1990a, 1990b). This neurotoxicity that occurred during neonatal brain developmental stages resulted in behavioral deficits in the adult mouse and correlated with changes in muscarinic acetylcholine receptors in the cerebrum. Behavioral deficits in treated adult animals have also been reported (Sobotka 1971); however, doses were 50-fold higher than those given to neonates. Information clarifying the mechanism of action in the neonate and in the adult mouse as well as data to describe the dose-response relationship for these effects would be extremely useful in further identifying sensitive subpopulations. A battery of neurotoxicity

tests would provide additional information on the neurotoxicity in animals, which might then be related to possible neurotoxic effects in humans. No data were located on neurological effects after inhalation exposure in humans or animals or after dermal exposure in humans. A single study reported neurological effects in animals after dermal exposure to DDT (Cameron and Burgess 1945). However, since levels in environmental media are not expected to be high enough to result in high inhalation or dermal exposures, additional inhalation or dermal exposure studies assessing neurological effects do not seem to be warranted at this time.

Epidemiological and Human Dosimetry Studies. Known acute health effects in humans at high exposure levels of DDT, DDE, or DDD are irritation of the eyes, nose, and throat, sweating, nausea, headache, tremors, and convulsions. This information comes from clinical studies and from studies in which volunteers ingested measured amounts of DDT and DDE (Hayes 1982; Hsieh 1954; Velbinger 1947a, 1947b). Effects in animals include liver alterations, developmental and reproductive effects, and neurological effects. More information on the effects of DDT, DDE, or DDD could be obtained from epidemiological studies of people who, because of proximity to areas where high concentrations of DDT, DDE, or DDD have been found, may have higher exposure to DDT, DDE, or DDD. Also, more insight could be gained through future monitoring at National Priorities List (NPL) sites. Because of the virtually ubiquitous distribution of DDT in the environment, some DDT would be expected to be detected in tissues of the majority of the general population. Studies have monitored human tissue and blood for DDT and its metabolites, but no correlation has been made between the levels found in these tissues and specific disease states. Based on the known estrogenic effects of the *o,p'*-isomers and antiandrogenic properties of *p,p'*-DDE in animals, monitoring these chemicals in human tissues seems important. Identification and follow-up of cohorts who may have been exposed to DDT before its ban would be useful for evaluation of health conditions that have long latency, such as cancer. Also of interest is the assessment of long-term low level exposure as well as potential delayed effects of acute high exposure. Pharmacokinetic studies to characterize the appropriate measurement of absorbed dose would be useful in future epidemiological studies.

Biomarkers of Exposure and Effect.

Exposure. DDT, DDE, and DDD can be measured in numerous body tissues and fluids including blood, serum, urine, feces, adipose tissue, amniotic fluid, breast milk, and semen. The presence of these compounds in blood or urine can be used to determine the relative amount of exposure of an individual, but total exposure cannot be quantitated. Methylsulfonyl metabolites of DDT have also been identified in

samples of human adipose and breast milk. Other potential biomarkers of exposure have been identified, but again, exposure cannot be quantified. More information could be provided by studies designed to correlate biomarkers of exposure with the temporal aspect of exposure.

Effect. No biomarkers of effect specific for DDT, DDE, or DDD have been identified in the literature. Nonspecific biomarkers of effect include tremors, convulsions, and an increase in hepatic microsomal enzymes. Studies designed to identify specific biomarkers of effect for DDT, DDE, and DDD would be useful.

Absorption, Distribution, Metabolism, and Excretion. Qualitative information from occupational and ingestion studies indicates that humans absorb DDT via inhalation, dermal, and oral routes of administration (Laws et al. 1967; Morgan and Roan 1971, 1974; Morgan et al. 1980). No quantitative information exists concerning the rate or extent of absorption following inhalation or dermal exposure, although some information exists that quantifies the extent of absorption following oral administration. The bioavailability of DDT from environmental media, such as soil or food, is not well characterized. Quantitative data (e.g., absorption rates) from animals exposed by oral and dermal routes in different environmental media would be useful in providing information on absorption of DDT, DDE, and DDD to be used in estimating absorption in humans following exposure by these routes in these environmental media.

Information exists on the distribution of DDT, DDE, and DDD and on the storage and release from storage of these compounds (Hayes et al. 1971; Morgan and Roan 1971, 1974). However, there is limited information on the long-term release rates from adipose tissue. This information would be helpful in determining the retention time of DDT in humans.

The ultimate metabolites of DDT, which can be isolated from animals, have been well described. However, two models have been proposed for the intermediate products between parent and ultimate metabolites. These models differ in the quantity of potential electrophilic intermediates (an epoxide and an acylating agent) produced. These electrophilic metabolites have not been isolated, but their presence may be confirmed if DNA adducts are found. Some species-specific metabolic differences, especially in the area of efficiency of the conversion of DDT to DDE in hamsters relative to other species, have been identified (Gold and Brunk 1982, 1983). However, the role of these metabolic differences in species-specific sensitivity to toxicity, especially carcinogenicity, is not well characterized. Further information

concerning the species-specific metabolic differences would be useful to provide more specific information when comparing the toxicokinetics of these substances in humans and animals.

Comparative Toxicokinetics. Metabolism studies indicate that the metabolism of DDT is qualitatively similar among several species, but that the efficiency of formation of certain metabolites and the proportion of metabolites excreted may be quantitatively different (Gingell 1976; Gold and Brunk 1982, 1983, 1984; Peterson and Robison 1964). Comparisons of elimination rates of DDT from fat showed that the process is the slowest in humans, followed by monkeys, dogs, and rats (Morgan and Roan 1974). Rats eliminate DDT 10 to 100 times faster than humans. Morgan and Roan (1974) suggested that the differences in elimination in rats could be due to differences in liver metabolism, gut bacterial metabolism, enterohepatic recirculation, or factors related to the accessibility of plasma-transported pesticide to the excretory cells of the liver. Differences in the metabolism of DDT among species may account for differences in toxic responses, especially cancer. The potential for DDT to produce toxic effects has been investigated in rats, dogs, mice, guinea pigs, and nonhuman primates, but the animal species that serves as the best model for extrapolating results to humans has not been determined. Ethical considerations limit the amount of information that can be obtained in humans, but analysis of urine of persons with known exposure to DDT to determine levels of parent compound and metabolites could provide more information on the metabolic pathways in humans. This information could help to identify the most appropriate animal model for extrapolation to humans.

Methods of Reducing Toxic Effects. The available data indicate some ways in which peak absorption of DDT, DDE, and DDD might be reduced following oral or dermal exposure (Dreisbach 1983; HSDB 2002a, 2002b, 2002c, 2002d). Studies that examine the efficacy of gastric lavage with mannitol versus other cathartics or of combinations of cathartics would be useful. Also, studies that evaluate the effectiveness of intestinal absorbants such as sodium muconate or cholestyramine would provide valuable information. No data were located regarding methods for reducing absorption following inhalation exposure.

In humans, DDT and its fat soluble metabolites are stored in adipose tissue, where they are retained for long periods of time. During that time, short term toxicity may be expected to be minimal since exposure to target organs would be via the circulation and the equilibrium between blood and adipose tissue is such that blood levels are about 1:280 of fat levels; however, there is still the potential for chronic toxicity and interactions with other environmental chemicals. If very large amounts of DDT or its metabolites are stored in adipose tissue, then rapid release of DDT or its metabolites into the circulation during

mobilization of fat stores, such as during rapid weight loss, may result in certain types of toxic effects. Release of DDT into the blood of a pregnant woman during gestation-related fat mobilization might result in toxicity to the developing fetus. Also, mobilization of adipose fat for lactation might provide an increased amount of DDT to a breast-feeding infant. Development of methods to enhance excretion of adipose-sequestered DDT, while minimizing toxicity would be beneficial in reducing the body burden.

DDT induces neurotoxicity (tremors and convulsions) by various mechanisms (see Section 3.5). No method is currently available for directly reducing the neurotoxic effects of DDT, DDE, or DDD by interfering with the mechanisms of action, although there are a variety of pharmacological methods for alleviating nonspecific symptoms.

Children's Susceptibility. The information on health effects of DDT and analogues in humans is derived mainly from accidental exposure and from controlled studies in volunteers, and the main adverse effect is neurotoxicity. No reports on exposed children were found, but it is reasonable to assume that children will exhibit signs and symptoms similar to those in adults under similar exposure conditions. There is no information on whether the developmental process is altered in humans exposed to DDT. Studies in animals have shown that DDT and analogues can alter the development and maturation of the male and female reproductive system (Bitman and Cecil 1970; Clement and Okey 1972; Duby et al. 1971; Gellert et al. 1972; Gray et al. 1999; Kelce et al. 1995, 1997; Loeffler and Peterson 1999; Singhal et al. 1970; You et al. 1998, 1999a). Hormone-like effects have been reproduced in many types of *in vitro* test systems, but there is a need for standardized operating procedures. *In vivo* tests are preferred over *in vitro* assays because they take into account pharmacokinetic and pharmacodynamic interactions.

There are no adequate data to evaluate whether pharmacokinetics of DDT in children are different from adults. DDT and analogues can cross the placenta and are transferred to offspring via breast milk. It is unknown whether the efficiency of gastrointestinal absorption of DDT and analogues in nursing neonates differs from adults and what influence the fat content of human milk might make. Further information on the dynamics of DDT and analogues during pregnancy and lactation, such as further refinement of PBPK models to include humans would be useful. Important information was recently published on estimates of body burden of DDE that result from nursing by using a model that incorporates a wide array of variables (LaKind et al. 2000). The only existing PBPK model for DDT is that of You et al. (1999b), which focuses on pregnant and lactating Sprague-Dawley rats. There is no information to evaluate whether metabolism of DDT is different in children than in adults since the specific phase I and II enzymes

involved in DDT metabolism have not been identified; it is unknown which phase I P-450 isozymes metabolize DDT.

There is little evidence about whether children or young animals differ in their susceptibility to the health effects from DDT from adults. In fact, some animal studies found that young rats are less susceptible than older ones to the acute neurotoxic effects produced by a single dose of DDT (Lu et al. 1965). However, the relevance of this information to human health is unknown. There is evidence that acute perinatal exposure to DDT in mice results in altered behavioral responses in the mice tested as adults (Eriksson et al. 1992, 1993; Johansson et al. 1995, 1996; Talts et al. 1998). Research efforts should focus on the possible underlying mechanism(s) that are responsible for such long-lasting postexposure alterations.

Continued research into the development of sensitive and specific biomarkers of exposure and effect for DDT and analogues would be valuable for both adults and children. There are no data on the interactions of DDT with other chemicals in children or adults. There are no pediatric-specific methods to reduce peak absorption for DDT following exposure, to reduce body burdens, or to interfere with the mechanism of action. Based on the information available, it is reasonable to assume that methods recommended for treating adults will also be applicable to children; however, these methods need to be validated in children.

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

3.13.3 Ongoing Studies

A number of studies concerning health effects associated with DDT, DDE, and DDD have been identified in the Federal Research in Progress (FEDRIP 2001) database and are listed in Table 3-7.

Table 3-7. Ongoing Studies on the Health Effects of DDT and DDT Analogues

Investigator	Affiliation[a]	Research description	Sponsor
Bhatia, R	Public Health Institute, Berkeley, CA	DDT and other organochlorine pesticides and male genital anomalies	NIEHS
Charles, MJ	University of California, Environmental Toxicology Center, Berkeley, CA	Exploration of linkages among organochlorines (DDT, DDE), oxidative DNA damage and breast cancer	USDA
Clark, DR	Columbia Environmental Research Center Columbia, MO	Ecotoxicological investigations of effects of DDT on native wildlife species	USGS
Cohn, BA	Public Health Institute, Berkeley, CA	Breast cancer and organochlorines	NCI
Cohn, BA	Public Health Institute, Berkeley, CA	Prenatal exposure to organochlorine and fecundity	NIEHS
Darvill, T	SUNY at Oswego, Oswego, NY	Prenatal exposure to PCBs, DDE and other contaminants on cognitive development in school-age children	NIEHS
Denslow, ND	University of Florida, Gainesville, FL	Chlorinated hydrocarbons and non ionic surfactants effect on endocrine functions	NIEHS
Fraser, PA	Center for Blood Research, Boston, MA	Genes and chemical exposure associated with SLE risk	NIEHS
Gammon, MD	Columbia University Health Sciences, New York, NY	Breast cancer and the environment on Long Island	NCI
Gross, TS	University of Florida, Gainesville, FL	Endocrine, reproductive, and developmental disrupting effects of chlorinated hydrocarbons on wildlife	USGS

Table 3-7. Ongoing Studies on the Health Effects of DDT and DDT Analogues *(continued)*

Investigator	Affiliation	Research description	Sponsor
Gross, TS	Florida Caribbean Science Center Gainesville, FL	Immuno-toxicity and endocrine disruption in Lake Apopka alligator neonates	USGS
Gurpide, E	Mount Sinai School of Medicine of CUNY, New York, NY	Hormonal activity of chlorinated hydrocarbons from New York harbor sediments	NIEHS
Hauser, RB	Harvard University, School of Public Health, Boston, MA	Environmental organochlorines and semen quality	NIEHS
Henny, CJ	Forest and Rangeland Ecosystem Science Center Corvallis, OR	Effects of persistent organochlorines on nesting success of Osprey along the Columbia River system	USGS
Hothem, R	Western Ecological Research Center Sacramento, CA	Contaminant residues and their effects on reproductive success of aquatic birds nesting at Edwards Air Force Base	USGS
Hothem, R	Western Ecological Research Center Sacramento, CA	Reproduction by black-crowned night-herons and snowy egrets on Alcatraz Island, California	USGS
Korrick, SA	Harvard University, School of Public Health, Boston, MA	In utero PCB, pesticide and metal exposure and childhood cognition	NIEHS
Landrigan, PJ	Mount Sinai School of Medicine of CUNY, New York, NY	Environmental distribution and toxic effects on human health of PCBs and DDT in New York City	NIEHS
Longnecker, MP	NIEHS	Human health effects of exposure to organochlorine compounds	NIEHS
Matsumura, F	University of California, Davis, CA	Mechanism of insecticides on the development of prostate cancer	USDA
Remington, P	University of Wisconsin Madison, Madison, WI	Regional variation of breast cancer rates in Wisconsin	NCI
Rice, RH	University of California, Environmental Toxicology Center, Davis, CA	Toxic and mutagenic responses of keratinocytes to heterocyclic amines and polycyclic aromatic hydrocarbons	USDA

Table 3-7. Ongoing Studies on the Health Effects of DDT and DDT Analogues *(continued)*

Investigator	Affiliation	Research description	Sponsor
Rogan, WJ	NIH, NIEHS	Human exposure to halogenated aromatic compounds	NIEHS
Schwartz, S	Fred Hutchinson Cancer Research Center, Seattle, WA	Risk associated with exposure to phytoestrogens, organochlorines, and fibroids	NIEHS
Seegal, RF	Wadsworth Center, Albany, NY	Developmental effects of fish borne toxicants in the rat	NIEHS
Stellman, SD	American Health Foundation, Valhalla, NY	Epidemiology of breast cancer	NCI
Terranova, PF	University of Kansas Medical Center, Kansas City, KS	Effects of dioxin, xenoestrogen, and estradiol on reproductive processes	NSF
Trosko, JE	Michigan State University, East Lansing, MI	Cell-cell communication carcinogenesis	NCI
Trosko, JE	Michigan State University, East Lansing, MI	Epigenic effects of environmental toxicants on cellular communication pathways	NIEHS
Weston, A	Mount Sinai School of Medicine of CUNY, New York	Effects of PCB-containing river sediments on carcinogen metabolism	NIEHS
Windham, GC	Public Health Institute, Berkeley, CA	Organochlorine compounds and menstrual cycle function	NIEHS
Wolff, MS	Mount Sinai School of Medicine of CUNY, New York	PBC and DDE body burden and breast cancer	NIEHS

[a]Post office state abbreviations used

DNA = deoxyribonucleic acid; NCI = National Cancer Institute; NIEHS = National Institute of Environmental Health Sciences; NSF =National Science Foundation; PCB = polychlorinated biphenyl; USDA = United States Department of Agriculture; USGS = United States Geological Survey