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CHAPTER 2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective 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. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.

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

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 by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

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), data for other isomers of these compounds will be discussed when available and 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 to indicate the total sum of DDT, DDE, and DDD.

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), and DDE is the principal metabolite of DDT (Chapter 3). 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 the *o,p'*- and *p,p'*- isomers of DDT and technical-grade DDT.

2. HEALTH EFFECTS

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to DDT, DDE, and DDD, but may not be inclusive of the entire body of literature.

Most laboratory animal toxicity studies of DDT, DDE, or DDD have involved oral exposure; there are only a small number of available inhalation or dermal contact studies (see Figure 2-1). The most widely studied health effects examined in human and animal studies were reproductive, neurological and developmental effects, and cancer (Figure 2-1). Considerable focus also has been given to effects on body weight and the liver in animal studies, endocrine, and immunological effects in human and animal studies, and human studies of risk for DMT2 (Figure 2-1). The human study counts in Figure 2-1 are principally for epidemiological studies examining possible associations between adverse health outcomes and levels of DDT, DDE, or DDD in samples of tissues or body fluids. Oral exposure through food and drinking water is the assumed principal route of exposure of the subjects in these studies. Studies that looked for associations between adverse health outcomes and more subjective measures of exposure (e.g., self-reported exposure history or work history records) were not included in the analyses described in this chapter. This chapter also discusses the small number of controlled-exposure human studies principally conducted in the 1940s through the 1950s, in which human subjects ingested, inhaled, or were dermally exposed to measured doses of DDT for acute or intermediate durations (principally technical DDT).

Levels of significant exposure (LSEs) for each route and duration of animals orally exposed are presented in Table 2-1 and illustrated in Figure 2-2. 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 endpoint 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 endpoints. 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 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.

A User's Guide has been provided at the end of this profile (see Appendix C). This guide should aid in the interpretation of the tables and figures for LSEs and MRLs.

The early controlled-exposure studies of human adult subjects given acute- or intermediate-duration exposure to oral doses of DDT provide adequate descriptions of exposure-response relationships for self-reported neurological symptoms and NOAELs for liver effects assayed by serum enzyme levels. In contrast, none of the epidemiological studies provide adequate evidence to describe LSEs to DDT, DDE, or DDD. For most of the health outcomes evaluated in multiple epidemiological studies, inconsistent evidence is available for associations with levels of DDT, DDE, or DDD in tissues or biological fluids, with the exception of consistent evidence for associations with: increased risk for abortions or preterm births (see Section 2.16); increased prevalence for wheeze in infant or child offspring (see Section 2.14); increased prevalence of DMT2 (see Section 2.18); and increased risk for liver cancer (see Section 2.19). In addition, consistent evidence for no associations was found in studies of breast cancer in women, pancreatic cancer, and endometrial cancer (see Section 2.19). Although epidemiological studies provide consistent evidence of associations (or no associations) between DDT and some health outcomes, these data do not establish causality. Other factors, particularly co-exposure to other highly lipophilic compounds (e.g., PCBs, CDDs, CDFs), may have influenced the study results. Some of the epidemiological studies have statistically adjusted for exposure to one or more non-DDT compound to decrease the uncertainty; however, most studies did not include this adjustment.

Health effects of DDT, DDE, DDD, and their related isomers have been evaluated in many animal studies (see Figure 2-1). Nearly all of the studies evaluated were oral exposure studies; no animal inhalation studies were identified. The most examined noncancer endpoints were reproductive, neurological, developmental, body weight, and hepatic effects. The most reliable health effects data come from oral studies of animals administered DDT (metabolites or isomers). Limited animal data for dermal exposure studies indicated that DDT and related compounds are not dermal irritants. Results from the oral animal studies identify the following targets of DDT, DDE, and DDD toxicity.

- **Hepatic effects:** Acute-, intermediate-, and chronic-duration oral exposures of laboratory animals to DDT, DDE, or DDD have been associated with mild-to-severe hepatic effects, such as induction of microsomal CYP450 xenobiotic metabolizing enzymes, liver hypertrophy, hepatocellular eosinophilic foci, and, less frequently, hepatocellular necrosis.

- **Neurological and neurodevelopmental effects:** Tremors, convulsions, and intermittent myoclonic movements have been observed in mature laboratory animals after acute-, intermediate- and chronic-duration exposure to technical DDT, *p,p'*-DDT, or *p,p'*-DDE at relatively high exposure levels. Young laboratory mice appear to be particularly sensitive to brain neurochemical changes and associated neurobehavioral changes from acute exposure to low doses of technical DDT during critical windows of neurodevelopment (PND 10, but not PND 3 or 18).

- **Reproductive and developmental reproductive effects:** Reproductive effects of DDT and related compounds in laboratory animals have been observed at relatively high dose levels. The observed effects include decreased male reproductive tissue weight or increased weight of the uterus after acute-duration exposures and decreased fertility after intermediate- or chronic-duration exposures. Gestational exposure to *p,p'*-DDT or *p,p'*-DDE has been associated with decreased prostate weight and decreased AGD in male offspring, decreased fertility in male and female offspring, and increased resorptions in female offspring after impregnation. Gestational exposure to *o,p'*-DDD or *p,p'*-DDT has been associated with delayed vaginal opening and increased ovary weight in female offspring. Exposure during gestation and lactation was associated with decreased fertility in female offspring at a high dose level of *o,p'*-DDT, but not at 5–6-fold lower doses of *o,p'*-DDT or *p,p'*-DDT.

- **Body weight effects:** Decreased body weight or body weight gain have been observed in laboratory animals orally exposed to DDT and related compounds after acute-, intermediate-, or chronic-duration exposures at relatively high dose levels.

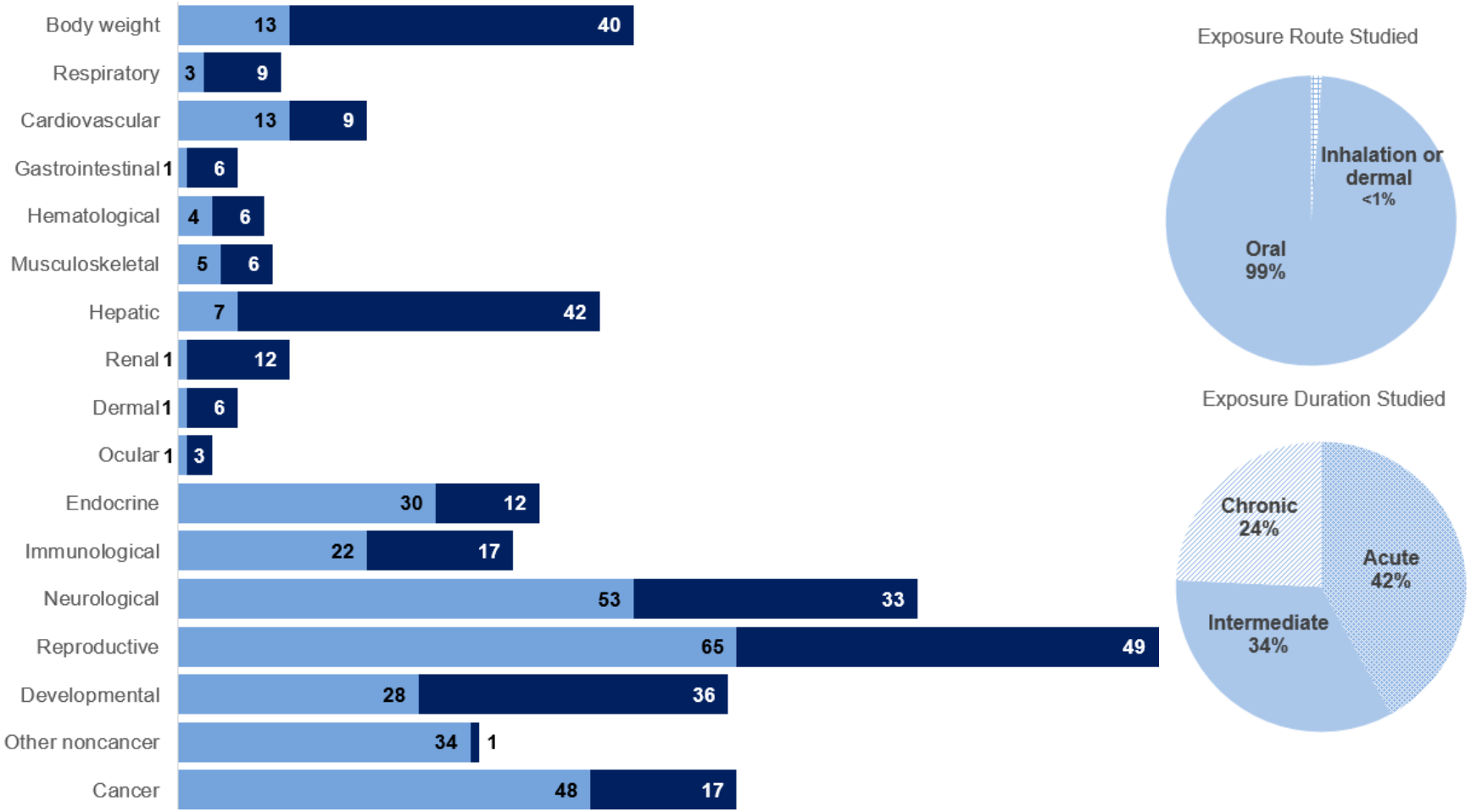
- **Immunological effects:** Suppression or stimulation of various immune system responses have been observed in rats and mice exposed to dietary doses of technical DDT, *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, or *o,p'*-DDD, but evidence is weak for weight changes or histological changes in immune system organs or tissues in laboratory animals after intermediate- or chronic-duration exposures.

- **Cancer:** The liver appears to the primary cancer target for isomers of DDT, DDE, and DDD in laboratory animals.

Figure 2-1. Overview of the Number of Studies Examining DDT, DDE, and DDD Health Effects

Most studies examined the potential reproductive, developmental, and cancer effects of DDT, DDE, and DDD

More studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 636 studies (including those finding no effect) have examined toxicity; most animal studies examined multiple endpoints.

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
ACUTE EXPOSURE									
1	Human 7 M, 4 F	Once (F)	5.1–120.5	CS	Neuro	10.3		16	Convulsions
DDT (NS) **Hsieh 1954**									
2	Monkey (Rhesus) 4 NS	Once (G)	0, 150	OW GN BI	Hepatic		150		Increased serum LDH, AP, and aminotransferases
DDT (NS) **Agarwal et al. 1978**									
3	Monkey (Rhesus) NS M	Once (G)	0, 150	BW GN HP BI	Neuro		150		Decreased CNS total lipids, phospholipids, and cholesterol
DDT, technical grade **Sanyal et al. 1986**									
4	Rat (Sprague-Dawley) 5–6 F	GD 13.5–17.5 (G)	0, 50, 100	CS MX BI BW HP BC DX	Bd wt	100 F			
					Repro	100 M			No change in plasma corticosterone, LH, or intratesticular testosterone levels
					Develop	50 M	100 M		Fetal alterations of steroidogenic cells; histological and ultrastructural alterations in fetal-type Leydig cells on ED 19.5 (vacuolated and reduced number of lipid droplets in Leydig cells, partially degenerated mitochondria in adrenal - incidence data not reported; no change in fetal plasma corticosterone, LH, or intratesticular testosterone levels; no effect on male or female embryo weights on ED 19.5
***p,p′*-DDE** **Adamsson et al. 2009**									
5	Rat (NS) NS	Once (NS)	NS	LE	Death			400	LD50
DDD (NS) **Ben-Dyke et al. 1970**									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
6	Rat (NS) NS	Once (NS)	NS	LE	Death			300	LD50
DDT (NS) Ben-Dyke et al. 1970									
7	Rat (NS) 5 NS	Once (G)	NS	LE	Death			800	LD50
DDT (NS) Cameron and Burgess 1945									
8	Rat (Wistar) 20 F	7 days, PNDs 23–30 (F)	0, 50, 100, 200, 300	HP	Develop	50 F	100 F		Increased uterus weight; premature vaginal opening
***o,p'*-DDT Clement and Okey 1972**									
9	Rat (Wistar) NS	5 or 12 days (G)	0, 40	GN OW	Hepatic		40		18% increase in relative liver weight; increased liver GSH and AHH enzyme activities
DDT (NS) DeWaziers and Azais 1987									
10	Rat (DA/Han) 6 F	3 days 1 time/day (G)	0, 10, 100, 500	OW BI	Repro	10 F	100 F		Significant increase in wet uterine weight
***o,p'*-DDT Diel et al. 2000**									
11	Rat (Sherman) NS B	Once (G)	NS	LE	Death			4,000	LD50
DDD-technical Gaines 1969									
12	Rat (Sherman) B	Once (G)	NS	LE	Death			880	LD50
DDT, technical grade Gaines 1969									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
13	Rat (Sherman) B	Once	NS	LE	Death			113	LD50
***p,p′*-DDT** **Gaines 1969**									
14	Rat (Sprague-Dawley) 15 F	GDs 15–19 (G)	0, 28	OW BW	Develop		28 F		Delayed vaginal opening (2 days)
***o,p′*-DDD** **Gellert and Heinrichs 1975**									
15	Rat (Sprague-Dawley) 15 F	GDs 15–19 (G)	0, 28	BW OW	Develop		28		11.9% Increase in body weight and no effects on estrous cycle, vaginal opening, or ovary, adrenal, or anterior pituitary weights in adult offspring
***o,p′*-DDE** **Gellert and Heinrichs 1975**									
16	Rat (Sprague-Dawley) 15 F	GDs 15–19 (G)	0, 28	BW OW	Develop		28		13% increase in body weight and no effects on estrous cycle, vaginal opening, or ovary, adrenal, or anterior pituitary weights in offspring
***o,p*'-DDT** **Gellert and Heinrichs 1975**									
17	Rat (Sprague-Dawley) 15 F	GDs 15–19 (G)	0, 28	OW BW	Develop		28 F		26% Decrease in ovary weight; 9% increase body weight in offspring; no effects on estrous cycle, or vaginal opening
***p,p′*-DDT** **Gellert and Heinrichs 1975**									
18	Rat (Sprague-Dawley) 10 M	Once (GO)	0, 25, 50, 100, 200	CS OF	Endocr	25	50		Reduced capacity to concentrate iodine in thyroid
DDT, technical grade **Goldman 1981**									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
19	Rat (Long-Evans) 8 F	5 days GDs 14–18 (GO)	0, 100	OW HP GN DX	Develop		100 M		At 10 months of age, significant decrease in ventral prostate weight; percent of animals with areolas; and mean number of retained nipples; no effect on pup weight or body weight as adults
***p,p′*-DDE Gray et al. 1999**									
20	Rat (Sprague-Dawley) 11 F	5 days GDs 14–18 (GO)	0, 100	OW HP DX GN	Develop		100 M		At 15 months of age, decreased weight of glans penis, epididymis, and ventral prostate; reduced AGD; increased percent with areolas and number with retained nipples; no effect pup weight or body weight as adults
***p,p′*-DDE Gray et al. 1999**									
21	Rat 32 M	Once (G)	0, 75		Neuro			75	Tremors
DDT (NS) Herr and Tilson 1987									
22	Rat 12 M	Once (G)	0, 50, 75, 100		Neuro			50	Tremors
DDT (NS) Herr et al. 1985									
23	Rat (Wistar) 40 M	Once (GO)	160	GN HP BI	Neuro			160 M	Tremors
DDT (NS) Hietanen and Vainio 1976									
24	Rat (Fischer-344) 4 M	Once (G)	0, 25, 50, 75, 100	BI CS	Neuro	25 M	50 M		Tremors, more severe at 75 and 100 mg/kg/day; increased brain 5-HIAA, aspartate, and glutamate
***p,p′*-DDT Hong et al. 1986; Hudson et al. 1985**									
25	Rat (Albino Sprague-Dawley) 18 M	Once (G)	0, 100-600	GN BI	Neuro		100 M	200 M	Serious LOAEL: severe myoclonus, tremors, seizures; increased brain 5-HIAA LOAEL: intermittent myoclonic movement
***p,p′*-DDT Hwang and Van Woert 1978**									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
26	Rat (Sprague-Dawley) 6 M	10 days (G)	0, 25, 50, 100	CS OW BC BW	Bd wt	100 M			
					Hepatic		25 M		Increased absolute liver weight (42%)
					Renal	100 M			
					Repro	25 M	50 M		Inhibited regrowth of TP-inhibited accessory sex organs; decreased seminal vesicle weight (34%)
***p,p′*-DDE Kang et al. 2004**									
27	Rat (Long-Evans) 8 F	1 time/day GDs 14–18 (GO)	0, 100	DX	Develop		100 M		Males: reduced AGD at birth; PND 13 retained thoracic nipples
***p,p′*-DDE Kelce et al. 1995**									
28	Rat (Long-Evans) 6 M	1 time/day 4 days (GO)	0, 200	CS OW	Bd wt		200 M		Decreased body weight (29.8%)
					Repro		200 M		Reduced seminal vesicle and ventral prostate weight; no effect on serum testosterone
***p,p′*-DDE Kelce et al. 1995**									
29	Rat (Sprague-Dawley) 10 M	1 time/day 5 days (GO)	0, 200	CS OW	Repro		200 M		Reduced seminal vesicle and ventral prostate weight; no effect on serum testosterone
***p,p′*-DDE Kelce et al. 1997**									
30	Rat (Wistar) 6 M	14 days 1 time/day (GO)	0, 12	BI HP OW	Hepatic		12 M		Increased relative liver weight; necrotic changes; increased cell proliferation peaked at exposure day 3
DDT, technical grade Kostka et al. 2000									
31	Rat 6 M	2 days, PNDs 4 and 5 (G)	0, 500	BW OW HP	Repro		500		Decreased number of fetuses and implantations in non-exposed dams mated with exposed males
DDT (NS) Krause et al. 1975									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
32	Rat (Long- Evans) 5 M	4 days (G)	0, 5, 12.5, 25, 50, 100	CS OW BI BW	Hepatic Repro	12.5 M 100 M	25 M		Increased relative liver weight (32%) No changes in relative ventral prostate or seminal vesicles weight, serum testosterone, or dihydrotestosterone levels
***p,p'*-DDE** **Leavens et al. 2002**									
33	Rat (Sprague-Dawley) 6 M	Once or 5 days (GO)	50	OW HP OF	Repro	50 M			No histopathological changes to testes or epididymis; no effect on testes, epididymis, prostate, or seminal vesicle weights, epididymal sperm counts, motility, or morphology
***p,p'*-DDT** **Linder et al. 1992**									
34	Rat (Sprague-Dawley) 6 M	Once (GO)	100	OW, HP, OF	Repro	100 M			No histopathological changes to testis or epididymis; no effect on testis, epididymis, prostate, or seminal vesicle weights; increased sperm counts in cauda only; no effect on sperm motility or morphology
***p,p'*-DDT** **Linder et al. 1992**									
35	Rat (Holtzman) 3–6 F	1 time/day GDs 14–18 (GO)	0, 1, 10, 50, 100, 200	DX OW BW	Develop	10 M	50 M		Reduced AGD on PND 1 and relative ventral and dorsolateral prostate weights on PND 21; increased nipple retention starting at 100 mg/kg/day; delayed age at preputial separation at 200 mg/kg/day; no effect on weights of seminal vesicles, testes, or epididymides; prostate weight changes were transient; no effect on sperm production
***p,p'*-DDE** **Loeffler and Peterson 1999**									
36	Rat (NS) 10 B	Once (G)	346.3–553.9	LE	Death			437.8	
DDT, technical grade **Lu et al. 1965**									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
37	Rat (NS) 10 B	4 days (G)	216.8–359.2	LE	Death			279.2	4-Day LD50, preweanling; cumulative dose
DDT, technical grade Lu et al. 1965									
38	Rat (NS) 10 B	Once (G)	4,000	LE	Death			4000	
DDT, technical grade Lu et al. 1965									
39	Rat (NS) 10 B	Once (G)	317.2–397.8	LE	Death			355.2	LD50, weanling rats
DDT, technical grade Lu et al. 1965									
40	Rat (NS) 10 B	4 days (G)	225.6–364.8	LE	Death			285.6	4-Day adult LD50; cumulative dose
DDT, technical grade Lu et al. 1965									
41	Rat (NS) 10 B	Once (G)	158.7–238.3	LE	Death			194.5	LD50, adult rats
DDT, technical grade Lu et al. 1965									
42	Rat (Fischer-344) 3/dose M	2 weeks (F)	0, 0.85, 2.6, 7.7, 23, 69, 200	BC BW EA GN BH	Bd wt	200 M			No change in body weight or body weight gain
					Hepatic	69 M	200 M		Increased relative liver weight
					Neuro	200 M			No muscle tremors or hyperexcitability
***p,p′*-DDD Nims et al. 1998**									
43	Rat (Fischer-344) 3/dose M	2 weeks (F)	0, 0.85, 2.5, 7.6, 23, 69, 200	BC BW EA GN BH	Bd wt	200 M			
					Hepatic	7.6 M	23 M		Increased relative liver weight
					Neuro	200 M			No muscle tremors or hyperexcitability
***p,p′*-DDE Nims et al. 1998**									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
44	Rat (Fischer-344) 3/dose M	2 weeks (F)	0, 0.94, 2.8, 8.5, 25, 76, 200	BC BW EA GN BH	Bd wt	200 M			
					Hepatic	8.5 M	25 M		Increased relative liver weight
					Neuro	200 M			No muscle tremors or hyperexcitability
***p,p'*-DDT** **Nims et al. 1998**									
45	Rat 8 M	Once (GO)	200, 600, 1,000	CS BI	Neuro	200		600	Convulsions, myoclonus
***p,p'*-DDT** **Pranzatelli and Tkach 1992**									
46	Rat NS M	Once (G)	0, 50, 100, 200, 400, 600		Neuro	50 M	200 M	400 M	LOAEL: Intermittent myoclonus Serious LOAEL: Continuous myoclonus
***p,p'*-DDT** **Pratt et al. 1986**									
47	Rat (Fischer-344, Albino) 6 M	Once (G)	0, 75	BI	Neuro		75 M		Tremors and increased brain 5-HIAA
***p,p'*-DDT** **Tilson et al. 1986**									
48	Rat (Fischer-344) M	Once (G)	0, 25, 50, 100	BH	Neuro	25 M	50 M	100 M	Serious LOAEL: severe tremors and death in some rats LOAEL: Hyperirritability and tremors; more severe at 100 mg/kg/day NOAEL: No tremors; no effects on learning a conditioned response at up to 100 mg/kg/day
***p,p'*-DDT** **Tilson et al. 1987**									
49	Rat (Fischer-344) 5–6 M	2 weeks (F)	0, 0.5, 5.0, 50	HE HP BC	Hemato	0.5 M	5 M		Increase in total iron binding capacity
***p,p'*-DDT** **Tomita et al. 2013**									
50	Rat (Fischer-344) 33 M	7 days (F)	0, 106	BI BC OW CS	Hepatic		106 M		Increased absolute and relative liver weight
***p,p'*-DDT** **Tomiyama et al. 2003**									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
51	Rat (Fischer-344) 36 M	Once (G)	0, 106	BI BC OW	Neuro			106 M	Tremors and convulsions, hyperactivity
***p,p′*-DDT Tomiyama et al. 2003**									
52	Rat (Fischer-344) 5 M	14 days (F)	0, 5, 16, 50		Hepatic		5 M		
***p,p′*-DDT Tomiyama et al. 2004**									
53	Rat (Sprague-Dawley) 8–11 F	1 time/day GDs 14–18 (GO)	0, 10, 100	CS BI DX	Develop	100 F			No effect on postnatal body weight or AGD on PND 2, no effect on age of vaginal opening
					Develop		10 M		PND 13 males retained thoracic nipples; no effect on postnatal body weights, AGD, age of preputial separation, no effect on reproductive organ weights or serum testosterone
***p,p′*-DDE You et al. 1998**									
54	Rat (Long-Evans) 8–11 F	1 time/day GDs 14–18 (GO)	0, 10, 100	CS BI DX	Develop		100 F		No effect on postnatal body weights, AGD, or age of vaginal opening
						10 M	100 M		Reduced anogenital distance on PND 2; retained thoracic nipples on PND 13; no effect on postnatal body weight, age of preputial separation, or AGD; no effect on reproductive organ weights; no change in serum testosterone
***p,p′*-DDE You et al. 1998**									
55	Rat (Long-Evans) 5–8 M	4 days (F)	0, 70	BW OW	Bd wt	70 M			
					Renal	70 M			
					Repro		70 M		Decreased ventral prostate weight (30%); epididymis (12.7%), and seminal vesicle (47%) weights on PND 85; no significant changes in serum testosterone or LH
***p,p′*-DDE You et al. 1999a**									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
56	Mouse (NMRI) 12 M	Once PND 10 (GO)	0, 0.5	BI	Develop		0.5 M[b]		7 days after exposure: increased muscarinic receptor binding, decreased high affinity, and increased low affinity binding; no effect on sodium-dependent choline uptake; no changes 24 hours after exposure
DDT (NS) Eriksson and Nordberg 1986									
57	Mouse (NMRI) 12 M	Once PND 10 (GO)	0, 0.5	BH	Develop		0.5 M[b]		Delayed habituation observed as increased motor activity
DDT (NS) Eriksson et al. 1990a									
58	Mouse (NMRI) 12 B	Once PND 10 (GO)	0, 0.5	DI	Develop		0.5 M[b]		Increased motor activity (reduced habituation) at 4 months; increased potassium evoked Ach release; reduced density of muscarinic receptors in cerebral cortex at 3 months; no change in choline acetyltransferase activity
DDT (NS) Eriksson et al. 1990b									
59	Mouse (NMRI) 12 M	Once at either PND 3, 10, or 19 (GO)	0, 0.5	BI CS BW BH	Develop		0.5 M[b]		At 4 months of age in males dosed at 10 days: decrease in cerebral cortex muscarinic acetylcholine receptor binding; delayed habituation; no change in proportion of HA and LA binding sites or affinity constants; no differences in body weight gain; no changes in mice dosed on PND 3 or 19
DDT, technical grade Eriksson et al. 1992									
60	Mouse (NMRI) M	Once PND 10 (GO)	0, 0.5	BI BH	Develop		0.5 M[b]		At 5 months of age: delayed habituation (increased motor activity); decrease in cortical muscarinic acetylcholine receptors; no change in high affinity or low affinity muscarinic binding sites
DDT (NS) Eriksson et al. 1993									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
61	Mouse (albino) 15 M	Once (GO)	0, 160	CS	Neuro			160 M	Tremors
***p,p'*-DDT** **Hietanen and Vainio 1976**									
62	Mouse (C57BL/6H) 10 M/dose	5 days (G)	0, 0.4, 2	BC BW	Bd wt	2 M			
					Other noncancer	0.4 M	2 M		Fasting Hyperglycemia 7 days after last exposure; no effect on serum insulin, glucagon, leptin, or resistin levels of glucose tolerance
***p,p'*-DDE** **Howell et al. 2014**									
63	Mouse (NMRI) NS M	Once PND 10 (GO)	0, 0.5	BI DX BH	Develop		0.5 M[b]		Decreased muscarinic receptors in cerebral cortex; increased spontaneous activity at 5 months
DDT, technical grade **Johansson et al. 1995**									
64	Mouse (NMRI) NS M	Once PND 10 (GO)	0, 0.5	BI DX BH	Develop		0.5 M[b]		Decreased muscarinic receptors in cerebral cortex; increased spontaneous activity at 5 and 7 months
DDT, technical grade **Johansson et al. 1996**									
65	Mouse (Inbred Swiss) NS	Once (G)	NS	LE	Death			300 M	LD50
DDT, technical grade **Kashyap et al. 1977**									
66	Mouse (Albino) 10 M	Once (G)	0, 200, 400, 600	CS BI	Neuro			200	Convulsions
***p,p'*-DDT** **Matin et al. 1981**									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
67	Mouse (C3H) NS B	6 days (F)	M: 87.5; F: 85.7	LE	Death			85.7 F	50% of mice died after a 6-day feeding period
***p,p'*-DDT Okey and Page 1974**									
68	Mouse (CD-1) NS B	7 days GDs 11–17 (GO)	0, 0.018, 0.18	DX BH	Develop	0.018 M			No significant effect on behavior or aggression
***o,p'*-DDT Palanza et al. 1999**									
69	Mouse (CF-1) 6–10 F/dose	GDs 11–17 (G)	0, 0.02, 0.2, 2, 20, 100	MX BW DX	Bd wt	100 F			No effect on maternal body weight
***o,p'*-DDT Palanza et al. 2001**									
70	Mouse (CF1) 8 NS	1 week (F)	0, 42.9	BW BI OW	Hepatic	42			29% increase absolute liver weight; increase cytochrome-c reductase and P-450
DDE (NS) Pasha 1981									
71	Mouse (CF1) 4 M, 4 F	Once (G)	NS	LE	Death			251.3 F 237 M	LD50 LD50
DDT, technical grade Tomatis et al. 1972									
72	Mouse (CF1) 8 B	Once (G)	NS	LE	Death			810	LD50
***o,p'*-DDE Tomatis et al. 1972**									
73	Mouse (CF1) 8 B	Once (G)	NS	LE	Death			1466	LD50
***p,p'*-DDD Tomatis et al. 1972**									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
74	Guinea pig (NS) 5 NS	Once (G)	NS	LE	Death			400	LD50
DDT (NS) Cameron and Burgess 1945									
75	Guinea pig (NS) 10 M	Once (G)	0, 160	GN HP BI	Neuro			160	Paralysis of hind legs
DDT (NS) Hietanen and Vainio 1976									
76	Hamster (NS) 8 F	Once (G)	0, 160	GN HP BI	Neuro	160			
DDT (NS) Hietanen and Vainio 1976									
77	Dog (NS) NS	14 days	0, 50	BW HP CS	Cardio			50	Decrease in contractile force
					Endocr		50		Decreased plasma glucocorticoids
***o,p'*-DDD Cueto 1970**									
78	Dog (NS) NS	10 days (C)	0, 138.5	HP BC	Endocr			138.5	Adrenal hemorrhage
***o,p'*-DDD Kirk et al. 1974**									
79	Dog (mongrels and beagles) 10 NS	Once (C)	0, 200	OW HP	Endocr		200		Adrenal vacuolization and necrosis
***p,p'*-DDD Powers et al. 1974**									
80	Rabbit (NS) 5 NS	Once (G)	NS	LE	Death			300	LD50
DDT (NS) Cameron and Burgess 1945									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
81	Rabbit (New Zealand) 10 F	GDs 4–7 (G)	0, 1.0	BW	Develop		1		On GD 28, 33% decreased fetal weight; decreased fetal brain and kidney weights
DDT (NS) Fabro et al. 1984									
82	Rabbit (New Zealand) 6–15 F	GDs 7–9 or 21–23 (GO)	0, 10, 50	BW HP OF	Repro			10 F	Exposure on GDs 7–9: increased resorptions, 1.3% in controls, 9.5% in treated; increased incidence of prematurity 22%
					Develop		10 F	50 F	LOAEL: 11% decreased fetal weight on day 28 Serious LOAEL: GDs 7–9 exposure: 19% decreased fetal weight on day 28; 40% deliveries premature; GDs 21–23 exposure: no effect on prematurity, resorptions or fetal weight
***p,p′*-DDT Hart et al. 1972**									
83	Rabbit (New Zealand) 6–15 F	GDs 7–9 (GO)	0, 50	BW HP OF	Repro			50 F	Increased resorptions, 1.8% in controls, 25% in treated
					Develop			50 F	22% decreased offspring weight
***p,p′*-DDT Hart et al. 1971**									
84	Rabbit (New Zealand) 30 M	10 days (G)	0, 4.3	BC	Immuno	4.3			No effect on antibody titres to Salmonella typhimurium
DDT (NS) Shiplov et al. 1972									
INTERMEDIATE EXPOSURE									
85	Monkey (Squirrel) 12 M, 18 F	2, 4, or 6 months (G)	0, 0.05, 0.5, 5, 50	BC BI	Death			50	Death of 6/6 in 14 weeks
***p,p′*-DDT Cranmer et al. 1972**									
86	Monkey (Squirrel) 12 M, 18 F	2, 4, or 6 months (G)	0, 0.05, 0.5, 5, 50	BC BI CS	Neuro	5		50	Staggering, weakness, loss of equilibrium
***p,p′*-DDT Cranmer et al. 1972**									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
87	Monkey 12–18 F	2, 4, or 6 months (G)	0, 0.05, 0.5, 5, 50	BC BI CS	Hemato Hepatic	50 F 5 F			
***p,p′*-DDT Cranmer et al. 1972**									
88	Monkey (Rhesus) M	100 days (G)	0, 10	BW GN HP BI	Neuro		10		15–20% decrease in brain lipids, CNS phospholipids, and cholesterol
DDT, technical grade Sanyal et al. 1986									
89	Rat (albino) 10–12 M	8–22 weeks (F)	0, 2.2, 5.5, 11	BW FI BC CS	Immuno	2.2 M	5.5 M		Decreased relative spleen weight (17%) at 22 weeks; increased serum albumin/globulin ratio and reduced IgG titers after tetanus toxoid stimulation; no effect on IgM titers, relative thymus weight, or body weight
***p,p′*-DDT Banerjee 1987b**									
90	Rat (Wistar) 10–12 M	4 weeks (F)	0, 2.3, 5.7, 11.4	BW CS FI OW LE	Immuno	2.3 M	5.7 M		Decreased IgG and IgM, increased albumin/globulin ratio
***p,p′*-DDT Banerjee et al. 1995**									
91	Rat (Wistar) 8–12 M	6 weeks (F)	0, 20.2	LE FI BW OW OF	Hepatic Immuno	20.2 M	 20.2 M		 After ovalbumin immunization: decreased serum IgG and IgM, and ovalbumin antibody titre; increased % migration of leucocytes and macrophages; decreased footpad thickness; decreased relative spleen weight; no effect on thymus weight
***p,p′*-DDD Banerjee et al. 1996**									
92	Rat (Wistar) 8–12 M	6 weeks (F)	0, 20.2	LE FI BW OW OF	Bd wt Hepatic	20.2 M	 20.2 M		 Increased relative liver weight (17.1%)
***p,p′*-DDE Banerjee et al. 1996**									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
93	Rat (Wistar) 8–12 M	6 weeks (F)	0, 20.2	LE FI BW OW OF	Bd wt	20.2 M			
					Hepatic		20.2 M		Increased relative liver weight (14.2% increase)
***p,p'*-DDT Banerjee et al. 1996**									
94	Rat (Wistar) NS B	7 months (F)	0, 2.6, 26, 128	BW HP LE	Repro	26 F		128 F	Decreased fertility in F1 females bred with nonexposed males
***o,p'*-DDT Clement and Okey 1974**									
95	Rat (Wistar) NS B	Through breeding GDs 1–21 LDs 1–21 (F)	0, 1.7, 16.8, 84	BW LE	Develop	16.8	84		Decreased body weights and growth of nursing pups 17% less body weight than controls at age 21 days; reduced fertility in F1 females (25% produced litters versus 100% in control)
***o,p'*-DDT Clement and Okey 1974**									
96	Rat (Wistar) NS B	Through breeding, GDs 1–21 LDs 1–21 (F)	0, 1.7, 16.8, 42.1	BW LE	Repro	16.8 F			No effect on fertility of F1 female progeny bred to nonexposed males
					Develop	1.7	16.8	42.1	LOAEL: Decreased body weights and growth of nursing pups; no effect on F1 fertility Serious LOAEL: All F1 offspring dead by 10 days after birth
***p,p'*-DDT Clement and Okey 1974**									
97	Rat (albino) 5 F	31 days 24 hours/day (F)	0, 2.3, 23	BW FI GN BC	Immuno		2.3		Decreased severity of anaphylactic shock, decreased mast cells response to diphtheria toxoid
DDT (NS) Gabliks et al. 1975									
98	Rat (Wistar) 12 M	3 weeks (GO)	0, 15	BW BI OW	Hepatic		15 M		Increase in liver weight and in cytochrome P450 enzymes
***p,p'*-DDT Gupta et al. 1989**									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
99	Rat (F344/DuCrj) 20 M, 20 F	26 weeks (F)	Male: 0, 0.17, 1.7, and 19.1; females: 0, 0.21, 2.2, 25.2	HP BW FI CS	Hepatic	0.21 F	2.2 F		Hepatocellular hypertrophy
							0.17 M[c]		Hepatocellular hypertrophy
***p,p'*-DDT** **Harada et al. 2003, 2006**									
100	Rat (F344/DuCrj) 30 M	4 weeks (F)	0, 4.8, 15.4, 45.7	HP BW FI CS	Bd wt	45.7 M			
					Hepatic		4.8 M		Increased absolute and relative liver weight; decreased gap junctional intercellular communication protein Cx32; increased hepatocyte proliferation (% PCNA labeling index) at ≥15.4 mg/kg/day
***p,p'*-DDT** **Harada et al. 2003; Tomiyama et al. 2004**									
101	Rat (Sprague-Dawley) P and F1, each 24 M, 24 F/dose	2-generation P generation: 10 weeks from before mating, through lactation. F1 generation: during rearing for 10 weeks, through mating, gestation, and lactation F2 generation: through weaning (F)	P- males: 0, 0.343, 3.44, 25; P- females: 0.73, 3.75, 27.7	CS BW OW MX DX TG OF HP GN FX FI BC	Bd wt	25 M	27.7 F		P and F1 females: decreased body weight
					Hepatic	0.73 F	3.75 F		Centrilobular hypertrophy and increased relative liver weights in P and F1 females
						0.343 M	3.44 M		Centrilobular hypertrophy, fatty change of hepatocytes; increased absolute and relative liver weight in P and F1 males.
					Renal	3.44	25		Parental males and females and F1 females: increased kidney weight (no histopathology)
					Neuro		27.7 F		Increased incidence of tremors in P and F1 parental females
						25 M			No incidence of tremors in males
					Repro	25			Estrous cyclicity, estrous cycle length, reproductive capability; testicular and epididymal sperm parameters (males); reproductive organ weights
						0.73 F	3.75 F		F0 females: decreased estradiol levels at 3.75 and 27.7 mg/kg/day; increased progesterone at 27.7 mg/kg/day, but no effects on F0 or F1 indices of mating and fertility, or viability of F1 and F2 offspring in any exposure group

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
						25 M			No effects on F0 male sex hormone levels, F0 and F1 indices of mating and fertility, or F1 and F2 viability
					Develop	3.75	27.7		Decreased pup viability index on PND 21 in F1 pups; Delayed preputial separation in F1 males and decreased body weight; increased kidney weight (no histopathology) in F1 females
***p,p'*-DDT** **Hojo et al. 2006**									
102	Rat (Sprague-Dawley) 6 F	36 weeks 7 days/week (F)	0, 6.6, 13.2	GN HP BC	Hepatic		6.6		Focal necrosis/regeneration
DDT (NS) **Jonsson et al. 1981**									
103	Rat (Sprague-Dawley) 6 F	36 weeks (F)	0, 6, 12		Repro	6		12	Decreased fertility
DDT, technical grade **Jonsson et al. 1976**									
104	Rat (Long-Evans) 12 M	PNDs 21–57 (GO)	0, 100	CS	Bd wt		100 M		Increased body weight (18%)
					Repro			100 M	Delayed onset of puberty by 5 days; no changes in serum testosterone
***p,p'*-DDE** **Kelce et al. 1995**									
105	Rat (Wistar) 8–10 M	8 weeks (F)	0, 10.3, 20.6	OF	Immuno	10.3 M	20.6 M		Decreased serum antibody titer to SRBC
DDT, technical grade **Koner et al. 1998**									
106	Rat (Sprague-Dawley) 110 F	5 weeks, 5 days/week (G)	0, 10	CS BW OW HP BC	Repro	10			No effects on reproductive functions (including fertility, body weights, development, or size of litters)
***p,p'*-DDE** **Kornbrust et al. 1986**									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
107	Rat (Wistar) 6 M	3 weeks 3 times/week (GO)	0, 100, 200	BW OW HP BC	Repro		100		Marginal, but significant decrease in testosterone in the testis
DDT (NS) Krause 1977									
108	Rat (NS) 6 M	PNDs 4–23 (G)	0, 200	BW OW HP	Bd wt	200 M			No effect on body weight up to 50 days after exposure
					Repro		200 M		Decreased absolute testis weight; decreased tubular diameter; reduced number of Sertoli cells, A-spermatagonia, and Leydig cells 6–12 days after exposure; significant reduction in number of fetuses and implants after two matings
DDT (NS) Krause et al. 1975									
109	Rat (Osborne-Mendel) 15 M, 15 F	15–27 weeks (F)	0, 0.05, 0.25, 0.5, 2.5	BW OW GN HP	Hepatic	0.05	0.25		Minimal centrilobular hypertrophy, cytoplasmic oxyphilia
DDT, technical grade Laug et al. 1950									
110	Rat (Fischer-344) 6 M	42 days (F)	0, 10	OW BW HP BC BI DX FI	Bd wt	10 M			
					Hepatic	10 M			
					Renal	10 M			
					Immuno	10 M			No significant effect on relative spleen and thymus weight or histology
					Repro	10 M			No changes in sperm count, serum levels of sex hormones, relative testis, epididymis, prostate, or seminal vesicle weights
***p,p'*-DDE Makita et al. 2003a**									
111	Rat (Osborne-Mendel) 5 M, 5 F	6 weeks (F)	M: 49, 88, 160, 280, 490; F: 54, 96, 170, 300, 540		Bd wt		170 F		39% reduction in body weight in females at 170 mg/kg/day
							160 M		Reduced body weight 10% in males at 160 mg/kg/day
DDD-technical NCI 1978									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
112	Rat (Osborne-Mendel) 5 M, 5 F	6 weeks (F)	M: 0, 16, 28, 50, 88, 157; F: 0, 17, 31, 54, 97, 172	BW LE	Bd wt		97 F 50 M		Reduced body weight (45% in females) Reduced body weight (16% in males)
DDT, technical grade NCI 1978									
113	Rat (Osborne-Mendel) 20–50 F	26 weeks (GO)	F: 0, 30, 61	CS	Neuro			30 F	By week 26, tremors in 8% at 30 mg/kg/day and 90% at 61 mg/kg/day; hunched appearance by week 6 at 61 mg/kg/day. Tremors also observed in males, but accurate doses could not be determined
DDT, technical grade NCI 1978									
114	Rat (Osborne-Mendel) 5 M, 5 F	6 weeks (F)	M: 0, 28, 49, 88, 160, 280; F: 30, 50, 96, 170, 300		Death Bd wt	 300 F	 49 M	300 F	All female rats died by 6 weeks 11% body weight depression in males
***p,p'*-DDE NCI 1978**									
115	Rat (Sherman) 4–20 B	2–6 months (F)	0, 0.5, 1.7, 5, 20, 40	GN HP BI	Hepatic	5 F 0.5 M	20 F 1.7 M		Mild hepatocellular hypertrophy, more severe in males than females More severe effects at 5 mg/kg/day in male. No quantitative data provided
DDT, technical grade Ortega 1956									
116	Rat (Sprague-Dawley) 11 M (treated); 24 M (control)	104 days; 14 days *in utero*, 20 lactational days, 70 days directly (G)	0, 35	DX BW OW HP BC	Develop		35 M		Increased liver mass, relative liver weight; testicular mass and relative testis weight. Decreased seminiferous tubule diameter, epithelium thickness, and lumen diameter; increased serum testosterone; no effect on AGD, body mass, seminal vesicle or epididymal mass
DDE (NS) Patrick et al. 2016									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
117	Rat (Wistar) 36 M, 36 F	9 weeks (F)	M: 0, 34.1; F: 0, 37	BW FI WI GN HP CS	Neuro			34.1 F	Tremors in 80% of females after 9 weeks of treatment
DDT, technical grade Rossi et al. 1977									
118	Rat (Fischer-344) 7 M	13 and 26 weeks (F)	0, 0.17, 1.7, 19.1	HE HP BC	Hemato	1.7	19.1		Significantly decreased hematocrit and hemoglobin levels (at 13 and 26 weeks) and erythrocyte counts (at 13 weeks only) coupled with increased bone marrow hematopoiesis on week 26
***p,p'*-DDT Tomita et al. 2013**									
119	Rat (Wistar) 46 F	20 weeks (F)	0, 0.1, 1.0, 2.0, 4.0	BW OW	Repro	4			No effects on reproductive organ weights, birth weights, or weaning weights, vaginal opening, age of first littering, body weight, ovarian weight, or uterus weight; no adverse effects on fertility, fecundity, or pup viability
***o,p'*-DDT Wrenn et al. 1971**									
120	Rat (Sprague-Dawley) 10 F	GD 6–PND 20 (G)	0, 5, 15, and 50	BW DX CS MX OW	Bd wt	50 F			
					Hepatic	15 F	50 F		Increased relative liver weight (20%) in dams
					Develop	15 F	50 F		Reduced weaning index and number of pups live on PND 21; prolonged preputial separation and early vaginal opening; no significant effects on number of litters, gestation index, gestational length, number of pups born, delivery index, birth index, or viability on PND 4
***p,p'*-DDE Yamasaki et al. 2009**									
121	Mouse (Hissar) 12–15 M	3–12 weeks (F)	0, 4.2, 10.5, 21	FI GN BC CS BI	Immuno	4.2	10.5		Decreased splenic PFC response to T-antigen independent LPS at weeks 6–12; decreased IgM antibody titer at 21 mg/kg/day
DDT (NS) Banerjee 1987a									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
122	Mouse (Rockfeller) 8–12 M	24 weeks (F)	0, 4.3, 10.7, 21.4	OF	Immuno	4.3	10.7		Increased growth of Mycobacterium leprae in footpad
***p,p′*-DDT** **Banerjee and Koner 1997a**									
123	Mouse (Hissar albino) 25–30 M	3–12 weeks (F)	0, 4, 10, 20	BW FI BC CS OW	Bd wt Hepatic	20 4	 10		 Increased relative liver weight (14.7%)
***p,p′*-DDT** **Banerjee et al. 1986**									
124	Mouse (Hissar) 8–10 M	4 weeks (F)	0, 4.1, 10.1, 20.3	LE BW FI OW OF	Immuno	10.1	20.3		Decreased splenic PFC response to SRBC (in restraint-stressed mice only)
***p,p′*-DDT** **Banerjee et al. 1997b**									
125	Mouse (C-57) 9 B	60–90 days (F)	0, 34.3, 51.4		Repro	34.3		51.4	78% decreased fertility
DDT, technical grade **Bernard and Gaertner 1964**									
126	Mouse (CF1) NS F	GDs 1–21 LDs 1–21 (F)	0, 34.3	DX LE BH	Develop		34.3		Decreased maze performance learning at 1 and 2 months in survivors
DDT, technical grade **Craig and Ogilvie 1974**									
127	Mouse (C57BL/6H) 30 M	5 days, 7-day rest, then 1 time weekly for 13 weeks (G)	0, 2.0	BW FI BC DI OW	Bd wt	2 M			Increased body weight compared with LFD animals, but no change compared to HFD-CTL
***p,p′*-DDE** **Howell et al. 2015**									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
128	Mouse (C57BL/6J) 14–15 F	15 days, GD 12–PND 5 (G)	0, 1.7	DX OF BI	Develop	1.7 M	1.7 F		NOAEL: No effect on timing of puberty, gestation length, gestation weight gain, number of pups/litter. body mass or fat mass in male offspring to 6 months, or no significant indication of metabolic syndromes after 12 weeks on a high-fat diet LOAEL: In female offspring on high fat diets for 12 weeks: Metabolic syndrome (impaired glucose tolerance, hyperinsulinemia, dyslipidemia), impaired cold tolerance, altered bile acid metabolism; no effect on timing of puberty. No effect on gestation length, gestation weight gain, or number of pups/litter
***p,p'*-DDT; prepared mixture of 77.2% p,p'-DDT and 22.8% o,p'-DDT** **La Merrill et al. 2014a, 2014b**									
129	Mouse (C57BL/6J) 14–15 F	15 days, GD 12–PND 5 (G)	0, 1.7	DX OF BI	Develop		1.7		Increased systolic and diastolic blood pressure in male offspring at 5 months; increase systolic in males and females at 7 months; cardiac hypertrophy (increased left ventricular wall thickness) in females, but not males; no histological effects on kidneys
***p,p'*-DDT; prepared Mixture of 77.2% p,p'-DDT and 22.8% o,p'-DDT** **La Merrill et al. 2016**									
130	Mouse (B6C3F1) 20 M, 20 F	86–130 days (F)	0, 0.86, 1.7, 3.4, 5.1,10.2,20.4	OF	Repro	3.4	5.1		Decreased number of pups/litter at birth or PND 1, decreased fertility
DDT, technical grade **Ledoux et al. 1977**									
131	Mouse (NMRI) 13 F	72–74 days 7 days/week (F)	0, 2.0	BI OF	Repro		2		Prolonged length of estrus cycle; decreased number of implants (223 versus 250 in controls)
***p,p'*-DDT** **Lundberg 1973**									
132	Mouse (NMRI) 10–14 F	28 days (G)	0, 1.77	GN BI OF	Repro		1.77 F		Decreased corpora lutea 17.2%
***p,p'*-DDT** **Lundberg 1974**									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
133	Mouse (B6C3F1) 5 M, 5 F	6 weeks (F)	M: 0, 45, 72, 114, 180, 287; F: 0, 49, 78, 123, 195, 310	BW LE	Bd wt	310 F 287 M			
DDD-technical NCI 1978									
134	Mouse (B6C3F1) 5 M, 5 F	6 weeks (F)	M: 0, 3, 6, 10, 18, 32; F: 0, 4, 6, 11, 20, 35	BW LE	Death Bd wt	 35 F 32 M		35 F	4 out of 5 died
DDT, technical grade NCI 1978									
135	Mouse (B6C3F1) 5 M, 5 F	6 weeks (F)	M: 0, 25, 35, 49, 66, 94; F: 0, 27, 38, 53, 71, 101		Death Bd wt	 101 F 94 M		66	Death of 4/5 males and 2/5 females
***p,p′*-DDE NCI 1978**									
136	Mouse (NMRI) 10–15 M	28 days (G)	0, 6.25	BW OW GN BI	Hepatic Repro		6.25 6.25		Increased absolute and relative liver weight Reduced seminal vesicle weight (28%) in castrated males only
***p,p′*-DDT Orberg and Lundberg 1974**									
137	Mouse (CF1) 60 B	15–30 weeks (F)	0, 42.8	BW OW GN HP	Cancer			42.8	CEL: Liver hepatomas
***p,p′*-DDT Tomatis et al. 1974b**									
138	Mouse (BALB/c) 53 M, 53 F	120 days (F)	0, 1.3	OF	Repro	1.3			No effects on fertility or litters per producing pair
DDT, technical grade Ware and Good 1967									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
139	Dog (NS) 14 M	36-150 days (C)	0, 50	OW HP CS	Endocr			50	Adrenocortical necrosis
***p,p′*-DDD Kirk and Jenson 1975**									
140	Rabbit (New Zealand) 5 F	3 times/week 12 weeks (GO)	0, 3	OF HP	Repro		3		Reduced ovulation rate and slight decrease circulating progesterone post-insemination
DDT, technical grade Lindenau et al. 1994									
141	Rabbit (New Zealand) 5 F	12–15 weeks 3 days/week (GO)	0, 3	DX	Repro	3			No fertility effects or effects on embryonic development observed
DDT, technical grade Seiler et al. 1994									
142	Rabbit 8 M	8 weeks (F)	0, 0.184, 0.92, 2.1, 6.54	BW HP BC	Immuno	2.1			
***p,p′*-DDT Street and Sharma 1975**									
CHRONIC EXPOSURE									
143	Human 51 M	12–18 months (F)	0, 0.05, 0.5	BC CS BW	Bd wt	0.5			
					Cardio	0.5			
					Hemato	0.5			
					Hepatic	0.5			
					Neuro	0.5			No effect on coordination, tremors, other neurological tests
DDT, technical grade Hayes et al. 1956									
144	Monkey (Rhesus) 22 B	3.5–7 years (F)	0, 0.1, 1, 3.9, 98	GN CS	Hepatic		3.9		N=3 at 3.9 mg/kg/day; slight variation in liver cell size and mild hydropic changes histopathology (n=1); severe hydropic and hyaline changes of liver cytoplasm with focal acute hepatitis (n=1); no liver changes noted (n=1); no bromsulfalein retention in any animals at any dose
DDT (NS) Durham et al. 1963									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
145	Monkey (Cynomolgus) 13 M, 11 F	130 months (F)	0, 6.4-15.5	HP OW CS BW HE GN	Death			6.9 F	
					Hepatic		6.4 F		Fatty changes in the liver
					Neuro			6.9 F	Severe tremors
***p,p'*-DDT** **Takayama et al. 1999**									
146	Rat (MRC Porton) 30–38 B	Life (F)	0, 6, 12, 24	BW GN HP CS	Bd wt	24			
					Resp	24			
					Neuro	24			
					Cancer			12 F	CEL: Liver-cell tumors (6.6 and 18.4% at 12 and 24 mg/kg/day)
DDT, technical grade **Cabral et al. 1982b**									
147	Rat (Osborne Mendel) 30 B	27 months (F)	0, 20	BW HP GN	Resp	20			
					Hemato		20		Hemolysis in spleen
					Hepatic		20		Focal hepato-cellular necrosis
					Renal			20	Some tubular epithelial necrosis and polycystic degeneration; small hemorrhages
DDT (NS) **Deichmann et al. 1967**									
148	Rat (Sprague-Dawley) F1, 5 M, 10 F	2-generation (F)	0, 0.5, 1.5	OF	Repro	1.5			No effect on fertility, time of vaginal opening, fecundity
DDT, technical grade **Duby et al. 1971**									
149	Rat (Sprague-Dawley) F1, 5 M, 10 F	2-generation (F)	0, 0.1, 0.3	OF	Repro	0.3			No effect on fertility, time of vaginal opening, fecundity
***o,p'*-DDT** **Duby et al. 1971**									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
150	Rat (Sprague-Dawley) F1, 5 M, 10 F	2-generation (F)	0, 0.4, 1.2	OF	Repro	1.2			No effect on fertility, time of vaginal opening, fecundity
***p,p'*-DDT Duby et al. 1971**									
151	Rat (Osborne-Mendel) 12 M, 12 F	2 years (F)	0, 7, 14, 28, 42, 56	GN	Hepatic		7		Focal hepatocellular necrosis
DDT, technical grade Fitzhugh and Nelson 1947									
152	Rat (F344/DuCrj) 40 M, 40 F	1–2 years (52, 78, and 104 weeks) (F)	Male: 0, 0.17, 1.7, and 19.1; females: 0, 0.21, 2.2, 25.2	HP BW FI CS BI	Bd wt	2.2 F		25.2 F	25% decreased mean body weight in females
						1.7 M	19.1 M		12% decreased mean body weight in males
					Hepatic	0.21 F	2.2 F		Increased incidence of hepatocellular hypertrophyclose to 100% from week 26 to 104
							0.17[d] M		Increased incidence of hepatocellular hypertrophy (close to 100% from week 52 to 104) $BMDL_{10}$ of 0.01 mg/kg/day
					Neuro	2.2 F	25.2 F		Whole body tremors weeks 70–104
						1.7 M	19.1 M		
					Cancer			1.7 M	CEL: Hepatocellular adenoma in males at ≥1.7 mg/kg/day and in females at 25.2 mg/kg/day; hepatocellular carcinomas in males (19.1 mg/kg/day)
***p,p'*-DDT Harada et al. 2003, 2006**									
153	Rat (Osborne-Mendel) 20–50 M, 20–50 F	78 weeks (F)	M: 0, 116, 231; F: 0, 66, 131	BW CS GN HP	Bd wt			66 F	28% decrease in body weight gain
					Resp	231 M			
					Cardio	231 M			
					Gastro	231 M			
					Musc/skel	231 M			
					Hepatic	231 M			

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Hepatic	231 M			
					Renal		66 F		Chronic inflammation of the kidney
					Dermal	231 M			
					Endocr	231 M			
					Immuno	231 M			No histological alterations in spleen, thymus or lymph nodes in males or females
					Neuro	131 F			
						231 M			No tremors or hunched appearance during last 6 months
					Repro	231 M			No significant alterations in uterus, mammary glands, ovaries, or prostate; reproductive function not assessed
					Cancer			116	CEL: thyroid follicular cell adenoma and carcinoma
DDD-technical NCI 1978 (Rat)									
154	Rat (Osborne-Mendel) 20–50 M, 20–50 F	78 weeks (F)	M: 0, 23, 45; F: 0, 16, 32	BW CS GN HP	Bd wt		32 F 45 M		20% decrease in body weight gain in males and females
					Resp	45 M			
					Cardio	45 M			
					Gastro	45 M			
					Musc/skel	45 M			
					Hepatic		23 M		Fatty metamorphosis
					Renal	45 M			
					Dermal	45 M			
					Endocr	45 M			
					Immuno	45 M			No significant alterations in spleen, thymus, or lymph nodes in males or females immunological function not assessed
					Repro	45 M			No significant alterations in uterus, mammary glands, ovaries, or prostate; reproductive function not assessed
DDT, technical grade NCI 1978									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
155	Rat (Osborne-Mendel) 20–50 M, 20–50 F	78 weeks (F)	M: 0, 31, 59; F: 0, 19, 36	BW CS GN HP LE	Death			19 F	16% death rate compared to 0% in controls
					Bd wt			19 F	21% decrease in body weight gain
					Resp	59 M			
					Cardio	59 M			
					Gastro	59 M			
					Musc/skel	59 M			
					Hepatic	36 F	31 M		Fatty metamorphosis
					Renal	59 M			
					Dermal	59 M			
					Endocr	59 M			
					Immuno	59 M			No histological alterations in spleen, thymus or lymph nodes
					Repro	59 M			No histopathological effects in males or females
***p,p'*-DDE NCI 1978**									
156	Rat (Sprague-Dawley) 6 M, 12 F	2-generation (F)	0, 1, 10	BW FI	Repro	10			No effect on fertility, fecundity, or offspring viability
DDT, technical grade Ottoboni 1969									
157	Rat (Sprague-Dawley) 6 M, 12 F	2-generation (F)	0, 1.9, 18.6	BW FI	Repro	18.6			No effect on litter size or sex ratios; no clear exposure effects on fertility or pup survival
					Develop	1.9	18.6		Tail abnormalities, constriction rings in 13.2–25.5%; no effect on birth weights or body weights at weaning
DDT, technical grade Ottoboni 1969									
158	Rat (Sprague-Dawley) 12 M, 12 F	7 days/week life (F)	0, 1.6	BW FI	Repro	1.6			
DDT, technical grade Ottoboni 1972									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
159	Rat (Wistar) 36 M, 36 F	120 weeks (F)	M: 0, 34.1; F: 0, 37	BW FI WI GN HP CS	Cancer			34.1 M	CEL: Liver cell tumors (33.3%)
DDT, technical grade Rossi et al. 1977									
160	Rat (Fischer-344) 5–10 M	Up to 104 weeks (F)	0, 0.17, 1.7, 19.1	HE HP BC	Hemato	0.17	1.7		Reduced hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin and increased hematopoiesis in bone marrow at week 78
***p,p′*-DDT Tomita et al. 2013**									
161	Rat (Sprague-Dawley) F0 12 B	3 generations (F)	0, 0.13, 0.63, 1.25		Repro	1.25			
DDT, technical grade Treon et al. 1954									
162	Mouse (ICR) 400 F	70 weeks (conception through death); multi-generation (F)	0, 16.5	GN	Resp	16.5			
					Cardio	16.5			
					Hepatic		16.5		Acute congestion in the liver
					Renal	16.5			
					Develop	16.5			Increased neonatal death (lactation index only), but decreased relative risk of postweaning death compared to controls
DDT, technical grade Del Pup et al. 1978									
163	Mouse (C57BL/6N) 36 M, 36 F	81 weeks (F)	0, 28	GN HP	Cancer			28	CEL: Liver tumors - primarily in males
***p,p′*-DDT Innes et al. 1969**									
164	Mouse (Swiss) 30 M, 30 F	80 weeks (F)	0, 16.5	BW GN HP CS OF	Cancer			16.5	Lymphomas; lung and liver tumors NS
DDT, technical grade Kashyap et al. 1977									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
165	Mouse (Swiss) 30 B	80 weeks (F)	0, 16.5	BW HP CS	Neuro			16.5	Tremors
DDT, technical grade Kashyap et al. 1977									
166	Mouse (Swiss) 30 B	80 weeks (F)	0, 13	HP CS	Ocular		13		Unilateral and bilateral corneal opacity
DDT, technical grade Kashyap et al. 1977									
167	Mouse (Swiss-Webster) 4 M, 14 F/ generation	3 generations (F)	0, 5, 20, 50	OF	Repro			20	Decreased fertility
DDT (NS) Keplinger et al. 1970									
168	Mouse (B6C3F1) 20–50 M, 20–50 F	78 weeks (F)	M: 0, 71, 141; F: 0, 71, 142	BW CS GN HP	Bd wt		71 F	142 F	Serious LOAEL: 28% decrease in body weight gain LOAEL: 17% decrease in body weight gain
						141 M			
					Resp	142 F			
					Cardio	142 F			
					Gastro	142 F			
					Musc/skel	142 F			
					Hepatic	142 F			
					Renal	142 F			
					Dermal	142 F			
					Ocular	142 F			
					Endocr	142 F			
					Immuno	142 F			No histological alterations in spleen, thymus, or lymph nodes

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Neuro	142 F			Neurological clinical signs in males and females were comparable to controls
					Repro	142 F			No histological alterations in male or female reproductive tissues
DDD-technical NCI 1978									
169	Mouse (B6C3F1) 20–50 M, 20–50 F	78 weeks (F)	M: 0, 3.7, 7.4; F: 0, 15.0, 30.2	BW CS GN HP LE	Death			15 F	10% mortality compared to 0% in controls
					Bd wt	30.2 F			
					Resp	30.2 F			
					Cardio	30.2			
					Gastro	30.2 F			
					Musc/skel	30.2 F			
					Hepatic	30.2 F			
					Hepatic		3.7 M		Amyloidosis
					Renal	30.2			
					Dermal	30.2 F			
					Endocr	30.2 F			
					Immuno	30.2 F			No histological alterations in spleen, thymus or lymph nodes
					Neuro	30.2 F			Neurological clinical signs in males and females were comparable to controls.
					Repro	7.4 F			No histological alterations in reproductive tissues
DDT, technical grade NCI 1978									
170	Mouse (B6C3F1) 20–50 M, 20–50 F	78 weeks (F)	M: 0, 27, 47; F: 0, 28, 49	BW CS GN HP LE	Death			49 F	40% death rate compared to 5% in controls
					Bd wt			28 F	29% decrease in body weight gain; no effect in males
						47 M			
					Resp	49 F			
					Cardio	49 F			
					Gastro	49 F			
					Musc/skel	49 F			
					Hepatic	49 F			
					Renal	49 F	27 M		Chronic inflammation of the kidney

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Dermal	49 F			
					Ocular	49 F			
					Endocr	49 F			
					Immuno	49 F			No histological alterations in spleen, thymus or lymph nodes
					Repro	49 F			No histological alterations in male or female reproductive tissues
					Cancer			28 F	CEL: hepatocellular carcinomas; 0/19, 19/47, 34/48
								27 M	CEL: hepatocellular carcinomas; 0/19, 7/41, 17/47
***p,p′*-DDE**									
NCI 1978									
171	Mouse (A strain) NS	5 generations (G)	0, 1.7, 8.7	GN HP	Death			8.7 F	F0 dams: 14 out of 30 animals died before 6 months (lung adenomas in 3/14)
					Cancer			1.7	Lung tumors, NS, lung adenomas
DDT, technical grade									
Shabad et al. 1973									
172	Mouse (BALB/c) 683 B	Life 6 generations (F)	0, 0.4, 0.7	BW GN HP BC CS OF LE	Cancer			0.4	Lung adeno-carcinomas in F2, leukemia in F3
***p,p′*-DDT**									
Tarjan and Kemeny 1969									
173	Mouse (BALB/c) 60 M, 60 F	Life 2-generation (F)	0, 0.4, 4, 50	BW GN HP OF	Cancer			50 F	Liver tumors in F0 and F1
DDT, technical grade									
Terracini et al. 1973									
174	Mouse (CF1) 30 B	2 years (F)	0, 15.8	GN HP CS	Cancer			15.8	Liver tumors, NS
***p,p′*-DDT**									
Thorpe and Walker 1973									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
175	Mouse (CF1) 50 M, 50 F	Life multi-generation (F)	M: 0, 0.38, 1.91, 9.5, 47.6; F: 0, 0.36, 1.82, 9.1, 45.5	BW GN HP OF	Cancer			45.5 F 0.38 M	Liver tumors in F0 and F1 Liver tumors in F0 and F1
DDT, technical grade Tomatis et al. 1972									
176	Mouse (CF-1) 20 M, 20 F	2-generation (F)	0, 0.4, 2, 10, 50		Develop	10		50	Increased preweanling death at 50 mg/kg/day; increased tremors, convulsions
***p,p′*-DDT Tomatis et al. 1972**									
177	Mouse (CF1) 60 M, 60 F	130 weeks (F)	M: 0, 42.6; F: 0, 45.8	BW GN HP CS	Cancer			42.6 M	CEL: lung and liver tumors
***p,p′*-DDD Tomatis et al. 1974a**									
178	Mouse (CF1) 60 M, 60 F	130 weeks (F)	M: 0, 42.6; F: 0, 45.8	BW OW GN HP LE	Cancer			42.6	CEL: liver tumors (males: 74 versus 35% in controls; females: 98 versus 1% in controls)
***p,p′*-DDE Tomatis et al. 1974a**									
179	Mouse (CF1) 60 NS	6 generations (F)	0, 0.33, 1.7, 8.3, 41.3	BW GN HP	Cancer			0.33	Liver tumors, NS
DDT, technical grade Turusov et al. 1973									
180	Mouse (CF1) 60 B	life (F)	0, 0.33, 1.65, 8.26, 41.32	GN HP CS	Develop	8.3		41.3	Increased in preweanling death
DDT, technical grade Turusov et al. 1973									
181	Mouse (CF1) 60 B	130-140 weeks (F)	0, 0.33, 1.7, 8.3, 41.3	GN HP CS	Neuro	1.7		8.3	Tremors
DDT, technical grade Turusov et al. 1973									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
182	Mouse (NS) 12 M, 12 F	15 months (F)	0, 0.24, 2.4	RE LE	Repro	2.4			
DDT, technical grade Wolfe et al. 1979									
183	Hamster (Syrian) 30-40 B	Life (F)	0, 10, 20, 40	BW GN HP	Bd wt	40			
					Hepatic	20 F	40 F		Hepatocyte hypertrophy; fatty change
						10 M	20 M		Focal necrosis, hepatocyte hypertrophy
DDT, technical grade Cabral et al. 1982a									
184	Hamster (NS) 60 B	Life (F)	0, 33, 67, 133	GN BI	Hepatic		67		50% increase in relative liver weight
DDT, technical grade Graillot et al. 1975									
185	Hamster (Syrian) 48 M, 48 F	128 weeks (F)	0, 95	BW LE HP CS	Bd wt		95		Decreased body weight gain
					Neuro	95			No tremors or convulsions
					Cancer			95	CEL: adrenal neoplasms; 14% in controls, 34% in treated
DDT, technical grade Rossi et al. 1983									
186	Hamster (Syrian) 87 M, 88 F	128 weeks (F)	0, 47.5, 95	BW LE HP CS	Bd wt		95 M		11% decrease in body weight gain
					Hepatic		47.5		Liver necrosis
					Neuro	95			No tremors or convulsions
					Cancer			47.5	CEL: hepatocellular tumors; 0/73, 11/69, 14/78
***p,p'*-DDE Rossi et al. 1983**									
187	Dog (NS) 1–10 NS	39–40 months (F)	0, 16, 80, 160	GN HP BI	Hepatic	16	80	160	LOAEL: Focal or diffuse liver alterations Serious LOAEL: Severe liver damage
DDT, technical grade Lehman 1965									

Table 2-1. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
188	Dog (Beagle) 4 M, 7–8 F	7 days/week, F2 generation (F)	0, 1, 5, 10	BW OW GN HP OF	Repro	10			
DDT, technical grade Ottoboni et al. 1977									

[a]The number corresponds to entries in Figure 2-2; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented, with the exception of the neurological and developmental endpoints for which levels of effect for both males and females are presented.
[b]Used to derive a provisional acute-duration oral minimal risk level (MRL) for DDT, DDE, or DDD of 0.0005 mg/kg/day based on the LOAEL of 0.5 mg technical DDT/kg on PND 10 for neurodevelopmental effects and an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).
[c]Used to derive a provisional intermediate-duration oral MRL for DDT, DDE, or DDD of 0.0002 mg/kg/day based on the LOAEL of 0.17 mg *p,p'*-DDT/kg/day for hepatocyte hypertrophy and an uncertainty factor of 1,000 (10 for the use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).
[d]Used to derive a provisional chronic-duration oral MRL for DDT, DDE, or DDD of 0.0001 mg/kg/day based on a $BMDL_{10}$ of 0.01 mg *p,p'*-DDT /kg/day for hepatocyte hypertrophy and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Principal studies for the MRLs

5-HIAA = 5-hydroxy-indoleacetic acid; AGD = anogenital distance; AP = alkaline phosphatase; b = both male, female; BC = serum (blood) chemistry; Bd Wt or BW = body weight; BH = behavioral; BI = biochemical changes; (C) = capsule; cardio = cardiovascular; CEL = cancer effect level; CNS = central nervous system; CS = clinical signs; develop = developmental; DI = distribution; DX = developmental toxicity; EA = enzyme activity; endocr = endocrine; (F) = feed; F = female(s); FI = food intake; FX = fetal toxicity; (G) = gavage, not specified; GD = gestation day; gen = generation(s); GN = gross necropsy; (GO) = gavage, oil; HE = hematology; hemato = hematological; HP = histopathology; immuno = immunological; LD = lactation day ; LD50 = dose producing 50% death; LDH = lactate dehydrogenase; LE = lethality; LOAEL = lowest-observed-adverse-effect-level; LPS = lipopolysaccharide; M = male(s); musc/skel = muscular/skeletal; MX = maternal toxicity; neuro = neurotoxicology; NOAEL = no-observed-adverse-effect-level; NS = not specified; OF = organ function; OW = organ weight; PCNA = proliferating cell nuclear antigen; PFC = plaque forming cell; PND = postnatal day; repro = reproductive; SRBC = sheep red blood cell; TG = teratogenicity; WI = water intake

Figure 2-2. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Acute (≤14 days)

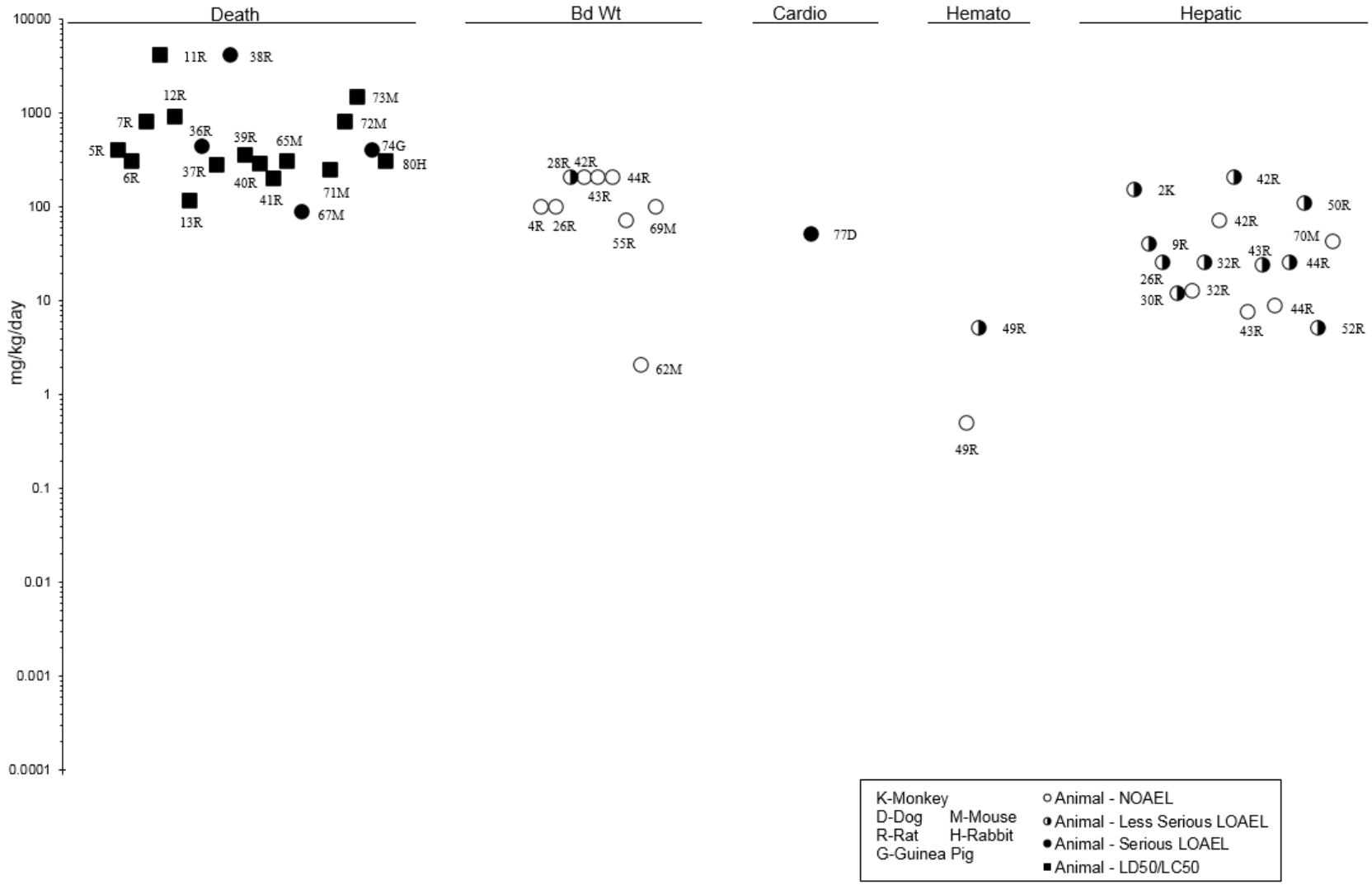


Figure 2-2. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Acute (≤14 days)

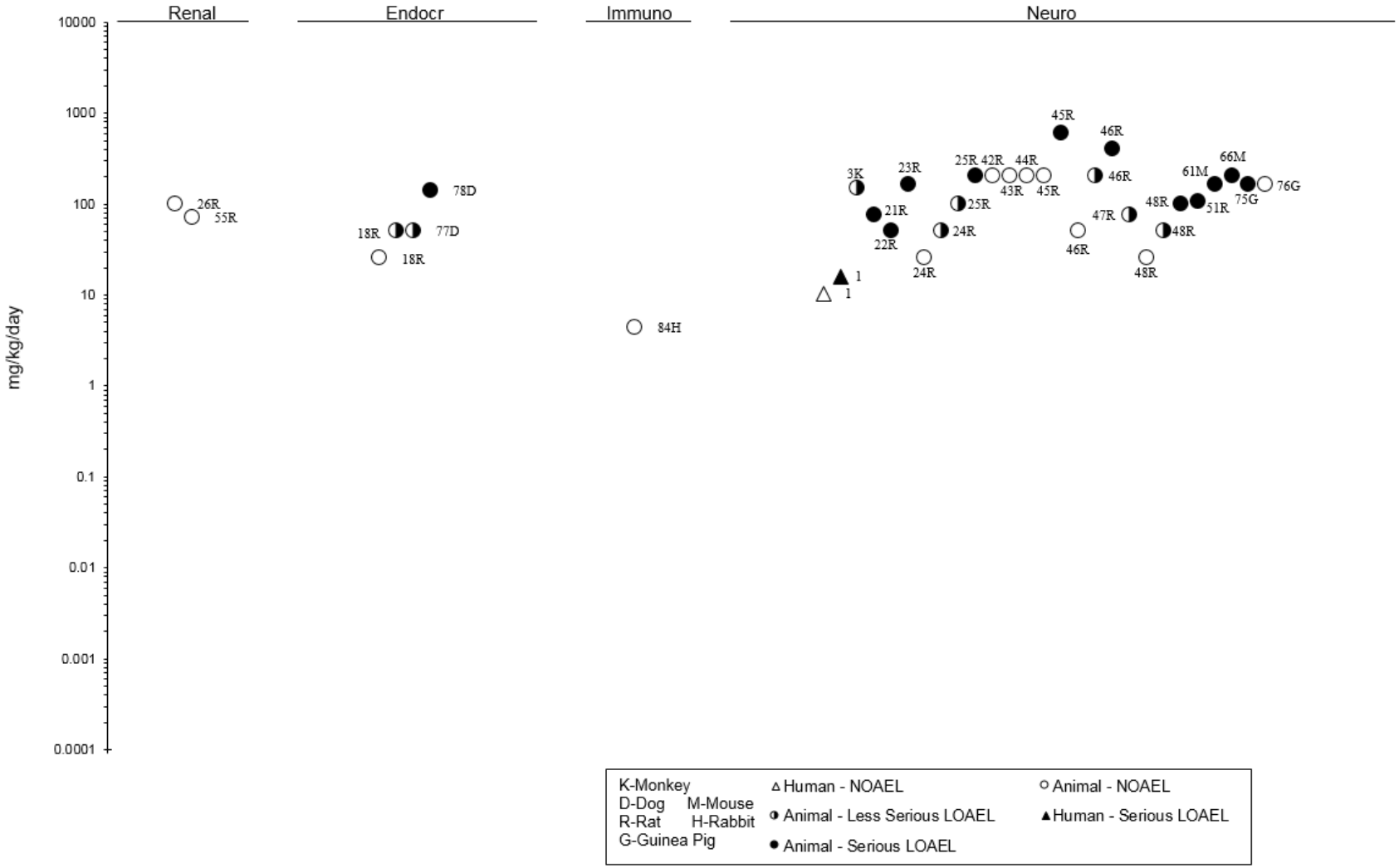


Figure 2-2. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Acute (≤14 days)

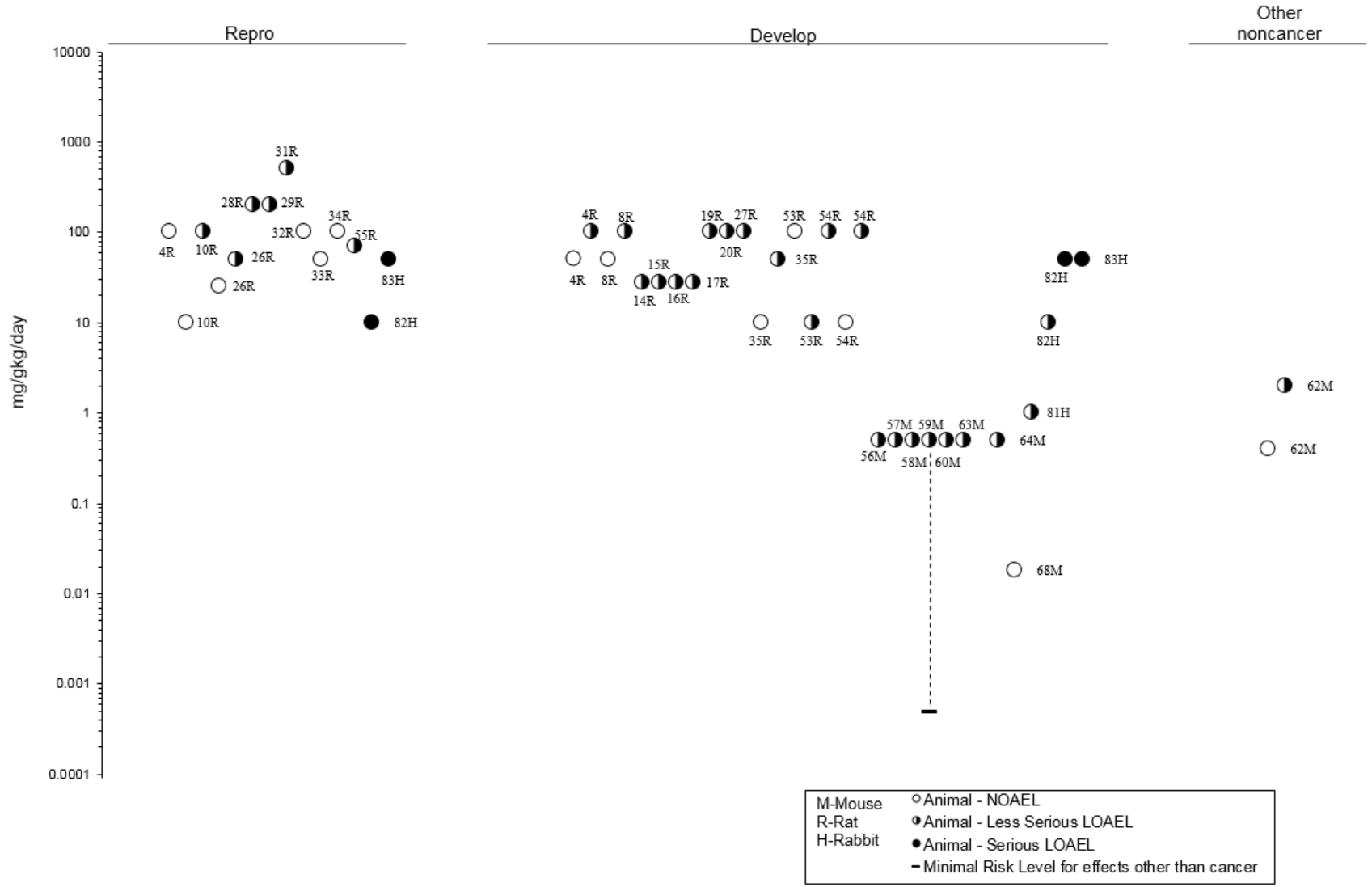


Figure 2-2. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Intermediate (15-364 days)

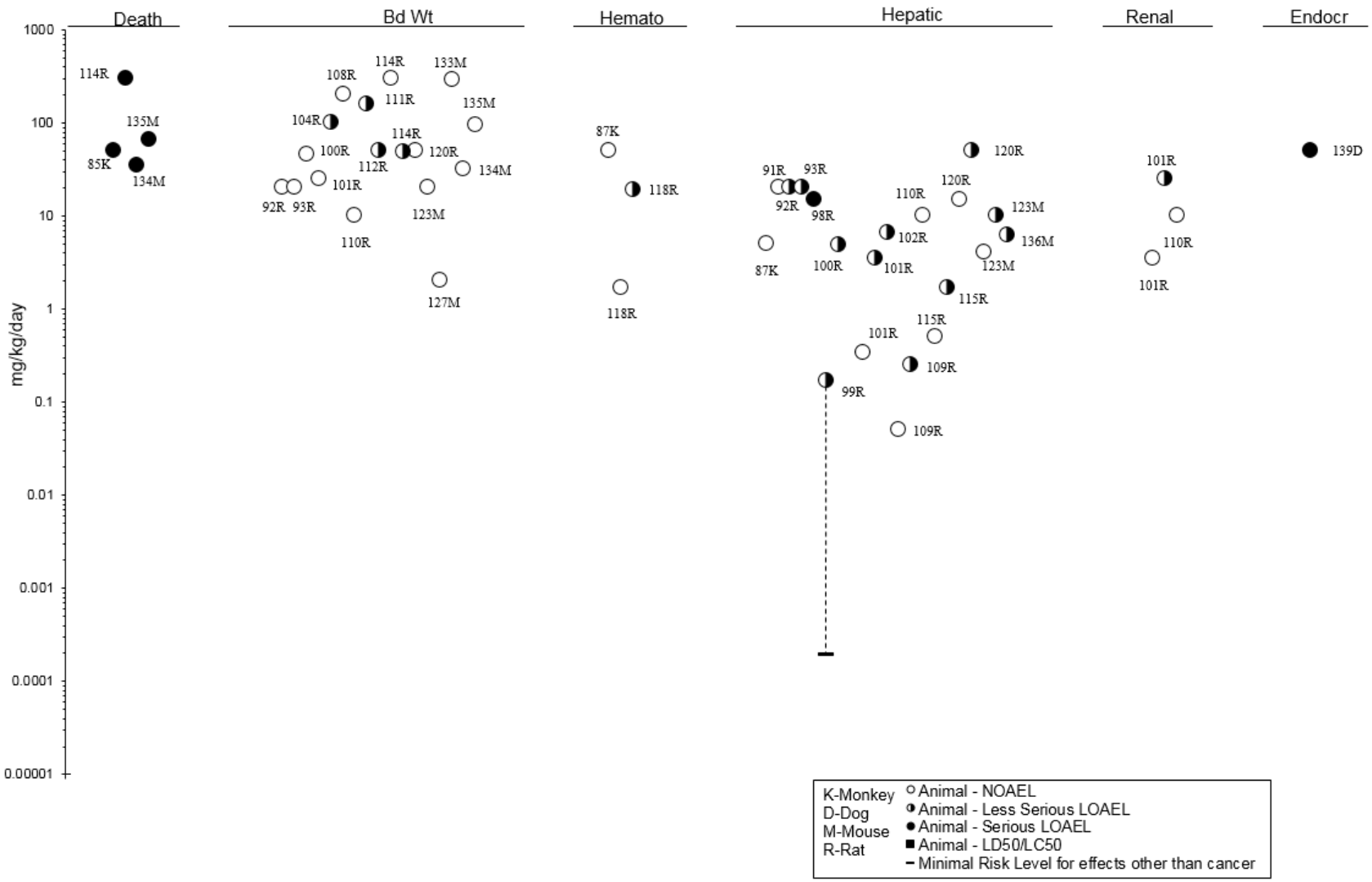


Figure 2-2. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Intermediate (15-364 days)

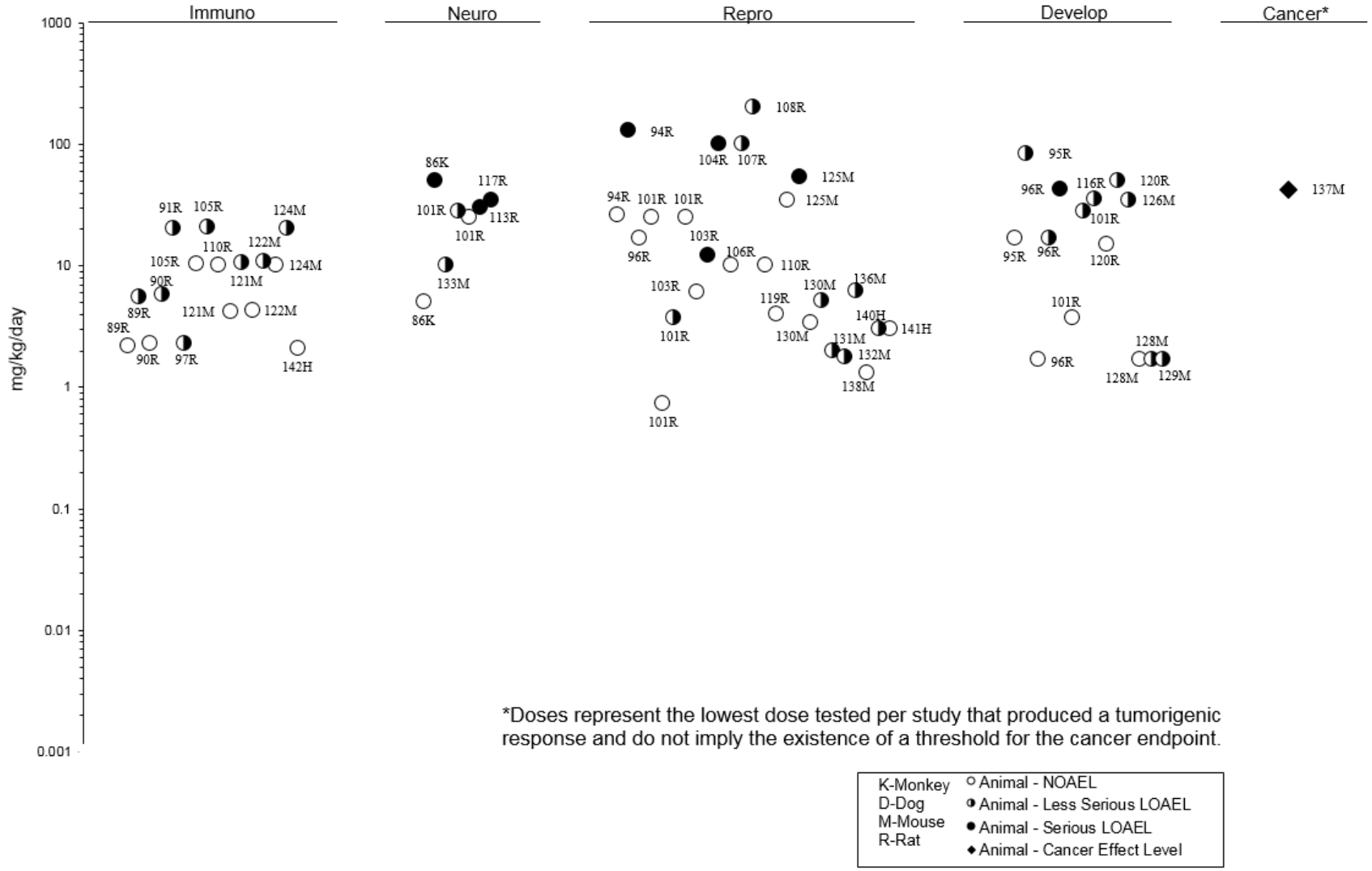


Figure 2-2. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Chronic (≥365 days)

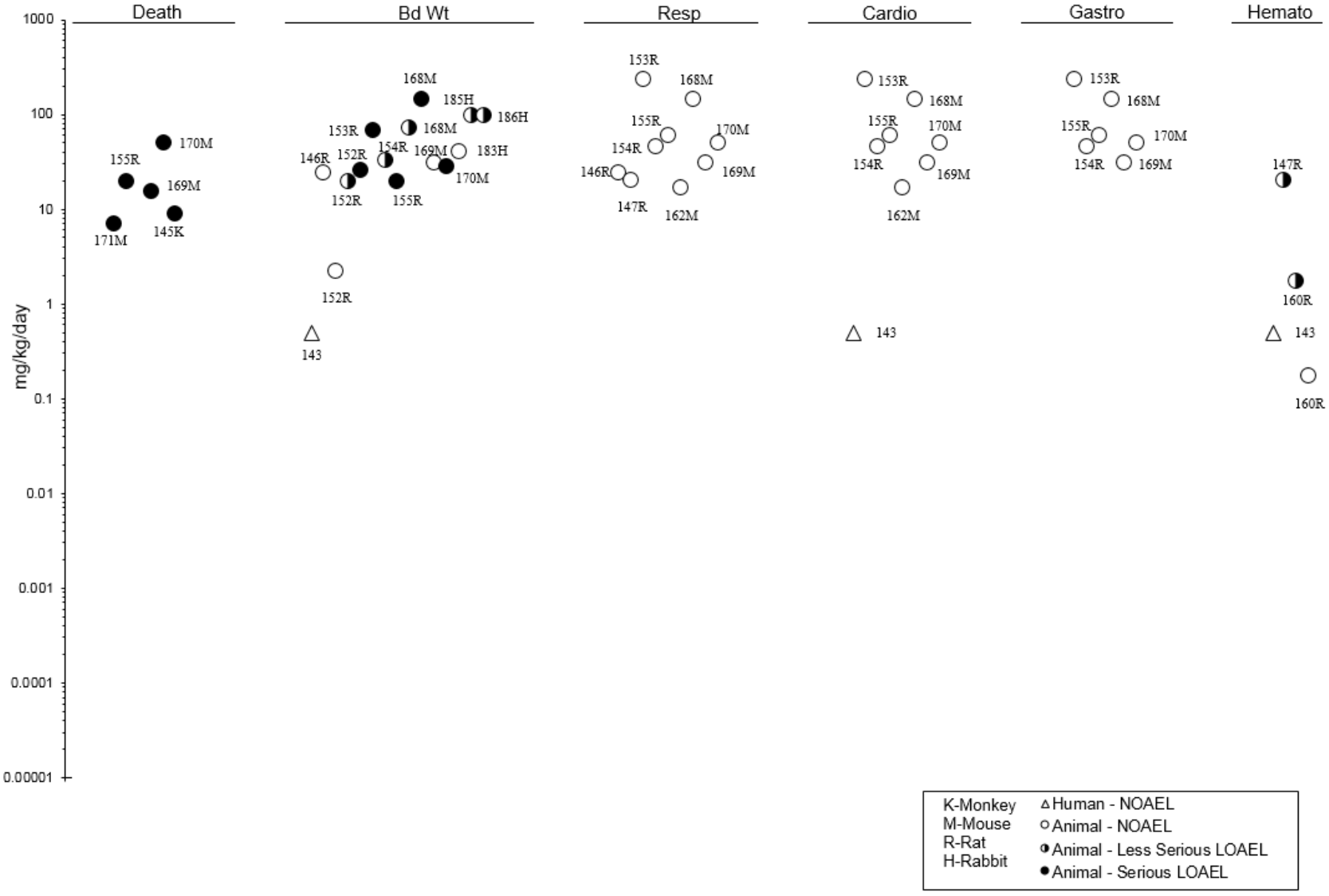


Figure 2-2. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Chronic (≥365 days)

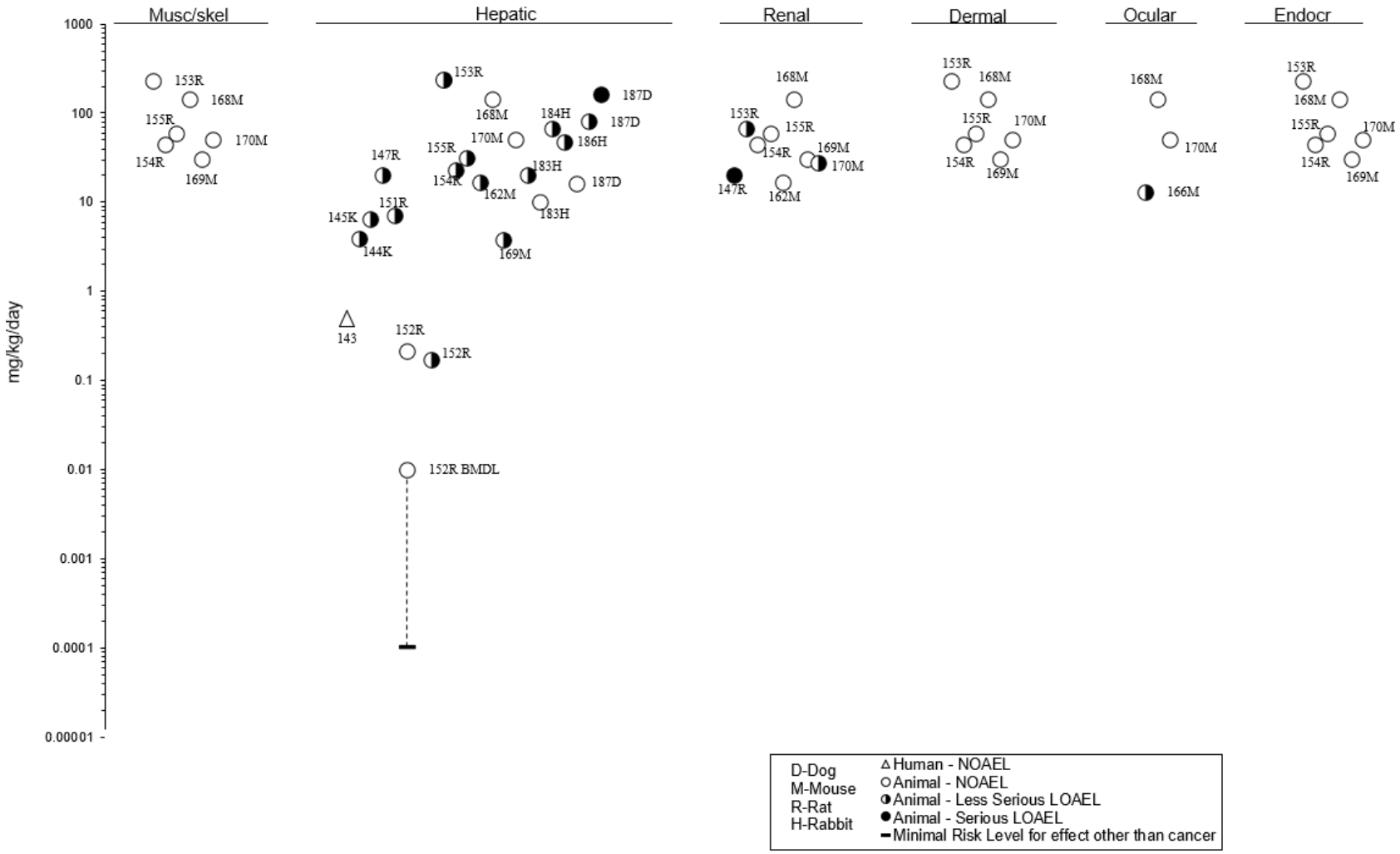
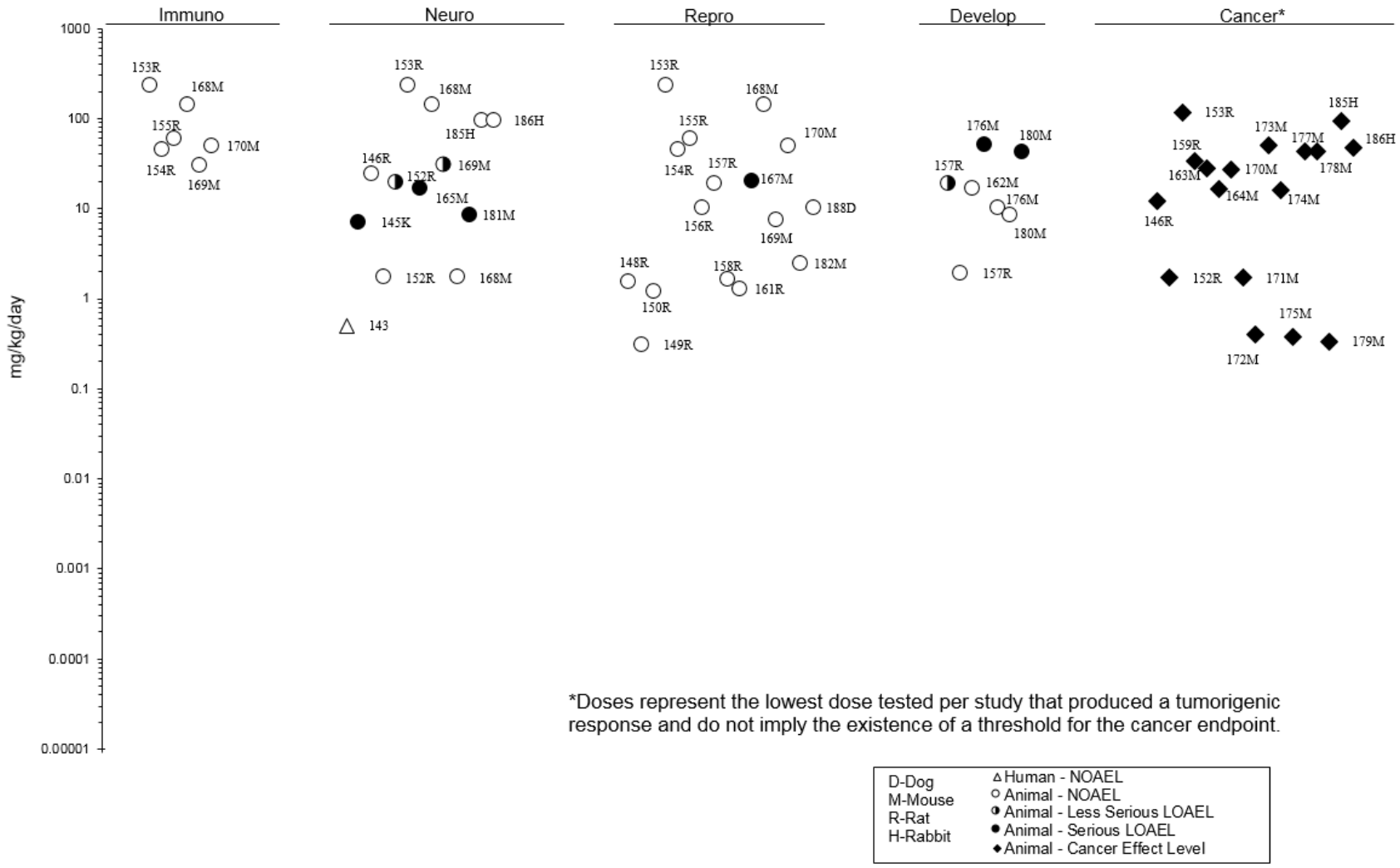


Figure 2-2. Levels of Significant Exposure to DDT, DDE, and DDD – Oral

Chronic (≥365 days)



2.2 DEATH

Evidence of Death Effects of DDT, DDD, or DDE in Humans. Only one case of fatal poisoning in humans after accidental oral exposure to DDT has been documented (Hill and Robinson 1945). One ounce (approximately 30 mL) of 5% DDT in kerosene was ingested by a 1-year-old child. Clinical signs included coughing and vomiting followed by convulsions, which were characterized as generalized fine tremors. The child then became comatose and died 4 hours post-exposure; however, the contribution of the kerosene solvent to DDT toxicity was not addressed. In 1947, kerosene, not DDT, was determined to be the cause of death of a patient who fatally swallowed an approximate 150 mL solution of commercial DDT (~4% DDT, 4% lethane, and 92% kerosene) (Reingold and Lasky 1947). Doses as high as 285 mg DDT/kg body weight have been accidentally ingested by 28 men with no fatal results (Garrett 1947).

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. Similar results were reported by Beard et al. (2003) with no significant increase in total mortality (SMRs) in occupationally exposed workers compared to the general population (Beard et al. 2003); in the exposed group, there were increases in mortality due to ischemic heart disease, respiratory disease, and pancreatic cancer. However, deaths from ischemic heart disease and respiratory disease were proposed to reflect smoking patterns, as comparisons with a control population of outdoor workers did not result in elevated deaths. This was not the case for pancreatic cancer; increases in deaths (SMR 5.27, 95% confidence interval [CI] 1.09–15.40) were found for workers with <3 years of DDT exposure, as compared the control population; no association was found in workers exposed to DDT for ≥3 years.

Brown (1992) conducted an update of a historical prospective mortality study of workers in five pesticide manufacturing plants. In the plant that manufactured DDT (230 persons and 90 deaths since 1964), there was a significant excess of deaths (11) from cerebrovascular disease. The SMR was 2.38. The study is limited by insufficient exposure data (with the exception of DDT exposure information for 35 workers employed in 1967), possible confounding exposures, and relatively small numbers of deaths from stroke.

A study of 4,552 male workers exposed to DDT and followed for 45 years reported no statistically significant increase in the relative risk of total mortality, or mortality due to various diseases or cancers in workers with estimated cumulative doses ranging from 0.01 to ≥2,755.1 mg DDT, between 1946 and

1950 (Cocco et al. 2005); the study did find lower risks of cardiovascular disease and liver cirrhosis. A group of 633 women with breast cancer with available blood DDT (n=622), DDE (n=632), chlordane (n=622), and lipid levels; the women were followed for 15 years (Parada et al. 2016). At year 5, *p,p'*-DDT exposure was associated with all causes of mortality and breast cancer-specific mortality. The respective hazard ratios (HRs) and 95% CI for T2 (serum *p,p'*-DDT levels of ≥5,638–<91.2 ng/g) versus T1 (<56.8 ng/g) were 2.19 (1.02, 4.67) and 2.72 (1.04, 7.13), respectively. At 15 years, there were no associations, other than for all-cause mortality, in women with DDT levels in the second tertile and with lower body mass index (BMI) values (<25 kg/m^2). The highest tertile of *p,p'*-DDE (≥1,058.2 ng/g) was inversely associated with all mortality (HR 0.66, 95% CI 0.44, 0.99) at 5 years, and no associations were observed at 15 years (Parada et al. 2016). Although these data may indicate increased risk of mortality with DDT exposure in these subjects, no comparisons were done with a control/non-breast cancer group, and data may not translate to risks to the general public.

Evidence of Death Effects of DDT, DDD, or DDE in Animals. The oral LD_{50} values for the various isomers and technical-grades of DDT, DDE, and DDD, as well as exposure levels associated with decreased survival in repeated-dose toxicity animal studies, are recorded in Table 2-1 and plotted in Figure 2-2. No acute inhalation studies were identified.

The LD_{50} values reported in rats exposed to single oral gavage doses of *p,p'*-DDT ranged from 113 to 800 mg/kg (Ben-Dyke et al. 1970; Cameron and Burgess 1945; Gaines 1969). The LD_{50} values for guinea pigs and rabbits after oral exposure to *p,p'*-DDT were 400 and 300 mg/kg, respectively (Cameron and Burgess 1945). The LD_{50} for technical-grade DDT in male Sherman rats in one study was 217 mg/kg (Gaines, 1969). Results from another study by Lu et al. (1965) revealed age-dependent LD_{50} values for technical-grade DDT in rats. The LD_{50} values in newborn, preweanling, weanling, and adult rats were >4,000, 438, 355, and 195 mg technical DDT/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 1969, 1970). The LD_{50} values for single oral doses of technical-grade DDT in mice from two studies were 237 and 300 mg/kg (Kashyap et al. 1977; Tomatis et al. 1972). In a short-term feeding experiment,

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).

In *p,p'*-DDE mortality studies, a LD_{50} values of 880 and 1,240 mg/kg were reported for male and female Sherman rats, respectively (Gaines 1969). Death occurred in mice after single oral doses of *o,p'*-DDE ranging from 810 to 880 mg/kg (Tomatis et al. 1974a).

In *p,p'*-DDD mortality studies, reported LD_{50} values for rats and mice ranged from about 400 to >4,000 mg/kg/day (Ben-Dyke et al. 1970; Gaines 1969; Tomatis et al. 1974a).

In dermal exposure studies, the dermal LD_{50} of DDT in rats was reported by Ben-Dyke et al. (1970) and Cameron and Burgess (1945) as 2,500 and 3,000 mg DDT/kg, respectively. The LD_{50} was 2,510 mg of technical-grade DDT/kg in female Sherman rats (Gaines 1969). In guinea pigs, a single dermal dose of 1,000 mg DDT/kg resulted in death of 50% of the animals (Cameron and Burgess 1945). Dermal LD_{50} values in rabbits were 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, ether, dimethyl phthalate, or dibutyl phthalate. It is uncertain what contribution these solvents made to the toxic effects observed; the authors stated that kerosene itself may have caused some deaths.

After intermediate-duration oral exposure to *p,p'*-DDT or technical DDT, significantly increased mortality has been observed in animals exposed to doses ≥~25–35 mg/kg/day. Increased mortality occurred shortly after mating in F0 (3/24) and F1 (6/23) female rats (not observed in males) exposed to 27.7 mg *p,p'*-DDT/kg/day in the diet for 10 weeks prior to mating, and throughout mating, gestation, and lactation (Hojo et al. 2006). Four out of five female B6C3F1 mice fed a diet that provided ~35 mg technical DDT/kg/day for 6 weeks died (NCI 1978). Gavage exposure to 50 mg *p,p'*-DDT/kg/day produced deaths in four of six monkeys after 4 weeks of treatment; the remaining monkeys died during weeks 9 and 14 of treatment (Cranmer et al. 1972).

After chronic-duration exposure to *p,p'*-DDT, *p,p'*-DDE, or technical DDT, reduced survival has been observed in monkeys, rats, and mice. Mortality rates of 10% and 28% were observed in female B6C3F1 mice exposed to 15 and 30.2 mg technical DDT/kg/day, respectively, in the diet for 78 weeks (NCI 1978). Early mortalities were not observed in male mice exposed to the same dietary concentrations of technical DDT (NCI 1978). Following dietary exposure to 49 mg *p,p'*-DDE/kg/day in the diet for 78 weeks,

female B6C3F1 mice had a 40% mortality rate (NCI 1978). A mortality rate of 16% was observed in female Osborne-Mendel rats exposed to 19 mg *p,p'*-DDE/kg/day in the diet for 78 weeks (NCI 1978). In a 130-month study that administered approximately 6.4–15.5 mg of *p,p'*-DDT/kg/day to Rhesus and Cynomolgus monkeys, there were 6/24 early deaths; the lowest dose associated with death was approximately 6.9 mg/kg/day (Takayama et al. 1999). Exposure-related reduced survival was not observed in male and female F344/DuCrj rats exposed for up to 104 weeks to 19.1 and 25.2 mg/kg/day *p,p'*-DDT, respectively, in the diet (Harada et al. 2003).

2.3 BODY WEIGHT

Evidence of Body Weight Effects of DDT, DDD, or DDE in Humans. Epidemiological studies described in Table 2-2 examined associations between serum or adipose levels of DDT, DDE, or total DDT and weight status markers including BMI, abdominal obesity, or measurements of visceral or subcutaneous abdominal tissue in older adults ≥50 years of age (Arrebola et al. 2014; De Roos et al. 2012; Dhooge et al. 2010; Lee et al. 2012a, 2012b; Roos et al. 2013; Schildkraut et al. 1999), young adults <50 years old (Lee et al. 2011b; Perry et al. 2005), and children or adolescents (Burns et al. 2012; Dhooge et al. 2010; Tang-Peronard et al. 2015a).

Consistent evidence for significant positive associations with BMI was found in most studies of adults ≥50 years of age with mean serum DDE levels lower than ~500 ng/g lipid (Arrebola et al. 2014; De Roos et al. 2012; Dhooge et al. 2010; Lee et al. 2012a; Schildkraut et al. 1999). Other studies of older adults have reported positive associations with body weight at 50 years of age and measures of intra-abdominal fat (but not subcutaneous fat) in a group of postmenopausal women (De Roos et al. 2012) and with abdominal areas of visceral and subcutaneous adipose tissue (VAT and SAT) in a group of 70-year-old Swedish men and women (Roos et al. 2013). Inconsistent results were reported in studies evaluating sex differences in adults (Elobeid et al. 2010; Lee et al. 2012a; Tang-Peronard et al. 2015a). For example, Elobeid et al. (2010) reported an inverse association between serum DDT and BMI in men and a positive association in women, and Lee et al. (2012a) reported no significant association with incidence of abdominal obesity at age 70 years in women and a significant positive association in men.

Table 2-2. Summary of Studies of Associations between DDT Exposure Biometrics and Body Weight Status[a]

Reference	Exposure metric[b]	Outcome evaluated	Results[c]	Notes
Arrebola et al. 2014 298 adults (median age 51 years old; 152 women, 146 men) Granada Spain	DDE, adipose	BMI	↑	Adjusted betas, continuous exposure variable Associations with total cholesterol and total lipids were NS
Burns et al. 2012 350 peri-pubertal boys 8–9 years old at study initiation; annual follow-ups for 4 years Chapaevsk Russia	DDE, serum	WHO age-adjusted BMI z-score WHO age-adjusted height z-score Change in height (cm/year over 4 years)	↓ (Q2–Q5) ↓ (Q4–Q5) ↓ (Q4–Q5)	Adjusted betas, categorical exposure levels versus Q1 Significant p-trends for all endpoints
De Roos et al. 2012 173 sedentary and overweight/obese, post-menopausal women, ~60 years old Seattle, Washington	DDE, serum	BMI Current body composition Fat mass (percent total body mass) Subcutaneous fat (per 100 units) Intra-abdominal fat (per 100 units) Waist circumference Hip circumference Waist:hip ratio Weight at 50 years Maximum weight Net weight change since 35 years Weight loss episodes Number of weight loss episodes	NS NS NS NS ↑ (beta 36.5) NS NS NS ↑ (50 years) NS NS NS NS	Adjusted betas, continuous exposure levels
Dhooge et al. 2010 775 men, 808 women, 50–65 years old 1,679 adolescents, 14–15 years old, 887 boys, 792 girls Flanders	DDE, serum	BMI (male adults) BMI (female adults) BMI (boys) BMI (girls)	↑ ↑ ↓ ↓	Adjusted betas for DDE doubling, continuous exposure levels Data for adolescents were not shown

Table 2-2. Summary of Studies of Associations between DDT Exposure Biometrics and Body Weight Status[a]

Reference	Exposure metric[b]	Outcome evaluated	Results[c]	Notes
Elobeid et al. 2010 2,464 adults (BMI), 2,448 adults (waist circumference) NHANES 1999–2002	DDT, serum	Overall adiposity: BMI (overall) Male Female Waist circumference (overall) Male Female	 ↑ ↓ ↑ NS ↓ ↑	Adjusted betas per SD unit increase of ln DDT
Lee et al. 2011b 5,115 U.S. adults at study initiation (18–30 years old at initiation)	DDE and DDT, serum; no exposure levels were provided	BMI at 20-year follow-up (ages 38–50 years)	↑ (p-trend)	Increasing p-trend of adjusted means of BMI across exposure quartiles for both DDE and DDT For DDE, adjusted means increased up to 3rd quartile, then decreased in 4th DDE quartile to levels equal to the 2nd DDE quartile For DDT, adjusted means steadily increased across quartiles
Lee et al. 2012a 970 adults (49% men); age 70 years at study initiation Abdominal obesity: waist circumference >102 cm men; >88 cm women Uppsala, Sweden	DDE, serum; serum levels provided in supplementary tables	Abdominal obesity at 70 years old Women Men Abdominal obesity developed between 70 and 75 years old Women Men	 NS (Q2–Q5) ↑ (Q4, Q5) NS NS	Adjusted ORs – Categorical exposure levels versus Q1 Significant positive p-trend across quintiles (women and men) for prevalence at 70 years old
Lim et al. 2011 1,099 adults ≥40 years old NHANES population 1999–2002	DDE, serum, no exposure levels were provided	Categories of 10-year changes in weight: -- - 0 + ++ Weight loss Stable Weight gain	↓	Adjusted correlation coefficients r= -0.16 (p<0.01) for decreasing DDE serum across weight change categories

Table 2-2. Summary of Studies of Associations between DDT Exposure Biometrics and Body Weight Status[a]

Reference	Exposure metric[b]	Outcome evaluated	Results[c]	Notes
Perry et al. 2005 466 nulliparous women, mean age ~25 years Anhui China	Total DDT, serum (high exposure levels: mean DDE 5,940 ng/g lipid)	BMI	↓	Adjusted betas, continuous exposure levels; each kg/m^2 increase in BMI was associated with -1.34 ng/g lipid decrease in serum ΣDDT
Roos et al. 2013 287 adults (aged 70 years) Uppsala, Sweden	DDE, serum	Areas of the abdomen measured by MRI: Visceral Adipose Tissue (VAT) Subcutaneous AT (SAT) VAT/SAT	 ↑ ↑ NS	Adjusted betas (95% CI), continuous exposure variable
Schildkraut et al. 1999 99 Women (42 black, 57 white) Mean age ~57 years North Carolina	DDE, serum	BMI Waist:hip ratio (4th versus 1st quartile) Weight loss in past year (>5 lbs) Weight gain in past year (>5 lbs)	↑ NS NS NS	Adjusted betas for lnDDE, categorical outcomes
Tang-Peronard et al. 2015a 509 children (8–9 years old) at study initiation Odense, Denmark	DDE, serum	At 14–16 years: BMI z-score Waist circumference Percent body fat At 20–22 years: BMI z-score Waist circumference Percent body fat	 NS (M, F) ↓ (F) NS (M) NS (M, F) ↓ (F) ↑ (M) NS (M, F) NS (M, F)	Adjusted betas (95% CI), both continuous and categorical exposure variables

[a]Studies in this table were selected because they: (1) measured DDT-related metrics in biological fluids or tissues in each subject, and (2) evaluated associations using linear regression, logistical regression, or correlation techniques, adjusting for possible confounding factors.
[b]DDE = *p,p'*-DDE, unless otherwise specified; DDT = *p,p'*-DDT, unless otherwise specified.
[c]Arrows "↑ or ↓" indicate statistical significance ($p<0.05$) and direction of change observed for a given outcome (↑ association and ↓ inverse association).

BMI = body mass index; CI = confidence interval; F = female(s); M = male(s); MRI = magnetic resonance imaging; NHANES = National Health and Nutrition Examination Survey; NS = not statistically significant; OR = odds ratio; Q = quintile(s); SAT = subcutaneous adipose tissue; SD = standard deviation; VAT = visceral adipose tissue; WHO = World Health Organization

In contrast to the consistent evidence of a positive association from studies of BMI in older adults, a negative (inverse) association was reported in a study of young (mean age ~25 years) Chinese women with very high serum levels of total DDT (32 ng/g) (Perry et al. 2005), and a positive association was observed in a group of U.S. adults, ages 38–50 years (Lee et al. 2011b). Lim et al. (2011) found an inverse relationship between weight change over a 10-year period and increased serum DDE, albeit at serum levels in the range of NHANES studies. It is possible that the apparent inverse association of very high serum levels on BMI in the group of Chinese women (~10-fold >500 ng/g lipid) may be an entirely different response to DDT or DDE, than that inferred from the studies of people with serum total DDT or DDE levels <500 ng/g lipid, reflecting some non-monotonic response, but this possible explanation is based on very limited data. In a systematic review and meta-analysis, Cano-Sancho et al. (2017) concluded that there was a moderate level of epidemiological evidence for an association between *p,p′*-DDE and BMI and calculated a beta of 0.13 (95% CI 0.01–0.25).

Three studies evaluating DDT biometrics in children and adolescents provide inconsistent evidence for an inverse association between serum DDE and weight status markers (Burns et al. 2012; Dhooge et al. 2010; Tang-Peronard et al. 2015a). Inverse associations were reported for BMI z-score in Russian boys (Burns et al. 2012) and for BMI in boys and girls from Flanders (Dhooge et al. 2010), but no significant associations for BMI z-scores were found in Danish boys and girls at 14–16 years of age (Tang-Peronard et al. 2015a); at 20–22 years, the Danish study reported a positive association in the males and an inverse association in the females (Tang-Peronard et al. 2015a).

In a single controlled exposure study, 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).

Evidence of Body Weight Effects of DDT, DDT, or DDE in Animals. Effects on body weight have been observed in animals orally exposed to DDT and related compounds for acute, intermediate, and chronic durations of exposure.

Following acute oral exposure of adult animals, reported body weight effects include transiently decreased body weight on GDs 17–21 (9–17 % decreased compared with controls; returned to control levels by postpartum day 1) in rat dams exposed to 200 mg *p,p'*-DDE/kg/day, but not 100 mg/kg/day, on GDs 14–18 (Loeffler and Peterson 1999), and in adult males treated for with 200 mg *p,p'*-DDE/kg/day for 4 days (Kelce et al. 1995). No significant exposure-related body weight changes were noted in rat dams

exposed to gavage doses of 50 or 100 mg *p,p'*-DDT/kg/day during pregnancy on GDs 13.5–17.5 (Adamsson et al. 2009); mouse dams exposed by gavage to up to 100 mg *o,p'*-DDT/kg/day on GDs 11–17 (Palanza et al. 2001); castrated male rats exposed to up to 100 mg *p,p'*-DDE/kg/day for 10 days (Kang et al. 2004); or male mice exposed to up to 2 mg *p,p'*-DDE/kg/day for 5 days (Howell et al. 2014).

After intermediate duration exposure to technical DDT, *p,p'*-DDE, or technical DDD, decreased body weight or body weight gain have been observed in rats and mice. Significantly decreased body weight or body weight gain (≥10% decreased, compared with control values) were reported in male albino rats exposed by gavage to 0.2 mg technical DDT/kg/day for 120 days (Chowdhury et al. 1990); male and female Osborne-Mendel rats fed ≥50 or 97 mg technical DDT/kg/day, respectively, in the diet for 6 weeks (NCI 1978); male Osborne-Mendel rats fed ≥49 mg *p,p'*-DDE/kg/day in the diet for 6 weeks (NCI 1978); and female and male Osborne-Mendel rats exposed to 97 or 279 mg technical DDD/kg/day, respectively, in the diet for 6 weeks (NCI 1978). Increased body weight (18% increase) occurred in pubertal male Long-Evans rats dosed with 100 mg *p,p'*-DDE/kg/day from PNDs 21 to 57 (Kelce et al. 1995). No significant changes in body weight (compared with control values) were observed in male and female B6C3F1 mice fed 35 mg technical DDT/kg/day in the diet for 6 weeks (NCI 1978); male and female F344/DuCrj rats exposed to up to 45.7 mg *p,p'*-DDE/kg/day in the diet for 4 weeks (Harada et al. 2003); F0 and F1 parental female Sprague-Dawley rats exposed to up to 27.7 mg *p,p'*-DDE/kg/day for 10 weeks prior to mating and then throughout gestation and lactation (Hojo et al. 2006); Sprague-Dawley rat dams exposed to gavage doses as high 50 mg *p,p'*-DDE/kg/day from GD 6 to PND 20 (Yamasaki et al. 2009) or in prepubertal male F344/DuCrlCrlj rats receiving dietary doses of 10 mg *p,p'*-DDE/kg/day for 42 days (Makita et al. 2003a).

Following chronic-duration exposures to technical DDT, *p,p'*-DDT, *p,p'*-DDE, or technical DDD, decreases in body weight have been observed in rats, mice, and hamsters. After 78-week exposures, consistent decreases in body weight or body weight gain ≥20% were observed in female Osborne-Mendel rats exposed to 32 mg technical DDT/kg/day, 66 mg technical DDD/kg/day (lowest dose tested), and 19 mg *p,p'*-DDE/kg/day (lowest dose tested) (NCI 1978). Male rats exhibited a 16% decrease in weight gain at 45 mg technical DDT/kg/day (NCI 1978) and a 28% decrease with 116 mg technical DDD/kg/day (NCI 1978). The chronic 2-year study by Harada et al. (2003, 2006) reported a 12% decrease in body weight in F344/DuCrj male rats orally exposed to 19.1 mg *p,p'*-DDT/kg/day and a 25% decrease at 25.5 mg/kg/day in females. Female B6C3F1 mice exposed to 28 mg *p,p'*-DDE/kg/day or 71 mg technical DDD/kg/day in their diets for 78 weeks had decreases in body weight gain of 29 and 17%, respectively (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 with controls (Rossi et al. 1983), but hamsters fed 40 mg technical DDT/kg/day for life were reported to have comparable body weights to control hamsters (Cabral et al. 1982a).

Mechanisms of Body Weight Effects of DDT, DDD, or DDE. The human epidemiological studies are consistent with the hypothesis that endocrine disrupting compounds (EDCs), including DDT, may act as obesogens that display non-monotonic dose-response relationships, leading to weight gain at lower exposure levels, but to growth restriction or weight loss at higher exposure levels (Tang-Peronard et al. 2011). Reduced body weights in the described animal studies are likely the result of high-dose exposure levels. Several studies focusing on potential mechanisms behind DDT-associated obesity and obesity-related diseases are discussed in Section 2.18. Further studies may increase understanding of the complexities between the timing of DDT exposure, differences between DDT metabolites, dose, and gender, as well as the influences of initial weight status and significant weight change on serum levels of DDT and DDT toxicity (La Merrill et al. 2013).

2.4 RESPIRATORY

Evidence of Respiratory Effects of DDT, DDD, or DDE in Humans. Epidemiological evidence of respiratory effects that are mediated by immunological function (e.g., asthma, wheezing, bronchitis, respiratory tract infections, and hypersensitivity) is discussed in detail in Section 2.14.

Two studies provide inconsistent evidence for associations between serum levels of *p,p'*-DDT or *p,p'*-DDE and measures of lung function (Hansen et al. 2016 [see Section 2.14]; Ye et al. 2015). In a study of 1,696 Canadian adults, serum levels of DDT and DDE were inversely associated with forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1) in models adjusted for age, sex, ethnicity, height, smoking status, and daily energy expenditure; associations with FEV1/FVC and forced expiratory flow (FEF) at 25–75% were not statistically significant (Ye et al. 2015). In the other study, maternal serum levels of DDE showed no associations with reduced lung function (FEV1 percent of predicted value <90%), but a positive association with airway obstruction (FEV1/FVC <75%) was noted in offspring at 20 years of age (Hansen et al. 2016).

In a single controlled exposure study, 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. The investigators provided some information on exposure levels, but noted that the DDT quickly settled, and thus, the actual exposure levels were lower than predicted.

Evidence of Respiratory Effects of DDT, DDD, or DDE in Animals. No studies were located regarding the respiratory effects in animals after acute or intermediate oral exposure to DDT, DDD, or DDE.

In chronic oral studies, rats fed a diet containing 20 mg commercial 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 the National Cancer Institute (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, or in 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). No histopathological changes or tumors in the lung were noted at 55 weeks of age of ICR mice exposed from conception through death to 16.5 mg/technical-DDT/kg/day (Del Pup 1978); however, development of lung tumors has been reported in other studies following oral exposure of mice to DDT isomers and is discussed in Section 2.19.

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).

Mechanisms of Respiratory Effects of DDT, DDE, or DDD. There is inconsistent evidence supporting associations between exposures to DDT isomers and impaired lung function. However, some mechanistic studies have begun to evaluate the relationship between serum levels of DDT isomers and respiratory effects mediated by immunological dysfunction (see Section 2.14).

2.5 CARDIOVASCULAR

Evidence of Cardiovascular Effects of DDT, DDD, or DDE in Humans. A number of epidemiological studies have examined associations between serum or adipose levels of DDT, DDD, or DDE and cardiovascular outcomes, including general hypertension in adults (Arrebola et al. 2015b; Goncharov et al. 2011; Henriquez-Hernandez et al. 2014; Lee et al. 2007b; La Merrill et al. 2013; Lind et al. 2014; Park et al. 2016; Valera et al. 2013a, 2013b), gestational hypertension (Savitz et al. 2014), stroke (Lee et al. 2012a, 2012b), cardiovascular disease (Ha et al. 2007), and peripheral arterial disease (Min et al. 2011).

The results provide inconsistent evidence for associations between serum or adipose levels of DDE or DDT and cardiovascular effects.

Three out of six epidemiological studies on hypertension (Lind et al. 2014; Valera et al. 2013a, 2013b) and one meta-analysis (Park et al. 2016) reported some evidence for associations with serum or adipose levels of *p,p'*-DDE. *p,p'*-DDE levels were associated with increased risk of hypertension in an Inuit community in Quebec (Valera et al. 2013a) and in a population of older adults in Sweden (Lind et al. 2014), but not in a population of 1,614 Inuit adults in Greenland with similarly high exposure levels (Valera et al. 2013b). Despite inconsistencies among individual studies, meta and random-effects analysis across all six studies suggested that an overall small, but significant, association between DDE serum levels and hypertension may exist (Park et al. 2016). Fewer studies have looked for associations with levels of DDT, and report both positive (association found in 18–39 year olds, but not in adults ≥40 years of age) (Valera et al. 2013b) and inverse relationships between *p,p'*-DDT serum levels and hypertension (Valera et al. 2013a). A single study examined possible associations between *p,p'*-DDT, *o,p'*-DDT, or DDE serum levels sampled from pregnant women in 1959–1967 and incidence of hypertension in adult daughters (as defined as medication for the treatment of hypertension) in 2005–2008 and found significant associations with *p,p'*-DDT, but not with *o,p'*-DDT or *p,p'*-DDE (La Merrill et al. 2013).

In other epidemiological studies, no statistically significant positive associations with gestational hypertension (Savitz et al. 2014), cardiovascular disease (Ha et al. 2007), or systolic or diastolic blood pressure levels (Goncharov et al. 2011; Henriquez-Hernandez et al. 2014; Valera et al. 2013a, 2013b) were observed (Table 2-3). Significant associations were reported between serum DDT or DDE levels and incidences of stroke in elderly men (Lee et al. 2012b) and peripheral arterial disease in obese adults (Min et al. 2011). Another study (Mills et al. 2009) found a significant association between DDT pesticide use and incidence of nonfatal myocardial infarction, although exposure levels of DDT were not reported; no associations were found for fatal myocardial infarction.

In a controlled exposure study, no clear effects on cardiovascular performance (resting and exercise heart rate, systolic blood pressure, and pulse pressure) were found in male volunteers orally administered 3.5 or 35 mg DDT/day by capsule for 12–18 months either as recrystallized DDT administered via a capsule or technical-grade DDT administered via a milk emulsion (about 0.05–0.063 or 0.36–0.5 mg/kg/day) (Hayes et al. 1956).

Table 2-3. Summary of Studies of Associations between DDT Exposure Biometrics and Cardiovascular Endpoints[a]

Reference	Exposure metric[b]	Outcome evaluated	Results[c]	Notes
Arrebola et al. 2015b 297 adults (median age 48 years) Granada, Spain	DDE, adipose	Hypertension Total BMI ≤26.3 BMI >26.3	 NS NS NS	Adjusted HRs (95% CI), per 1 log-unit increase in exposure
Goncharov et al. 2011 394 adults (median age 47 years) Anniston, Alabama	*p,p'*-DDT, *p,p'*-DDT, *p,p'*-DDE	Systolic blood pressure Diastolic blood pressure	NS (all compounds) NS (all compounds)	Linear regression analysis, categorical exposure levels
Ha et al. 2007 889 adults NHANES 1999–2002	DDE, serum	Cardiovascular disease	NS	Adjusted ORs (95% CI), categorical Exposure levels (percentiles) p-trend = NS
Henriquez-Hernandez et al. 2014 428 adults (mean age 47.2 years) Canary Islands	DDE, serum	Hypertension SBP ≥140 mmHg DBP ≥90 mmHg	NS NS NS	Adjusted OR (95% CI), categorical exposure levels (tertiles)
Lee et al. 2007b 721 adults NHANES 1999–2002	DDE, serum	BP ≥130/85	NS	Adjusted ORs (95% CI), categorical exposure levels p-trend = NS
Lee et al. 2012b 898 adults (mean age 70 years) Uppsala, Sweden	DDE, serum	Incidence of stroke	↑ (Q2, Q4)	Adjusted ORs (95% CI), categorical exposure levels p-trend NS
La Merrill et al. 2013 457 normotensive, 111 adult women self-reporting hypertension, 70 adult women using hypertension medication San Francisco 2005–2008	*p,p'*-DDT, *o,p'*-DDT, DDE, serum from mothers' sampled during pregnancy (1959–1967)	Medicated hypertension Self-reported hypertension	↑ (T2, T3) *p,p*'-DDT NS *o,p'*-DDT NS DDE ↑ (T2) NS (T3) *p,p*'-DDT	Adjusted ORs (95% CI), categorical exposure levels

Table 2-3. Summary of Studies of Associations between DDT Exposure Biometrics and Cardiovascular Endpoints[a]

Reference	Exposure metric[b]	Outcome evaluated	Results[c]	Notes
Lind et al. 2014 1,016 adults (mean age 70 years) Uppsala, Sweden	DDE, serum	Prevalent hypertension	↑	Adjusted ORs (95% CI), per doubling of DDE
Min et al. 2011 2,032 adults NHANES 1999–2004	DDE, serum	Peripheral arterial disease	↑ (obese) NS (non-obese)	Adjusted ORs (95% CI) per unit increase in exposure in obese and non-obese subjects
Park et al. 2016 Meta-analysis of six studies (Arrebola et al. 2015b Heriquez-Hernandez et al. 2014; Lee et al. 2007b; Lind et al. 2014; Valera et al. 2013a, 2013b)	DDE	Hypertension	↑	Overall OR (95% CI); only one (out of six) individual studies (Lind et al. 2014) with significant OR
Savitz et al. 2014 1,933 women total 364 with gestational hypertension; 151 with preeclampsia	DDE; DDT, serum	Gestational hypertension	↓ (DDT, Q3, Q5) NS (DDE)	Adjusted ORs (95% CI), categorical exposure levels
		Preeclampsia	NS (DDT, DDE)	Risk for gestational hypertension was inversely associated with increasing DDT
Valera et al. 2013a 315 Inuit adults; mean age 32.7 years Quebec	DDE; DDT, serum	Risk of hypertension		Adjusted ORs (95% CI) per 1 log unit increase in exposure levels
		Model 1	↓ (DDT)	
		Model 2	↑ (DDE) ↓ (DDT)	
		Model 3	↑ (DDE) ↓ (DDT)	Model 1: standard adjustments Model 2: additional adjustment for specific polyunsaturated fatty acids, eicosapentaenoic acid, and docosahexaenoic acid Model 3: model 1 plus additional adjustments for toxic metals (lead and mercury)
		SBP (DDE or DDT)	NS	Adjusted betas for change per 1 log-unit increase in exposure levels
		DBP (DDE or DDT)	NS	

Table 2-3. Summary of Studies of Associations between DDT Exposure Biometrics and Cardiovascular Endpoints[a]

Reference	Exposure metric[b]	Outcome evaluated	Results[c]	Notes
Valera et al. 2013b	DDE; DDT, serum	Hypertension (DDE)	NS	Adjusted ORs (95% CI) per 1 log-unit
1,614 Inuit adults; mean age		Ages 18–39 years	NS	increase in exposure levels
44.6 years		Ages ≥40 years	NS	
Greenland		Hypertension (DDT)	NS	
		Ages 18–39 years	↑	
		Ages ≥40 years	NS	
		SBP (DDE or DDT)	NS	Adjusted betas for change per 1 log-unit
		DBP (DDE or DDT)	NS	increase in exposure levels

[a]Studies in this table were selected because they: (1) measured DDT-related metrics in biological fluids or tissues in each subject, and (2) evaluated associations using linear regression, logistical regression, or correlation techniques, adjusting for possible confounding factors.
[b]DDE = *p,p'*-DDE, unless otherwise specified; DDT = *p,p'*-DDT, unless otherwise specified.
[c]Arrows "↑ or ↓" indicate statistical significance ($p<0.05$) and direction of change observed for a given outcome (positive or inverse associations, respectively).

BMI = body mass index; CI = confidence interval; DBP = diastolic blood pressure; NHANES = National Health and Nutrition Examination Survey; NS = not statistically significant; OR = odds ratio; HR = hazard ratio; Q = quartile(s) and quintile(s); SBP = systolic blood pressure; T = tertile(s)

Evidence of Cardiovascular Effects of DDT, DDD, or DDE in Animals. In a developmental toxicity study, increased systolic blood pressure was measured at 5 and 7 months of age in the offspring of C57BL/6J mice exposed to 1.7 mg/kg/day mixture of *p,p'*-DDT (77.2%) and *o,p'*-DDT (22.8%) from GD 12 to PND 5 (La Merrill et al. 2016). Cardiac hypertrophy was also observed in 8.5-month-old mice. No other studies evaluating cardiovascular effects following acute- or intermediate-duration studies in rodents orally exposed to DDT, DDD, or DDE were identified. A 14-day study in dogs exposed to *o,p'*-DDD, resulted in decreased contractile force at a LOAEL of 50 mg/kg/day (Cueto et al. 1970), but no effects were observed with *p,p'*- isomers in dogs at any exposure duration.

In chronic oral exposure studies, no significant chemical-related adverse effects on the cardiovascular system were observed in Osborne-Mendel rats treated in the diet for up to 78 weeks with up to 45 mg technical DDT/kg/day, 59 mg *p,p'*-DDE/kg/day, or 231 mg technical DDD/kg/day or in 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). No histopathological changes in the heart were noted at 55 weeks of age of ICR mice exposed from conception through death to 16.5 mg/technical-DDT/kg/day (Del Pup 1978).

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 muscle fibers of the heart.

Mechanisms of Cardiovascular Effects of DDT, DDD, or DDE. In men, DDT has known anti-androgenic effects and has been inversely associated with serum testosterone (Blanco-Muñoz et al. 2012). Low levels of testosterone have been linked to hypertension and is a known risk factor for the development of major cardiac events, supporting the hypothesis that the anti-androgenic effects of DDE may impact cardiac health (Lind et al. 2014); see Section 2.16 for discussion of anti-androgenic effects. Although plausible, more experimental studies are needed to elucidate potential mechanistic relationships between DDT anti-androgenic activity and cardiovascular effects. In a mouse developmental toxicity study, DDT-induced increased systolic and diastolic blood pressure could be partially reversed with the angiotensin converting enzyme (ACE) inhibitor, captopril (La Merrill et al. 2016). The results are consistent with the idea that overactivation of ACE may be involved in DDT-induced hypertension (La Merrill et al. 2016). Biochemical studies on kidney tissue showed the overactivation of the renin-angiotensin system to be associated with increased renal expression of sodium transporter mRNA (La Merrill et al. 2016). Whether a similar mechanism may operate in humans is unknown.

2.6 GASTROINTESTINAL

Evidence of Gastrointestinal Effects of DDT, DDD, or DDE in Humans. A single human study of 199 mother-infant pairs found no significant associations between gastrointestinal infections in 7-month-old offspring and maternal serum levels of DDE (Dallaire et al. 2004; see Section 2.14).

Evidence of Gastrointestinal Effects of DDT, DDD, or DDE in Animals. No evaluation of gastrointestinal effects following acute or intermediate oral exposure studies have been reported.

In chronic studies, no significant chemical-related adverse effects on the gastrointestinal system were observed in Osborne-Mendel rats treated for up to 78 weeks 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 or in 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).

2.7 HEMATOLOGICAL

Evidence of Hematological Effects of DDT, DDD, or DDE in Humans. Epidemiological studies evaluating associations between hematological effects and DDT exposure biometrics are limited (Table 2-4). A Brazilian study of adults with markedly high levels of serum DDE (>10-fold higher than the general population) found no significant associations between serum DDE levels and risks for abnormal distributions of various blood cell types (Freire et al. 2015a, 2015b). Analysis of a general population NHANES cohort (2003–2004) found associations between serum DDT levels and increased number of lymphocytes and decreased number of segmented neutrophils (Serdar et al. 2014).

In a controlled exposure study, 51 male volunteers were exposed to 0.05–0.063 or 0.36–0.5 mg DDT/kg/day for 12–18 months (Hayes et al. 1956). Although some variation among individuals in hemoglobin levels, red and white blood cell counts, and percentage of polymorphonuclear leukocytes was noted, these variations did not correlate with increased dosage of DDT or with duration of exposure.

Table 2-4. Summary of Studies of Associations between DDT Exposure Biometrics and Hematological Endpoints[a]

Reference	Exposure metric[b]	Outcome evaluated	Results[c]	Notes
Freire et al. 2015a, 2015b 415 males; 432 females Brazil	DDE, serum	Anemia Leukopenia Leukocytosis Neutropenia Neutrophilia Eosinophilia Thrombocytopenia	NS NS NS NS NS NS NS	Adjusted ORs (95% CI), continuous and categorical exposure variables Results NS in both males and females
Serdar et al. 2014 NHANES 2003–2004	DDT, serum	Lymphocyte number Segmented neutrophils number	↑ (Q2, Q4) ↓ (Q4)	Adjusted betas (95% CI), categorical exposure variables

[a]Studies in this table were selected because they: (1) measured DDT-related metrics in biological fluids or tissues in each subject, and (2) evaluated associations using linear regression, logistical regression, or correlation techniques, adjusting for possible confounding factors.
[b]DDE = *p,p'*-DDE, unless otherwise specified; DDT = *p,p'*-DDT, unless otherwise specified.
[c]Arrows "↑ or ↓" indicate statistical significance ($p<0.05$) and direction of change observed for a given outcome (positive or inverse associations, respectively).

CI = confidence interval; NHANES = National Health and Nutrition Examination Survey; NS = not statistically significant; OR = odds ratio; Q = quartile(s)

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 re-divided 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) was significantly greater in the high-DDE group than in the low-DDE group; however, the changes were within the range of normal clinical values. No other differences were seen in other hematological parameters.

Evidence of Hematological Effects of DDT, DDD, or DDE in Animals. There is little evidence that hematological parameters are sensitive targets for DDT, DDE, or DDD toxicity.

Some evidence for microcytic anemia has been reported in rats fed *p,p'*-DDT in the diet at doses ≥5.0 mg/kg/day for 13–78 weeks (Tomita et al. 2013). The changes in hematocrit, hemoglobin, and red blood cell counts were small (approximately 3% lower than controls), but statistically significant. The magnitude of these decreases did not markedly change with longer durations of exposure and some were not significant at all time points. With 2 weeks of exposure, these effects were not seen, but small, statistically significant decreases (compared with controls) were found only at 50 mg/kg/day for hemoglobin content of reticulocytes (3%) and mature erythrocytes (1%), along with a decrease in transferrin saturation, but no changes in plasma iron levels. There was, however, a marked increase in unsaturated iron binding capacity (115% increase at 50 mg/kg/day) and total iron binding capacity (5 and 53% at 5 and 50 mg/kg/day, respectively) (Tomita et al. 2013).

In other intermediate- to chronic-duration studies, rats exposed to commercial DDT at 20 mg/kg/day for 27 months had congestion and hemolysis of the spleen (Deichmann et al. 1967). No hematological changes were observed in squirrel monkeys exposed orally to doses of 0.05–50 mg *p,p'*-DDT/kg/day for up to 6 months; however, all monkeys in the highest dose group (six animals) died by week 14 (Cranmer et al. 1972); the cause of death was not determined.

Cameron and Burgess (1945) exposed rats, guinea pigs, and rabbits to acute dermal doses ranging from 50 to 200 mg technical DDT/kg. A decrease in hemoglobin and leukocytosis was reported.

Mechanisms of Hematological Effects of DDT, DDD, or DDE. Due to the lack of strong evidence that DDT exposure is associated with consistent hematological effects, mechanistic investigations are limited.

Tomita et al. (2013) hypothesized that microcytic anemia from repeated dietary DDT exposure in rats may be due to impaired iron utility.

Several *in vitro* incubation studies indicated that DDT isomers can induce apoptosis in multiple blood cell-types including human primary peripheral blood mononuclear cells (PBMCs) (Alegria-Torres et al. 2009; Perez-Maldonado et al. 2004, 2005, 2006). In a preliminary study, exposed children in Chiapas, Mexico, had an increased percentage of PBMC apoptotic cells compared to a non-exposed group of controls (Perez-Maldonado et al. 2004). In a follow-up study with more participants, significant correlations between DDT or DDE exposure and DNA damage were reported; however, no significant associations between DDT or DDE exposure and oxidative DNA damage were observed (Perez-Maldonado et al. 2011). A correlation between DDE exposure and PBMC apoptosis was also reported.

2.8 MUSCULOSKELETAL

Evidence of Musculoskeletal Effects of DDT, DDD, or DDE in Humans. Inconsistent evidence is provided by a limited number of epidemiological studies for associations between serum levels of DDT, DDD, or DDE and bone mineral density in men (Glynn et al. 2000; Wallin et al. 2005; Table 2-5) and peri- or post-menopausal women (Beard et al. 2000; Bohannon et al. 2000; Rignell-Hydbom et al. 2009; Wallin et al. 2005; Table 2-5). No clear evidence for associations with DDE serum levels were found in two studies of Swedish men (Glynn et al. 2000; Wallin et al. 2005), despite the known anti-androgenic effects of DDT and the association between androgen deprivation and bone loss and osteoporosis in men (Taylor et al. 2009). In studies of post-menopausal women, a significant association with decreased bone mineral density was found in one study (Beard et al. 2000), another study found an association with increased bone mineral density (Rignell-Hydbom et al. 2009), and two studies found no associations with bone mineral density (Bohannon et al. 2000; Wallin et al. 2005).

Evidence of Musculoskeletal Effects of DDT, DDD, or DDE in Animals. Limited information exists from studies in animals. In chronic-duration oral exposure studies, 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, or in 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). Fisher 344 rats treated for 104 weeks to 19.1 mg/kg/day DDT showed no histopathology of the femur and sternum (Tomita et al. 2013).

Table 2-5. Summary of Studies of Associations between DDT Exposure Biometrics and Musculoskeletal Endpoints

Reference	Exposure metric	Outcome evaluated	Results	Notes
Beard et al. 2000 68 sedentary women (mean 52 years) Australia	DDE, serum	Bone Mineral Density	↓	Adjusted betas, continuous exposure variable (coefficients, data not shown, only p-values provided)
Bohannon et al. 2000 103 peri- and post-menopausal women (mean age 54.5 years); 50 black, 53 white United States	DDE, serum	Bone mineral density (baseline/rate of change) in lumbar spine and radius: Whites Blacks All	 NS/NS NS/NS NS/NS	Adjusted betas for T1 and T3 versus T2 DDE All p-trends = NS
Glynn et al. 2000 115 men (mean age 63 years) Sweden	DDE, DDT, DDD, serum	Bone mineral density FNBMD, LSBMD, WBBMD Ultrasound bone endpoints BUA and SOS	 All NS ↓ (DDE Q3)	Adjusted betas (±SE) – continuous exposure levels Adjusted betas – categorical exposure levels. Q4 versus Q1 was NS
Rignell-Hydbom et al. 2009 908 women (60–70 years old) General population, Sweden	DDE, serum	 Osteocalcin Bone mineral density	 NS ↑	Adjusted betas (95% CI) – Continuous exposure variables: Beta for effects on bone mineral density, although statistically significant, was very small.
Wallin et al. 2005 196 men (59 years old); 184 women (62 years old) Sweden	DDE, serum	Bone mineral density Men Women	 NS NS	Adjusted betas (95% CI) – both continuous and categorical exposure variables

[a]Studies in this table were selected because they: (1) measured DDT-related metrics in biological fluids or tissues in each subject, and (2) evaluated associations using linear regression, logistical regression, or correlation techniques, adjusting for possible confounding factors.
[b]DDE = *p,p'*-DDE, unless otherwise specified; DDT = *p,p'*-DDT, unless otherwise specified.
[c]Arrows "↑ or ↓" indicate statistical significance ($p<0.05$) and direction of change observed for a given outcome (positive or inverse associations, respectively).

BUA = broad-band attenuation; CI = confidence interval; FNBMD = femoral neck bone mineral density, LSBMD = lumbar spine bone mineral density; NS = not statistically significant; Q = quartile(s); SE = standard error; SOS = speed of sound in os calcis; T = tertile(s); WBBMD = whole body bone mineral density

2.9 HEPATIC

Evidence of Hepatic Effects of DDT, DDT, or DDE in Humans. Inconsistent evidence is provided by four studies examining serum or cord blood levels of DDT or DDE and serum or urinary markers of liver damage or dysfunction (Table 2-6). No clearly significant associations were found with serum enzymes or chemicals indicative of liver damage (e.g., increased AST, ALT, or bilirubin) in subjects residing in a heavily contaminated region in Brazil (Freire et al. 2015a, 2015b) or in U.S. workers exposed to pesticides and monitored between 1969 and 1973 (Morgan and Lin 1978), but an analysis of NHANES data from 2003 to 2004 reported increased adjusted mean serum levels of ALT, gamma-glutamyl transferase (GGT), AST, and bilirubin in higher exposure quartiles, compared with the lowest exposure quartile (Serdar et al. 2014; see Table 2-6); however, the changes do not appear to be dose-related. Significant associations between cord blood DDE or DDT levels and urinary levels of total porphyrins, coproporphyrin I, and coproporphyrin III (indicators of altered hepatic heme synthesis in the liver) were reported in a group of 52 4-year-old children from Ribera D'Ebre Spain (Sunyer et al. 2008).

Studies of workers involved in the manufacture and formulation of DDT for many years found no evidence of hepatotoxicity, hepatic enlargement, or dysfunction (as measured by the bromsulphalein test, also known as sulfobromophthalein sodium) in one group (Laws et al. 1973), and no changes in hepatic metabolism of phenylbutazone or cortisol in another group of workers (Poland et al. 1970).

In a single controlled-exposure study, Hayes et al. (1956) exposed 51 male volunteers to about 0.05–0.063 or 0.36–0.5 mg DDT/kg/day administered via a capsule for 12–18 months. The background dose from 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) reported were considered to be related to DDT exposure to humans.

Evidence of Hepatic Effects of DDT, DDD, or DDE in Animals. In animals, the liver appears to be one of the primary targets of toxicity for DDT and related compounds. Acute-, intermediate-, and chronic-duration oral exposures have been shown to cause dose-related mild-to-severe hepatic effects in numerous animal studies, with chronic exposure leading to the development of liver tumors in some animals (see Section 2.19).

Table 2-6. Summary of Studies of Associations between DDT Exposure Biometrics and Hepatic Endpoints[a]

Reference	Exposure metric[b]	Outcome evaluated	Results[c]	Notes
Freire et al. 2015a, 2015b 415 men; 432 women Brazil (2003–2004; ages 14–>60 years)	DDE, serum	Outcomes measured in serum Elevated indirect bilirubin Elevated total and direct bilirubin, elevated AST, ALT, and GGT	 ↑ (women only) All NS	Adjusted ORs (95% CI), continuous exposure variables Significance lost with categorical analysis
Morgan and Lin 1978 ~2,480 male adults Nationwide United States (1969–1973) workers exposed to pesticides	DDE and DDT, serum	Serum AP, LDH Serum AST, ALT	↑ (weak) NS	Partial correlation coefficients, log-transformed DDT metrics Significant trends for increased serum AST and LDH, but not AP or ALT, with increasing serum DDE No adjustments for confounding factors
Serdar et al. 2014 ~1955 subjects, age range 12–>60 years NHANES 2003–2004	DDE and DDT, serum	Serum ALT DDE DDT Serum GGT DDE DDT Serum AST (DDT only) Serum total bilirubin (DDE only)	 ↑ (Q3, Q4) ↑ (Q2–Q4) ↑ (Q2) ↑ (Q2, Q3) ↑ (Q2, Q4) ↑ (Q3, Q4)	Adjusted means, categorical exposure variables Associations with increased glucose (for both DDT and DDE) and bilirubin (for DDE), data not shown

Table 2-6. Summary of Studies of Associations between DDT Exposure Biometrics and Hepatic Endpoints[a]

Reference	Exposure metric[b]	Outcome evaluated	Results[c]	Notes
Sunyer et al. 2008 52 children (4 years old) Ribera d'Ebre Spain, birth between 1997 and 1999	DDE and DDT, cord blood	(Outcomes measured in urine) Total porphyrins UPI CPI CPIII	 ↑ (DDE, DDT) NS ↑ (DDE, DDT) ↑ (DDE, DDT)	Adjusted betas, continuous exposure variables, significant for T3 only Significance for DDE remained with additional adjustments for other pollutants (DDT not reported)

[a]Studies in this table were selected because they: (1) measured DDT-related metrics in biological fluids or tissues in each subject, and (2) evaluated associations using linear regression, logistical regression, or correlation techniques.
[b]DDE = *p,p'*-DDE, unless otherwise specified; DDT = *p,p'*-DDT, unless otherwise specified.
[c]Arrows "↑ or ↓" indicate statistical significance ($p<0.05$) and direction of change observed for a given outcome (positive or inverse associations, respectively).

AP = alkaline phosphatase; AST = aspartate amino transferase (formerly known as glutamic oxaloacetic transaminase); ALT = alanine aminotransferase (formerly known as glutamic pyruvic transaminase); CI = confidence interval; CPI = coproporphyrin I; CPIII = coproporphyrin III; GGT = gamma-glutamyl transferase; LDH = lactate dehydrogenase; NS = not statistically significant; OR = odds ratio; Q = quartile(s); T = tertile; UPI = uroporphyrin I

After acute oral exposure to technical DDT or unspecified DDT, *p,p'*-DDT, *p,p'*-DDE or unspecified DDE, or unspecified DDD, a number of liver effects in animals have been observed including induction of liver microsomal xenobiotic metabolizing enzymes (often associated with increased liver weight), increased serum levels of liver enzymes (suggestive of liver injury), changes in the appearance of the liver, and necrosis. Increased liver activities of various microsomal enzymes (e.g., CYP2B, CYP2B1, CYP3A1, CYP3A2, GGT, glutathione-S-transferase) have been observed after acute oral exposure in rats given 200 mg *p,p'*-DDT/kg (Garcia and Mourelle 1984). Rats given 5–50 mg *p,p'*-DDT/kg/day for up to 14 days accompanied by increases in relative liver weights exceeding 10% (Tomiyama et al. 2004); rats given 40 mg DDT/kg/day for 12 days, accompanied with 18% increased relative liver weight (de Waziers and Azais 1987); rats given ≥4.2 mg DDT/kg/day, but not 0.17 mg/kg/day, for 14 days, accompanied by increased relative liver weight (Nims et al. 1998); and mice given 42.9 mg DDT(NS) or DDE(NS)/kg/day for 1 week (but not 42.9 mg DDD(NS)/kg/day), accompanied with increased liver weight after DDE exposure (Pasha 1981). Increased liver weight was also reported in rats given 25 mg *p,p'*-DDE/kg/day for 4 days (Leavens et al. 2002) or 10 days (Kang et al. 2004), and rats given 106 mg *p,p'*-DDT/kg once or for 7 days (Tomiyama et al. 2003). Rhesus monkeys exposed once to 150 mg *p,p'*-DDT/kg had increased alkaline phosphatase (AP), lactate dehydrogenase (LDH), AST, and ALT activities in serum, indicative of liver damage (Agarwal et al. 1978). Necrotic liver changes, accompanied with increased liver weight, were observed in rats exposed to 12 mg *p,p'*-DDT/kg/day for 14 days (Kostka et al. 2000).

After intermediate-duration exposure to technical DDT, *p,p'*-DDT, or *p,p'*-DDE, an array of liver effects, similar to those observed after acute exposure, have been observed in rats and mice. The lowest reliable intermediate-duration LOAEL for liver effects is 0.17 mg technical DDT/kg/day in the diet reported for cellular hypertrophy in F344/DuCrj rats exposed for 26 weeks (Harada et al. 2003, 2006). Observations of liver effects include increased liver weight and induction of CYP enzymes in Wistar rats exposed by gavage to 15 mg *p,p'*-DDT/kg/day for 3 weeks (Gupta et al. 1989) and NMRI mice exposed to 6.25 mg *p,p'*-DDT/kg/day for 28 days (Orberg and Lundberg 1974); hepatic focal necrosis and regeneration in Sprague-Dawley rats exposed to 6.6 mg DDT/kg/day in the diet for 36 weeks (Jonsson et al. 1981); minor vacuolation, hypertrophy and cell margination in livers of Sherman rats exposed to technical DDT in food for 2–18 months at 5 mg/kg/day in males and 20 mg/kg/day in females (Ortega 1956); increased relative liver weight (20% increase compared with control) in Sprague-Dawley rat dams exposed by gavage to 50 mg *p,p'*-DDE/kg/day (but not 15 mg/kg/day) between GD 6 and PND 20 (Yamasaki et al. 2009); centrilobular hypertrophy, fatty hepatocytes, and increased liver weight in F1 and F2 Sprague-Dawley rats exposed to 3.44 (males) or 3.75 (females) mg *p,p'*-DDT/kg/day in the diet in a 2-generation reproductive toxicity study (NOAELs of 0.34 and 0.73 mg/kg/day), and enlarged and darkened livers in

F0 rats at 25 (males) or 27.7 (females) mg/kg/day, but not at 3.44 or 3.75 mg/kg/day (Hojo et al. 2006); and increased absolute and relative liver weight and liver levels of CYP2B1 and decreased levels of liver GJIC protein in male F344/DuCrj rats exposed to ≥5 mg *p,p'*-DDT/kg/day for 28 days (Harada et al. 2003, 2006; Tomiyama et al. 2004). Cellular hypertrophy and cytoplasmic eosinophilia were also reported in livers of Osborne-Mendel rats exposed to 0.25 mg technical DDT/kg/day (but not 0.05 mg/kg/day) in the diet for 15–27 weeks (Laug et al. 1950). Laug et al. (1950) however, provided no incidence data or statistical analysis, and only noted that at 0.25 mg/kg/day, "some of the rats were unaffected," and the liver effects "were truly minimal." It is unknown, therefore, whether hepatic changes at this level would have reached statistical significance; the LOAEL for this study was therefore considered to be unreliable. Minor microscopic changes in hepatocytes (cytoplasmic vacuolation, mitochondrial changes, and lipid droplets) were described in male C57BL/6N mice treated by gavage with *p,p'*-DDT for 8 weeks, but only qualitative data were provided (Liu et al. 2017a, 2017b). No exposure-related changes in liver weight, liver histology, or serum levels of AST and ALT were reported in immature, prepubertal F344/DuCrl male rats exposed to 10 mg *p,p'*-DDE/kg/day in the diet for 42 days (Makita et al. 2003a).

Nonneoplastic liver lesions, and in some cases liver tumors, have been observed in rats, mice, hamsters, monkeys, and dogs after chronic oral exposure to DDT and related compounds. LSEs in the liver from chronic oral exposure are summarized in Table 2-1 and Figure 2-2, and the following three paragraphs, which first present the results for rats, followed by results for mice and then other laboratory animal species.

Chronic exposure to DDT and related compounds has been associated with liver necrosis, centrilobular hypertrophy, hyperplasia, and fatty metamorphosis in rats (Deichmann et al. 1967; Fitzhugh and Nelson 1947; Harada et al. 2003, 2006; NCI 1978), including effects in F1 males and females in a 2-generation study (Hojo et al. 2006). In rats, the lowest reliable chronic-duration LOAELs for nonneoplastic histological changes in the liver are 0.17 mg *p,p'*-DDT/kg/day for hepatocellular hypertrophy in male F344/DuCrj rats exposed in the diet for 2 years (the lowest dose tested by Harada et al. 2003, 2006) and 7 mg technical DDT/kg/day for focal hepatocellular necrosis in Osborne-Mendel rats exposed in the diet for 2 years (the lowest dose tested by Fitzhugh and Nelson 1947) (see Table 2-1 and Figure 2-2). Similar to intermediate exposure durations, increased CYP-450 content and microsomal activities and decreased GJIC protein Cx32 were observed in rats (Harada et al. 2003, 2006), as well as indicators of oxidative stress including increased lipid peroxide at ≥1.7 mg/kg/day, and 8-hydroxydeoxyguanosine (8-OHdG)

levels at 19.1 mg *p,p'*-DDT/kg/day in males (Harada et al. 2003, 2006). Increased incidences of liver tumors have been reported in rats (see Section 2.19).

Noncancer liver effects have been less consistently observed in chronically exposed mice. Liver effects in B6C3F1 mice exposed for 78 weeks were restricted to amyloidosis at dietary doses ≥3.7 mg technical DDT/kg/day (NCI 1978); however, NOAELs for noncancer liver histological changes of 49 mg *p,p'*-DDE/kg/day and 142 mg technical DDD/kg/day were reported for mice exposed for 78 weeks (NCI 1978). Increased incidences of liver tumors have been observed in mice (see Section 2.19).

Nonneoplastic changes in the liver have also been reported in monkeys, hamsters, and dogs chronically exposed to DDT and related compounds. In Rhesus and Cynomolgus monkeys exposed to *p,p'*-DDT in the diet for up to 130 months, fatty changes in the liver were observed at doses as low as 6.4 mg *p,p'*-DDT/kg/day (Takayama et al. 1999), mild to severe hydropic changes in liver cells, assessed by periodic biopsies, occurred in two of three Rhesus monkeys, but no functional liver changes, assessed by bromsulfalein retention, were observed when exposed to 3.9 mg technical DDT/kg/day in the diet for 3.5–7 years (Durham et al. 1963). Reported nonneoplastic liver effects in hamsters include focal necrosis after lifetime dietary exposure to 40, but not 20, mg technical DDT/kg/day (Cabral et al. 1982a); increased relative liver weight (with no increase in serum ALT, lactate dehydrogenase [LDH], or AP) after exposure to 67–133 mg technical DDT/kg/day in the diet for life (Graillot et al. 1975) and liver necrosis after 128-week dietary exposure to 47.5 mg *p,p'*-DDE/kg/day (Rossi et al. 1983). Rossi et al. (1983) also observed increased incidences of liver tumors at levels ≥47.5 mg *p,p'*-DDE/kg/day, but increased incidences of liver tumors were not observed in the other hamster chronic-duration studies. In dogs given technical DDT in the diet for 39–40 months, focal or diffuse liver changes occurred at 80 mg/kg/day and severe liver damage at 160 mg/kg/day; no liver changes were seen at 16 mg/kg/day (Lehman et al. 1965).

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 of the liver.

Mechanisms of Hepatic Effects of DDT, DDD, or DDE. DDT is considered to be an animal liver carcinogen with a nongenotoxic mitogenic mechanism of action (see Section 2.19). Studies in animals indicate that initial inductions of microsomal liver xenobiotic metabolizing enzymes (e.g., CYP monooxygenases) and transient bursts in DNA synthesis and cell proliferation are key initial hepatic responses to DDT exposure (Harada et al. 2003, 2006). Increased liver weights, particularly in acutely

exposed animals, likely reflects this increased mitogenic, proliferative activity. Although cell proliferation generally ceases within days, it has been hypothesized that these initiated cells may contribute to the generation of eosinophilic abnormal hepatic foci (AHF) whose number and size correlate with dose and time of exposure (Harada et al. 2003). An observed decrease in the GJIC protein Cx32, which plays an important role in cell-cell communication, may contribute to the isolation of AHF cells from growth regulatory signals from neighboring cells (Harada et al. 2003). Additionally, increases in 8-OHdG and LPO are indicative of hepatic oxidative stress and damage to DNA, which may contribute to liver nonneoplastic changes and eventual tumor formation.

In *in vitro* studies, increased oxidative stress and reactive oxygen species (ROS) due to DDT exposure in Hep2 cells is thought to activate the Jak/STAT3 pathway, ultimately resulting in impaired expression of E-cadherin, which is known to be associated with hepatocellular carcinogenesis and poor prognosis in humans (Nakagawa et al. 2014). The induction of microsomal liver xenobiotic metabolizing enzymes may be involved in proliferation of smooth-surfaced endoplasmic reticulum that are observed with longer durations of exposure, contributing to hypertrophy (Harada et al. 2006). In addition to CYP induction, DDT has been shown to activate both the constitutive androgen receptor (*CAR*) and *ERα* transcription factors, which increase transcription of target genes related to hepatocyte proliferation, cell-cycle progression, and apoptosis inhibition in the mouse liver (Kazantseva et al. 2013). Several animal and *in vitro* studies have demonstrated activation of microsomal enzymes in response to DDT-isomer exposure, presumably through activation of the *CAR* (Harada et al. 2016). Aberrant expression of genes within these functional categories were observed in micro-dissected tissues including hypertrophic tissue, eosinophilic AHF, and tumors in rats, from DDT-treated animals versus controls (Harada et al. 2016). *In vitro* studies in isolated hepatocytes also showed increases in expression of genes associated with hepatic estrogen, lipid, and sugar metabolism (Jellali et al. 2018).

2.10 RENAL

Evidence of Renal Effects of DDT, DDD, or DDE in Humans. In a case-control study of 270 chronic kidney disease patients and 270 age- and sex-matched controls from a hospital in Delhi India, serum levels of DDE, but not DDT, were significantly associated with risk for chronic kidney disease (Siddarth et al. 2014). The odds ratio (OR) (95% CI) (adjusted for age, sex, BMI, and serum lipid content) for DDE was 2.70 (1.04–7.02) for the third tertile, compared with the first tertile. However, no association was found with further adjustment for serum levels of other pesticides including endosulfan, dieldrin, aldrin,

and hexachlorocyclohexanes and glutathione-S-transferase (GST) genotype (e.g., further adjusted OR 2.15 [95% CI 0.63–6.82] for third tertile versus first tertile).

No other epidemiological studies were located that examined possible associations between serum levels of DDT, DDD, or DDE and kidney outcomes.

Evidence of Renal Effects of DDT, DDD, or DDE in Laboratory Animals. Limited evidence is available for kidney effects in laboratory animals after acute- or intermediate-duration oral exposure to DDT and related compounds. In a two-generation study of Wistar rats exposed to *p,p'*-DDT in the diet before mating and during mating, gestation, and lactation, increased kidney weight was observed in F0 parental and F1 female rats at 25 mg/kg/day, but not at 3.44 mg/kg/day (Hojo et al. 2006). No significant changes in kidney weight or serum levels of creatinine or urea nitrogen were found in sexually immature male Wistar rats (6 weeks old) fed 10 mg *p,p'*-DDE/kg/day in the diet for 42 days (Makita et al. 2003a). Significant decreases (~36%) in kidney weights were observed in the offspring of pregnant rabbits given gavage doses of 1 mg DDT(NS)/kg/day on GDs 4–7 (Fabro et al. 1984). No significant changes in kidney weights were observed in Sprague-Dawley offspring, or their dams, exposed during GD 6–PND 20 to up to 50 mg *p,p'*-DDE/kg/day (Yamasaki et al. 2009) or in male rat offspring exposed during GDs 14–18 and then on PNDs 80–83 to 100 mg *p,p'*-DDE/kg/day (You et al. 1999a); no histological changes were observed in kidneys of offspring from C57BL/6J mouse dams treated with DDT (77.2% *p,p'*-DDT and 22.8% *o,p'*-DDT) during GD 12–PND 5 (La Merrill et al. 2016). No changes to kidney weight were found in castrated male Sprague-Dawley rats (Hershberger Assay) given gavage doses of *p,p'*-DDE doses up to 100 mg/kg/day for 10 days after castration, compared with control rats (Kang et al. 2004). Histology of the kidney was not examined in this study.

The kidney does not appear to be a sensitive target for histological changes in laboratory animals chronically exposed to DDT and related compounds. Histological kidney lesions were observed in Osborne-Mendel rats exposed to 20 mg DDT(NS)/kg/day in the diet for up to 27 months (tubular epithelial necrosis and polycystic degeneration; Deichmann et al. 1967) and 66 mg technical DDD/kg/day in the diet for 78 weeks (chronic inflammation; NCI 1978), but no histological changes in the kidney were reported in Osborne-Mendel rats exposed to up to 45 mg technical DDT/kg/day or 59 mg *p,p'*-DDE for 78 weeks (NCI 1978). After 78-week exposures, chronic inflammation of the kidney was observed in male B6C3F1 mice exposed to ≥27 mg *p,p'*-DDE/kg/day, but no histological kidney changes were observed in female B6C3F1 mice exposed to up to 49 mg *p,p'*-DDE/kg/day or male or female B6C3F1 mice exposed to up to 30.2 mg technical DDT/kg/day or 142 mg technical DDD/kg/day (NCI 1978). No

histopathological changes in the kidney were noted at 55 weeks of age in ICR mice exposed from conception through death to 16.5 mg/technical-DDT/kg/day (Del Pup 1978).

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.

2.11 DERMAL

Evidence of Dermal Effects of DDT, DDD, or DDE in Humans. No correlation was found between DDT exposure in clinical laboratory workers, via dermal or inhalation routes, and the frequency and distribution of skin abnormalities, except for a few cases of minor skin irritation (Ortelee et al. 1958).

Cameron and Burgess (1945) conducted a series of experiments on volunteers wearing clothing and undergarments impregnated with 1% DDT for 18–26 days in order to determine whether this treatment would protect soldiers against body lice. Several individuals had transient dermatitis, but no other symptoms were observed; however, the investigators did not attribute the dermatitis to DDT exposure.

Evidence of Dermal Effects of DDT, DDD, or DDE in Animals. No studies were located indicating adverse dermal effects in animals after oral 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 inflammation, edema, and destruction of the epidermis. Guinea pigs were dermally dosed 5 days/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.

2.12 OCULAR

Evidence of Ocular Effects of DDT, DDD, or DDE in Humans. Reports of ocular effects in humans exposed to DDT in the air are limited to the studies by Neal et al. (1944) and Ortelee et al. (1958). In the Neal et al. (1944) study, moderate, nonspecific eye irritation was reported by two 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. The investigators provided limited information on exposure levels, but noted that the DDT quickly settled; thus, the actual exposure levels were lower than

predicted. The ocular effects were only observed at the higher of the two tested concentrations. Red, itching, and inflamed eyes and/or excessive tearing was reported in 8 workers involved in the manufacture and/or formulation of DDT and exposed to "heavy" concentrations of dust; DDT air concentrations associated with these effects were not reported (Ortelee et al. (1958). The study examined 40 workers, although DDT exposure was limited for 30 of the workers; it is unclear from the paper whether any of the cases of eye irritation were in the limited exposure group of workers.

Evidence of Ocular Effects of DDT, DDD, or DDE in Animals. Unilateral (11 exposed versus 1 control) and bilateral (9 exposed versus 2 controls) corneal opacity was described in a single study in mice exposed to 13 mg technical-DDT/kg/day in the diet for 80 weeks (Kashyap et al. 1977). In a second oral exposure study, minute darkened areas were observed during the ophthalmologic examination of the retina of 5 of 10 dogs administered capsules containing 50 mg/kg *o,p'*-DDD for 120–147 days; vision did not appear to be affected (Kirk and Jensen 1975). No evidence of vascular or cellular changes were observed during the histologic examination of the retina, and the darkened areas were not evident.

2.13. ENDOCRINE

Reported endocrine effects related to thyroid hormone dysregulation in humans and effects on endocrine-related tissues (pituitary, adrenals, thyroid, parathyroid) in laboratory animals are discussed in this section. Other possible hormonal effects of DDT, DDD, or DDE in humans and laboratory animals are described in Sections 2.14 (immunological effects), 2.15 (neurological effects), 2.16 (reproductive effects), 2.17 (developmental effects), and 2.18 (other noncancer effects).

Thyroid Hormone Dysregulation, Human Studies. Epidemiological studies provide inconsistent evidence for associations between levels of DDT, DDE, or DDD in biological fluids or tissues and changes in serum levels of thyroid hormone levels in humans (Tables 2-7 and 2-8). Table 2-7 describes summary results from epidemiological studies that examined possible associations between DDT exposure biometrics in adolescents or adults and changes in serum levels of thyroid hormones (thyroid stimulating hormone [TSH]; triiodothyronine [T3]; thyroxine [T4]). An additional study in Table 2-7 only examined a possible association between serum DDE levels and serum levels for thyroid peroxidase antibody, TPOAb (Schell et al. 2009). Table 2-8 describes results from epidemiological studies looking

Table 2-7. Summary of Studies of Associations Between DDT Exposure Biometrics in Adolescents or Adults and Serum Thyroid Hormone Levels[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Studies finding at least one statistically significant association between any DDT metric and any thyroid hormone level			
Abdelouahab et al. 2008 211 consenting adults, general population (124 males, 87 females)	Serum DDE quartiles, ng/g lipid 25th 50th 75th Men 139 211 387 Women 157 240 459	Serum levels: TSH, TT3, TT4	**Adjusted betas with DDE** Women Men **TT3 -0.12*↓** NS TT4 NS NS **TSH** NS **0.20*↑**
Alvarez-Pedrerol et al. 2008 259 4-year-old children, general population	Serum exposure quartile ranges, ng/mL DDE DDT Q2 0.436–0.807 0.026–0.049 Q3 0.808–1.75 0.050–0.103 Q4 1.76–43.9 0.104–0.657	Serum levels: TSH, TT3, fT4	**Adjusted TT3 betas with** Q2 Q3 Q4 Overall **DDT -9.25*↓ -10.75*↓** NS **-2.51*↓** DDE NS NS NS NS Unadjusted betas were NS for: fT4 with DDE or DDT TSH with DDE or DDT ALL fT4 and TSH p-trends >0.05 (NS)
Blanco-Muñoz et al. 2016 84 male floriculture workers	Serum tertiles, DDE, ng/mL Tertile 1: <0.37 Tertile 2: 0.37–7.96 Tertile 3: >7.96	Serum levels: TSH, TT3, TT4	**Adjusted betas with DDE** **Total T3** **Total T4** Overall NS **0.08*↑** Tertile 2 NS NS **Tertile 3 0.07*↑** **0.20*↑** **p-trend 0.03*** **0.03*** TSH associations with DDE were NS.
Bloom et al. 2014 114 consenting adults, general population (66 males, 48 females)	Mean serum levels, ng/mL ΣDDT (DDE + DDT) Men 4.50 Women 3.59	Serum levels: TSH, TT3, fT4, TT4	**Adjusted change per 2-fold change in ΣDDT** Men Women TSH NS NS fT4 NS NS **TT4** NS **0.34*↑** **TT3** NS **2.78*↑**

Table 2-7. Summary of Studies of Associations Between DDT Exposure Biometrics in Adolescents or Adults and Serum Thyroid Hormone Levels[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Croes et al. 2015 Adolescents (aged 14–15 years) with parental consent FLEHS I cohort (n=1,679) FLEHS-II cohort (n=210)	Serum levels of DDE. Geometric mean FLEHS I FLEHS II (ng/g lipid) 94 70 (ng/mL) 0.418 0.307	fT4	**Adjusted betas per IQR increase in DDE** fT4: **1.003***↑
Freire et al. 2012 193 children with parental consent, general population	80th percentiles in serum, ng/mL *o,p'*-DDT: 2.20 *p,p'*-DDT: 17.7 *p,p'*-DDD: 2.63 *p,p'*-DDE: 35.7	Serum levels, TSH, TT3, fT4	**p-trends from adjusted quintile linear regression analysis** TSH NS with all DDT metrics **TT3** ***o,p'*-DDT** **0.05***↑ ***p,p'*-DDT** **<0.01***↑ ***p,p'*-DDD** **<0.01***↑ ***p,p'*-DDE** **0.02***↑ **fT4** *o,p'*-DDT NS *p,p'*-DDT NS ***p,p'*-DDD** **0.04***↑ *p,p'*-DDE NS
Freire et al. 2013 608 consenting adults, general population (303 males, 305 females)	Median serum levels, ng/mL Men Women *p,p'*-DDE 8.32 9.64 *o,p'*-DDT 0.30 0.42 *p,p'*-DDT 3.09 3.20 *p,p'*-DDD 0.61 0.66	Serum levels, TSH, TT3, fT4, TPOAb	**Adjusted betas** TSH: NS with all DDT metrics (M & F) **TT3: 0.40*↑ with *p,p'*-DDT (F only)** TT3: NS with other DDT metrics (M & F) **fT4: 0.003*↓ with *p,p'*-DDT (M)** **fT4: 0.003*↑ with *p,p'*-DDT (F)** **fT4: 0.02*↑ with *o,p'*-DDT (F)** fT4: NS with other DDT metrics (M & F) ORs for elevated TPOAb ≥10 U/Ml; NS for all DDT metrics

Table 2-7. Summary of Studies of Associations Between DDT Exposure Biometrics in Adolescents or Adults and Serum Thyroid Hormone Levels[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Kim et al. 2013 105 consenting pregnant women, hospital-based	Median serum levels, ng/g lipid *p,p'*-DDE: 57.37 *p,p'*-DDT: 5.20 ΣDDT: 64.40	Serum levels, TSH, fT3, TT3, fT4, TT4	**Adjusted betas** TSH: *p,p'*-DDE NS; *p,p'*-DDT NS; ΣDDT NS **fT3**: *p,p'*-DDE NS; *p,p'*-DDT NS; ΣDDT **-0.52*↓** **TT3**: *p,p'*-DDE NS; *p,p'*-DDT NS; ΣDDT **-0.096*↓** **fT4**: *p,p'*-DDE NS; *p,p'*-DDT **-0.045*↓**; ΣDDT NS TT4: *p,p'*-DDE NS; *p,p'*-DDT NS; ΣDDT NS
Lopez-Espinosa et al. 2009 157 consenting pregnant women, hospital-based	Geometric means DDE in serum 2.0 ng/mL 200 ng/g lipid	Serum levels, TSH, TT3, fT4	**Adjusted betas with DDE** TSH: NS TT3: NS **fT4: -0.03*↓** Adjusted OR (95% CI) with DDE **TSH ≥2.5 mIU/L** **2.53*↑ (1.36–4.73)**
Meeker et al. 2007 341 consenting men, hospital-based	DDE in serum 5th percentile: 87.7 ng/g lipid; 0.38 ng/Ml 95th percentile: 1,230 ng/g lipid; 5.94 ng/mL	Serum levels, TSH, TT3, fT4	**Adjusted betas with DDE** **TSH: 0.92*↓** **TT3: 0.033*↑** **fT4: 0.036*↑**
Rylander et al. 2006 196 consenting fishermen, general population	DDE in serum, ng/g lipid 5th percentile: 110 95th percentiles: 2,140	Serum levels, TSH, fT3	Adjusted betas with DDE (per 100 ng/g lipid increase) **TSH: 0.03*↑** fT3: NS Categorical linear regression analysis indicated NS association between DDE and TSH
Schell et al. 2009 115 youth with consent from parents, general population (61 males, 57 females; 47 subjects were breastfed)	Geometric mean DDE in serum, ng/mL Breastfed: 0.37 Non-breastfed: 0.28	Serum levels, TPOAb	**Adjusted betas with DDE** **Breastfed: 0.34*↑** Non-breastfed: NS

Table 2-7. Summary of Studies of Associations Between DDT Exposure Biometrics in Adolescents or Adults and Serum Thyroid Hormone Levels[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Takser et al. 2005 101 consenting pregnant women, hospital-based	DDE, DDT in serum at delivery, ng/mL 5th percentile / 95th percentile DDE 0.20 / 1.20 DDT ND / 0.07	Serum levels, TSH, TT3, fT4	**Adjusted change with change in** **DDE** / **DDT** TSH NS / NS **TT3** NS / **-0.54*↓** fT4 NS / NS
Turyk et al. 2006 56 consenting male adults, general population 25–29 sport-caught fish eaters 23–27 referents	Mean (range) DDE in serum, ng/g lipid Fish eaters: 602 (99–9,499) Referents: 290 (43–4,554)	Serum levels, TSH, TT3, T4, FTI	**Adjusted correlation coefficients with DDE** TSH NS TT3 NS **T4** **-0.03*↓ (adjusted for smoking)** **FTI** NS fT4 significance lost after further adjustment for fish eating or serum PCBs or dioxins
Turyk et al. 2007 1,021 men, 740 women, general population 1999–2000: 454 males, 350 females 2001–2002: 667 males, 490 females	Geometric means DDE in serum ng/mL / ng/g lipid 1999–2000 1.82 / 293 2001–2002 2.12 / 337	Serum levels, TSH, TT4	**Adjusted betas with DDE** 1999–2000 / 2001–2002 TSH, all women NS / NS TSH, all men NS / NS TT4, all women NS / NS TT4, all men NS / NS **TT4, women <60 years** **0.33*↑** / NS **TT4, women >60 years** **-0.47*↓** / NS
Studies finding no associations between any DDT metric and any serum thyroid hormone level			
Alavarez-Pedrerol et al. 2009 1,090 consenting pregnant women from two regions (A and B)	25th, 50th, and 75th percentiles DDE in serum, ng/g lipid Region A: 69.8, 112,1, 174.8 Region B: 59.9, 89.9, 139.4	Serum levels TSH TT3, fT4	Adjusted betas with DDE TSH: NS TT3: NS fT4: NS

Table 2-7. Summary of Studies of Associations Between DDT Exposure Biometrics in Adolescents or Adults and Serum Thyroid Hormone Levels[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Chevrier et al. 2008 334 consenting pregnant women	Geometric mean serum, ng/g lipid *p,p'*-DDE: 1,302.1 *p,p'*-DDT: 18.8 *o,p'*-DDT: 1.7	Serum levels, TSH, TT3, fT4, TT4	Adjusted betas for change per 10-fold change in DDT metric TSH: NS (*p,p'*-DDE, *p,p'*-DDT, *o,p'*-DDT) TT3: NS fT4: NS TT4: NS
Dallaire et al. 2009 623 consenting adults, general population	Geometric mean serum DDE level: 0.478 ng/mL	Serum levels, TSH, TT3, fT4, T4BG	**Adjusted betas with DDE** TSH: NS TT3: NS fT4: NS T4BG: **- 0.030***↓ T4BG changed to NS with further adjustment for alcohol consumption
Darnerud et al. 2010 160 infants at 3 weeks of age and 138 at 3 months of age; 325 consenting primiparous women	Median serum levels DDE 3-week-old infants: 95 ng/g lipid Mothers: 91 ng/g lipid	Serum levels, TSH, TT3, fT4	**Adjusted betas with DDE** TSH: NS (3-week), NS (3-month) TT3: NS, NS fT4: NS, NS Unadjusted associations of DDE with mother thyroid hormone levels were NS
Pelletier et al. 2002 16 consenting men in a weight loss program	Mean serum levels, ng/g lipid DDE: Before 430, After 547.2 DDT: Before 8.4, After 9.2	Serum level, TT3	Adjusted correlation coefficient TT3: DDE NS, DDT NS

Table 2-7. Summary of Studies of Associations Between DDT Exposure Biometrics in Adolescents or Adults and Serum Thyroid Hormone Levels[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Schell et al. 2008 232 youth with consent from parents	Geometric mean serum DDE, ng/mL Breastfed: 0.31 Non-breastfed: 0.42	Serum level, TSH, fT4	Adjusted betas with DDE TSH: NS fT4: NS

[a]See Table 1 in the *Supporting Document for Epidemiological Studies for DDT, DDE, and DDD* for more detailed descriptions of studies. Studies in this table were selected from those described in Table 1, because they: (1) measured DDT-related metrics in biological fluids or tissues in each maternal subject, and (2) evaluated associations using linear regression, logistical regression, or correlation techniques, adjusting for possible confounding factors.
DDE = *p,p'*-DDE, unless otherwise specified; DDT = *p,p'*-DDT, unless otherwise specified.
[c]**Bold text,** "significant" and asterisk indicate statistically significant ($p<0.05$). Adjusted OR or adjusted β indicates adjustment for standard confounding variables for thyroid hormone levels, such as BMI, serum lipids, medications, smoking, and age. Further adjustments for other analyzed chlorinated compounds or pesticides in biological fluid samples are expressly noted in the table. Arrows "↑ or ↓" indicate direction of change observed for a given outcome (positive or inverse associations, respectively).

BMI = body mass index; CI = confidence interval; F = female(s); FLEHS = Flemish Environment and Health studies; fT3 = free triiodothyronine; fT4 = free thyroxine; FTI = free T4 index; IQR = interquartile range; M = male(s); ND = not determined; NS = not statistically significant; OR = odds ratio; PCB = polychlorinated biphenyl; Q = quartile or quintile; RBP = retinol-binding protein; T3 = triiodothyronine; T4 = thyroxine; T4BG = thyroxine-binding globulin (the major transport protein for T4); TPOAb = thyroid peroxidase antibody; TSH = thyroid stimulating hormone; TT3 = total T3; TT4 = total T4

Table 2-8. Summary of Studies of Associations Between Maternal Serum, Cord Blood, or Milk DDT Exposure Metrics and Thyroid-Related Outcomes in Offspring[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Arrebola et al. 2016 200 mother-infant pairs	Cord blood serum percentiles (ng/mL) 25th 50th 75th *p,p'*-DDE 0.26 1.01 2.52 *o,p'*-DDT 0.10 0.22 0.37	Serum levels: TSH	Adjusted betas for log-dependent variables DDE *o,p'*-DDT TSH NS NS
Darnerud et al. 2010 150 mother-infant pairs (3 weeks) 115 mother-infant pairs (3 months)	Median maternal serum, milk, infant serum, and calculated postnatal exposure levels of DDE Maternal serum: 91 ng/g lipid Milk: 95 ng/g lipid Infant serum: 113 ng/g lipid Postnatal exposure: 329 ng/g x days Postnatal exposure-quartiles (ng/g x days) Q1: 0–190 Q2: 191–329 Q3: 330–503 Q4: 504–2,199	TT3, fT4, and TSH at 3 weeks and 3 months of age	Adjusted 3-month means for Q2–Q4 versus Q1 postnatal DDE (ng/g x days) TSH TT3 fT4 Q2 NS NS NS Q3 NS NS NS Q4 NS NS NS Adjusted betas for change per unit DDE in child's serum at 3 weeks and 3 months TSH TT3 fT4 3 weeks NS NS NS 3 months NS NS NS
de Cock et al. 2014 83 mother-infant pairs 53 males, 31 females	Mean cord blood, milk, and total exposure levels of DDE, mean (ng/g lipid) Cord blood: 114.48 Milk: 2,379.58 Total (ng/mL): 100,000 Quartiles for total DDE Q1: <41.8 Q2: 41.8–74.5 Q3: 74.51–107.5 Q4: 0.107.5	T4 (4–7 days after birth)	Adjusted betas for T4 change per unit total DDE, Q2–Q4 versus Q1 DDE Boys Girls Q2 NS NS Q3 NS NS Q4 NS **24.8***↑

Table 2-8. Summary of Studies of Associations Between Maternal Serum, Cord Blood, or Milk DDT Exposure Metrics and Thyroid-Related Outcomes in Offspring[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Freire et al. 2011 220 mother-infant pairs	Geometric mean placental DDT metrics (ng/g placenta) *p,p'*-DDE: 2.01 *p,p'*-DDT: 1.25 *o,p'*-DDT: 0.86 *o,p'*-DDD: 1.91 ΣDDT: 4.16	TSH in cord blood	Adjusted betas for continuous TSH and ORs for TSH ≥5 mU/L Adjusted betas / Adjusted ORs *p,p'*-DDE: NS / NS *p,p'*-DDT: NS / NS *o,p'*-DDT: NS / NS *o,p'*-DDD: NS / NS ΣDDT: NS / NS
Kim et al. 2015d 102 mother-infant pairs TSH measured in cord blood and in infant bloodspot within 2 days after birth	Maternal serum and cord blood DDE. Median (IQR) (ng/g lipid) Serum: 55.2 (38.7–73.9) Cord blood: 63.0 (44.0–91.5)	fT4, TT4, fT3, TT3, TSH in cord blood, or TSH in newborn infant blood	Adjusted betas (95% CI) per IQR increase in cord blood or maternal serum DDE Cord blood / Serum fT3: NS / **-0.035**↓ TT3: **-0.038**↓ / NS fT4: NS / **-0.020**↓ TT4: NS / **-0.034**↓ TSH: NS / NS TSH (bloodspot): **0.208***↑ / **0.264***↑
Li et al. 2014 247 mother-infant pairs	Median maternal serum and cord blood DDT and DDE (ng/g lipid) Maternal / Cord blood DDE: 333.951 / 193.513 DDT: 7.456 / <LOD	TSH in cord blood	Adjusted betas for TSH change per unit DDE in cord blood NS Adjusted ORs, less than median versus greater than median in cord blood NS
Lopez-Espinosa et al. 2010 453 mother-infant pairs	Geometric mean in cord blood (ng/g lipid) *p,p'* -DDE: 197 *p,p'* -DDT: 8.0 Tertiles, DDE Tertile 1: <50th percentile Tertile 2: ≥50th –90th percentile Tertile 3: ≥90th percentile	TSH in cord blood	Adjusted betas for TSH change per 10-fold increase in DDE or for T2 or T3 versus T1 *p,p'*-DDT / *p,p'*-DDE Tertile 2: NS / NS Tertile 3: NS / NS 10-fold increase: NS / NS

Table 2-8. Summary of Studies of Associations Between Maternal Serum, Cord Blood, or Milk DDT Exposure Metrics and Thyroid-Related Outcomes in Offspring[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Maervoet et al. 2007 198 mother-infant pairs	Mean cord blood DDE ng/mL ng/g lipid DDE 0.37 189	fT3, fT4, and TSH in cord blood	Adjusted betas for change per unit DDE in cord blood fT3: NS fT4: **-0.10***↓ TSH: NS
Ribas-Fito et al. 2003 98 mother-infant pairs	Median cord serum DDE 0.85 ng/mL	TSH	Adjusted OR for TSH ≥10 mU/L per doubling of DDE NS

[a]See Table 2 in the *Supporting Document for Epidemiological Studies for DDT, DDE, and DDD* for more detailed descriptions of studies. Studies in this table were selected from those described in Table 2, because they: (1) measured DDT-related metrics in biological fluids or tissues in each subject, and (2) evaluated associations using linear regression, logistical regression, or correlation techniques, adjusting for possible confounding factors.
[b]DDE = *p,p'*-DDE, unless otherwise specified; DDT = *p,p'*-DDT, unless otherwise specified.
[c]**Bold text,** "significant" and asterisk indicate statistically significant ($p<0.05$). Arrows indicate an increase or decrease in thyroid hormone levels. Adjusted OR or adjusted β indicates adjustment for standard confounding variables for thyroid hormone or DDT exposure levels, such as age at time of sampling, sex, serum lipids, smoking, alcohol consumption, prematurity, maternal BMI, fish consumption, economic levels, birth weight, weight gain during pregnancy, education, parity, use of thyroid medication, nursing, sampling season, and small for gestational age.

BMI = body mass index; CI = confidence interval; fT3 = free triiodothyronine; fT4 = free thyroxine; = interquartile range; LOD = limit of detection; ND = not determined; NS = not statistically significant; OR = odds ratio; Q = quartile(s); T = tertile(s); T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone; TT3 = total T3; TT4 = total T4

for associations between maternal serum, cord blood, or breast milk DDT exposure metrics and levels of thyroid hormones in offspring.

Associations with any DDT metric in studies of adults and adolescents (Table 2-7) were found in:

- 9/18 studies for T3 (4 with negative and 5 with positive T3 regression coefficients; 8 with no associations);
- 10/18 studies with T4 (3 with negative, 6 with positive, and 1 study with age-dependent negative or positive T4 regression coefficients; 8 with no associations);
- 4/18 studies with TSH (1 with negative and 3 with positive TSH regression coefficients; 14 with no associations).

Evidence for possible associations between DDT serum biometrics and serum thyroid hormone levels in adolescent and adult humans is considered to be inconsistent due to: (1) the inconsistency of finding associations across studies; (2) the variable direction of association across those studies finding an association (some found increasing, and others found decreasing, levels of thyroid hormone levels with increasing DDT biometric level); and (3) the variability across studies in the DDT metric showing an association with serum thyroid hormone levels (some found associations with DDE, others with DDT).

Two studies summarized in Table 2-7 used multiple logistic regression analyses to examine possible associations with thyroid hormone dysregulation in adults (Freire et al. 2013; Lopez-Espinoza et al. 2009), but this statistical technique did not provide information that clarified inconsistencies in the available data. No associations were found between several DDT biometrics (DDT, DDD, and DDE) and prevalences of adult subjects with serum levels of TPOAb ≥10 U/mL (Freire et al. 2013), but an elevated OR was found for DDE in pregnant subjects with TSH levels ≥2.5 mIU/L (Lopez-Espinoza et al. 2009). However, the Lopez-Espinoza et al. (2009) study did not find an association between DDE and TSH levels.

The inconsistent evidence for associations between DDT, DDD, or DDE in maternal serum, cord blood, or breast milk and levels of thyroid hormones in offspring is presented in Table 2-8. Associations with any maternal DDT metric were found in:

- 1/3 studies for T3 (1 inverse, 0 positive, and 2 with no association);
- 3/4 studies for T4 (2 inverse, 1 positive, and 1 with no association); and
- 0/6 studies for TSH (no associations in all studies measuring TSH in cord blood or blood of newborn infants) (Table 2-8).

The inconsistency of the evidence is emphasized by the observations that: (1) one study reported no associations between changes in children's serum DDE levels at 3 weeks or 3 months with changes in children's serum levels of TSH, total T3, or free T4 (Darnerud et al. 2010) and (2) another study reported no associations between cord blood DDE and cord blood TSH and T4 levels, an inverse association with total T3 levels, and associations for increased levels of TSH in newborn infants' blood with increasing levels of DDE in cord blood or maternal serum (Kim et al. 2015d).

Effects on Non-Sexual, Endocrine-Related Organs in Laboratory Animals. Non-sexual endocrine system organs (e.g., pituitary, adrenal gland, thyroids) do not appear to be sensitive toxicity targets in laboratory animals orally exposed to DDT and related compounds.

No exposure-related nonneoplastic histological changes were found in three non-sexual endocrine system organs in the NCI (1978) studies of rats and mice exposed to technical DDT, *p,p'*-DDE, or technical DDD: pituitary, thyroid, and parathyroid. In male and female offspring of Sprague-Dawley rats exposed to gavage doses of 5, 15, or 50 mg *p,p'*-DDE/kg/day on GD 6–PND 20, relative weights of pituitary and thyroid gland were not significantly different from control values; a 13% increase in relative adrenal weight in 50-mg/kg/day female offspring was observed (Yamasaki et al. 2009). Histology of the pituitary and thyroid glands were reported to be normal, but the adrenals were not examined histologically (Yamasaki et al. 2009). Adrenal gland changes were reported in dogs after administration of single oral doses of 200 mg technical DDD/kg via a capsule; the alterations consisted of vacuolation, inflammation, and necrosis (Powers et al. 1974). In some dogs, adrenal gland biopsies were taken prior to the terminal sacrifice. No histological alterations were observed 6 hours post-exposure, but were observed as early as 26 hours post-exposure. Powers et al. (1974) also conducted a repeated exposure study in which dogs were administered capsules containing 100 mg technical DDD/kg/day for 6 days or 200 mg/kg/day DDD every other day for 30 days. Although the study reported vacuolation, atrophy, and necrosis of the adrenal gland, conclusions cannot be drawn from this repeated exposure study due to the poor reporting of the study design (it appears that some of the dogs received two or three 6-day exposures) and the long recovery period (up to 32 weeks for some animals). Necrosis of the adrenal cortex was observed in dogs exposed to 138.5 mg *o,p'*-DDD/kg/day for 10 days (Kirk et al. 1974); adrenocortical necrosis, degeneration, and vacuolation also was reported in dogs exposed to 50 mg *o,p'*-DDD/kg/day for 120–156 days (Kirk and Jensen 1975).

In a series of reports by Yaglova and Yaglov (2014, 2015a, 2015b, 2017), a very low dose of *o,p'*-DDT administered to male Wistar rats in drinking water for 6 or 10 weeks (0.0019 mg *o,p'*-DDT/kg/day) was reported to increase serum levels of total T4, free total T4, T3, and free T3, decrease serum TSH levels, and produce histological changes in the thyroid (e.g., enlarged follicles, increased resorption of thyroglobulin, and decreased height of thyrocytes in peripheral lobes of the thyroid). The toxicological significance of these reports is uncertain because of the small magnitude of the changes in serum thyroid hormone levels and the absence of reporting of incidence data for the histological changes; thus, the apparent LOAEL of 0.0019 *o,p'*-DDE/kg/day was excluded from Table 2-1 and Figure 2-2. The only other study of thyroid effects in orally exposed laboratory animals reported reduced iodine concentrating capacity in Sprague-Dawley rats given single doses ≥50 mg/kg technical DDT (Goldman 1981).

Mechanisms of Endocrine Effects of DDT, DDE, or DDD. Although available studies of thyroid histology in laboratory animals orally exposed to DDT and related compounds do not clearly identify the thyroid as a sensitive toxicity target, the potential disruption of thyroid hormone homeostasis by environmentally persistent organochlorine chemicals, such as PCBs and DDT compounds, is an active area of *in vitro,* cell biology, and epidemiological research (for reviews of mechanistic hypotheses, see Liu et al. 2014; Rossi et al. 2017; Yaglova and Yaglov 2015b). To explain the observation of decreased serum levels of T4, T3, and TSH measured in Sprague-Dawley rats after 5 days of intraperitoneal co-exposure to PCB153 and *p,p'*-DDE (32 mg PCB153 + 20, 60 or 100 mg *p,p'*-DDE/kg/day), Liu et al. (2014) proposed that disruptive mechanisms could include decreasing levels of thyroglobulin, deiodinase 2, and serum transthyretin (TTR), inducing hepatic enzymes that metabolize thyroid hormones, and increasing levels of hormone receptors. Based on *in vitro* and cellular studies, Rossi et al. (2017) proposed that DDT may disrupt thyroid hormone homeostasis via inhibitory action on the TSH receptor via internalization of the TSH receptor from the plasma membrane by altering the structure of membrane lipid subdomains and that autoimmune responses to extracellular vesicles containing the TSH receptor could develop (Rossi et al. 2017). Yaglova and Yaglov (2015b) proposed that *o,p'*-DDT interferes with iodine anion transport into follicular thyrocytes, evidenced by decreased levels of the Na+/I- symporter (NIS) and increased thyroperoxidase (TPO) observed in exposed rats.

2.14. IMMUNOLOGICAL

Evidence of Immunological Effects of DDT, DDD, or DDE in Humans. Numerous epidemiological studies have examined associations between serum DDE levels and immune function biomarkers (e.g., immunoglobulin serum levels or counts of white blood cell or lymphocyte subtypes) or immune-related

conditions (e.g., asthma, bronchitis, eczema) in adults (Cooper et al. 2004; Miyake et al. 2011; Vine et al. 2001; Table 2-9) and children (Karmaus et al. 2001, 2003, 2005a, 2005b; Meng et al. 2016; Perla et al. 2015; Table 2-10). Thirteen epidemiological studies have examined associations between DDE levels in cord blood, maternal serum, or breast milk and levels of immune function markers or prevalence of immune-related conditions in offspring (see Table 2-11 for references and summaries of results).

The studies provide inconsistent evidence for associations between DDE serum levels and immune function biomarkers or immune conditions in adults or children. Consistent evidence comes from five studies for associations between levels of DDE in cord blood or maternal serum during pregnancy and prevalences of wheeze (or airway obstruction) in infant or child offspring. In other studies, inconsistent evidence was provided for associations between maternal DDE exposure biometrics (cord blood, maternal serum, or breast milk) and prevalence of asthma, blood levels of biomarkers associated with asthma, and prevalence of infections in offspring.

Results from studies of adults and children. In studies of adults (Table 2-9), increasing DDE serum levels were associated with decreased serum levels of IgG, but not IgA or anti-nuclear antibodies (Cooper et al. 2004) and increased total lymphocytes, CD3s, CD56s, and levels of IgA, but not with white blood cells or CD4s (Vine et al. 2001). In adult Japanese women, no significant associations were found between DDE levels in their breast milk and prevalences for asthma, wheeze, rhino-conjunctivitis, or eczema (Miyake et al. 2011).

Studies examining possible associations between serum DDE levels in children and immunological outcomes provide inconsistent evidence across studies (Table 2-10). Associations between increasing serum DDE levels and prevalence of asthma and serum IgE >200 kU/L were found in a group of German children, but the association was not apparent when the data were stratified by gender, breastfeeding status, or age, or when the logistic regression models included other organochlorine compounds analyzed in the children's serum (Karmaus et al. 2001). In a second analysis to examine the protective effects of breastfeeding and detrimental effects of DDE, no associations were found for increasing prevalences for several atopic outcomes (asthma, bronchial hyperactivity, atopic eczema, or hay fever), except for increased prevalence of children with serum IgE >200 kU/L (Karmaus et al. 2003). In a third analysis, elevated blood or serum levels of IgG, IgA, IgE, white blood cell, eosinophilic granula and IgE counts on basophils, but not lymphocyte counts, were associated with high-category DDE serum levels (0.30–

Table 2-9. Summary of Studies of Associations Between DDT Exposure Biometrics[a] in Adults and Immunological Endpoints

Reference and Study Population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Cooper et al. 2004 Consenting African-American male adult farmers (n=137)	Serum levels of DDE Quartiles (ng/mL) Q1: <3.0 Q2: 3.0–5.9 Q3: 6.0–11.9 Q4: ≥12.0	Serum IgA, IgG, and prevalence of anti-nuclear antibodies	Adjusted betas and ORs for Q2–Q4 DDE versus Q1, or per 1-unit increase in DDE (overall analysis) IgA: β / OR; IgG: β / OR Q2: NS / NS / NS / NS Q3: NS / NS / **-0.717*↓** / NS Q4: NS / NS / **-0.609*↓** / NS *p* -OT: NS / NS / **0.03*↓** / **0.05*↓** *p*-MT: NS / NS / NS / NS Overall: NS / NS / NS / NS Associations with prevalence of anti-nuclear antibodies were NS
Miyake et al. 2011 Consenting Japanese women (n=124) Samples collected 1 month after delivery	Milk levels of DDE Minimum–maximum (ng/g lipid) = 7.5–362	Wheeze, asthma, eczema, and rhinoconjunctivitis	Adjusted ORs (95% CI) for each 1 ng/g lipid increase in DDE in breast milk Wheeze: NS Asthma: NS Eczema: NS Rhinoconjunctivitis: NS

Table 2-9. Summary of Studies of Associations Between DDT Exposure Biometrics[a] in Adults and Immunological Endpoints

Reference and Study Population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Vine et al. 2001 Cross-sectional study, community based Consenting adults (n=302) Aberdeen residents (n=151); living near a pesticides dump sites. Control community outside of Aberdeen (n=151)	Serum levels of DDE. Quintiles (ng/mL) Q1: ≤1.0 Q2: >1.0–2.0 Q3: >2.0–4.3 Q4: >4.3–7.6 Q5: >7.6	Lymphocyte profiles (cell counts and % of total), mitogen induced lymphoproliferative activity (PHA, PKW, or ConA), IgA, IgG, IgM, cell mediated immune function (skin test)	Adjusted ordinal p-trends for change with increasing DDE quintiles WBC: 0.07 Total lymphocytes: **0.05***↑ CD3: **0.05***↑ CD4: 0.06 CD56: **0.05***↑ IgA: **0.04***↑ PHA: 0.08 ConA: **0.03***↓ Significance not apparent, when lymphocyte subsets expressed as percent of total No significant associations with other immune endpoints Adjusted ORs for skin test positivity were NS Clinical significance of alterations is uncertain due to the small magnitude of change

[a]See Table 3 in the *Supporting Document for Epidemiological Studies for DDT, DDE, and DDD* for more detailed descriptions of studies. Studies in this table were selected from those described in Table 3, because they: (1) measured DDT-related metrics in biological fluids or tissues in each subject, and (2) evaluated associations using linear regression, logistical regression, or correlation techniques, adjusting for possible confounding factors.
[b]DDE = *p,p'*-DDE, unless otherwise specified; DDT = *p,p'*-DDT, unless otherwise specified.
[c]**Bold text,** "significant" and asterisk indicate statistically significant (p<0.05). Arrows indicate an increase or decrease in the immunological endpoint or direction of p-trend. Adjusted Odds Ratio or Adjusted β indicates adjustment for standard confounding variables for Immunological outcomes, such as age, sex, serum lipids, smoking, and family history of allergic disorders.

CD(X) = cluster of differentiation X; CI = confidence interval; ConA = concanavalin A; Ig(X) = immunoglobin X; MT = median p-trend; NS = not statistically significant; OR = odds ratio; OT = ordinal p-trend; PHA = phytohemagglutinin; PKW = pokeweed mitogen; Q = quartile or quintile; WBC = white blood cell

Table 2-10. Summary of Studies of Associations between DDT Exposure Biometrics in Children and Immunological Endpoints[a]

Reference and study population	DDT exposure metric	Outcome evaluated	Results[b]
Karmaus et al. 2001 School-aged (second grade) children with parental consent (n=343)	Serum DDE Geometric mean: 0.32 ng/mL Exposure categories: (low) <0.3 Q1: ≤0.2 (high) ≥0.3 Q2: 0.21–0.29 Q3: 0.30–0.43 Q4: 0.44–4.02	Otitis media, pneumonia, whooping cough, asthma, and IgE	Adjusted ORs (95% CI) for DDE high versus low exposure levels Otitis media: NS Pneumonia: NS Whooping cough: NS Asthma: **3.71 (1.10–12.56)***↑ IgE ≥200: **2.28 (1.20–4.31)***↑ Associations lost in models including other analytes Adjusted betas for change in IgE with Q2–Q4 DDE versus Q1 p-value Q2 NS 0.49 Q3 NS 0.08 Q4 **62.1***↑ **0.01**
Karmaus et al. 2003 School-age children (second-grade) with parental consent (n=323) Breastfed 1–12 weeks (n=134) Breastfed >12 weeks (n=142) Not breastfed (n=47)	Serum DDE quartiles, ng/mL Q1: <0.21 Q2: 0.21–0.29 Q3: 0.29–0.44 Q4: ≥0.44	IgE, specific IgE aeroallergens, bronchial hyper-reactivity, atopic eczema, hay fever, and asthma	Adjusted ORs for atopic outcomes across DDE quartiles (Q1=reference <0.21 ng/mL) Asthma NS NS NS BroncHR NS NS NS Atopic eczema NS NS NS Hay fever NS NS NS IgE >200 kU/L NS NS **5.63*** IgE aeroallergen NS NS **3.82*** In separate analyses, the beneficial effects of breastfeeding were shown to be countered by DDE levels ≥0.29 ng/mL, especially for atopic eczema

Table 2-10. Summary of Studies of Associations between DDT Exposure Biometrics in Children and Immunological Endpoints[a]

Reference and study population	DDT exposure metric	Outcome evaluated	Results[b]
Karmaus et al. 2005a, 2005b Children (aged 7–10 years) with parental consent (n=331)	Serum DDE Geometric mean (ng/mL) 0.32 Quartiles Q1: ≤0.2 Q2: 0.21–0.29 Q3: 0.30–0.43 Q4: >0.43	WBC count, EG content, IgE count on basophils, IgE, IgG, IgM, and IgA, and lymphocyte cell counts (T-cells, T-helper cells, cytotoxic T-cells, memory T-helper cells, NK, NK subset, and B-cells)	Adjusted betas for Q2–Q4 DDE versus Q1 IgG: Q1 NS, Q2 NS, Q3 NS, Q4 **1,295***↑ IgA: Q1 NS, Q2 NS, Q3 **138***↑, Q4 **141***↑ IgM: Q1 NS, Q2 NS, Q3 NS, Q4 NS IgE: Q1 NS, Q2 NS, Q3 NS, Q4 **80***↑ WBC: Q1 NS, Q2 NS, Q3 NS, Q4 **8,564***↑ EG: Q1 NS, Q2 NS, Q3 NS, Q4 **895***↓ IgE(bas): Q1 NS, Q2 NS, Q3 **896***↑, Q4 NS Associations with various lymphocyte counts were NS
Meng et al. 2016 Asthmatic cases (n=620); control cases (n=218); ages 3–6 years	Serum DDT metrics Mean (ng/g lipid) *p,p'*-DDE: Controls 36.97, Cases 166.52 *p,p'*-DDT: Controls 10.13, Cases 12.13 *p,p'*-DDD: Controls 42.06, Cases 33.71 *o,p'*-DDT: Controls 69.42, Cases 38.32	Asthma, severe asthma	Adjusted ORs (95% CI) for asthma per 1 ng/g lipid increase in DDT exposure metric *p,p'*-DDE: **1.02(1.01–1.03)***↑, p-trend **0.0004** *p,p'*-DDT: NS, p-trend NS *p,p'*-DDD: NS, p-trend NS *o,p'*-DDT: NS, p-trend NS ORs for severe asthma were NS for all exposure metrics, but trends were significant for *p,p'*-DDD and *o,p'*-DDT

Table 2-10. Summary of Studies of Associations between DDT Exposure Biometrics in Children and Immunological Endpoints[a]

Reference and study population	DDT exposure metric	Outcome evaluated	Results[b]		
Perla et al. 2015	Serum levels of DDE	Asthma prevalence	Adjusted PRRs, T2 or T3 versus T1		
Consenting children (n=962);	Geometric mean (95% CI) (ng/g)			Current wheeze	Ever asthma
ages 12–15 years	105 (93.0–1,185)		T2	NS	NS
	Tertiles		T3	NS	NS
	T1: <40th percentile		p-trend	NS	NS
	T2: 40th–80th percentiles				
	T3: >80th percentile				

[a]See Table 4 in the *Supporting Document for Epidemiological Studies for DDT, DDE, and DDD* for more detailed descriptions of studies. Studies in this table were selected from those described in Table 4, because they: (1) measured DDT-related metrics in biological fluids or tissues in each subject, and (2) evaluated associations using linear regression, logistical regression, or correlation techniques, adjusting for possible confounding factors.
[b]DDE = *p,p'*-DDE, unless otherwise specified; DDT = *p,p'*-DDT, unless otherwise specified.
[c]**Bold text,** "significant" and asterisk indicate statistically significant (p<0.05). Arrows indicate an increase or decrease in the immunological endpoint. Adjusted OR or adjusted β indicates adjustment for standard confounding variables for Immunological outcomes, such as age, sex, serum lipids, smoking, atopy, education, gestational age, and number of recent infections. Further adjustments for other analyzed chlorinated compounds or pesticides in biological fluid samples are expressly noted in the table.

BronHR = bronchiolar hyperactivity; CI = confidence interval; EG = eosinophilic granula; Ig(X) = immunoglobin X; NK = natural killer; NS = not statistically significant; OR = odds ratio; PRR = prevalence rate ratio; Q = quartile; SD = standard deviation; T = tertile

Table 2-11. Summary of Studies of Associations Between Maternal Serum, Cord Blood, or Milk DDT Exposure Metrics and Immunological Endpoints in Offspring[a]

Reference and study population	DDT exposure metric	Outcome evaluated	Results[b]
Ashley-Martin et al. 2015 Consenting mother-infant pairs (n=1,258) Immunological parameters measured in cord blood	Maternal serum DDE (ng/mL) T1: ≤0.23 (referent) T2: 0.24–0.39 T3: >0.39	IgE, Ig33/TSLP ratio in cord blood	Adjusted ORs (95% CI) for T2–T3 versus T1 or per doubling of DDE IL33/TSLP (≥80%) / IgE (≥1.2 ng/mL) Log_{10}: **0.81*↓** / NS T2: NS / – T3: NS / – p-trend: 0.06
Bilrha et al. 2003 Consenting mother-infant pairs (n=112) fishing group (n=47) Reference group (n=65)	Cord blood DDE; geometric mean (ng/g lipid) (Groups) Fishing Reference DDE 144 84	TNF-α and IL-10 in mitogen (PHA) induced CBMCs	Pearson's coefficient (*R)* for DDE IL-10: NS TNF-α: **-0.289*↓** Adjusted betas Il-10: NS TNF-α: ** ↓ (but value not reported)
Cupul-Uicab et al. 2014 Consenting mother-male infant pairs (n=747)	Maternal serum DDE and DDT Quartiles (ng/g lipid) DDE / DDT Q1 ≤3.0 / ≤0.25 Q2 3.01–6.00 / 0.26–0.75 Q3 6.01–9.00 / 0.76–1.99 Q4 >9.00 / ≥2.00	IRR of LRTIs in children under 2 years of age	Adjusted IRR (CI%) of LRTIs for Q2–Q4 versus Q1 or per interquartile increase in DDT metrics DDE / DDT Q2: NS / NS Q3: NS / NS Q4: NS / NS p-trend: NS / NS Interquartile increase: NS / NS

Table 2-11. Summary of Studies of Associations Between Maternal Serum, Cord Blood, or Milk DDT Exposure Metrics and Immunological Endpoints in Offspring[a]

Reference and study population	DDT exposure metric	Outcome evaluated	Results[b]
Dallaire et al. 2004 Consenting Inuit mother-infant pairs (n=199) Infant plasma collected at 7 months	Maternal and infant serum DDE Quartiles (ng/g lipid) Maternal / Infant Q1 <183 / <100 Q2 183–281 / 100–355 Q3 281–472 / 355–618 Q4 >472 / >618	URTIs, LRTIs, otitis media, GI infections, or all infections within the first 12 months of age	Adjusted RRs for maternal Q2–Q4 DDE versus Q1 6 months: Q2 / Q3 / Q4; 12 months: Q2 / Q3 / Q4 URTIs: **1.56*↑** / NS / NS; **1.34*↑** / NS / NS Otitis: NS / **1.83*↑** / NS; NS / NS / NS GI infections: NS / NS / NS; **1.59*↑** / NS / NS LRTIs: NS / NS / NS; NS / NS / NS All Infections: **1.38*↑** / **1.33*↑** / NS; NS / NS / NS All p-trends for continuous exposure levels were NS
Dewailly et al. 2000 Consenting mother-infant pairs (n=98)	Maternal milk DDE Tertiles (ng/g lipid) T1: <730 T2: 730–1,320 T3: >1,320	Acute during 0–3, 4–7, 8–12 months ≥1 or ≥3 otitis media episodes during 1st year of age	RRs for T2–T3 versus T1 1 year of age: T2 / T3 ≥1 episode: **1.55*↑** / **1.52*↑** ≥3 episode: **4.64*↑** / NS Acute otitis media 0–3 months / 4–7 months / 8–12 months T2: NS / NS / **1.63*↑** T3: NS / **1.87*↑** / NS Similar results for adjustments (NR)
Gascon et al. 2012 Consenting mother-infant pairs: Spanish mothers (n=1,342) Latin-American mothers (n=79)	Maternal serum DDE Quartiles and tertiles (ng/g lipid) Spanish / Latin **Q1** <72.6 / **T1** <197.9 **Q2** 72.6–115.9 / **T2** 197.9–595.9 **Q3** 115.9–191.7 / **T3** >595.9 **Q4** >191.7	LRTI and wheezing during the first 12–14 months of age	Adjusted RRs for Q2–Q4 DDE versus Q1 Spanish: LRTI / Wheeze; Latin-American: LRTI / Wheeze Q2: 1.20 / NS; T2: **2.59*↑** / NS Q3: **1.40*↑** / **1.37*↑**; T3: **2.89*↑** / **3.54*↑** Q4: **1.28*↑** / NS Cont.: **1.11↑** / **1.14*↑**; NS / 1.20 RRs adjusted for multiple chemical (hexachlorobenzene and PCB) exposure for Spanish cohort

Table 2-11. Summary of Studies of Associations Between Maternal Serum, Cord Blood, or Milk DDT Exposure Metrics and Immunological Endpoints in Offspring[a]

Reference and study population	DDT exposure metric	Outcome evaluated	Results[b]
Gascon et al. 2014 Consenting mother-infant cohorts. Duisburg (n=204) FLEHS I (n=133) HUMIS (n=386) PCB COHORT (n=720) RHEA (n=996) INMA, Menorca (n=395) INMA, Gipuzkoa (n=540) INMA, Sabadell (n=543) INMA, Valencia (n=505) PELAGI (n=186)	Estimated cord-serum levels of DDE. Geometric mean (ng/mL) Duisburg: 0.201 FLEHS I: 0.285 HUMIS: 0.052 PCB: 0.934 RHEA: 0.641 INMA, Menorca: 1.067 INMA, Gipuzkoa: 0.208 INMA, Sabadell: 0.229 INMA, Valencia: 0.503 PELAGIE: 0.165 Tertile levels not reported: T1, T2, and T3 = low, medium, and high exposure levels	Ever bronchitis (Br) and/or wheezing (Wz) before or after 18 months of age (maximum age ~3.5 years); Br/Wz indicates prevalence for bronchitis or wheeze	Adjusted RRs per doubling of DDE ("Contin") or T2–T3 versus T1, all cohorts | | <18 months | | >18 months | | | | Br/Wz | Br | Wz | Wz | | Contin | **1.03*↑** | **1.05*↑** | NS | NS | | T2 | NS | NS | NS | NS | | T3 | **1.14*↑** | NS | **1.16*↑** | NS | Adjusted risk estimates for bronchitis/wheezing in individual cohorts were NS
Glynn et al. 2008 Consenting mother-infant pairs WBC counts group (n=81) Lymphocyte profile group (n=52) Infections group (n=190)	Maternal serum, milk, and estimated postnatal DDE levels Median (ng/g lipid) | | WBC | Lymphocyte | Infections | | Serum | 85 | 83 | 88 | | Milk | 289 | 306 | 311 | Quartile levels not reported.	Counts and percent total WBCs (neutrophils, eosinophils, lymphocytes, monocytes), lymphocyte subsets at 3 months of age; Incidence of respiratory infection during first 3 months after birth	Adjusted betas for DDE with Eosinophil percent **-1.62 (0.46)*↓** Adjusted betas for DDE with: all other WBC counts and % total WBCs = NS; all lymphocyte subsets = NS Adjusted ORs for Q2–Q4 DDE versus Q1 Respiratory infections | | Serum | Estimated postnatal | | Q2 | NS | **0.18*↓** | | Q3 | NS | NS | | Q4 | NS | NS |

Table 2-11. Summary of Studies of Associations Between Maternal Serum, Cord Blood, or Milk DDT Exposure Metrics and Immunological Endpoints in Offspring[a]

Reference and study population	DDT exposure metric	Outcome evaluated	Results[b]
Hansen et al. 2014 Consenting mother-offspring pairs (n=872) Offspring follow-up at 20 years of age	Maternal serum DDE Tertiles (ng/mL) T1: 0.20–1.86 T2: >1.86–3.24 T3: >3.25–38.77	Asthma: hospital diagnosis, asthma medication use for 20 years, self-reported lifetime diagnosis, and self-reported asthma medication use	Adjusted ORs for T2–T3 DDE versus T1 Self-reported diagnosis / Current medication use T2: NS / NS T3: NS / NS *p*-for trend: NS / NS Adjusted HRs for T2–T3 DDE versus T1 Hospital diagnosis 20-year medication use T2: NS / NS T3: NS / NS *p*-for trend: NS / NS
Hansen et al. 2016 Consenting mother-offspring pairs (n=421) Offspring age 20 years	Maternal serum DDE Tertiles (ng/mL) T1: 0.2–1.9 T2: 1.9–3.2 T3: 3.2–38.8	Allergic sensitization, airway obstruction, and reduced lung function	Adjusted ORs for T2–T3 DDE, versus T1 T2 / T3 / p-trend Allergic sensitization: NS / NS / NS Airway obstruction: NS / **2.87***↑ / **0.05***↑ Reduced lung function: NS / NS / NS
Huang et al. 2018 (Vhembe, South Africa) Consenting mother-child pairs (n=674)	Maternal serum *o,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDT geometric mean: 9.18, 292.95, and 70.04 µg/L, respectively	Maternally reported number of persistent fevers, ear infections, and severe sore throats between ages 1 and 2 years	IRRs (95% CI) per 10-fold increase in maternal serum level Persistent fever *o,p'*-DDT: NR *p,p'*-DDE: 1.21 (1.01-1.46) *p,p'*-DDT: NR No significant associations for ear infections or severe sore throat

Table 2-11. Summary of Studies of Associations Between Maternal Serum, Cord Blood, or Milk DDT Exposure Metrics and Immunological Endpoints in Offspring[a]

Reference and study population	DDT exposure metric	Outcome evaluated	Results[b]
Jusko et al. 2016a, 2016b Consenting mother-infant pairs (n=541)	Maternal serum, cord blood, and estimated 6-month DDE (ng/g lipid) Percentiles 25th / 75th Maternal 265 / 723 Cord blood 259 / 706 Estimate-6 months 115 / 847	BCG-specific IgA and IgG at 6 months of age	Adjusted beta per DDE increase from the 25th to 75th percentile BCG-IgG / BCG-IgA Maternal: NS / NS Cord blood: NS / NS 6-months: **-18.2***↓ / **-15.6***↓
Sunyer et al. 2005 Consenting mother-child pairs (n=405) Serum collected at 4 years of age for presence of IgE specific to house dust mite, cat and grass; positive value defined as atopy	Cord blood DDE Quartiles (ng/mL) Q1: <0.57 Q2: 0.57–1.03 Q3: 1.03–1.90 Q4: >1.90	Wheezing at 4 years of age	Adjusted RR (95% CI) for wheezing Q2–Q4, versus Q1 All children (4 years) / Non-atopic Q2: NS / NS Q3: NS / NS Q4: **2.63***↑ / **2.49***↑ Associations with wheezing in atopic children and with doctor diagnosed asthma were NS
Sunyer et al. 2006 Consenting mother-infant pairs (n=402) Blood samples from children at 4 years (n=285) Atopic status evaluated at 6 years; wheezing during a given year defined as one or more episodes of wheezing over 12 months (at years 1, 2, 3, 4, 6.5)	Cord blood and child serum (4 years) levels of DDE and DDT Minimum–maximum (ng/mL) DDE / DDT Cord blood 0.043–19.54 / 0.008–2.283 Serum 0.088–43.88 / 0.038–0.658	Asthma, wheezing	Adjusted ORs per 1 ng/mL increase in DDE All / Non-atopic Asthma At 6.5 years: **1.18***↑ / **1.25***↑ >1 wheeze event/year Year 1: NS / NS Year 2: NS / NS Year 3: NS / NS Year 4: **1.14***↑ / NS Year 6.5: NS / NS Persistent wheeze at 6.5 years: NS / NS No associations with DDT found

Table 2-11. Summary of Studies of Associations Between Maternal Serum, Cord Blood, or Milk DDT Exposure Metrics and Immunological Endpoints in Offspring[a]

Reference and study population	DDT exposure metric	Outcome evaluated	Results[b]			
Sunyer et al. 2010	Maternal serum DDE and DDT	LRTIs from birth to 6 months, or 6–14 months of age	Adjusted RRs for T2–T3, versus T1, or per log-unit increase DDE			
Consenting mother-infant pairs (n=520)	Tertiles (ng/g lipid)			At 6 months	At 14 months	Recurrent
	T1: <83.0		T2	**1.69*↑**	**1.59*↑**	**2.46*↑**
	T2: 83.0–149.5		T3	NS	**1.68*↑**	**2.56*↑**
	T3: >149.5		Log↑	**1.29*↑**	**1.25*↑**	**1.50*↑**

[a]See Table 5 in the *Supporting Document for Epidemiological Studies for DDT, DDE, and DDD* for more detailed descriptions of studies. Studies in this table were selected from those described in Table 5, because they: (1) measured DDT-related metrics in biological fluids or tissues in each subject, and (2) evaluated associations using linear regression, logistical regression, or correlation techniques, adjusting for possible confounding factors.
[b]DDE = *p,p'*-DDE, unless otherwise specified; DDT = *p,p'*-DDT, unless otherwise specified.
[c]**Bold text,** "significant" and asterisk indicate statistically significant (p<0.05). Arrows indicate an increase or decrease in the immunological endpoint or direction of p-trend. Adjusted Odds Ratio or Adjusted β indicates adjustment for standard confounding variables for Immunological outcomes, such as age, sex, serum lipids, smoking, and family history of allergic disorders.

BCG = *Mycobacterium bovis* bacilli Calmette-Guerin; Br = bronchitis; CBMC = cord blood mononucleocytes; CI = confidence interval; FLEHS = Flemish Environment and Health Studies; GI = gastrointestinal; HUMIS = Norwegian Human Milk Study; Ig(X) = immunoglobin X; IL-(X) = interleukin-(x); INMA = Infancia y Medio Ambiente; IQR = inter quartile range; IRR = incidence rate ratio; LRTI = lower respiratory tract infection; NR = not reported; NS = not statistically significant; OR = odds ratio; PCB = polychlorinated biphenyl; PELAGIE = Perturbateurs endocriniens, Étude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance; PHA = phytohemagglutinin; Q = quartile; RR = relative risk; T = tertile; TNF-α = tumor necrosis factor-alpha; TSLP = thymic stromal lymphopoietin; URTI = upper respiratory tract infection; WBC = white blood cell; Wz = wheezing

0.43 or >0.43 ng/mL), compared with children with low (≤0.2 ng/mL) DDE serum levels (Karmaus et al. 2005a, 2005b). In the other two children studies, a small, but elevated, adjusted OR for asthma [1.02 (95% CI 1.01–103)] for per 1 ng/g lipid increase in serum DDE was found in one study of children ages 3–6 years (Meng et al. 2016), and no association with asthma prevalence was found in a study of children ages 12–15 years (Perla et al. 2015).

Results relating maternal exposure and immunological outcomes in offspring. Consistent evidence for associations between levels of DDE in maternal serum and prevalence of wheeze (or airway obstruction) in infant or child offspring have been reported in five studies (Gascon et al. 2012, 2014; Hansen et al. 2016; Sunyer et al. 2005, 2006; Table 2-11). Each of these European cohort studies reported elevated relative risks (RRs) or ORs for this condition in infants of mothers with high DDE serum levels, compared with infants of mothers with low DDE levels or increasing risk for this condition in infants with increasing maternal serum DDE levels. For example, the Gascon et al. (2014) meta-analysis of 4,608 mother-infant pairs from 10 birth cohorts from 7 European countries reported elevated RRs of 1.14 for bronchitis or wheeze and 1.16 for wheeze in young (<18 months old) infants of mothers with the highest category of DDE serum levels, compared with referent infants of mothers in the low DDE serum level category (Table 2-11). This association was not significant with offspring older than 18 months (Gascon et al. 2014).

Inconsistent evidence comes from studies examining associations between maternal serum or cord blood DDE levels and prevalence for asthma or changes in blood immune function markers associated with wheezing or asthma. Hansen et al. (2014) found no association for asthma in 20-year-old offspring with maternal serum DDE levels at birth, but Sunyer et al. (2006) reported increased ORs for asthma in 6.5-year-old children per 1 ng/mL increase in cord blood DDE levels. One study (Ashley-Martin et al. 2015) reported an association between increased maternal serum DDE levels and decreased ratio of interleukin-33 and thymic stromal lymphopoietin (IL-33/TSLP) in cord blood, but no associations with cord blood levels of IL-33 or IgE; elevated levels of each of these individual immune function markers have been associated with wheezing or asthma in other studies. In other studies, associations were found between increasing cord blood DDE levels and decreased cord blood levels of tumor necrosis factor-alpha (TNF-α), but not IL-10, in 111 Canadian mother-infant pairs (Bilrha et al. 2003) and between increasing infant serum DDE levels and decreased IgG and IgA responses to vaccination, but no associations with maternal or cord blood DDE levels (Jusko et al. 2016a, 2016b).

Inconsistent evidence comes from five studies examining associations between maternal serum or breast milk levels of DDE and prevalences of infections in offspring (Table 2-11). No associations were found with increased prevalences of lower respiratory tract infections in a group of 747 Mexican <2-year-old children (Cupul-Uicab et al. 2014) or respiratory tract infections during the first 3 months after birth in a group of 190 Swedish infants (Glynn et al. 2008), but associations were found for increased prevalences for all infections (respiratory, ear, and gastrointestinal) during the first 6 months, but not 12 months, after birth in a group of 199 Canadian children (Dallaire et al. 2004), ear infections between 4 and 12 months, but not 0–3 months, after birth in 98 Inuit infants (Dewailly et al. 2000), lower respiratory tract infections between 6 and 14 months after birth in a group of 520 children from Catalonia Spain (Sunyer et al. 2010); lower respiratory tract infections between birth and 14 months in a group of 1,342 children from Gipuzkoa, Sabadell, and Valencia Spain (Gascon et al. 2012); and elevated rates of persistent fever between 1 and 2 months were associated with *p,p'*-DDE, but not *o,p'*-DDT or *p,p'*-DDT and no associations were found for the number of ear infections or severe sore throats (Huang et al. 2018).

Evidence of Immunological Effects of DDT, DDE, or DDD in Laboratory Animals

Summary. Studies of laboratory animals have provided evidence for suppression or stimulation of various immune system responses in rats and mice exposed to dietary doses technical DDT, *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, or *o,p'*-DDD as low as 2–20 mg/kg/day, but evidence is weak for weight changes or histological changes in immune system organs or tissues in laboratory animals after intermediate- or chronic-duration exposures.

Acute-duration evidence. Information on possible immunological effects in laboratory animals after acute oral exposure to DDT and related compounds is restricted to a report that gavage administration of New Zealand rabbits to 4.3 mg DDT(NS)/kg/day for 10 days produced no effects on serum antibody titers to *Salmonella typhi* infection (Shiplov et al. 1972). Additionally, a study in male NOD mice administered *p,p'*-DDE via intraperitoneal injection every other day for 10 days found increases in splenocyte proliferation in response to Concanavalin A exposure in mice exposed to 100 mg/kg DDE, but not at 1 mg/kg; increases in IL-6, tumor necrosis factor-α, and interferon-γ were also observed at 100 mg/kg (Cetkovic-Cvrlje et al. 2016). There were no effects on splenocyte viability or splenocyte immunophenotype.

Intermediate-duration evidence. The potential for intermediate-duration exposures to technical DDT, *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, or *o,p'*-DDD in the diet to suppress immune responses has been

examined in rats (Banerjee 1987b; Banerjee et al. 1995, 1996; Gabliks et al. 1975; Hamid et al. 1974; Koner et al. 1998), mice (Banerjee 1987a; Banerjee et al. 1986, 1997a, 1997b; Rehana and Rao 1992), and rabbits (Street and Sharma 1975). As shown in Table 2-1, Figure 2-2, and the following text, the lowest doses associated with immune system perturbations in intermediate-duration studies of laboratory animals ranged from about 2 to 20 mg/kg/day, and *p,p'*-DDT was the most widely used test material.

Evidence of immunosuppression comes from studies evaluating the response to various antigens:

- Response to sheep red blood cells (SRBC)
    - decreased splenic plaque forming cell (PFC) response and thymic rosette-forming cell response in Sprague-Dawley rats exposed to 121 mg *o,p'*-DDD/kg/day for 16–24 days (no statistical analysis performed) (Hamid et al. 1974);
    - decreased serum antibody titer response in Wistar rats exposed to 20.6 mg *p,p'*-DDT/kg/day, but not 10.3 mg/kg/day, in the diet for 8 weeks (Koner et al. 1998);
    - decreased splenic PFC response in Hissar mice fed 20 mg *p,p'*-DDT/kg/day, but not 4 or 10 mg/kg/day, for 12 weeks (Banerjee et al. 1986); and
    - decreased splenic PFC response in restraint-stressed Hissar mice fed 20.3 mg *p,p'*-DDT/kg/day, but not 4.1 or 10.1 mg/kg/day, for 4 weeks (Banerjee et al. 1997b).
- Response to tetanus or diphtheria toxoids
    - decreased antibody response to tetanus toxoid in Wistar rats exposed for 22 weeks to 11 mg *p,p'*-DDT/kg/day in the diet, but not doses ≤5.5 mg/kg/day, and decreased serum IgG levels and decreased relative spleen weight (17–20% decreased) and increased serum albumin/globulin ratio resulting from decreased IgG titers in tetanus toxoid-immunized rats exposed to ≥5.5 mg/kg/day, but not 2.2 mg/kg/day (Banerjee 1987b);
    - decreased serum levels of IgG and IgM and antibody titers in response to tetanus toxoid and increased serum albumin/globulin ratio in Wistar rats fed 5.7 mg *p,p'*-DDT/kg/day (but not 2.3 mg/kg/day) in a low (3%) protein diet for 4 weeks; these effects were not seen in similarly exposed rats fed diets containing 12 or 20% protein (Banerjee et al. 1995)
    - decreased severity of anaphylactic shock and number of mast cells in mesenteries in response to diphtheria toxoid (without effects on serum antitoxin titers) in albino rats exposed to 2.3 or 23 mg technical DDT/kg/day in the diet for 31 days (Gabliks et al. 1975)

- Response to *Escherichia coli* lipopolysaccharide
    - decreased splenic PFC response and reduced secondary haemagglutination titres in Hissar mice fed 10.5 or 21 mg *p,p'*-DDT/kg/day, but not 4.2 mg/kg/day, for 6–12 weeks (Banerjee 1987a);
- Response to ovalbumin
    - decreased serum levels of IgM, IgG and ovalbumin antibodies in Wistar rats exposed to 20.2 mg *p,p'*-DDT, *p,p'*-DDE, or *p,p'*-DDD/kg/day in the diet for 6 weeks and increased serum albumin/globulin ratio in *p,p'*-DDT and *p,p'*-DDE exposed animals (Banerjee et al. 1996)
- Resistance to leprosy bacilli
    - increased susceptibility to leprosy bacilli infections in Rockfeller mice exposed to 10.7 or 21.4 mg *p,p'*-DDT/kg/day, but not 4.3 mg/kg/day, in the diet for 24 weeks (Banerjee et al. 1997a).
- Response to tuberculin
    - Decreased skin reactivity to tuberculin challenge in New Zealand rabbits exposed for 8 weeks to 6.54 mg *p,p'*-DDT/kg/day, but not to doses ≤2.10 mg/kg/day, in the diet (Street and Sharma 1975).

Possible effects on weights or histology of immune system organs have also been examined in laboratory animals after intermediate-duration oral exposure to technical DDT, *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT, or *o,p'*-DDD, but the evidence from these studies for these types of immune effects is weak. Hamid (1974) observed decreased absolute weights of the thymus and spleen, along with decreased body weight and atrophy of the thymus and adrenal glands, in Sprague-Dawley rats exposed to 121 mg *o,p'*-DDD/kg/day for 16–24 weeks. Yaglova et al. (2013) reported changes in thymus morphology, such as increased counts of Hassall's corpuscles in the thymic medulla and increased ^{3}H-thymidine incorporation rates in thymus of Wistar rats exposed to 0.0019 or 0.0078 mg *o,p'*-DDT/kg/day in drinking water for up to 10 weeks. Observations from both studies were not included in Table 2-1 or Figure 2-2 due to lack of incidence data and statistical analysis (Hamid 1974) or the lack of corroborating evidence for immune system effects at such low exposure levels and deficiencies in reporting of methodological details (Yaglova et al. 2013). No exposure-related organ weight changes or histopathologies in the spleen or thymus were reported in F344/DuCrl rats exposed to 10 mg *p,p'*-DDE/kg/day for 42 days (Makita et al. 2003a). In a study of rabbits exposed to *p,p'*-DDT in the diet for up to 8 weeks at doses ranging from 0.18 to 6.54 mg/kg/day, several effects were reported in all exposed groups that were of uncertain adversity: 23–36% increase in relative spleen weight; decreased counts of splenic germinal centers (about

12 centers/4-mm diameter in all exposed groups versus about 19/4-mm diameter in control); and increased mean severity score for thymic atrophy (means were about 0.5, 1.9, 0.7, 0.9, and 1.1 for control through high-dose groups) (Street and Sharma 1975).

Chronic-duration evidence. Studies of laboratory animals orally exposed to DDT, DDE, or DDD for chronic durations do not identify the immune system as a sensitive toxicity target, but the scope of these investigations did not include possible perturbations of immune system function. In the 78-week chronic bioassays, no treatment-related histological changes in 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 or in B6C3F1 mice exposed to dietary doses 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). Similarly, F344/DuCrl rats treated for 104 weeks to up to 19.1 mg *p,p'*-DDT/kg/day showed no histopathology of the spleen (Tomita et al. 2013). In a 2-generation study, no exposure-related organ weight changes or histopathologies in the spleen or thymus were reported in Sprague-Dawley rats exposed to *p,p'*-DDT up to 25 mg/kg/day (males) or 27.7 mg/kg/day (females) (Hojo et al. 2006).

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

Mechanisms of Immunological Effects of DDT, DDE, or DDD. Although several DDT exposure-related immunomodulatory effects have been reported, the mechanisms behind these effects are still under investigation. There is currently no clear understanding of the pathophysiological mechanisms of immune-mediated respiratory (e.g., asthma, wheezing, bronchitis) or other immunological effects associated with exposure to DDT, DDE, or DDD.

Disruptions to humoral and cell-mediated immune responses could be due to a variety of cellular and system responses that have been observed *in vitro*. Exposure to DDT or to related compounds has been shown to increase ROS, nitric oxide (NO), or TNF-α production (Perez-Moldonado et al. 2005; Dutta et al. 2008); induce pro-inflammatory responses (Gaspar-Ramirez et al. 2015c; Kim et al. 2004); alter inflammatory mediator production (Mangum et al. 2016) and apoptotic pathways (Alegria-Torres et al. 2009; Perez-Moldonado et al. 2004, 2005); alter immune cell morphologies and activity (Dutta et al. 2008; Reed et al. 2004; Udoji et al. 2010); and lead to aberrant cytokine production (Alegria-Torres et al. 2009; Kim et al. 2004; Quaranta et al. 2006) and alterations in the complement system (Dutta et al. 2008). Microscopic observations of peripheral blood mononuclear cells (PBMCs) exposed to 10, 50, or 100

µg/mL technical DDT showed characteristic signs of cells undergoing apoptosis (cytoplasmic vacuolization, loss of pseudopodia, and presence of lipid bodies), as well as dose-related increases in the inflammatory cytokine, TNF-α and NO (51.7% increase at high dose) (Dutta et al. 2008). Other studies suggested that increased TNF-α, and pro-inflammatory responses following DDT exposure could be the result of activation of transcription factors including NF-κB and AP-1 (Kim et al. 2004); TNF-α in turn may regulate expression of the aryl hydrocarbon receptor (AhR), which can further mediate the inflammatory response (Gaspar-Ramirez et al. 2015c).

Natural killer (NK) cells are an important first-line immune response against tumor cells and viral infection; correlations between plasma *p,p'*-DDT levels and reduction of NK cell numbers has been documented in fish eaters from southeast Sweden (Svensson et al. 1994). *In vitro,* NK lytic function was decreased by 55.4% 24 hours following exposure to 2.5 µM *p,p'*-DDT for 60 minutes in culture (Udoji et al. 2010); determinations of whether DDT can interfere with the essential involvement of mitogen-activated protein kinase (MAPK) signaling in NK cell lytic activity is under investigation (Udoji et al. 2010). In a follow-up study, Hurd-Brown et al. (2013) found 22, 35, and 36% decreases in binding function in NK cells exposed to 2.5 µM DDT for 24 hours, 48 hours, or 6 days, respectively. Exposure to 2.5 µM DDT for 24 or 48 hours also resulted in decreases in CD16 expression in NK cells; no alterations were observed in CD2, CD11a, CD18, or CD56 cell-surface protein expression.

Despite several studies attempting to uncover possible mechanisms of DDT-related immunological effects *in vitro,* it is unclear whether the responses observed in various cultured cell types would occur *in vivo,* or on a scale large enough to elicit an adverse immunotoxic response. This may be reflected in the inconsistencies observed in human epidemiological studies (see Tables 2-9, 2-10, and 2-11).

2.15 NEUROLOGICAL

Summary. Volunteers given single oral doses of DDT reported mild neurological symptoms like perspiration, headache, and nausea at doses as low as 6 mg DDT/kg and transient convulsions or tremors at doses ≥16 mg/kg/day (Hayes 1982; Hseih 1954; Velbinger 1947a, 1947b), but no neurological effects were found in volunteers who ingested 0.05–0.063 or 0.36–0.5 mg/kg/day for 12–18 months (Hayes et al. 1956). In epidemiological studies, inconsistent evidence was provided for associations of serum levels of DDT, DDE, or DDD with deficits in cognitive or mental status tests or risks for neurological conditions, such as Alzheimer's, Parkinson's disease, or attention deficient disorder, in adults or adolescents or associations between DDT, DDE, or DDD levels in maternal serum at birth or during pregnancy, cord

blood, placenta tissue, or breast milk with adverse neurodevelopmental effects of offspring (for references, see Tables 2-12 and 2-13 and text below).

In laboratory animals orally exposed to DDT or metabolites, tremors, convulsions, or myoclonus (abrupt, repeated involuntary contractions of skeletal muscles) and increases in brain biogenic amine and neurotransmitter levels have been observed at acute doses ≥50 mg DDT/kg/day (see text below for references). Acute oral administration of DDT and related compounds *in utero* or to neonates during sensitive periods of neurodevelopment also has been associated with behavioral and neurochemical changes in mice (see Section 2.17 for references and more details).

Tremors, hyperactivity, or hunched appearance have been observed in mature laboratory animals after intermediate- or chronic-duration oral exposure to *p,p'*-DDT, technical DDT, or *p,p'*-DDE at intermediate-duration doses as low as 27 mg *p,p'*-DDE/kg/day and chronic-duration doses as low as 6.9 mg *p,p'*-DDT/kg/day, but these signs of neurological dysfunction were not observed in laboratory rats or mice exposed chronically to doses as high as 231 mg technical DDD/kg/day (see text below for references).

Evidence for Neurological Effects in Controlled-Exposure Human Studies. The nervous system appears to be one of the primary target systems for DDT toxicity in humans after acute, high exposures. Several investigators conducted experimental studies on humans in the 1940s and 1950s at controlled high doses that produced neurological effects (e.g., Hayes et al. 1956; Velbinger 1947a, 1947b). 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 were reported (Hayes 1982), and convulsions were reported at doses of 16 mg DDT/kg or higher (Hsieh 1954). In a controlled exposure study with volunteers given single oral doses of DDT suspended in oil, reported symptoms included prickly sensation of the mouth at 250 or 500 mg; uncertain gait, malaise, cold moist skin, and hypersensitivity to dermal contact within 6 hours of dosing with 750 or 1,000 mg; and prickly tongue, mouth, and nose, dizziness, confusion, tremors, headache, fatigue, and vomiting within 10 hours of dosing with 1,500 mg (about 22 mg/kg) (Velbinger 1947a, 1947b). Symptoms disappeared within 24 hours of dosing. Similar symptoms were reported in persons after accidental or intentional ingestion of DDT (Francone et al. 1952; Garrett 1947; Hsieh 1954; Mulhens 1946). No neurological effects were noted in 51 volunteers who ingested 3.5 or 35 mg DDT/day (0.05–0.063 or 0.36–0.5 mg/kg/day) for 12–18 months (Hayes et al. 1956). The subjects displayed no loss of

Table 2-12. Summary of Neurological Perturbations in Adult or Adolescent Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Kim et al. 2015a Consenting elders (n=644), aged 60–85 years, participants in 1999–2002 NHANES	Serum levels of DDE and DDT Quartile medians (ng/g lipid) (ng/g serum) DDE DDT DDE DDT Q1 280 <LOD 1.73 <LOD Q2 663 – 4.42 – Q3 1,290 12.9 8.28 0.09 Q4 2,660 36.3 18.3 0.23	Low DSST scores (<25th percentile)	Adjusted ORs for Q2–Q4 versus Q1 (ng/g lipid) (ng/g serum) DDE DDT DDE DDT Q2 NS NS NS NS Q3 NS NS NS NS Q4 **1.8*↑** **3.3*↑** **2.3*↑** **2.5*↑** p-trend **0.02*** **<0.01*** **<0.01*** **<0.01*** (increased association with low DSST)
Kim et al. 2015b Consenting elders (n=644), aged 60–85 years Age quintiles: Q1: (60–63 years) (n=134) Q2: (64–67 years) (n=121) Q3: (68–72 years) (n=124) Q4: (73–39 years) (n=128) Q5: (80–85 years) (n=137)	Serum levels of DDE and DDT Tertile medians (ng/g lipid) DDE DDT T1 324.5 5.7 T2 940.5 9.4 T3 2,200.0 25.6	Low DSST scores (<25th percentile)	Adjusted ORs for risk of low DSST with Q2–Q5 versus Q1 at (T1+T2) and T3 exposure levels DDE DDT (T1+T2) (T3) (T1+T2 (T3) Age Q2 NS NS NS NS Age Q3 NS NS NS NS Age Q4 NS NS NS NS Age Q5 **4.7*↑** 2.7 **4.2*↑** 3.1 p-trend **<0.01** 0.20 **<0.01** 0.25
Kim et al. 2015c Consenting elders (n=644), aged 60–85 years, participants in 1999–2002 NHANES	Serum levels of DDE and DDT Tertile medians (ng/g lipid) DDE DDT T1 324.5 5.7 T2 940.5 9.4 T3 2,200.0 25.6	Low DSST scores (<25th percentile)	Adjusted ORs (95% CI) for low DSST scores, hypertensive versus non-hypertensive (referents), across DDE or DDT tertiles. DDE DDT T1 0.8 (0.4–1.9) 1.6 (0.7–3.9) T2 2.1 (0.5–5.1) 1.2 (0.5–3.0) T3 **2.8 (1.2–6.5)*** **2.5 (1.1–5.5)***
Lee et al. 2016a, 2016b Consenting adults (n=989) aged 70 years Prospective follow-up at ages 75 and 80 years	Serum levels of DDE tertiles ng/g lipid ng/g serum T1 <162 <1.0 T2 162–551 1.0–3.5 T3 >551 >3.5	Risk of cognitive impairment diagnosis ranging from mild to overt Alzheimer's disease	Adjusted HRs for cognitive impairment, T2–T3 versus T1 DDE ng/g lipid ng/g serum T2 NS NS T3 NS NS p-trend NS NS

Table 2-12. Summary of Neurological Perturbations in Adult or Adolescent Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Medehouenou et al. 2014 Consenting adults (n=2,023) Dementia cases (n=574) Alzheimer's disease (AD) cases (n=399) Control (normal) cases (n=1,449)	Serum levels of DDE and DDT Median (IQR) (ng/mL) DDE DDT Dementia 4.40 0.06 AD 4.70 0.07 Control 3.90 0.07	Dementia, Alzheimer's disease (AD)	Adjusted ORs for diagnosis per 1-unit increase in log-DDT DDE DDT Dementia NS 0.82 Alzheimer's disease NS NS
Richardson et al. 2014 Consenting adults (n=165) Alzheimer's disease cases (n=86) Control (normal) cases (n=79)	Serum levels of DDE Tertiles (ng/g cholesterol) T1: 90–260 T2: 270–1,640 T3: 1,660–18,750	Alzheimer's diagnosis and MMSE score	Adjusted OR or beta for T2–-T3 DDE versus T1 T2 T3 Alzheimer's disease (OR) 0.54 **3.40***↑ MMSE (β) – **-0.84***↓
Steenland et al. 2014 Consenting adults (n=89)	Serum levels of DDE and DDT Quartiles (ng/mL) DDE DDT Q1 <0.27 <0.09 Q2 ≥0.27–0.69 ≥0.09–0.13 Q3 >0.69–1.26 >0.13–0.17 Q4 >1.26 >0.17	MMSE score and tremor-at-rest	Adjusted ORs for tremor-at-rest and betas for change in MMSE score, Q2–Q4 versus Q1 DDE and DDT MMSE Tremor-at-rest Q2 NS NS Q3 NS NS Q4 NS NS p-trend NS NS

Table 2-12. Summary of Neurological Perturbations in Adult or Adolescent Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Weisskopf et al. 2010 Consenting adults (n=171) Parkinson's disease cases (n=101) Matched controls (normal) (n=349)	Serum levels of DDE and DDT Geometric mean (IQR) (ng/g lipid) DDE: 1,087 (787–1,676) DDT: 257.9 (191.8–359.4)	Parkinson's disease	Adjusted OR's for Parkinson's disease per IQR increase in exposure DDE DDT Confirmed cases NS NS Confirmed cases (nonsmoker) NS NS All cases (nonsmoker) NS NS Adjusted ORs per IQR increase in exposure in subjects over at 66 years were similarly NS
Child/adolescent exposure			
Lee et al. 2007a Children (n=278) aged 12–15 years Participating in the United States 1999–2000 NHANES Learning disability cases (n=44) Attention deficit disorder cases (n=26)	Serum DDT Median (IQR) (ng/g lipid) <50th: 75.3 (49.0–98.4) ≥50th: 278 (186–528)	Learning disabilities, attention deficit disorder	Adjusted ORs for disorder in group with DDT above the (≥50th) versus below (<50th) median Learning disabilities: NS Attention deficit disorder: NS

Table 2-12. Summary of Neurological Perturbations in Adult or Adolescent Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]		
Rocha-Amador et al. 2009 Children with parental consent (n=73); ages 6–11 years living in a region in which DDT was used for malaria control	Serum levels of DDE and DDT Geometric mean±SD (ng/mL) DDE: 57.3±6.6 DDT: 5.5±6.4	Rey-Osterrieth Complex Figure Test: copy and immediate recall scores	Adjusted partial correlation coefficient (*r)* Copy Immediate recall	DDE NS **-0.25***↓	DDT NS NS

[a]See Table 6 in the *Supporting Document for Epidemiological Studies for DDT, DDE, and DDD* for more detailed descriptions of studies. Studies in this table were selected from those described in Table 6, because they: (1) measured DDT-related metrics in biological fluids or tissues in each subject, and (2) evaluated associations using linear regression, logistical regression, or correlation techniques, adjusting for possible confounding factors.
[b]DDE = *p,p'*-DDE, unless otherwise specified; DDT = *p,p'*-DDT, unless otherwise specified.
[c]**Bold text,** "significant" and asterisk indicate statistically significant ($p<0.05$). Arrows indicate an increase or decrease in the neurological endpoint or direction of p-trend. Adjusted OR, adjusted β, adjusted HR, or adjusted partial correlation coefficient indicates adjustment for standard confounding variables for neurological outcomes, such as age, sex, serum lipids, smoking, alcohol consumption, weight loss, BMI, ethnicity, poverty, heart disease, hypertension, diabetes, and region of study.

BMI = body mass index; CI = confidence interval; DSST = Digit Symbol Substitution Test (to assess cognitive function); HR = hazard ratio; IQR = interquartile range; LOD = limit of detection; MMSE = Mini Mental Status Exam (used to measure cognitive impairment); NHANES = National Health and Nutrition Examination Survey; NS = not statistically significant; OR = odds ratio; PIVUS = Prospective Investigation of the Vasculature in Uppsala Seniors; Q = quartile or quintile; SD = standard deviation; T = tertile

Table 2-13. Summary of Studies of Associations between Maternal Serum, Cord Blood, Breast Milk, or Placental DDT Exposure Biometrics and Neurological Outcomes in Offspring[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Early Development Assessments Such as Bayley Scales of Infant Development (BSID), the Brazleton Neonatal Behavior Assessment Scale (BNBAS), Mullen Scales of Early Learning (MSEL) or MacArthur Bates Communicative Development Index (CDI)			
Bahena-Medina et al. 2011 Consenting maternal-infant pairs (n=265) BSID at ~1 month	Maternal serum DDE. Geometric Mean (ng/mL) 1st trimester: 6.33 2nd trimester: NR 3rd trimester: 7.27	NSS, AR, PDI, and MDI	Adjusted betas or ORs per doubling of DDE (ORs) (ORs) (betas) (betas) AR NSS PDI MDI 1st NS NS NS NS 2nd NS NS NS NS 3rd NS NS NS NS
Eskenazi et al. 2006 Consenting mother-infant pairs (n=360) BSID assessed at 6 (n=330), 12 (n=327), and 24 (n=309) months	Maternal serum DDE, DDT, and *o,p'*-DDT Geometric mean (ng/g lipid) *p,p'*-DDE: 1,436 *p,p'*-DDT: 22.0 *o,p'*-DDT: 1.8	MDI, PDI	Adjusted betas for change in index per 1 log-unit increase in DDE metric 6 months 12 months 24 months PDI *p,p'*-DDT **-1.73***↓ **-2.33***↓ NS *o,p'*-DDT NS NS NS *p,p'*-DDE **-2.14***↓ NS NS MDI *p,p'*-DDT NS **-1.71***↓ **-2.12***↓ *o,p'*-DDT NS **-2.56*** **-3.06***↓ *p,p'*-DDE NS NS NS
Forns et al. 2012b Consenting mother-child cohort (n=1,391) BSID at 14 months	Maternal serum DDE and DDT Median (25th–75th percentile), ng/g lipid DDE: 119.06 (74.44–200.26) DDT: 6.21 (5.46–7.28)	MDI, PDI	Adjusted betas for change per 1-log unit increase in DDE or for DDE levels >120.42 ng/g lipid versus <120.42 ng/g lipid Unit increase >120.42 ng/g lipid MDI NS – PDI – NS

Table 2-13. Summary of Studies of Associations between Maternal Serum, Cord Blood, Breast Milk, or Placental DDT Exposure Biometrics and Neurological Outcomes in Offspring[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Gascon et al. 2013 Consenting mother-infant pairs (n=1,175) BSID assessed at 14 months	Maternal serum DDE and postnatal estimates of exposure/3-month increments Postnatal estimates (ng/g lipid) GM / Median 1st 3 months: 199.17 / 199.79 2nd 3 months: 199.11 / 230.94 3rd 3-months: 177.86 / 213.51 4th 3 months: 159.28 / 192.61	MDI and PDI	Adjusted betas for change per DDE levels >median versus <median MDI / PDI 1st 3 months: NS / NS 2nd 3 months: NS / NS 3rd 3-months: NS / NS 4th 3 months: NS / NS
Gladen et al. 1988 Consenting mother-child pairs (n=302) BSID assessed at 6 and 12 months	Maternal breast milk DDE exposure categories (ng/g lipid) a. 0–0.9 b. 1–1.9 c. 2–2.9 d. 3–3.9 e. 4–4.9 f. 5–5.9 g. 6+ Similar categories for transplacental exposure based on cord blood, placenta and maternal serum samples	MDI and PDI at 6 and 12 months	Adjusted betas for change per ng/g lipid DDE Transplacental: 6 months / 12 months MDI: **0.65***↑ / NS PDI: NS / NS Milk MDI: NS / NS PDI: NS / NS
Hoyer et al. 2015 Consenting mother-child cohort (n=1,103) Ukraine (n=492) Poland (n=520) Greenland (n=91) Assessment of early development milestones assessed by parental recall	Maternal serum DDE Tertiles (ng/g lipid) Greenland / Ukraine / Poland T1: 5–209 / 147–488 / 88–303 T2: 209–445 / 488–791 / 303–471 T3: 445–3,122 / 791–4,834 / 471–1,750	Crawl, stand-up, walking, developmental coordination disorder score	Adjusted betas for mean change (month) per 1 log-unit increase in DDE, or for T2 and T3 versus T1 in each cohort Adjusted betas were NS for all study groups

Table 2-13. Summary of Studies of Associations between Maternal Serum, Cord Blood, Breast Milk, or Placental DDT Exposure Biometrics and Neurological Outcomes in Offspring[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Jusko et al. 2012 Consenting mother-infant pairs (n=1,100) BSID assessed at 8 months, cognitive development (IQ) assessed at 7 years	Maternal serum DDE and DDT (ng/mL) DDE DDT Q1 <15 <5 Q2 15–29.9 5.0–9.9 Q3 30–44.9 10–14.9 Q4 45–59.9 15–19.9 Q5 >60 >20	MDI, PDI (at 8 months), and IQ (at 7 years)	Adjusted betas (95% CI) for change in score per 5 ng/mL increase in DDT, 15 ng/mL increase in DDE, or for Q2–Q5 versus Q1 DDT (5 ng/mL) DDE (15 ng/mL) MDI NS NS PDI NS NS IQ NS NS Q2–Q5 versus Q1 betas were all NS
Pan et al. 2009 Consenting mother-infant pairs (n=456) MSEL and CDI assessed at 12 months	Breast milk and lactational exposure metric (LEM) estimates of DDE and DDT to 1 year Median (ng/g lipid) DDE DDT Milk 121 5 (ng/g lipid-month) DDE DDT LEM 871 33	Below average Mullen Scales in: receptive language, expressive language, visual reception, fine motor, and gross motor early learning composite, and CDI	Adjusted ORs for below average scale per 2-fold increase in DDT metrics LEMs DDE DDT Fine motor NS **1.4***↑ Gross motor (all) NS NS (boys) **1.9***↑ – (girls) NS – Associations for all other outcomes were NS; adjusted betas for each outcome were similarly NS
Ribas-Fito et al. 2003 Consenting mother-infant pairs (n=92) BSID evaluated at 13 months of age	Cord blood levels of DDE Median: 0.85 (ng/mL)	Bayley scales (MDI and PDI) and Griffith Scales (locomotor, social, hearing and language, eye-hand coordination, performance)	Adjusted betas for changes per doubling of DDE MDI: **-3.44***↓ PDI: **-3.83***↓ Locomotor: **-4.02***↓ Social: **-4.66***↓ Hearing and language: NS eye-hand coordination: NS Performance: **-2.78***↓

Table 2-13. Summary of Studies of Associations between Maternal Serum, Cord Blood, Breast Milk, or Placental DDT Exposure Biometrics and Neurological Outcomes in Offspring[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Rogan and Gladen 1991 Consenting mother-child pairs (n=678) BSID assessed at 18 and 24 months	Estimated transplacental DDE Exposure categories (ng/g lipid) 1) 0–0.9 4) 3–3.9 2) 1–1.9 5) 4–4.9 3) 2–2.9 6) 5–5.9 7) 6+ Similar exposure categories for breast milk DDE	MDI and PDI at 18 and 24 months	Adjusted betas for PDI change per DDE transplacental exposure categories 2–7 versus 1 18 months 24 months 2–7 versus 1 all NS all NS Associations with MDI were also NS Associations of breast milk DDE with MDI and PDI were also NS
Torres-Sanchez et al. 2007 Consenting mother-infant pairs (n=244) BSID assessments at 1, 3, 6, and 12 months of age	Pre-pregnancy and trimester serum DDE Geometric mean (GSD) (ng/mL) Pre-pregnancy: 6.8 (2.8) 1st trimester: 6.4 (2.8) 2nd trimester: 6.8 (2.9) 3rd trimester: 7.8 (2.8)	PDI and MDI during first year of life	Adjusted betas for change in score in 1st year per doubling of DDE PDI MDI 1st trimester -**0.52***↓ NS 2nd trimester NS NS 3rd trimester NS NS
Torres-Sanchez et al. 2009 Consenting mother-child pairs (n=270) BSID assessments at 12, 18, 24, and 30 months of age	Trimester levels of DDE and DDT. Geometric mean (ng/mL) 1st 2nd 3rd DDE 6.3 6.5 7.9 DDT 0.008 0.006 0.006 DDT/DDE 0.003 0.002 0.002	MDI and PDI between 12 and 30 months of age	Adjusted betas for change in score after 1st year per 2-fold increase in DDE PDI MDI 1st trimester NS NS 2nd trimester NS NS 3rd trimester NS NS

Table 2-13. Summary of Studies of Associations between Maternal Serum, Cord Blood, Breast Milk, or Placental DDT Exposure Biometrics and Neurological Outcomes in Offspring[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Engel et al. 2007 Consenting mother-infant pairs (n=151) BNBAS assessments before hospital discharge	Maternal serum levels of DDE Median (IQR), ng/L: 0.6 (0.4–1.3)	Habituation, orientation, motor, range of state, regulation of state, autonomic stability, risk for abnormal reflexes	Adjusted betas per exposure level Habituation: NS Orientation: NS Motor: NS Range of state: NS Regulation of state: NS Autonomic stability: NS Adjusted RR for abnormal reflexes = NS
Fenster et al. 2007 Consenting mother-infant pairs (n=303) BNBAS assessments at ≤2 months of age	Maternal serum DDE, DDT, and *o,p*'-DDT Geometric mean (ng/g lipid) *p,p'*-DDE: 1,464 *p,p'*-DDT: 23.2 *o,p'*-DDT: 1.8	Habituation, orientation, motor, range of state, regulation of state, autonomic stability, reflex	Adjusted betas for score change per 10-fold increase in DDT metrics No significant associations were observed with any of the BNBAS outcomes
Sagiv et al. 2008 Consenting mother, infant pairs (n=542) BNBAS assessed in 408 infants at 1–3 days following birth and at ~2 weeks of age	Cord blood DDE Mean±SD (range) (ng/g serum): 0.48±0.85 (0–10.27) Quartile exposure levels not reported	Alertness, quality of alertness, cost of attention, consolability, self-quieting, hand-to-mouth, irritability, never in state for orientation items, elicited activity, spontaneous activity, motor maturity	Adjusted *p*-for-trend for Q2–Q4 DDE versus Q1 (coefficients displayed graphically) Irritability: **0.03***↑ Never in state for orientation items: **0.04***↑ All other associations were NS

Table 2-13. Summary of Studies of Associations between Maternal Serum, Cord Blood, Breast Milk, or Placental DDT Exposure Biometrics and Neurological Outcomes in Offspring[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Stewart et al. 2000 Consenting mother-child pairs (n=293) Fish-eating cases (n=141) Control (non-fish-eating cases (n=152) BNBAS assessments at 12–24, and 25–48 hours after birth	Cord blood DDE Exposure values (percentiles) (ng/g wet) 25th: 0.06 50th: 0.10 75th: 0.18	Habituation, autonomic, reflexes, percent poor NBAS scores	Adjusted betas for change in score per unit increase in DDE Habituation: NS Autonomic: NS Reflexes: NS % poor NBAS scores: NS
Behavioral problems, Attention and ADHD			
Strom et al. 2014 Consenting mother-offspring pairs (n=872) 22-year prospective follow-up: ADHD cases (n=27) Depression cases (n=104) Scholastic achievement below median cases (n=383) determined by standardized written examinations in 9th grade	Maternal serum DDE. Median (IQR) (ng/mL) Total: 2.5(2.2) ADHD cases: 2.9(2.3) Depression cases: 2.6(2.5) Scholastic achievement: 2.4 (2.1) Tertiles T1: 0.20–1.86 T2: >1.86–3.24 T3: >3.24–38.77	ADHD, depression, scholastic achievement below median	Adjusted HRs for conditions, T2 and T3 DDE versus T1 ADHD: T2 NS, T3 NS, p-trend NS Depression: T2 NS, T3 NS, p-trend NS Adjusted betas per ng/mL difference in DDE and for T3 versus T1 Scholastic achievement <median: ng/mL difference NS, T3 versus T1 NS

Table 2-13. Summary of Studies of Associations between Maternal Serum, Cord Blood, Breast Milk, or Placental DDT Exposure Biometrics and Neurological Outcomes in Offspring[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Forns et al. 2012a Consenting mother-child pairs (n=393) Child attention function evaluated at 11 years using the CPT-II	Cord blood and child serum (4 years) DDE Percentiles (ng/mL) 50th: Cord blood 1.08; Serum 0.85 25th–75th: Cord blood 0.56–1.85; Serum 0.46–1.81	tHRT (speed of correct response), errors of omission or commission	Adjusted beta (95% CI) for change per 1 log-unit increase in DDE levels at 4 years tHRT: **0.051 (0.014–0.088)***↓ (indicates delayed response) Significance lost with further adjustment for serum PCBs and breastfeeding. Adjusted IRRs for errors of omission or commission per log-unit increase in DDE were NS
Forns et al. 2016 Consenting mother-infant pairs (n=522) Children evaluated for behavioral problems at 12 and 24 months using the ITSC	Breast milk levels of DDE and DDT Mean (SD), ng/g lipid DDE: 62.93 (51.09) DDT: 2.55 (2.43)	Behavioral problems	Adjusted betas (95% CI) for behavioral problem score at 12 months per IQR increase in DDT Behavioral problems 12 months: **0.62 (0.45–0.79)***↑ 12 months: **0.35 (0.08–0.63)***↑ (with additional adjustments for other analytes) 24 months: NS
Sagiv et al. 2010 Consenting mother-child pairs (n=573) ADHD measured in children aged 7–11 years using Connors' Rating Scale for Teachers	Cord blood DDE Mean (SD) (ng/g serum): 0.50 (1.04) Quartile levels not provided	Conners' ADHD index, DSM-IV inattentive, hyperactive impulse, DSM-IV-total	Adjusted betas for log change with P95 versus P5 DDE ADHD: NS Inattentive: NS Hyper-impulse: **0.02***↑ DSM-IV Total: **0.01***↑ Adjusted Risk Ratios for atypical score Q4 DDE versus Q1 Conners' ADHD index: **1.80***↑ DSM-IV inattentive: NS DSM-IV hyperactive-impulse: NS DSM-IV total: **1.69***↑

Table 2-13. Summary of Studies of Associations between Maternal Serum, Cord Blood, Breast Milk, or Placental DDT Exposure Biometrics and Neurological Outcomes in Offspring[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Sioen et al. 2013 Consenting mother-child pairs (n=270); 130 males, 140 females Child behavioral problems assessed at 7–8 years of age; SDQ completed by parents	Cord blood DDE Median (25th, 75th percentile), ng/g fat All: 124.0 (67.2, 218.5) Boys: 128.0 (67.6, 226.0) Girls: 118.0 (66.7, 205.9)	Emotional symptoms, conduct problems, hyperactivity, total difficulties	Adjusted ORs for abnormal SDQ score per log-1 unit increase in DDE or for T3 versus T1 stratified by gender All / Girls (T3 versus T1) Emotional symptoms: NS / NR Conduct problems: NS / NR Hyperactivity: NS / NR Total difficulties: **3.061*↑** / **9.959*↑**
McCarthy Scales of Children's Abilities (MSCA), MacArthur Bates Communicative Development Inventories (CDI), Wide Range Assessment of Memory and Learning (WRAML), or Wechsler Intelligence Scale for Children (WISC)			
Gladen and Rogan 1991 Consenting mother-child pairs (n=370) MSCA assessment at 3, 4, and 5 years	7 DDE transplacental exposure categories (ng/g lipid) ranging from 0–0.9 to >6.0 5 DDE postnatal exposure categories (mg) ranging from 0–3 to >17	MSCA at 3, 4, and 5 years	Adjusted mean quantitative McCarthy scores: NS across transplacental DDE exposure categories Adjusted mean McCarthy scores at 4 years: NS across postnatal DDE exposure categories
Kyriklaki et al. 2016 Consenting mother-child cohort (n=689) MSCA assessments at 4 years	Maternal serum DDE Mean±SD (ng/mL) = 2.948±3.071	Neurodevelopment outcomes: verbal, perceptual, quantitative, cognitive, memory, motor scale, and subscales: executive function, working memory, memory span, and functions of posterior cortex	Adjusted betas (95% CI) for change in score, DDE ≥90th percentile versus ≤90th percentile All adjusted betas were NS

Table 2-13. Summary of Studies of Associations between Maternal Serum, Cord Blood, Breast Milk, or Placental DDT Exposure Biometrics and Neurological Outcomes in Offspring[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Orenstein et al. 2014 Consenting mother-child pairs (n=393) Memory and learning assessed at 8 years using WRAML	Cord blood DDE Mean±SD (ng/g serum): 0.5±01.1	Visual memory, verbal memory, learning indices	Adjusted betas (95% CI) for a change in score per 1-unit increase in DDE Visual memory: NS Verbal memory: NS Learning: NS
Osorio-Valencia et al. 2015 Consenting mother-child pairs (n=167) MSCA assessments at 5 years only for laterality and spatial orientation endpoints	Maternal trimester serum DDE Percentiles (ng/g lipid) 1st / 2nd / 3rd 10th: 260.8 / 152.7 / 149.0 50th: 1,331.1 / 1,138.1 / 826.3 90th: 4,253.9 / 2,983.4 / 2,767.6	Laterality and spatial orientation	Adjusted OR and beta (95% CI) per 2-fold increase in DDE (2nd trimester) Laterality (OR): NS Spatial orientation (β): NS

Table 2-13. Summary of Studies of Associations between Maternal Serum, Cord Blood, Breast Milk, or Placental DDT Exposure Biometrics and Neurological Outcomes in Offspring[a]

Reference and study population	DDT exposure metric[b]			Outcome evaluated	Results[c]			
Ribas-Fito et al. 2006 Consenting mother-child pairs (n=445) Ribera d'Ebre cohort (n=40) Menorca cohort (n=405) MSCA assessed at 4 years	Cord blood DDE Median (ng/mL)			General cognitive, memory, verbal, executive function, memory span, verbal memory	Adjusted betas per doubling of DDE			
		Ribera d'Ebre	Menorca			DDT	DDE	DDT/DDE
	DDT	0.05	0.08		General cognitive	**-1.99*↓**	NS	NS
	DDE	0.86	1.03		Memory	**-3.79*↓**	**-1.93*↓**	**-2.67*↓**
	DDT/DDE	0.04	0.09		Verbal	**-2.63*↓**	NS	**-0.98*↓**
					Executive function	**-2.61*↓**	NS	NS
					Memory span	**-2.82*↓**	NS	NS
	DDT quartiles (ng/mL)				Verbal memory	**-6.10*↓**	NS	**-4.64*↓**
	Q1: ≤0.05							
	Q2: >0.05–0.10				Adjusted betas for Q4 DDT versus Q1			
	Q3: >0.10–0.20					All	Boys	Girls
	Q4: >0.20				General cognitive	**-5.87*↓**	NS	**-8.89*↓**
					Verbal	**-7.86*↓**	NS	**-12.79*↓**
					Memory	**-10.56*↓**	NS	**-17.19*↓**
					No significant associations with Q2 and Q3 exposure levels			
					Further adjustments for cord blood HCB or PCBs did not change significance			

Table 2-13. Summary of Studies of Associations between Maternal Serum, Cord Blood, Breast Milk, or Placental DDT Exposure Biometrics and Neurological Outcomes in Offspring[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Ribas-Fito et al. 2007 Consenting mother-infant pairs (n=391) MSCA assessed at 4 years Companion study to Ribas-Fito et al. 2006	Cord blood levels of DDT Median (ng/mL) according to breastfeeding Duration (weeks) DDT DDE <2 (reference) 0.07 0.94 2–20 (short-term) 0.07 1.07 >20 (long-term) 0.06 1.01 DDT exposure groups (ng/mL) Low (<0.05) Medium (0.05–0.20) High (>0.20)	GCS, verbal scale, memory scale	Adjusted betas for change in neurodevelopmental scales per low, medium, and high DDT exposure levels in different breastfeeding groups DDT exposure GCS Low Medium High 2–20 weeks NS NS NS >20 weeks NS NS **13.04*↑** Verbal scale 2–20 weeks NS NS NS >20 weeks NS NS NS Memory scale 2–20 weeks NS NS NS >20 weeks NS NS NS Results may indicate a positive effect of breastfeeding on infant neurodevelopment
Torres-Sanchez et al. 2013 Consenting mother-child pairs (n=203) MSCA assessed at 42, 48, 54, and 60 months	Trimester levels of DDE and DDT Median (ng/mL) 1st 2nd 3rd DDE 7.65 8.22 8.95 DDT 0.0045 0.0045 0.0045 DDT/DDE 0.001 0.001 0.001	General cognitive index perceptual performance, quantitative, verbal, memory, and motor	Adjusted betas for change in score per doubling of DDE 3rd trimester (n=84) Average DDE General cognitive index **-2.01*↓** NS Perceptual performance NS NS Quantitative **-2.06*↓** **-1.78*↓** Verbal **-1.14*↓** NS Memory **-1.26*↓** NS Motor NS NS Association with 1st and 2nd trimester exposure levels were NS

Table 2-13. Summary of Studies of Associations between Maternal Serum, Cord Blood, Breast Milk, or Placental DDT Exposure Biometrics and Neurological Outcomes in Offspring[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Gaspar et al. 2015a, 2015b Consenting mother-child pairs (n=619) WISC assessment at ages 7 (n=316) and 10.5 years (n=595)	Maternal serum DDE and DDE Geometric mean (ng/g lipid) DDT: 21.4 DDE: 606.4	Working memory, perceptual reasoning, verbal comprehension, processing speed, full-scale IQ	Adjusted betas for change per 10-fold increase in DDT metrics at 7 years (ALL) DDT; (ALL) DDE; (Boys) DDE; (Girls) DDE Working memory: NS; NS; **2.9***↑; NS Perceptual reasoning: NS; NS; NS; NS Verbal comprehension: NS; NS; NS; **-3.1***↓ Processing speed: **-2.4***↓; NS; NS; **-4.2***↓ Full-scale IQ: NS; NS; NS; **-4.4***↓ All betas at 10.5 years and 7 and 10.5 years, analyzed together were NS, except for deficits in processing speed in girls
Lyall et al. 2016 Consenting mother-infant pairs (n=1,144) Autism spectrum disorder cases (n=545); intellectual disability cases (n=181); control (general population) (n=418)	Maternal serum DDE (ng/g lipid) Q1: <121.7 Q2: 121.7–212.5 Q3: 212.5–<505.4 Q4: ≥505.4	Autism spectrum disorder and intellectual disability	Adjusted ORs (95% CI) for Q2–Q4, versus Q1 Autism spectrum disorder; Intellectual disability Q2: NS; NS Q3: NS; **1.89***↑ Q4: NS; NS p-trend = 0.57

Table 2-13. Summary of Studies of Associations between Maternal Serum, Cord Blood, Breast Milk, or Placental DDT Exposure Biometrics and Neurological Outcomes in Offspring[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Sagiv et al. 2012 Consenting mother-child pairs (n=584); 254 females, 258 males CPT and WISC assessed at 8 years	Cord serum DDE Mean±SD (ng/g) = 0.50±1.03; IQR=0.27	Reaction time, reaction time variability, errors of commission (rate ratio), error of omission, processing speed, and freedom from distractibility	Adjusted betas per IQR change in DDE All / Girls / Boys Reaction time: NS / NS / NS Reaction time variability: NS / NS / NS Errors of commission: NS / NS / NS Error of omission: NS / NS / NS Processing speed: NS / NS / NS Freedom from distractibility: NS / NS / NS A significant sex interaction (p<0.05) was observed for reaction time and errors of omission
Other			
Cartier et al. 2014 Children with parental consent (n=150) Child serum collected at 5 and 11 years of age VEP evaluation at ~11 years of age	Cord blood and child serum DDE and DDT. Geometric mean (ng/g lipids), cord blood serum (11 years) DDE: 509.27, 268.54 DDT: 24.45, 6.93	VEPs: N150 and N75 amplitudes, and P100 latency	Adjusted betas per 1 log-unit increase in DDE from cord blood or 5-year serum N150 for DDE (cord blood): **0.721***↑ N75 for DDE (5 years): **-1.439***↓ P100 latency for DDT: NS
Riva et al. 2004 Consenting mother-infant pairs (n=25) VEP evaluation at 12 months	Colostrum and milk levels of DDT and DDE measured in each subject, but mean values were not reported	P100 wave latency	After controlling for infant's serum levels of polyunsaturated fat, partial correlation coefficients with colostrum DDT or DDE levels were NS

Table 2-13. Summary of Studies of Associations between Maternal Serum, Cord Blood, Breast Milk, or Placental DDT Exposure Biometrics and Neurological Outcomes in Offspring[a]

Reference and study population	DDT exposure metric[b]			Outcome evaluated	Results[c]
Ren et al. 2011 Consenting mother-infant pairs (n=130) Neural tube defect (NTD) cases (n=80 fetuses or newborns) Healthy matched controls (n=50 newborns)	Placental levels of all DDT metrics Median (ng/g lipid)			Anencephaly, spina bifida, any NTD	Adjusted ORs (95% CI) for Σ*o,p'*-DDTs levels above median level in control group Anencephaly: **9.52 (1.45–62.65)***↑ Spina bifida: **8.68 (1.94–38.76)***↑ Any NTDs: **5.19 (1.70–15.82)***↑ Adjusted OR for Q4 versus Q1 Σ*o,p'*-DDTs Any NTDs: **5.10 (1.66–15.70)***↑ Associations with Q2 and Q3 exposure levels were NS
		Cases	Controls		
	Σ*o,p'*-DDTs	4.3	2.7		
	Σ*p,p'*-DDTs	55	59		
	Σall DDTs	60	61		

[a]See Table 7 in the *Supporting Document for Epidemiological Studies for DDT, DDE, and DDD* for more detailed descriptions of studies. Studies in this table were selected from those described in Table 7, because they: (1) measured DDT-related metrics in biological fluids or tissues in each subject, and (2) evaluated associations using linear regression, logistical regression, or correlation techniques, adjusting for possible confounding factors.
[b]DDE = *p,p'*-DDE, unless otherwise specified; DDT = *p,p'*-DDT, unless otherwise specified.
[c]**Bold text,** "significant" and asterisk indicate statistically significant (p<0.05). Arrows indicate an increase or decrease in the neurological endpoint or direction of p-trend. Arrows "↑ or ↓" indicate direction of change observed for a given outcome (positive or inverse associations, respectively). Adjusted OR, adjusted beta, or adjusted HR indicates adjustment for standard confounding variables for neurological outcomes, such as maternal age, occupation, parity, smoking and alcohol during pregnancy, socio-economic status, depression, marital status, breastfeeding, birth weight, sex, type of birth, and age at evaluation.

ADHD = attention deficit hyperactivity disorder; AR = abnormal reflex; BNBAS or NBAS = Brazelton Neonatal Behavioral Assessment Scale (two components for evaluating behavior and reflex); BSID = Bayley Scales of Infant Development (for mental and psychomotor development); CDI = MacArthur-Bates Communicative-Development Inventories (to measure language comprehension); CI = confidence interval; CPT = Continuous Performance Test; CPT-II = Cognitive Performance Test-II; CRS-T = Conners' Rating Scale for Teachers; DSM-IV = Diagnostic and Statistical Manual of Mental Disorders, 4th edition; GCI = general cognitive index; GM = geometric mean; GCS = General Cognitive Score; GSD = geometric standard deviation; HCB = hexachlorobenzene; HR = hazard ratio; HRT = hit reaction time (measures speed of visual processing); IQ = intelligence quotient; IQR = interquartile range; IRR = incidence rate ratio; ITSC = infant toddler symptom checklist; LEM = lactational exposure metric; MDI = mental development index; MSCA = McCarthy Scales of Children's Abilities (to assess cognitive and motor development); MSEL = Mullen Scales of Early Learning; NBAS = Neonatal Behavioral Assessment Scale; NR = not reported; NS = not statistically significant; NSS = Neurological soft signs; NTD = Neural tube defect; OR = odds ratio; PBDE = polybrominated diphenyl; PCB = polychlorinated biphenyl; PDI = psychomotor developmental index; PP = perceptual performance; Q = quartile or quintile; RR = relative risk; SD = standard deviation; SDQ = Strengths and Difficulties Questionnaire; T= tertile; VEP = visual evoked potential (to assess visual brain function/visual processing); WISC = Wechsler Intelligence Scale for Children; WRAML = Wide Range Assessment of Memory and Learning

coordination and there was no indication of tremors. Other tests (over 20) conducted on the volunteers 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.

Neurological Adult or Adolescent Epidemiological Studies. Possible associations between serum levels of DDT, DDE, or DDD and deficits in cognitive or mental status tests or risks for neurological conditions, such as Alzheimer's, Parkinson's disease, or attention deficient disorder have been evaluated in studies described in Table 2-12. The studies provide inconsistent evidence for such associations. In adults, associations were found with low Digit Symbol Substitution Test scores in U.S. adults ages 60–85 years participating in the 1999–2002 NHANES (Kim et al. 2015a, 2015b, 2015c) and with increased risk for Alzheimer's disease and decreased Mini Mental Status Exam (MMSE) scores in a U.S. case-control study (Richardson et al. 2014), but no significant associations were found between serum biometrics and increased risk for cognitive impairment in 70-year-old Swedish adults (Lee et al. 2016a, 2016b); increased risk for dementia or Alzheimer's disease in Canadian adults (Medehouenou et al. 2014); decreased MMSE scores or increased risk for at-rest tremors in Costa Rican adults ages >65 years (Steenland et al. 2014), or increased risk for Parkinson's disease in Finnish adults ages 20–70 years (Weisskopf et al. 2010). No significant associations were found between serum DDT or DDE levels and increased risks for learning disability or attention deficient disorder in U.S. adolescents ages 12–15 years participating in the NHANES (Lee et al. 2007a), but serum levels of DDE were associated with decreased scores for a test of visual memory in 6–11-year-olds living in a Mexican region in which DDT was used for malaria control (Rocha-Amador et al. 2009).

Neurodevelopmental Epidemiological Studies. Possible associations between DDT, DDE, or DDD levels in maternal serum at birth or during pregnancy, cord blood, placenta tissue, or breast milk with adverse neurodevelopmental effects in offspring have been examined in numerous epidemiological studies. To date, these studies (summarized in Table 2-13) provide inconsistent evidence for such associations. Studies in Table 2-13 are presented in four groups of studies evaluating associations with: (1) neurobehavioral endpoints in infants ≤2 years of age using the Bayley Scales of Infant Development (BSID), the Brazleton Neonatal Behavioral Assessment Scale (BNBAS), Mullen Scales of Early Learning (MSEL), or the MacArthur-Bates Communicative Development Inventories (CDI); (2) behavioral problems, attention and attention deficit hyperactivity disorder (ADHD) in offspring; (3) neurobehavioral endpoints in older children using McCarthy Scales of Children's Abilities (MSCA), Wide Range Assessment of Memory and Learning (WRAML), Wechsler Intelligence Scale for Children (WISC), and related methods; and (4) other neurological endpoints in offspring. The following paragraphs summarize

the inconsistency of the evidence relating maternal biometrics for DDT, DDE, or DDD with related neurological outcomes in offspring.

Early neurodevelopment epidemiological studies. Using the BSID, no associations between maternal DDT, DDE, or DDD biometrics and adverse early developmental scores in children up to about 30 months after birth were found in a North Carolina cohort (Gladen et al. 1988; Rogan and Gladen 1991), a U.S. 12-center cohort (Jusko et al. 2012), and cohorts from Sabadell, Gipuzkoa, and Valencia Spain (Forns et al. 2012b; Gascon et al. 2013), but significant associations with age-dependent BSID deficits were reported for cohorts from Ribera d'Ebre and Menorca, Spain (Ribas-Fito et al. 2003); Salinas, California (Eskenazi et al. 2006); and Morelos, Mexico (Bahena-Medina et al. 2011; Torres-Sanchez et al. 2007, 2013). In another North Carolina birth cohort, significant associations were reported for MSEL motor deficits, but not for MSEL language scales or CDI scores, in 12-month-olds in a 2004–2006 North Carolina birth cohort (Pan et al. 2009). No significant associations were reported with time of achieving early development milestones (e.g., crawling or walking) in cohorts from Ukraine, Poland, and Greenland (Hoyer et al. 2015). No statistically significant associations with BNBAS deficits for infants <2 weeks of age were found in cohorts from New York City (Engel et al. 2007); Oswego, New York (Stewart et al. 2000); or Salinas, California (Fenster et al. 2007), but significant associations were reported for attention-related BNBAS deficits in a New Bedford, Massachusetts cohort (Sagiv et al. 2008).

Epidemiological studies of attention, behavioral problems, or ADHD in offspring. No associations with maternal biometrics were reported for prevalences of ADHD or depression in a 22-year follow-up of offspring from a Danish cohort (Strom et al. 2014) for scores from a Strength and Difficulties Questionnaire (SDQ) or ADHD test in 4-year-old children in a Greek cohort (Kyriklaki et al. 2016), or attention function measures in 11-year-olds from a Menorca Spain cohort (Forns et al. 2012a), but associations were reported for behavioral problem scores at 12 months, but not at 24 months, in infants from a Norwegian cohort (Forns et al. 2016); for Connors' ADHD Index in children ages 7–11 years in a New Bedford Massachusetts cohort (Sagiv et al. 2010); and for SDQ scores for total behavioral difficulties in children ages 7–8 years in a Flemish cohort (Sioen et al. 2013).

Epidemiological studies of neurological endpoints in non-infant children. No associations with maternal biometrics were found for MSCA-evaluated deficits in 3–5-year-old children in a North Carolina cohort (Gladen and Rogan 1991); MSCA deficits in 4-year-old children in a Greek cohort (Kyriklaki et al. 2016), or WRAML deficits in 8-year-olds in a New Bedford, Massachusetts cohort (Orenstein et al. 2014), but associations were reported for MSCA cognitive deficits, but not spatial orientation deficits, in 42–

60-month-old children in a Morelos, Mexico cohort (Osorio-Valencia et al. 2015; Torres-Sanchez et al. 2013) and MSCA general cognitive and memory deficits in 4-year-old children in cohorts from Ribera d'Ebre and Menorca, Spain (Ribas-Fito et al. 2006, 2007). In intelligence assessments, no associations were reported with IQ in 7-year-old children in a U.S. 12-center cohort (Jusko et al. 2012) or 8-year-old children in a New Bedford, Massachusetts cohort (Sagiv et al. 2012), but associations were reported with IQ deficits at 7 years of age, but not at 10.5 years of age, in children (especially girls) in a Salinas, California cohort (Gaspar et al. 2015a, 2015b) and for increased risk for intellectual disability, but not autism spectrum disorder, in a 2000–2003 Southern California birth cohort (Lyall et al. 2016).

Epidemiological studies of other neurodevelopmental endpoints in offspring. Associations between cord blood DDE levels and visual evoked potential (VEP) deficits were reported in a group of 150 11-year-old children (Cartier et al. 2014), but no significant associations were found for VEP deficits in a group of 25 12-month-old children (Riva et al. 2004). Placental levels of Σ*o,p'*-DDTs, but not *Σp,p'-DDTs,* were significantly associated with increased risk of neural tube defects in a study of 80 cases and 50 controls without neural tube defects (Ren et al. 2011).

Evidence for Neurological Effects in Laboratory Animals. The nervous system appears to be one of the primary targets in animals after acute-, intermediate-, and chronic-duration oral exposure to technical or *p,p'*-DDT. Several older acute- or intermediate-duration studies (Henderson and Wooley 1969; Hrdina and Singhal 1972; Hrdina et al. 1973; Khairy 1959; Sobotka 1971; Talts et al. 1998; vom Saal et al. 1995) are mentioned in the text below to reflect the breadth of supporting evidence, but were not included in Table 2-1 and Figure 2-2 for various reasons such as poor study design (e.g., low number of animals), lack of comprehensive endpoint evaluation, poor data reporting, outdated methodologies, or exposure levels well above exposure levels producing the most sensitive neurological endpoints. Clinical signs of neurological effects also have been observed in rats and mice after chronic dietary exposure to *p,p'*-DDE, but were not observed in rats or mice after chronic exposure to technical DDD (NCI 1978).

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, or tremors and convulsions in several species. These effects have been observed in rats after single high gavage doses of about 50–600 mg technical *p,p'*-DDT/kg/day (Herr and Tilson 1987; Herr et al. 1985; Hietanen and Vainio 1976; Hong et al. 1986; Hudson et al. 1985; Hwang and Van Woert 1978; Pranzatelli and Tkach 1992; Pratt et al. 1986; Tilson et al. 1987; Tomiyama et al. 2003; supported by Henderson and Wooley 1969; Hrdina and Singhal 1972; Hrdina et al. 1973). Mice receiving single gavage doses of 160 mg

DDT(NS)/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(NS)/kg, but hind leg paralysis occurred in guinea pigs (Hietanen and Vainio 1976).

Acute oral exposure of animals to DDT and related compounds also has been associated with increases in brain biogenic amine and neurotransmitter levels. Alterations in the metabolite 5-hydroxy-indoleacetic acid (5-HIAA), the degradation product of serotonin, have been reported to correlate with DDT-induced tremors; doses ≥50 mg *p,p'*-DDT/kg/day resulted in increases in the levels of 5-HIAA in the brain (Hong et al. 1986; Hudson et al. 1985; Hwang and Van Woert 1978; Tilson et al. 1986 supported by Hrdina et al. 1973). Alterations in the levels of other neurotransmitters have been found. The neurotransmitter changes observed are consistent with a mechanistic hypothesis that DDT and metabolites influence membrane ion fluxes and consequently potentiate neurotransmitter release. Acetylcholine and norepinephrine decreased in rats after acute oral exposure to 400 mg/kg DDT (Hrdina et al. 1973), and aspartate and glutamate were statistically significantly increased in brain tissue of F344 rats after administration of single oral doses ≥50 mg *p,p'*-DDT/kg (Hong et al. 1986; Hudson et al. 1985; Tilson et al. 1986).

Acute oral administration of DDT and related compounds *in utero* or to neonates during sensitive periods of neurodevelopment has been associated with behavioral and neurochemical changes in mice (see Section 2.17 for more details and references).

Behavioral effects have been examined in only a few studies of rodents exposed to DDT as adults. Administration of single oral doses of as high as 100 mg *p,p'*-DDT/kg to male F344 rats did not markedly impair their ability to acquire a conditioned behavioral response, although ≥50 mg/kg doses produced tremors, and deaths occurred in some rats in the 100 mg/kg group (Tilson et al. 1987). Two earlier reports provided inconsistent evidence of DDT effects on behavioral endpoints, but they examined different behavioral endpoints. Sobotka (1971) reported an impairment of habituation in open field activity in adult albino mice after administration of single oral doses of 25 mg DDT(NS)/kg, but not after 10 mg/kg. No statistically significant differences in tests of problem solving, locomotion speed, or reaction to stress were found between untreated rats and rats given oral doses up to 30 mg DDT (NS)/kg/day in food for 21 days (Khairy 1959). The doses in these adult animal studies were distinctly higher than the dose of technical DDT administered to neonates in the Eriksson (1990a, 1990b, 1992, 1993) studies discussed in Section 2.17.

Other neurological effects have been reported in animal studies after intermediate-duration oral exposure to DDT or DDE isomers, including body tremors and/or hunched appearance in female Osborne-Mendel rats after 26 weeks of dietary exposure to 30 mg technical DDT/kg/day (NCI 1978), female Wistar rats after 9 weeks of dietary exposure to 34 mg technical DDT/kg/day (Rossi et al. 1977), male B6C3F1 mice after 22 week of dietary exposure to 27 *p,p'*-DDE/kg/day (NCI 1978); parental and F1 parental females (but not males) exposed to 27.7 mg/kg/day *p,p'*-DDT for 10 weeks before mating, through gestation and lactation in a 2-generation study (Hojo et al. 2006); and male Osborne-Mendel rats after 8 weeks of dietary exposure to 59 mg of *p,p'*-DDE/kg/day (NCI 1978). Other observed effects include decreased brain levels of total lipids and the relative amount of cholesterol to phospholipid after oral exposure of Rhesus monkeys to 10 mg technical DDT/kg/day for 100 days (Sanyal et al. 1986) and staggering, weakness, and loss of equilibrium in monkeys treated for up to 14 weeks with a lethal dose of 50 mg *p,p'*-DDT/kg/day, but not with exposure to 5 mg/kg/day (Cranmer et al. 1972).

Effects reported in animals after chronic-duration oral exposure include severe tremors in F344/DuCrj rats at doses of 19.1 (males) and 25.2 (females) mg *p,p'*-DDT/kg/d in the diet after 70–104 weeks of exposure (Harada et al. 2003, 2006); severe tremors in some Rhesus and Cynomolgus monkeys exposed in the diet to doses ≥6.9 mg of *p,p'*-DDT/kg/day in a 130-month study (Takayama et al. 1999); and hyperactivity and tremors in chronically exposed mice at dietary doses ≥8.3 mg technical DDT/kg/day (Kashyap et al. 1977; Turusov et al. 1973). In contrast, no clinical signs of neurotoxicity were observed in hamsters fed diets at doses up to 95 mg technical DDT or *p,p'*-DDE/kg/day for life (Rossi et al. 1983). In the 78-week NCI (1978) chronic bioassays, by week 26, tremors or hunched appearance were observed in about 8% of female Osborne-Mendel rats exposed to 30 mg technical DDT/kg/day and in 90% of females exposed to 61 mg technical DDT/kg/day. Tremors were also reported in male rats exposed to technical DDT and *p,p'*-DDE, but due to changes in dosing early in the studies, accurate exposure levels are unclear; tremors ceased when doses were lowered. No tremors were observed in female rats at up to 36 mg *p,p'*-DDE/kg/day or in male or female rats exposed to up to 231 mg technical DDD/kg/day. In B6C3F1 mice, observations of tremors or hunched appearance in males and females were comparable to controls at dietary doses up to 30.2 mg technical DDT/kg/day and 142 mg technical DDD/kg/day. Male mice exposed to a time-weighted average dose of 47 mg *p,p'*-DDE/kg/day exhibited a hunched appearance in a cyclic fashion throughout the exposure period, but were comparable to controls during the last 12 weeks; no neurological effects were observed in female mice exposed to up to 49 mg *p,p'*-DDE/kg/day (NCI 1978).

Cameron and Burgess (1945) exposed rats, rabbits, and guinea pigs to acute, dermal doses ranging from 50 to 200 mg/kg DDT(NS) and reported tremors and nervousness.

Mechanisms of Neurological Effects of DDT, DDE, or DDD. There are several proposed mechanisms for the neurotoxic effects of DDT and its metabolites. DDT has been shown to disrupt nerve membrane ion fluxes through induced closure of sodium channels (Vijverberg et al. 1982), inhibition of potassium transport, and by targeting Na^+/K^+ and Ca^{2+}/Mg^{2+} ATPases (Janicki and Kinter 1971). There is also evidence that DDT can potentiate neurotransmitter release through interference with calcium calmodulin binding, which could then lead to central nervous system excitation and induction of tremors (Harada et al. 2016).

Evidence *in vitro* suggests DDT and its metabolites can also inhibit the plasma membrane dopamine transporter (DAT) and the vesicular monoamine transporter (VMAT2); these transporters are often disrupted in Parkinson's Disease patients (Hatcher et al. 2008). However, *in vivo*, mice exposed orally to 1, 3, or 6 mg DDT/kg every 3 days for 30 days demonstrated none of the expected nigrostriatal effects or evidence of neuronal dysfunction and DDT was not associated with Parkinson's Disease in a study in adults (Weisskopf et al. 2010). This suggests that the effects of DDT and its metabolites on the dopamine system *in vitro* may not translate into neurotoxicological outcomes in exposed individuals (Hatcher et al. 2008).

A study exploring potential mechanisms involved in DDT associations with Alzheimer's disease suggests that DDT may positively affect the amyloid-β (Aβ) synthesis pathway, and impair the clearance and degradation of Aβ peptides, potentially through impairment of the ATP-binding cassette transporter A1 (ABCA1) and insulin-degrading enzyme (IDE), both of which play roles in Aβ homeostasis (Li et al. 2015). Epigenetic changes, particularly alterations in methylation status of neuronal cells and tissues of the brain, are thought to contribute to various neuronal pathologies, including Alzheimer's disease (Shutoh et al. 2009). DNA from the hypothalamus of young rats dosed with 0.06 mg/kg DDT/day for 4 weeks was hypomethylated at CpG islands for six genes, including the estrogen-regulated neuropeptides *Gal, Sst,* and *Penk1*; mRNA levels of several genes including DNA methyltransferase, *Dnmt1*, were also significantly lower in exposed groups (Shutoh et al. 2009). Whether these changes could contribute to any pathologies is unknown. Further neuro-specific studies looking at epigenetic changes related to DDT exposure could further our understanding of possible relationships with adverse neurological effects.

2.16 REPRODUCTIVE

Evidence of Reproductive Effects in Humans. Tables 2-14, 2-15, and 2-16 summarize results from epidemiological studies that examined possible associations between exposure to DDT (isomers and metabolites), as assessed by levels of DDT in biological media (mostly blood), and reproductive outcomes. In the majority of the studies, levels of other persistent chemicals also were examined, including PCBs and other organochlorine pesticides. These tables only describe studies that included measurements of DDT metrics in biological fluid in each subject and examined possible associations with reproductive outcomes using correlation, logistic regression, or linear regression statistical techniques.

Not included in these tables are studies that (although they conducted analyses such as those mentioned previously) presented the results only textually, without providing quantitative information. Finally, some studies in which the studied population was heavily exposed to DDT (i.e., areas of endemic malaria) and consequently had extremely high DDT body burdens were not included in the tables. More detailed data regarding these studies are presented in Tables 8, 9, and 10 in the *Supporting Document for Epidemiological Studies for DDT, DDE, and DDD.*

Summary of human evidence. In epidemiological studies examining possible association between levels of DDT, DDE, or DDD in tissues or biological fluids (e.g., serum), inconsistent evidence across studies of adults was provided for associations with time to pregnancy (fecundity), menstrual cycle, uterine alterations, early menopause, levels of reproductive hormones in men or women, and semen parameters. However, consistent evidence was reported for associations for increased risk for abortions or preterm births in groups of women with serum levels above those currently found in samples from the U.S. general population (see text below and Table 2-14). Inconsistent evidence across studies was provided for associations with puberty onset in preadolescents and adolescents (see text below and Table 2-15). Consistent evidence for no association was reported for maternal levels of DDT, DDE, or DDD in serum, cord blood, breast milk, or placenta with risks for male birth defects (cryptorchidism [undescended testes] and hypospadias [condition in which the opening of the urethra is on the underside of the penis]) or adverse reproductive outcomes in adult offspring (see Table 2-16).

Table 2-14. Summary of Reproductive Outcomes in Adult Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Result[c]
Time to pregnancy (TTP)			
Studies providing some evidence for an association between blood DDT and TTP (n=3)			
Axmon et al. 2006 1,505 women 716 men Men and women from four different countries (A, B, C, and D) Participants' age ≥18 years	Median serum DDE (ng/g lipid) Men Women (A) 600 300 (B) 520 360 (C) 920 620 (D) 240 820 Exposure categories Low: <370 Medium: 370–750 High: >750	Fecundity, based on interview data	Adjusted fecundity OR (95% CI) for high versus low DDE: Women from (A) **0.68 (0.49–0.94)***↓ Significance lost after adjustment for maternal age at conception ORs for women from B-D were NS ORs for medium versus low DDE were NS
Buck-Louis et al. 2013 347 couples that achieved pregnancy (A) 154 couples that withdrew or did not achieve pregnancy (B) Mean age ~30 years old (men and women)	DDE geometric means, serum: men from (A): 0.77 ng/g men from (B): 0.82 ng/g DDE <LOD in women	Fecundity based on menstrual cycle data	Adjusted fecundity ORs (95% CI) for males DDE Males **0.83 (0.70–0.97)***↓ PCBs in men and women were associated with longer TTP
Chevrier et al. 2013 332 pregnant women (mean age 29 years)	DDE geometric mean in cord blood, 0.13 ng/mL T1: <0.13 T2: 0.13–0.249 T3: >0.250	Fecundity based on results from interview	Adjusted OR for T2–T3 versus T1 (95% CI), or per log_{10} change in DDE T2: NS T3: **0.60 (0.42–0.85)***↓ Log_{10}: **0.84 (0.71–0.99)***↓ p-trend: **0.003** Significance lost after further adjustment for shellfish consumption and mercury in hair

Table 2-14. Summary of Reproductive Outcomes in Adult Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Result[c]
Studies providing no evidence for a significant association between blood DDT and TTP (n=2)			
Harley et al. 2008 289 pregnant women (median age 25 years)	Geometric mean in serum (ng/g lipid) DDT: 24 *o,p'*-DDT: 2 DDE: 1,500	Fecundity based on menstrual cycle data	Adjusted fecundity ORs for log_{10} exposure levels DDT: NS DDE: NS *o,p'*-DDT: NS No significant association between ΣDDT and TTP
Law et al. 2005 390 pregnant women (median age 23 years)	Median DDE in serum: 22.6 ng/mL Quintiles Q1: 0–14 Q2:15–29 Q3: 30–44 Q4: 45–59 Q5: ≥60	Fecundity based on results from interview	Adjusted ORs for Q2–Q5 versus Q1 or per 16.6 ng/mL increase in DDE Q2–Q4: all NS Q5: NS p for trend: NS 16.6 ng/mL: NS Associations with lipid-adjusted levels were similarly NS

Table 2-14. Summary of Reproductive Outcomes in Adult Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Result[c]	
Semen parameters-sex organ function				
Studies providing evidence for a significant association between blood DDT and sperm parameters (n=3)				
Aneck-Hahn et al. 2007 311 adult males (23–40 years old)	Median levels in serum DDE: 697.0 ng/mL DDT: 249.0 ng/mL	Sperm parameters:	Adjusted betas for continuous exposures: DDE	 DDT
		Volume (β)	**-0.0003***↓	NS
		Total count (β)	**-0.003***↓	NS
		Beat cross frequency (β)	**0.0064***↑	**0.0138***↑
		Mean motility (β)	**-0.0175***↓	**-0.0494***↓
		Head displacement (β)	NS	**-0.0003***↓
		Tail defects (β)	NS	**-0.0007***↓
		Round cells (β)	**0.0005***↑	**0.0013***↑
		Cytoplasmic droplets	NS	**0.0013***↑
		Straight-line velocity (β)	NS	**-0.0018***↓
		Oligozoospermia (OR)	**1.001***↑	**–**
		Asthenozoospermia (OR)	–	**1.003***↑
			Other motility and morphological parameters, viability, and CASA were NS	

Table 2-14. Summary of Reproductive Outcomes in Adult Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Result[c]
De Jager et al. 2006 116 adult males (mean age 27 years old)	Arithmetic mean plasma DDE 45,000 ng/g lipid	Sperm parameters: Volume Count Concentration Velocity Mean motility Tail abnormalities Progressive motility Chromatin integrity Epididymal function Normal morphology Motility	Crude betas (95% CI) NS NS NS NS **-0.1 (-0.19 to -0.10)***↓ **0.0373 (0.007–0.067)***↑ **-0.09 (-0.18 to -0.007)***↓ NS NS Similar results using adjusted models (data not reported) Men with poor sperm characteristics: Pearson coefficient (r) = -0.408 Pearson coefficient (r) = -0.387
Messaros et al. 2009 336 adult males (18–60 years old)	Median levels in serum: DDT: 4.72 ng/g lipid DDE: 290.4 ng/g lipid ΣDDT=DDT+DDE	Sperm parameters: Motility Abnormal morphology Concentration	Adjusted OR (95% CI) for high ΣDDT (≥75th percentile) versus <75th percentile **2.91 (1.27–6.66)***↑ **3.23 (1.51–6.95)***↑ **2.53 (1.0–6.95)***↑ 75th percentile not specified
Studies providing mainly no evidence for a significant association between blood DDT and sperm parameters (n=5)			
Charlier and Foidart 2005 Cases: 83 subfertile/unfertile men (mean age 26 years) Controls: 75 fertile men (mean age 25 years)	Arithmetic mean DDE in blood Cases: 1,050 ng/g lipid Controls: 980 ng/g lipid	Sperm parameters: Concentration Motility Abnormal morphology	Adjusted OR (95% CI) combined groups: NS NS NS Similar results for separate analyses

Table 2-14. Summary of Reproductive Outcomes in Adult Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Result[c]
Dallinga et al. 2002 Cases: 34 men with poor sperm quality (mean age 35 years) Controls: 31 men with normal sperm (mean age 37 years)	Arithmetic mean (±SD) of DDE in serum (ng/g blood) Cases: 0.31 (0.42) Controls: 0.22 (0.22)	Sperm parameters: Sperm count Progressive motility Overall motility Sperm morphology	No significant associations between DDE exposure levels and sperm parameters were found (data not shown) Testing for potential confounders (smoking and polymorphisms) were not significant, and were therefore not included in the model
Hauser et al. 2003 212 adult males (mean age 36 years)	Geometric mean DDE in serum: 275.3 ng/g lipid DDE 3rd tertile range in serum: 302.5–8,991.8 ng/g lipid	Sperm parameters: Concentration Motility Morphology	Adjusted OR (95% CI) for T3 versus T1 NS NS NS
Rygnell-Hydbom et al. 2005a 157 adult men (mean age 47 years)	Median serum DDE, 231 ng/g lipid	Markers of secondary sex organ function: PSA Neutral α-glucosidase Fructose Zinc	Adjusted betas change in outcome with increasing DDE NS NS NS NS
Toft et al. 2006 763 men from 4 different countries (A, B, C, and D) (mean ages 28–47 years)	Arithmetic mean serum DDE (ng/g lipid) (A) 890 (B) 240 (C) 1,270 (D) 580 Quintiles: Q1 0–250 Q2 251–500 Q3 501–1,000 Q4 1,001–1,500 Q5 >1,500	Sperm parameters: Concentration Motility Motility Morphology	Adjusted betas (95% CI) for log DDE NS in separate or combined analysis **-3.2 (- 5.9 to -0.6)*↓ for group A** **-2.8 (-4.8 to -0.7)*↓ groups combined** NS in separate or combined analysis

Table 2-14. Summary of Reproductive Outcomes in Adult Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]			Outcome evaluated	Result[c]			
Studies of associations between sperm parameters and DDT in semen (n=1)								
Pant et al. 2007 50 infertile men (cases) 50 fertile men (controls)	Arithmetic mean in semen (ng/mL)				Pearson's correlation coefficient (r)			
		Cases	Controls	Sperm concentration	DDE **-0.39***↓	p-value <0.005	DDD **-0.38***↓	p-value <0.01
	DDT	2.17	3.07	Sperm motility	NS		NS	
	DDE	20.29	7.24					
	DDD	20.74	13.14		Similar low correlations reported for hexachlorocyclohexane			
	o,p'-DDT	2.92	0.12					
					No statistically significant confounders were identified, and therefore were not included in the analysis			
Menstrual cycle								
Studies finding statistically significant associations between DDT biometrics and menstrual cycle (n=3)								
Ouyang et al. 2005 466 adult women	Arithmetic quartile means for total DDT in serum Q1: 13.5 ng/g serum Q2: 23.5 ng/g Q3: 34.0 ng/g Q4: 57.1 ng/g Mean total DDT=DDT, DDE, DDD: 32 ng/g serum			Age at menarche (self report)	Adjusted beta for Q2–Q4 versus Q1 Q2 NS Linear (per 10 ng/g) = **-0.20***↓	Q3 NS	Q4 **-1.11***↓	p-trend **0.001**
				Short cycle	Adjusted ORs for Q2–Q4 versus Q1 NS	NS	**2.78***↑	**0.016**
				Long cycle	NS	NS	NS	NS
					Linear (per 10 ng/g) for both outcomes NS			

Table 2-14. Summary of Reproductive Outcomes in Adult Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Result[c]
Toft et al. 2008 1,494 adult women from four different countries (A, B, C, and D)	Tertile ranges for DDE in serum (ng/g lipid) T1: <370 T2: 370–750 T3: >750 Means ranged from 430 in C to 2,147 in D	Menstrual cycle length Long cycle	Adjusted beta in (C) for analysis restricted to minor cycle deviations **0.1 (95% CI 0.03–0.2**)*↓ Adjusted OR in (A) for analysis of DDE as continuous variable **0.7 (95% CI 0.5–0.99)***↓ Adjusted OR in (C) for T3 versus T1 analysis **3.1 (95% CI 1.1–8.6)***↑
Windham et al. 2005 49 adult women	Arithmetic mean serum concentration DDT: 1.77 ng/mL DDE: 20.8 ng/mL Quartiles: Q1: <0.5 Q2: 0.5–0.69 Q3: 0.70–1.39 Q4: >1.4	 Menstrual cycle length Follicular phase Luteal phase	Adjusted betas for change for Q4 versus Q1 Exposure levels NS (both DDE and DDT) NS (both DDE and DDT) **-1.5 (95% CI -2.7 to -0.30)***↓ for DDT **-1.4 (95% CI -2.6 to -0.20)***↓for DDE
Studies finding no statistically significant association between DDT biometrics and menstrual cycle (n=3)			
Cooper et al. 2005 2,314 adult women (Collaborative Perinatal Project)	Quintile ranges for DDE in serum Q1: <15 ng/g Q2: 15–29 ng/g Q3: 30–44 ng/g Q4: 45–59 ng/g Q5: >60 ng/g Arithmetic mean: 30 ng/mL	Self-report of: Bleeding duration Cycle irregularity Heavy bleeding Dysmenorrhea Cycle length	Adjusted OR (95% CI) for Q5 versus Q1 NS NS NS NS NS PCBs were associated with increasing menstrual cycle length

Table 2-14. Summary of Reproductive Outcomes in Adult Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Result[c]
Denham et al. 2005 138 young women (10–16.9 years old)	Geometric mean in blood DDE: 0.35 ng/mL	Self report of presence or absence of menarche	Adjusted beta per doubling of DDE NS Associations existed for some PCBs and lead
Gallo et al. 2016 140 adult women	Geometric mean in serum DDE: 0.30 ng/mL	Ovulatory status based on levels of estradiol and progesterone in saliva	Adjusted beta for ovulatory status per 1 log-unit increase in DDE = NS Association existed for low chlorinated PCBs
Abortion-preterm birth			
Studies finding significant associations between DDT biometric and abortion-preterm birth (n=3)			
Korrick et al. 2001 15 cases of spontaneous abortions 15 normal term pregnancy	Arithmetic mean DDE in serum Cases: 22 ng/g serum Controls: 12 ng/g serum	Clinically recognized spontaneous abortion	Adjusted ORs (95% CI) for increased risk of spontaneous abortion per ng/g increase in DDE and total DDT or per ng/100 g increase in other metrics DDE: **1.13 (1.02–1.2)***↑ *o,p'* -DDE: **1.56 (1.08–2.25)***↑ Total DDT: **1.13 (1.02–1.25)***↑ Other DDT metrics were NS
Longnecker et al. 2005 1,717 women (Collaborative Perinatal Project)	Quintile ranges for DDE in serum Q1: <15 ng/g Q2: 15–29 ng/g Q3: 30–44 ng/g Q4: 45–59 ng/g Q5: >60 ng/g Median: 24.5 ng/mL	Previous pregnancy outcome data assessed by interview	Adjusted OR (95% CI) for fetal loss for Q2–Q5 versus Q1 or per 60 ng/mL increase in DDE Q2: NS Q3: 1**.4 (1.0–1.9)***↑ Q4: **1.6 (1.1–2.4)***↑ Q5: NS 60 ng/mL: **1.4 (1.1–1.6)***↑

Table 2-14. Summary of Reproductive Outcomes in Adult Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Result[c]
Venners et al. 2005 388 newly married Chinese women (20–34 years old)	Tertiles for ΣDDT in serum T1: 5.5–22.9 ng/g T2: 23.0–36.5 ng/g T3: 36.6–113.3 ng/g Median: 27.9 ng/g	Conception monitored by daily urinary assay of human chorionic gonadotropin; pregnancies were followed to monitor losses	Adjusted OR (95% CI) for early pregnancy loss T3 versus T1 ΣDDT **2.12 (1.26–3.57)*↑** Adjusted OR (95% CI) for total pregnancy loss T3 versus T1 ΣDDT **2.01 (1.23–3.28)*↑**
Studies finding no significant associations between DDT biometric and abortion-preterm birth (n=4)			
Khanjani and Sim 2006 815 women assessed 6–12 weeks postpartum	Tertiles in breast milk DDT T1: 0–39 ng/g fat T2: 39–66 ng/g fat T3: >66 ng/g fat DDE T1: 0–400 ng/g fat T2: >400–730 ng/g fat T3: >730 ng/g fat	Reproductive outcome data collected as part of a previous breast milk study	Adjusted OR (95% CI) for preterm birth T3 versus T1 DDT: NS DDE: NS Adjusted OR (95% CI) for miscarriage or stillbirth T3 versus T1 DDT: NS DDE: NS
Ouyang et al. 2014 291 newly married Chinese women (mean age 24.9 years)	Mean ΣDDT in serum: 34.4 ng/g serum Women were categorized into low-DDT and high-DDT using a median split of 30.7 ng/g serum	Conception monitored by daily urinary assay of human chorionic gonadotropin; pregnancy and pregnancy loss confirmed clinically	Adjusted OR (95% CI) for early pregnancy loss high-exposure group versus low-exposure group 1.52 (0.96–2.41) = NS
Torres-Arreola et al. 2003 Cases: 100 preterm delivery Controls: 133 women full-term delivery	Median DDE in serum collected ≤24 hours after delivery: Cases: 189.5 ng/g lipid Controls: 152.8 ng/g lipid	Preterm delivery assessed clinically (<37 weeks of gestation)	Adjusted OR (95% CI) for increased risk of preterm delivery for T3 (>228.8 ng/g lipid) versus T1 (<111.6 ng/g lipid) 1.67 (0.84–3.31) = NS

Table 2-14. Summary of Reproductive Outcomes in Adult Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Result[c]
Wood et al. 2007 Cases: 26 women delivering preterm infants (mean age 29.2 years) Controls: 52 women delivering full term infants (mean age 29.7 years)	Median DDE in serum collected 1 day postpartum Cases: 67.02 ng/g lipid Controls: 69.29 ng/g lipid	Premature delivery (>23 weeks and <35 weeks) assessed clinically	Adjusted OR (95% CI) for premature delivery per 1-log unit increase in DDE 1.15 (0.47–2.78) = NS
Uterine and ovarian alterations			
Studies finding significant associations between DDT biometrics and female sex organ alterations (n=1)			
Trabert et al. 2015 Cases: 99 women with uterine fibroids Controls: 374 women without uterine fibroids Cohort age range:18–44 years	Geometric mean in serum (ng/g serum) Cases / Controls DDE 36.95 / 16.90 DDT 1.20 / 1.22 *o,p'*-DDE 0.61 / 0.69	Fibroids surgically diagnosed	Adjusted ORs (95% CI) for increased risk of uterine fibroids per 1 SD increase in DDT metrics DDE: **1.37 (1.05–1.80)***↑ DDT: NS *o,p'*-DDE: NS
Studies finding no significant associations between DDT biometrics and female sex organ alterations (n=3)			
Cooney et al. 2010 Cases: 29 women with endometriosis Controls: 51 women without endometriosis Cohort age range: 18–40 years	DDE tertiles in serum T1: <0.63 ng/g serum T2: 0.63–0.94 ng/g serum T3: >0.94 ng/g serum	Endometriosis diagnosed by laparoscopy	Adjusted ORs (95% CI) for increased risk of endometriosis T2 versus T1: NS T3 versus T1: NS 1 log-unit increase DDE: NS
Porpora et al. 2009 Cases: 80 women with endometriosis (mean age 31.6 years) Controls: 78 women without endometriosis (mean age 29.5 years)	DDE tertiles in serum T1: ≤231 ng/g lipid T2: 232–492 ng/g lipid T3: ≥493 ng/g lipid	Endometriosis diagnosed by laparoscopy	Adjusted ORs (95% CI) for increased risk of endometriosis T2 versus T1: NS T3 versus T1: NS

Table 2-14. Summary of Reproductive Outcomes in Adult Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Result[c]
Upson et al. 2013 Cases: 248 women with endometriosis Controls: 538 women without endometriosis Cohort age range: 18–49 years old	Quartiles in serum DDT Q1: ≤0.19 ng/g serum Q2: >0.19–0.28 ng/g serum Q3: >0.28–0.44 ng/g serum Q4: >0.44 ng/g serum DDE Q1: ≤0.91 ng/g serum Q2: >0.91–1.6 ng/g serum Q3: >1.6–2.8 ng/g serum Q4: >2.8 ng/g serum	Endometriosis confirmed by direct surgical visualization	Adjusted ORs (95% CI) for increased risk of endometriosis for Q2–Q4 versus Q1 DDE DDT Ovarian endometriosis NS NS All endometriosis NS NS Mirex and β-hexachlorocyclohexane were associated with endometriosis
Menopause (n=2)			
Cooper et al. 2002 Cases: 748 women with breast cancer (21–24 years old) Controls: 659 women without breast cancer	DDE deciles in serum (ng/g lipid) <50th: <620 50th–74th: 620–1,360 75th–89th: 1,370–2,760 ≥90th: ≥2,770 Median: 600 ng/g lipid	Age at time of the last menstrual period was used to define age at menopause among women who reported that their periods had stopped by themselves	Adjusted HR for rate of onset of menopause (all women) DDE 50th–75th versus <50th: NS 75th–89th versus <50th: NS ≥90th versus <50th: NS 1-log unit change: **1.1***↑
Grindler et al. 2015 Subset of 1,442 women from 31,575 participants in NHANES 1999–2008	Median DDE in serum: 243 ng/g lipid 90th percentile = 1,430 ng/g lipid	Age at last menstrual period determined by survey	Adjusted beta for average change in age of menopause - per 1-log DDE **-0.67***↓ **(*p*=0.046).** - per serum DDE subdivided into deciles and analyzed as a continuous variable: **-0.34***↓ **(*p*=0.043).** - per DDE >90th versus <90th percentile = NS Other organochlorine chemicals also associated with early menopause

Table 2-14. Summary of Reproductive Outcomes in Adult Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Result[c]
Sex hormones			
Studies reporting mixed results for associations between DDT biometrics and sex hormones (n=8)			
Blanco-Muñoz et al. 2012 84 men (18–52 years old)	Geometric mean DDE in serum: 864 ng/g lipid	FSH, LH, prolactin, testosterone, E2, and inhibin B in serum	Adjusted betas per doubling of DDE Prolactin: **-0.040***↓ Inhibin: **0.114***↑ No significant associations between DDE and other outcomes measured
Emeville et al. 2013 277 adult men (45–69 years old)	Geometric mean concentration of DDE in serum: 1.77 ng/mL	Dehydroepiandrosterone (and sulfate), androstenedione, DHT, androstenediol, estrone (and sulfate), E2, testosterone (total, free, and bioavailable), SHBG, LH, and FSH in serum	Adjusted betas per 1-ln-unit increase in DDE DHT: **-0.063***↓ LH: **0.057***↑ Testosterone/LH: **-0.061***↓ No significant associations between DDE and other outcomes measured
Freire et al. 2014 304 men (15–94 years old) 300 women (17–92 years old)	Median concentration in serum (ng/mL) DDE DDT DDD *o,p'*-DDT Men 8.3 3.1 0.6 0.3 Premenopausal women 7.9 3.0 0.6 0.4 Peri/postmenopausal women 20.6 4.7 0.9 0.4	Men: testosterone in serum Women: estradiol, progesterone, prolactin, LH, and FSH in serum	Adjusted betas for log-transformed sex hormones and DDT metrics Peri/postmenopausal Men Women T LH FSH *o,p'*-DDT **-0.02***↓ NS NS DDT NS **-0.01***↓ NS DDE NS NS NS DDD NS **-0.09***↓ **-0.15***↓ No significant associations with other hormones Premenopausal women: no significant associations between DDT isomers and metabolites and outcomes measured

Table 2-14. Summary of Reproductive Outcomes in Adult Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Result[c]
Giwercman et al. 2006 258 men from Greenland (18–50 years old) 198 men from Ukraine (19–45 years old) 113 men from Poland (20–46 years old) 184 men from Sweden (24–68 years old)	Mean serum concentrations of DDE (ng/g lipid) Greenland: 480 Ukraine: 1,100 Poland: 530 Sweden: 250	Free testosterone (fT), SHBG, E2, LH, inhibin B, and FSH in serum	Adjusted betas for 1-log unit increase in DDE Ukraine / Greenland / All fT: **0.008*↑** / **0.011*↑** / NS SHBG: **3.57*↑** / NS / NS Ln LH: **0.227*↑** / NS / ND Ln inhibin B: **-17.0*↓** / NS / NS LN FSH: NS / NS / **0.056*↑** No significant associations between DDE and E2; no significant associations in Sweden or Poland cohorts
Hagmar et al. 2001 110 men (23–79 years old)	Plasma levels (ng/g lipid) DDE / DDT 50th %: 828 / 50 90th %: 3,152 / 185	FSH, LH, prolactin, fT in serum	Adjusted Pearson correlation coefficient: **0.41 (p<0.001)*↑** for DDT/FSH **0.41 (p<0.001)*↑** for DDE/FSH **0.35 (p=0.003)*↑** for DDE/fT No significant correlation between DDT/DDE and LH or prolactin
Perry et al. 2006 287 women (24–25 years old)	Mean serum concentration (ng/g serum) ΣDDT (isomers and metabolites) Low exposure group: 19.6 High exposure group: 46.4	Urinary concentration of major metabolite of progesterone (PdG) and estrogen (E_1C)	Adjusted betas per 10 ng/g increase in DDT metrics (two-sided p): log E_1C: POD / LD; logPdG: POD / LD Total DDT: **-0.04*** / **-0.04*** / **-0.06↓** / **-0.05*** *p,p'*-DDT: **-0.43*** / NS / NS / **-0.57*** *p,p'*-DDE: **-0.05*** / **-0.05*** / **-0.06*↓** / **-0.06*** *p,p'*-DDD: **-5.33*** / NS / **-10.71*** / **-6.53*** *o,p'*-DDT: **-4.99↓** / NS / NS / NS *o,p'*-DDE: **-8.84*** / **-8.15*** / ND / **-13.42*** (* indicates statistical significance, inverse associations)

Table 2-14. Summary of Reproductive Outcomes in Adult Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Result[c]
Rylander et al. 2006 196 men (48–82 years old)	Median DDE in serum: 580 ng/g lipid 1st Q: 300 2nd Q: >300–600 3rd Q: >600–1,100 4th Q: >1,100	FSH, LH, E2, total testosterone, and SHBG in serum and FAI	Adjusted β (95% CI) for change in E2 per 100 ng/g increase in DDE or for Q2–Q4 versus Q1 100 ng/g: **-0.57 (-1.0 to -0.12)***↓ Q2: NS Q3: **-10 (-19 to -1.2)***↓ Q4: **-12 (-21 to -2.8)***↓ No significant associations with other outcomes measured
Windham et al. 2005 49 women (18–40 years old)	Arithmetic mean concentration in serum DDT: 1.8 ng/mL DDE: 21 ng/mL DDE quartiles Q2: 7–12.9 ng/mL Q3: 13–23.9 ng/mL Q4: ≥24 ng/mL	Main urinary metabolites of E2 and progesterone monitored during follicular and luteal phases of the menstrual cycle	Adjusted beta (95% CI) for change luteal phase length (days) and in progesterone during total luteal phase for Q2–Q4 DDE versus Q1 Luteal length / Progesterone Q2: NS / NS Q3: **-1.1***↓ / **-18.5***↓ Q4: **-1.4***↓ / **-25.5***↓ No significant association between DDT and E2 or progesterone
Studies reporting inverse associations between DDT biometrics and sex hormones (n=8)			
Ferguson et al. 2012 341 men (18–51 years old)	Geometric mean DDE concentration in serum: 236 ng/g lipid	FSH, LH, inhibin B, total testosterone, fT, E2, SHBG, FAI, T/E2, and T/LH in serum	No significant association between DDE and any of the outcomes measured in adjusted models; data for FSH, LH, or inhibin B not shown
Goncharov et al. 2009 257 men (18–82 years old) 436 women (18–95 years old)	Arithmetic mean DDE concentration in serum: 2.9 ng/g serum Tertile interval was not provided	Total testosterone in serum	Adjusted OR (95% CI) for comparison of highest tertile with lowest tertile: 1.18 (0.37–3.69) = NS PCBs were associated with lower testosterone

Table 2-14. Summary of Reproductive Outcomes in Adult Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Result[c]
Martin et al. 2002 137 men (30–88 years old)	Median DDE concentration (interquartile range) in serum: 1,213 (558–2,136) ng/g lipid	tT, bT, DHT, and FAI in serum	Adjusted betas for percentage change in ln-hormone levels per increase in interquartile distance of ln-DDE tT: NS bT: NS DHT: NS FAI: NS
Rignell-Hydbom et al. 2004 194 fishermen	Median (range) serum DDE: 240 (33.4–2,251) ng/g lipid	FSH, LH, E2, serum testosterone, inhibin B, and SHBG	No significant associations between hormones and DDE exposure levels were observed using adjusted linear and logistic regression analysis (data was not provided)
Rignell-Hydbom et al. 2005a 157 fishermen	Median (range) serum DDE: 231 (40–2,252) ng/g lipid	PSA, NAG, zinc, and fructose levels in semen	Adjusted betas for change in PSA with Q1–Q4 DDE versus Q5 (referent) Q1: NS Q2: NS Q3: NS Q4: NS Associations with other outcomes were NS using univariate analysis
Schell et al. 2014 127 young men (10–<17 years old)	Arithmetic mean DDE in serum: 0.45 ng/g serum	tT in serum	Adjusted β (95% CI) for change in tT per 10% increase in DDE = NS Other organochlorines compounds were associated with lower testosterone

Table 2-14. Summary of Reproductive Outcomes in Adult Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Result[c]
Turyk et al. 2006 56 men (27–70 years old)	Geometric mean DDE concentration in serum Fish eaters: 602 ng/g lipid Referents: 290 ng/g lipid	tT, fT, SHBG, SHBG-T, LH, FSH, and estrone sulfate in serum	Pearson's correlation coefficients (r) (p values) in adjusted models that included total PCBs: tT: -0.15 (0.30) = NS Ln fT: -0.04 (0.78) = NS SHBG-T: -0.17 (0.23) = NS Estrone sulfate: -0.32 (>0.05) = NS SHBG: -0.11 (0.32) = NS Ln LH: -0.14 (0.32) = NS Ln FSH: 0.14 (0.32) = NS

[a]See Table 8 in the *Supporting Document for Epidemiological Studies for DDT, DDE, and DDD* for more detailed descriptions of studies.
[b]DDT, DDE, and DDD refers to the *p,p'*- substituted compounds, other substitutions are explicitly noted throughout the table.
[c]**Bold text,** "significant" and asterisk indicate statistically significant (p<0.05). Arrows "↑ or ↓" indicate direction of change observed for a given outcome (positive or inverse associations, respectively). Adjusted OR or adjusted β indicates adjustment for standard confounding variables for reproductive outcomes, such as age, BMI, serum triglycerides and cholesterol, and smoking. Further adjustments for other analyzed chlorinated compounds or pesticides in biological fluid samples are expressly noted in the table.

BMI = body mass index; bT = bioavailable testosterone; CASA = Computer Assisted Sperm Analysis; CI = confidence interval; DHT = dihydrotestosterone E2 = estradiol; FAI = free androgen index; FSH = follicle-stimulating hormone; fT = free testosterone; HR = hazard ratio; LD = luteal day; LH = luteinizing hormone; LOD = limit of detection; NAG = neutral α-glucosidase; NHANES = National Health and Nutrition Examination Survey; NS = not statistically significant; OR = odds ratio; PCB = polychlorinated biphenyl; POD = post-ovulation day; PSA = prostate specific antigen; Q = quartile or quintile; SD = standard deviation; SHBG = sex hormone-binding globulin; SHBG-T = SHBG-bound testosterone; T = tertile; tT = Total testosterone; TTP = time to pregnancy

Table 2-15. Summary of Studies of Associations between Human DDT Exposure Metrics in Child and Adolescent Serum and Reproductive Endpoints[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Croes et al. 2015 Adolescents, (aged 14–15 years) (n=1,889) FLEHS I cohort (n=1,679) FLEHS II cohort (n=210)	Adolescent serum DDE Geometric mean FLEHS I / FLEHS II (ng/g lipid) 94 / 70 (ng/mL) 0.418 / 0.307	Breast development (girls), genital development (boys), pubic hair (boys)	Adjusted ORs (per IQR (53.3 ng/g lipid) increase in DDE FLEHSII — OR — p-value Breast development **0.74*↓** **0.03** (delayed breast development) FLEHSI Pubic hair (ORs NR) **0.002*↑** Genital development (ORs NR) **0.001*↑** (faster sexual maturation)
Den Hond et al. 2011 Consenting adolescents, 14–15 years old (n=1,679) 887 males; 792 females	Adolescent serum DDE Median (ng/g lipid) Boys: 104 (47–404) Girls: 84 (39–247)	Tanner genitalia growth (G1–G5), and Tanner pubic hair growth (P1–P5)	Adjusted ORs (95% CI) per 2-fold increase in DDE Boys — Girls Reaching G3 **1.52*↑** – Reaching P3 **1.50*↑** NS Breast development – NS (DDE associated with faster sexual maturation in boys) Association with G3 remained significant with adjustments for other pollutants (p=0.035)
Dhooge et al. 2011 887 male adolescents (14–15 years old)	Median concentration of DDE in serum: 104 ng/g lipid	LH, total and free testosterone, FSH, SHBG, total and free E2, and aromatase index (total testosterone/E2) in serum	Adjusted beta for ln E2 per doubling of DDE **3.3 (95% CI 1.6–5.0)*** No significant associations between DDE and other outcomes assessed (data not shown)

Table 2-15. Summary of Studies of Associations between Human DDT Exposure Metrics in Child and Adolescent Serum and Reproductive Endpoints[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Lam et al. 2014 Boys, aged 8–9 years old with parental consent	Prepubertal serum concentrations of DDE. Wet-weight quartiles (ng/g serum) Q1: 0.261–0.907 Q2: 0.908–1.406 Q3: 1.407–2.237 Q4: 2.238–41.301 Median: 287 ng/g lipid; 1.41 ng/g serum	Tanner genitalia growth (G1–G5), and Tanner pubic hair growth (P1–P5), testicular volume (TV)	Adjusted mean shifts (months) for Q2–Q4 versus Q1 DDE Q2 Q3 Q4 G2+ NS NS NS TV >3 mL NS NS NS P2+ NS NS NS p for trend NS for each outcome
Lam et al. 2015 Boys, aged 8–9 years old with parental consent	Prepubertal serum concentrations of DDE. Wet-weight quartiles (ng/g serum) Q1: 0.261–0.907 Q2: 0.908–1.406 Q3: 1.407–2.237 Q4: 2.238–41.301 Median: 287 ng/g lipid; 1.41 ng/g serum	Age at sexual maturity (G5, TV >20 mL, and P5)	Adjusted mean shifts (months) for Q2– Q4 versus Q1 DDE. Q2 Q3 Q4 p trend G5 -1.68 -1.34 2.52 NS TV >20 mL -0.32 -0.09 2.45 NS P5 -0.30 1.67 **6.19*↑** **0.04*↑** DDE was associated with later attainment of Tanner stage 5 for pubic hair growth

[a]See Table 9 in the *Supporting Document for Epidemiological Studies for DDT, DDE, and DDD* for more detailed descriptions of studies. Studies in this table were selected from those described in Table 9, because they: (1) measured DDT-related metrics in biological fluids or tissues in each subject, and (2) evaluated associations using linear regression, logistical regression, or correlation techniques, adjusting for possible confounding factors.
[b]DDE = *p,p'*-DDE, unless otherwise specified; DDT = *p,p'*-DDT, unless otherwise specified.
[c]**Bold text,** "significant" and asterisk indicate statistically significant (p<0.05). Arrows indicate an increase or decrease in the reproductive endpoint or direction of p-trend. Adjusted OR or adjusted β indicates adjustment for standard confounding variables for reproductive outcomes, such as total serum lipids, birth weight, blood lead levels, age of pubertal onset, smoking, BMI, and height z-scores.

BMI = body mass index; CI = confidence interval; E2 = estradiol; FLEHS = Flemish Environment and Health Studies; FSH = follicle stimulating hormone; G1–5 = Tanner genitalia growth, stages 1–5; LH = luteinizing hormone; NS = not statistically significant; OR = odds ratio; P1–5 = Tanner pubic hair growth, stages 1–5; Q = quartile; SHBG = sex-hormone binding globulin; TV = testicular volume

Table 2-16. Summary of Studies of Associations between Human DDT Exposure Metrics in Maternal Serum, Cord Blood, or Breast Milk and Reproductive Endpoints in Offspring Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Bhatia et al. 2005 Consenting mother-child pairs Cryptorchidism cases (n=75) Hypospadias cases (n=66) Both cryptorchidism and hypospadias (n=4) Normal controls (n=283) Infants followed at least 2 years from birth	Maternal serum DDE and DDT Exposure quartiles (ng/mL) DDE / DDT Q1 <27.0 / <10.0 Q2 27.0–43.9 / 10.0–14.9 Q3 44.0–60.9 / 15.0–19.9 Q4 ≥61.0 / ≥19.9 Median range: 4,600–5,300 ng/g lipid	Cryptorchidism and hypospadias	Adjusted ORs for Q4 DDE and DDT versus Q1 DDE / DDT All: NS / NS Cryptorchidism: NS / NS Hypospadias: NS / NS Associations for Q2 and Q3 were also NS p-values for trend across exposure levels were NS
Brucker-Davis et al. 2008 Consenting mother-infant pairs (n=164) Infants with cryptorchidism (n=78) Control infants (n=86)	Cord blood and milk DDE Median (ng/g lipid-milk; ng/mL cord blood) Cord blood / Milk Controls 0.2 / 80 Cryptorchidism 0.2 / 119.4 Total 0.2 / 98.7	Cryptorchidism status at birth and 3 months	Adjusted ORs for DDE ≥median versus <LOD Birth: NS 3 months: NS
Carmichael et al. 2010 Mother-infant pairs (n=48) Hypospadias cases (n=20) Non-malformed controls (n=28)	Maternal serum DDT and DDE Mean (ng/g lipid) DDT / DDE Cases 20.5 / 344.5 Controls 34.4 / 624.3	Hypospadias	Adjusted ORs for a 1 ng/g lipid change in DDT or a 10 ng/g lipid change in DDE DDT: NS DDE: NS
Cohn et al. 2003 Consenting mother-infant pairs (n=289) Daughters born 1960–1963	Maternal serum levels of *p,p'*-DDT, *p,p'*-DDE, and *o,p'*-DDT in each subject Median (ng/mL): *o,p'*-DDT: 0.49 *p,p'*-DDT: 13.05 *p,p'*-DDE: 48.19	Time to pregnancy in daughters	Adjusted FRs per 10 ng/mL or SD DDE and DDT (data are presented in figures): DDE / DDT Per 10 ng/mL: >**1.0*** / **<1.0***

Table 2-16. Summary of Studies of Associations between Human DDT Exposure Metrics in Maternal Serum, Cord Blood, or Breast Milk and Reproductive Endpoints in Offspring Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Damgaard et al. 2006 Consenting mother-child pairs (n=130) Cryptorchidism boy cases (n=29 Danish; n=33 Finnish) Control cases (n=36 Danish; n=32 Finnish)	Maternal serum DDT metrics Mean (ng/g lipid) Cases / Controls *p,p'*-DDT 4.63 / 3.98 *p,p'*-DDE 97.32 / 83.76 *p,p'*-DDD 0.36 / 0.34 *o,p'*-DDT 0.35 / 0.34 *o,p'*-DDE 0.08 / 0.08 *o,p'*-DDD 0.03 / 0.03 DDE/DDT 17.88 / 19.31 ΣDDT 140.41 / 116.6	Cryptorchidism	Adjusted *p*-values from logistic regression analysis (cases versus controls; adjusted ORs were not reported) *p,p'*-DDT: NS *p,p'*-DDE: NS *p,p'*-DDD: NS *o,p'*-DDT: NS *o,p'*-DDE: NS *o,p'*-DDD: NS DDE/DDT: NS ΣDDT: NS
Fernandez et al. 2007 Cases (n=48); boys with hypospadias and/or cryptorchidism at 1 month of age Healthy control boys (n=114)	Placental levels of DDT metrics Mean±SD (ng/g lipid) *o,p'*-DDD: 28.7±43.7 *p,p'*-DDE: 10.8±28.0 *o,p'*-DDT: 3.6±8.3 *p,p'*-DDT: 2.0±5.2 ΣDDT: 28.7±47.3	Cryptorchidism and/or hypospadias	Adjusted ORs for exposure levels ≥LOD versus <LOD *o,p'*-DDT: NS *p,p'*-DDT: NS
Giordano et al. 2010 Consenting mother-child pairs (n=160) Hypospadias cases (n=80) Healthy controls (n=80)	Maternal serum DDE Mean±SD (ng/g lipid) Cases (n=37): 128±0.71 Controls (n=21): 0.96±0.56 All subjects: 1.16±0.67	Hypospadias	Adjusted ORs for DDE ≥Median versus <median: NS Per 10 pg/g increase: NS
Han et al. 2016 Two-generation cohort of maternal fish-eaters Angler mother-daughter (aged 20–50 years) pairs (n=151) Daughter pregnancies (n=288)	Estimated birth serum DDE Exposure tertiles (ng/mL) T1: 0–2.4 T2: 2.5–7.4 T3: ≥7.4	Time to pregnancy in daughters (stratified by: all times of unprotected intercourse (UI), time of unprotected intercourse (TUI) ≥1 month, and women who planned a baby)	Adjusted FRs for T2 and T3 DDE versus T1 T2 / T3 All times of UI 1.61 / 1.67 TUI ≥1 month 1.36 / 1.35 Planned baby 1.62 / 1.72

Table 2-16. Summary of Studies of Associations between Human DDT Exposure Metrics in Maternal Serum, Cord Blood, or Breast Milk and Reproductive Endpoints in Offspring Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Kristensen et al. 2016 Consenting mother daughter-pairs (n=244) Follow-up of female offspring at ~20 years of age) Users of hormonal contraceptives cases (n=170); non-users of hormonal contraceptives cases (n=74)	Maternal serum DDE Median (ng/mL) = 2.466 Tertiles (nmol/mL) T1: 0.00024–0.00154 T2: 0.00154–0.00216 T3: 0.00216–0.01097	Cycle length, follicle number/ovary, and reproductive hormones: TT, SHBG, FAI, FSH, LH, E2, and AMH	Adjusted betas for T2 and T3 DDE versus T1 T2 T3 Cycle length, days NS NS # follicles/ovary NS **-28*↓** serum TT NS NS SHBG NS NS FAI NS NS FSH NS NS LH NS NS E2 NS NS AMH NS NS Adjusted p-values for trend were NS
Longnecker et al. 2002 Consenting mother-child pairs Control cases (n=552) Cryptorchidism cases (n=219) Hypospadias cases (n=199) Polythelia (extra nipples) cases (n=167)	Maternal serum DDE Exposure categories (ng/mL) (1) <15.0 (2) 15.0–29.9 (3) 30.0–44.9 (4) 45.0–59.9 (5) ≥60 Median ranges: 23.6–31.9 ng/mL blood	Cryptorchidism, hypospadias, polythelia	Adjusted OR per 15.0 ng/mL increase in DDE or for exposure categories 2–5 versus 1 (5 versus 1 DDE) (15 ng/mL↑) Cryptorchidism NS NS Hypospadias NS NS Polythelia NS NS
Longnecker et al. 2007 Consenting mother-infant pairs (n=781)	Maternal serum DDE and DDT Median ng/g lipid ng/mL DDT 250 1.9 DDE 2,700 19.5 DDT:DDE 0.12	AGD1, AGD2, ASD, penis length, penis width	Adjusted betas (95% CI) mm change per ng/g DDE AGD1: NS AGD2: NS ASD: NS Penis length: NS Penis width: NS

Table 2-16. Summary of Studies of Associations between Human DDT Exposure Metrics in Maternal Serum, Cord Blood, or Breast Milk and Reproductive Endpoints in Offspring Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Torres-Sanchez et al. 2008 Mother-infant pairs (n=71; 37 males and 34 females)	Maternal trimester serum levels DDE and DDT Median (ng/g lipid) Boys / Girls Baseline 2,456.6 / 1,688.2 1st 1,714.8 / 1,407.9 2nd 1,276.5 / 1,083.0 3rd 1,274.2 / 1,040.1 *p,p'*-DDT in both boys and girls at each sampling time point=0.0123 (ng/g lipid)	Anal position index, PA, PA/W	Adjusted betas for a doubling of DDE in boys Base / 1st / 2nd / 3rd API NS / **-0.02*↓** / NS / NS PA NS / NS / NS / NS PA/W NS / NS / NS / NS No significant associations were observed in girls No significant associations with levels of DDT (data not shown)
Vasiliu et al. 2004 Two-generation cohort of maternal fish-eaters Angler mother-daughter (aged 20–50 years) pairs (n=151)	Estimated maternal DDE serum medians at birth: 3.8–7.0 ng/mL	Age at menarche	Adjusted beta per ng/mL serum DDE = –0.07, p=0.038 Significance was lost after controlling for body size at menarche

Table 2-16. Summary of Studies of Associations between Human DDT Exposure Metrics in Maternal Serum, Cord Blood, or Breast Milk and Reproductive Endpoints in Offspring Biometrics[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Vested et al. 2014 Maternal-son pairs (n=166) Follow-up with male offspring aged ~20 years	Maternal serum DDE Median (ng/mL): 2.55 Median (ng/g lipid): 288 Tertile exposure levels (nmol/mL) T1: 0.0007–0.0063 T2: >0.0063–0.0106 T3: >0.0106–0.055	Sperm concentration, total sperm count, semen volume, percent progressive spermatozoa, percent motile spermatozoa, percent morphologically normal spermatozoa, mean testicular volume, testosterone, free testosterone, E2, LH, FSH, inhibin B, and SHBG	Adjusted betas for % difference from T1 DDE All adjusted beta coefficients were NS Adjusted trend *p*-values calculated using DDE concentrations as a continuous variable were NS

[a]See Table 10 in the *Supporting Document for Epidemiological Studies for DDT, DDE, and DDD* for more detailed descriptions of studies. Studies in this table were selected from those described in Table 10, because they: (1) measured DDT-related metrics in biological fluids or tissues in each subject, and (2) evaluated associations using linear regression, logistical regression, or correlation techniques, adjusting for possible confounding factors.
[b]DDE = *p,p'*-DDE, unless otherwise specified; DDT = *p,p'*-DDT, unless otherwise specified.
[c]**Bold text,** "significant" and asterisk indicate statistically significant (p<0.05). Arrows indicate an increase or decrease in the reproductive endpoint or direction of p-trend. Adjusted OR or adjusted β indicates adjustment for standard confounding variables for reproductive outcomes, such as total serum lipids, birth weight, blood lead levels, age of pubertal onset, smoking, BMI, and height z-scores.

AGD = anogenital distance; AMH = anti-mullerian hormone; API = anal position index; BMI = body mass index; CI = confidence interval; FAI = free androgen index; FR = fecundability ratio; FSH = follicle stimulating hormone; LH = luteinizing hormone; LOD = limit of detection; NS = not statistically significant; OR = odds ratio; PA = perineal distance; PA/W = perineal distance/weight; Q = quartile; SD = standard deviation; SHBG = sex hormone-binding globulin; T = tertile; TT = total testosterone; TUI = time of unprotected intercourse; UI = unprotected intercourse

Reproductive Effects in Adults (Table 2-14)

Time to pregnancy (TTP) (fecundity). Inconsistent evidence comes from five studies of the association between serum levels of DDT, DDD, or DDE and TTP (Table 2-14). Only one study reported an inverse association between serum DDE (in male partners) and longer TTP in models adjusted for potential confounders (Buck Louis et al. 2013). The geometric mean concentration of DDE was relatively low, 0.82 ng/g serum, compared with approximately 1.46 ng/g serum in contemporaneous surveys of U.S. adult males (CDC 2018). DDE levels in women were below the levels of detection. In two studies, inverse associations (found in preliminary analyses) lost statistical significance after models were adjusted for maternal age at conception (Axmon et al. 2006) or shellfish consumption and mercury in the women's hair (Chevrier et al. 2013). Relatively high levels of DDE in women's serum (Law et al. 2005) and of DDE and DDT (*p,p'*- and *o,p'*- isomers) (Harley et al. 2008) were not associated with TTP. Recently, Buck Louis (2014) reviewed the issue of fecundity and environmental pollutants and noted that subtle changes in human fecundity may be easily missed without continued research specifically aimed at the preconception enrollment of couples for longitudinal measurement of sensitive outcomes such as TTP and pregnancy loss. The investigator also noted the necessity to consider male-mediated exposures when assessing couple-dependent outcomes because failure to do so may lead to the wrong conclusions, particularly in the absence of female exposures.

Menstrual cycle. Six studies provide inconsistent evidence for associations between menstrual cycle changes and serum DDE or DDT levels: three reported associations and three reported no association (Table 2-14). High mean total DDT levels (~20–30 ng/g serum compared with <2 ng/g serum) reported in contemporaneous surveys of women from the U.S. general population [CDC 2018]) were associated with increased prevalence of short cycles and reduced age at menarche (Ouyang et al. 2005) and reduced luteal phase length (Windham et al. 2005). However, in a larger study of women with similar mean serum DDE levels (30 ng/mL), no association was found between DDE and menstrual cycle parameters (Cooper et al. 2005). Evaluations of four different populations by Toft et al. (2008) also showed inconsistent results between studied groups. In Inuit women from Greenland, DDE was associated with decreased prevalence of long menstrual cycles, whereas in Polish women, DDE was associated with an increased risk for long cycles; both cohorts had similar mean serum DDE concentrations: 430 ng/g lipid in Polish women and 444 ng/g lipid in the Inuit group. In the same study, DDE was not associated with long cycles in a cohort of Swedish fishermen's wives who had a significantly higher mean serum DDE concentration (2,147 ng/g lipid) (Toft et al. 2008). No associations between DDE and menstrual cycle

parameters were reported among women with low (≤0.35 ng/mL blood) DDE levels (i.e., Denham et al. 2005; Gallo et al. 2016).

Uterine alterations. Inconsistent evidence comes from four studies examining associations between serum DDE or DDT levels and uterine alterations (Table 2-14). In one study, women with high serum levels of *p,p'*-DDE (36.95 ng/g serum) had an increased risk for uterine fibroids compared to women with lower levels of *p,p'*-DDE (16.9 ng/g serum); no association was found with *p,p'*-DDT (Trabert et al. 2015). A small study of only 18 endometriosis cases and 8 controls (not shown in Table 2-14) reported a higher concentration of DDE (and PCBs) in serum from cases (770 ng/g lipid) than in controls (310 ng/g lipid); no further analysis was conducted (Quaranta et al. 2006). Studies of women with relatively low serum levels of total DDT (DDT + DDE) did not find associations between serum levels of DDE or DDT and prevalence of endometriosis (Cooney et al. 2010; Porpora et al. 2009; Upson et al. 2013).

Abortion-preterm birth. Evidence from seven studies suggests that serum levels of DDT, DDD, or DDE currently found in the U.S. general population (CDC 2018) may not present increased risks for abortion or premature delivery, but increased risk may exist in countries where DDT is still being used (Table 2-14). Early fetal loss has been associated with relatively high serum levels of DDE (Korrick et al. 2001; Longnecker et al. 2005; Venners et al. 2005). However, the reported increased ORs were marginally significant in two of these studies (Korrick et al. 2001; Longnecker et al. 2005; see Table 2-14). In the latter study, the odds for fetal loss were increased in the third and fourth DDE quintiles compared to the first, but not when comparing the second (15–29 ng/mL) or fifth (≥60 ng/mL) with the first quintile. However, in a model fitting DDE as a continuous variable, a 60 ng/mL increase in serum DDE level increased the risk for fetal loss (OR 1.4 [95% CI 1.1–1.6]). Two studies from India reported higher levels of DDE in placental tissue from women who had preterm delivery compared with women who gave birth to full-term babies (Anand et al. 2015; Saxena et al. 1980; described in Table 8 in the *Supporting Document for Epidemiological Studies for DDT, DDE, and DDD*, but not in Table 2-14, because regression analysis was not conducted). Serum ΣDDT was not associated with abortions or preterm birth in three modern studies (Ouyang et al. 2014; Torres-Arreola et al. 2003; Wood et al. 2007). In the Ouyang et al. (2014) study, the mean ΣDDT serum concentration in Chinese women (34.4 ng/g serum) was higher than those levels measured in the studies mentioned above that reported associations between DDE and fetal loss (22–28 ng/g serum) (Korrick et al. 2001; Longnecker et al. 2005; Venners et al. 2005). Ouyang et al. (2014) noted that they classified the total DDT concentration as low and high by using a median split (30.7 ng/g serum), which may have influenced the results. Serum concentrations of DDE in the Torres-Arreola et al. (2003) and Wood et al. (2007) studies were low (~190 and 67 ng/g lipid,

respectively), and in both studies, cases and controls had comparable levels. Neither DDT nor DDE in breast milk collected between 6 and 12 weeks postpartum was associated with preterm birth in a study of Australian women; no analysis of the pesticides in blood was conducted (Khanjani and Sim 2006).

Menopause. Inconsistent evidence for an association between serum DDE levels and early age at menopause comes from two studies (Table 2-14). Cooper et al. (2002) found no association between serum DDE and early menopause in a study of 1,407 women when serum DDE was categorized into deciles; the median concentration of DDE in serum was 600 ng/g lipid. Analysis of DDE as a continuous variable, however, yielded a marginally higher HR for early menopause (1.1 [95% CI 1.0–1.3]). In an evaluation of 1,442 women participants in NHANES 1999–2008, with a median DDE serum concentration of 243 ng/g lipid, serum DDE was associated with early menopause in analyses of serum DDE categorized into deciles or when DDE was analyzed as a continuous variable (Grindler et al. 2015).

Reproductive sex hormones. Inconsistent evidence for associations between serum levels of DDT, DDD, or DDE and serum or urine levels of sex hormones or their metabolites is provided by 15 studies described in Table 2-14. Most of the studies (n=13) collected data from men, and only four studies collected data from women (Freire et al. 2014; Goncharov et al. 2009; Perry et al. 2006; Windham et al. 2005). A wide variety of sex hormones and related chemicals were measured across the studies, including testosterone (total, free, or bioavailable), sex-hormone-binding globulin (SHBG), estradiol (E2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, progesterone, and inhibin B (IHB, which inhibits synthesis and secretion of FSH), as well as androgenic/estrogenic indices such as free androgen index (FAI, ratio of testosterone:SHBG), testosterone/estradiol ratio (marker of aromatase activity) and testosterone/LH ratio (marker of Leydig cell function). The inconsistency of the evidence is illustrated by Table 2-17 showing the number of studies reporting positive associations, inverse associations, and no associations for each examined sex hormone and related endpoints. For example, among the 13 studies in men measuring serum levels of testosterone and related endpoints, 2 found positive associations (Giwercman et al. 2006; Hagmar et al. 2001), 2 found inverse associations (Emeville et al. 2013; Freire et al. 2014), and 9 found no associations (Blanco-Muñoz et al. 2012; Ferguson et al. 2012; Goncharov et al. 2009; Haugen et al. 2011; Martin et al. 2002; Rignell-Hydbom et al. 2004; Rylander et al. 2006; Schell et al. 2014; Turyk et al. 2006).

Table 2-17. Number of Studies Finding Statistically Significant Associations and No Significant Associations Between Serum Levels of DDT, DDD, or DDE and Levels of Sex Hormones in Serum or Urine[a]

Men	Testosterone[b]	SHBG	E2[c]	LH	FSH	PL	IHB
	2 ↑ 2 ↓ 9 NS	1 ↑ 0 ↓ 6 NS	1 ↑ 0 ↓ 6 NS	2 ↑ 0 ↓ 7 NS	2 ↑ 0 ↓ 7 NS	0 ↑ 1 ↓ 2 NS	1 ↑ 1↓ 3 NS
Women	Testosterone[b]	E2[c]	PG[d]	FSH	PL		
	0 ↑ 0 ↓ 1 NS	0 ↑ 1 ↓ 2 NS	0 ↑ 2 ↓ 0 NS	0 ↑ 0 ↓ 1 NS	0 ↑ 0 ↓ 1 NS		

[a]Studies counted are from the 15 studies with results described in Table 2-14.
[b]Endpoints included in this count were serum testosterone (total, free, or bioavailable), FAI (free androgen index), and testosterone/LH ratio. Only one study evaluated testosterone levels in women (Goncharov et al. 2009).
[c]In all seven studies of men, serum levels of E2 were measured. In studies of E2 in women, Freire et al. (2014) measured serum E2 (association NS), and the major urinary metabolite of E2 was measured by Perry et al. (2006) (association NS) and Windham et al. (2005) (↓ association).
[d]Perry et al. (2006) and Windham et al. (2005) reported significant inverse associations with the major urinary metabolite of progesterone.

↑ = positive association; ↓ = inverse association; E2 = estradiol; FAI = free androgen index; FSH = follicle-stimulating hormone; IHB = inhibin B; LH = luteinizing hormone; NS = not statistically significant; PG = progesterone; PL = prolactin; SHBG = sex hormone-binding globulin

The inconsistency of the evidence is further illustrated by stratification of the studies into high- (mean or median DDE or ΣDDT >5 ng/mL or >600 ng/g lipid) and low-level categories. Seven high-level studies collected data from men (Blanco-Muñoz et al. 2012; Freire et al. 2014; Giwercman et al. 2006; Hagmar et al. 2001; Martin et al. 2002; Rylander et al. 2006; Turyk et al. 2006) and three collected data from women (Freire et al. 2014; Perry et al. 2006; Windham et al. 2005). All three high-level women studies evaluated E2 levels, but two found no association between serum DDE levels and E2 levels (Friere et al. 2014; Windham et al. 2005) and the third found an inverse association (Perry et al. 2006). In the seven high-level studies of men, five found associations with at least one sex hormone (Blanco-Muñoz et al. 2012; Freire et al. 2014; Giwercman et al. 2006; Hagmar et al. 2001; Rylander et al. 2006), but sex hormones showing associations differed among the studies. For example, Blanco-Muñoz et al. (2012) found no association between DDE and serum testosterone, FSH, LH, or E2, an inverse association with prolactin, and a positive association with inhibin B in a group of Mexican men, whereas Giwercman et al. (2006) reported positive associations with testosterone, SHBG, and LH, inverse associations with inhibin B, and no associations with FSH or E2 in a group of Ukrainian men. Two of seven high-level studies of men found no associations: Martin et al. (2002) evaluated several testosterone-related endpoints and Turyk et al. (2006) evaluated several testosterone-related endpoints, as well as SHBG, LH, FSH, and estrone sulfate. Contributing to the general inconsistency of the evidence, Emeville et al. (2013), in a low-level

study of men, reported inverse associations between serum DDE and dihydrotestosterone and testosterone:LH ratio and a positive association with LH. Other low-exposure studies reported no associations between DDE and reproductive sex hormones (Ferguson et al. 2012; Goncharov et al. 2009; Haugen et al. 2011; Rignell-Hydbom et al. 2004, 2005a; Schell et al. 2014).

Semen parameters. Inconsistent evidence for associations between serum levels of DDT, DDD, or DDE and changes in semen parameters (e.g., sperm count or concentrations, sperm motility) comes from results of nine studies described in Table 2-14. Associations with changes in a number of sperm parameters were found in a study of men with high serum DDE levels (mean of 697 ng/mL) (Aneck-Hahn et al. 2007) and in a study of men with relatively low serum ΣDDT levels (mean of ~300 ng/g lipid) (Messaros et al. 2009). However, the risk for low sperm concentration was elevated in the low-level study, but not in the high-level study. Charlier and Foidart (2005) found no associations in sperm parameters at serum DDE levels 4–5 times higher than those associated with sperm alterations in the Messaros et al. (2009) study. Toft et al. (2006) reported associations with decreased sperm motility in a group of men with a mean serum DDE concentration of 890 ng/g lipid, but not in a group whose mean serum DDE was 1,270 ng/g lipid. De Jaeger et al. (2006) evaluated a wide range of sperm parameters in a Mexican population living in malaria endemic areas in which DDT was sprayed annually and found associations for decreasing sperm motility and increasing sperm tail abnormalities, but none for sperm count or concentration. The mean plasma concentration of DDE in the men was 45,000 ng/g lipid, which is approximately 200 times higher than levels reported in the most recent survey of men from the U.S. general population (CDC 2018). Other low-level studies (mean or median DDE or Σ DDT <5 ng/mL or <600 ng/g lipid) did not find associations between DDE and changes in sperm parameters (Dallinga et al. 2002; Hauser et al. 2003; Rignell-Hydbom et al. 2005b). Associations between semen levels of DDE or DDD and sperm concentration, but not sperm motility, were reported in a single study using this biomarker of DDT exposure (Pant et al. 2007).

Reproductive Effects in Preadolescents/Adolescents (Table 2-15). Inconsistent evidence is provided by five studies examining possible associations between serum DDE levels and puberty onset outcomes in Russian preadolescent boys, ages 8–9 years (Lam et al. 2014, 2015) and adolescent Belgian boys and girls, ages 14–15 years (Croes et al. 2015; Den Hond et al. 2011; Dhooge et al. 2011). Outcomes were Tanner indices of genitalia and pubic hair growth in males (Croes et al. 2015; Den Hond et al. 2011; Lam et al. 2014, 2015), breast development in females (Croes et al. 2015; Den Hong et al. 2011), and serum levels of sex hormones in male adolescents (Dhooge et al. 2011). Belgian subjects had relatively low levels of serum DDE (geometric mean 105 ng/g lipid), lower or comparable to those measured in the most

recent survey of U.S. teenagers (CDC 2018), whereas the Russian boys had higher serum DDE levels (median 287 ng/g lipid). In Russian boys, no associations were found for shifts in attaining early milestones for genitalia growth, testicular volume, or pubic hair growth (Lam et al. 2014), but at later stages of development, the highest DDE exposure quartile showed later attainment of Tanner pubic hair growth stage five (P5) than the first quartile (Lam et al. 2015). In contrast, data for Belgian boys showed associations between DDE serum levels and faster attainment of genitalia growth and pubic hair growth milestones (Croes et al. 2015; Den Hond et al. 2011). In Belgian boys, an association was found between DDE levels and increasing E2 levels, but no associations with other levels of reproductive sex hormones (Dhooge et al. 2011). In Belgian girls, an association between DDE levels and delayed development was observed in one study group (Croes et al. 2015), but not in another study group (Den Hond et al. 2011).

Maternal Exposure and Effects in Neonates/Infants/Adults (Table 2-16). Results from six studies described in Table 2-16 provide consistent evidence for no association between maternal DDT, DDE, or DDD levels in serum, cord blood, breast milk, or placenta and risk for the male birth defects, cryptorchidism (undescended testes) or hypospadias (condition in which the opening of the urethra is on the underside of the penis) (Bhatia et al. 2005; Brucker-Davis et al. 2008; Damgaard et al. 2006; Fernandez et al. 2007; Giordano et al. 2010; Longnecker et al. 2002). Two of these studies looked for associations in study groups with high maternal serum levels (Bhatia et al. 2005: mean of 5,200 ng DDE/g lipid; Longnecker et al. 2002: case medians of 24–32 ng DDE/mL), but reference groups for the statistical comparisons in these studies, although 2–4-fold lower than the high-level groups, were still high, compared with U.S. general population values (CDC 2018). Markers of androgen action such as AGD were not associated with ΣDDT in two studies (Longnecker et al. 2007; Torres-Sanchez et al. 2008), except for a decrease in anal position index (a non-age-dependent measurement) in boys and DDE from maternal serum collected in the first trimester (but not 2^{nd} or 3^{rd} trimesters) in the Torres-Sanchez et al. (2008) study.

Four studies provided consistent evidence for no associations between maternal exposures to DDT and adverse reproductive outcomes in their adult offspring. Two studies examined age at menarche and time to pregnancy (fecundity) in daughters from mothers exposed through consumption of Great Lakes fish (Han et al. 2016; Vasiliu et al. 2004); the other two studies measured sex hormones and menstrual cycle length in adult Danish daughters (Kristensen et al. 2016) and sperm parameters and sex hormones in adult Danish sons (Vested et al. 2014).

Evidence of Reproductive Effects of DDT, DDD, or DDE in Laboratory Animals

Overview. The principal reproductive effects of DDT and related compounds in laboratory animals have been observed at dose levels >1 mg/kg/day, and are thought to involve anti-androgenic activities (e.g., androgen-receptor binding and impaired male reproductive tissue development) of *p,p'* isomers of DDT, DDE, or DDD and estrogenic activities (e.g., estrogen-receptor binding and promotion of female reproductive tissue development) of *o,p'*-DDT (see Harada et al. 2016; Hojo et al. 2006; Kelce et al. 1995, 1997; Yamasaki et al. 2009; You et al. 1998, 1999a).

Reliable acute-duration oral LOAELs for adverse effects on reproductive tissues or reproductive function in laboratory animals range from 50 to 200 mg/kg/day for decreased weights of male reproductive tissues from *p,p'*-DDT, technical DDT, technical DDD, or *p,p'*-DDE and from 100 to 500 mg/kg/day for increased uterine weight from *o,p'*-DDT (see Table 2-1, Figure 2-2, and text below).

After intermediate-duration exposure, decreased fertility has been observed in adult laboratory animals at doses ranging from 5.1 to 51.4 mg technical DDT/kg/day (Bernard and Gaertner 1964; Jonsson et al. 1976; Ledoux et al. 1977), but was not observed in other studies at dose levels up to 4 mg *o,p'*-DDT/kg/day (Wrenn et al. 1971), 10 mg *p,p'*-DDE/kg/day (Kornbrust et al. 1986), or 27.7 mg *p,p'*-DDT/kg/day (Hojo et al. 2006). The lowest apparent intermediate-duration LOAELs for other male and female reproductive effects are 3.75 mg/kg/day for decreased estradiol levels in female rats (Hojo et al. 2006), 6.25 mg *p,p'*-DDT/kg/day for decreased seminal weight in castrated mice (no effects were found in normal mice) (Orberg and Lundberg 1974) and 2 mg *p,p'*-DDT/kg/day in female mice exposed for 72–74 days before mating to nonexposed males for small decreases in the number of implants and decreased corpus luteum (Lundberg 1973, 1974).

No adverse effects on indices of reproduction in laboratory animals were observed in several chronic oral multiple generation studies, which identified NOAELs of 0.5–18.6 mg technical DDT/kg/day in rats (Duby 1971; Ottoboni 1969, 1972; Treon et al. 1954), 10 mg technical DDT/kg/day in dogs (Ottoboni et al. 1977), 0.3 mg *o,p'*-DDT/kg/day (Duby et al. 1977), and up to 27 mg *p,p'*-DDT/kg/day in rats (Duby et al. 1977; Hojo et al. 2006), but decreased fertility was observed in a multiple-generation study of mice at 20 mg technical DDT/kg/day (Keplinger 1970). Histological examination revealed no exposure-related abnormalities in the ovaries, uterus, mammary glands, adrenals, or prostate of Osborne-Mendel rats or B6C3F1 mice fed dietary doses for 78 weeks up to 45 mg technical DDT/kg/day, 59 mg

p,p'-DDE/kg/day, or 231 mg technical DDD/kg/day (rats) and 30.2 mg technical DDT/kg/day, 49 mg *p,p'*-DDE/kg/day, or 142 mg technical DDD/kg/day (mice) (NCI 1978).

Acute-duration studies. As shown in Table 2-1 and Figure 2-2, decreased weights of male reproductive tissues (e.g., seminal vesicles and ventral prostate) and decreased reproductive function have been observed after acute-duration exposure of male rats to DDT (NS), *p,p'*-DDT or *p,p'*-DDE, and increased uterine weights have been observed after acute exposure of female rats to *o,p'*-DDT.

Decreases in weights of seminal vesicles or ventral prostate were observed in male adult Long-Evans or Sprague-Dawley rats given gavage doses of 200 mg *p,p'*-DDE/kg/day for 4 or 5 days, without changes in serum testosterone levels, but not at doses up to 100 mg *p,p'*-DDE/kg/day (Kelce et al. 1995, 1997; Leavens et al. 2002); castrated Sprague-Dawley 6-week-old rats supplemented with subcutaneous testosterone and co-exposed to gavage doses ≥50 mg *p,p'*-DDT/kg/day for 10 days (Kang et al. 2004); and adult male rats given gavage doses of 70 mg *p,p'*-DDE/kg/day for 4 days (You et al. 1999a). Other reported male reproductive effects include decreases in levator ani plus bulbocanvernosus muscles and Cowper's gland in castrated rats supplemented with subcutaneous testosterone and administered 100 mg *p,p'*-DDT /kg/day via gavage for 10 days (Kang et al. 2004); decreased serum testosterone, but not LH or FHS, in male rats treated with 200 mg *p,p'*-DDE for 2 weeks by gavage (Krause 1977), and significantly decreased number of fetuses and implantations in non-exposed female rats mated with male rats given 500 mg DDT(NS)/kg/day on PNDs 4 and 5 (Krause et al. 1975). No significant changes in reproductive organ weights, histology of testis or epididymis, or sperm morphology or motility were observed in adult male Sprague-Dawley rats exposed once to 100 mg *p,p'*-DDT/kg, or to 50 mg *p,p'*-DDT/kg/day for 5 days (Linder et al. 1992).

Increased uterine weight as a result of *o,p'*-DDD exposure was observed in immature (23-day-old) female Wistar rats after 3 or 7 days exposure to dietary doses ≥100 mg *o,p'*-DDT/kg/day, accompanied by increased glycogen content and premature vaginal opening (Clement and Okey 1972) and in ovariectomized female DA/Han rats given 3 daily gavage doses ≥100 mg *o,p'*-DDT/kg/day, but not 10 mg/kg/day (Diel et al. 2000).

Acute-duration oral exposure of laboratory animals to *p,p'*-DDT, *p,p'*-DDE, *o,p'*-DDT, or *o,p'*-DDD during gestation has produced effects on developing reproductive tissues and reproductive functions in adults (see Section 2.17 for more details and references).

Intermediate-duration studies. Fertility has been assessed in adult laboratory animals after intermediate-duration exposures to technical DDT, *p,p'*-DDE, *p,p'*-DDT, or *o,p'*-DDT (see Table 2-1 and Figure 2-2). An early study (Green 1969) reported decreased fertility when parental male and female Sprague-Dawley rats were fed diets of approximately 0.56 mg DDT/kg/day (only level tested, presumably technical DDT) for 60 days before mating, but this apparent LOAEL for decreased fertility was not included in Table 2-1 and Figure 2-2 due to insufficient reporting of study details.

Decreased fertility after intermediate exposure to technical DDT has been reported for female Sprague-Dawley rats exposed to 12 mg technical DDT/kg/day in food, but not to 6 mg/kg/day, for 36 weeks before mating to nonexposed males (Jonsson et al. 1976); female C-57 mice exposed to 51.4 mg technical DDT/kg/day in food, but not to 34.3 mg/kg/day, for up to 60–90 days before mating to non-exposed males (Bernard and Gaertner 1964); and pairs of male and female B6D2F1 mice exposed to ≥5.1 mg technical DDT/kg/day in food for 130 days before mating, but not in pairs exposed to up to 3.4 mg/kg/day for 86 days before mating (Ledoux et al. 1977).

Other studies reported no effects on fertility in female Sprague-Dawley rats exposed to up to 4 mg *o,p'*-DDT/kg/day in food for up to 20 weeks before mating to nonexposed males (Wrenn et al. 1971); female Sprague-Dawley rats given gavage doses of 10 mg *p,p'*-DDE/kg/day for 5 weeks before mating to nonexposed males (Kornbrust et al. 1986); pairs of male and female BALB/c mice exposed to 1.3 mg technical DDT/kg/day in food for 30 days before mating and 90 days beyond mating (Ware and Good 1967); New Zealand rabbits given gavage doses of 3 mg technical DDT/kg/day, 3 times/week for 12–15 weeks before artificial insemination, but a decreased ovulation rate and slight decrease in circulating progesterone levels (Lindenau et al. 1994; Seiler et al. 1994); and F0 parental male and female Sprague-Dawley rats exposed for 10 weeks before mating to dietary doses up to 25 or 27.7 mg *p,p'*-DDT/kg/day, respectively, but altered circulating levels of sex hormones in F0 females, but not in F0 males (Hojo et al. 2006).

Other findings for male reproductive effects after intermediate-duration exposure include decreased testis weight and Sertoli cell numbers in male rats exposed to gavage doses of 200 mg *p,p'*-DDT/kg/day on PNDs 4–23 and mated to nonexposed female rats on PND 60 or 90, as well as decreased number of fetuses and implants in the pregnant dams (Krause et al. 1975); and decreased seminal vesicle weight in castrated adult NMRI mice supplemented with testosterone and exposed to 6.25 mg *p,p'*-DDT/kg/day in food for 28 days, but not in similarly exposed nonsurgically modified mice (Orberg and Lundberg 1974). No significant changes in serum levels of sex hormones, sperm counts, and relative weights or histology

of reproductive organs were observed in sexually immature male F344 rats exposed to 10 mg *p,p'*-DDE in food from 6 to 12 weeks of age (Makita et al. 2003a).

Other female reproductive effects include small (~12%) decreases in the number of implants and decreased number of corpus luteum in female NMRI mice exposed to 2 mg *p,p'*-DDT/kg/day for 72–74 days before mating to nonexposed males (Lundberg 1974) and decreased serum estradiol levels and increased progesterone (with no effects on fertility) in F0 female Sprague-Dawley rats fed dietary doses ≥3.75 or 27.7 mg *p,p'*-DDT/kg/day, respectively, for 10 weeks before mating with exposed males (Hojo et al. 2006).

Chronic-duration studies. In chronic multi-generation exposure-duration studies, no adverse effects on reproduction functions 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). Hojo et al. (2006) found no effects on reproduction functions in F0 and F1 Sprague-Dawley rats exposed to dietary doses up to 25 (males) and 27.7 (females) mg *p,p'*-DDT/kg/day.

The results of a chronic-duration dietary study showed no treatment-related adverse effects on the ovaries, uterus, mammary glands, prostate, or adrenals of 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 (NCI 1978). 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 the NCI (1978) study.

In mice, no adverse effects on reproduction were observed in field mice fed 2.4 mg technical DDT/kg/day in food for 15 months (Wolfe et al. 1979), but in multiple-generation studies of laboratory mice, decreased fertility was observed in Swiss Webster mice fed 20 mg technical DDT/kg/day, but not 5 mg/kg/day (Keplinger 1970). No significant reproductive effects were reported in a 3-generation study in dogs dosed with up to 10 mg technical DDT/kg/day (Ottoboni et al. 1977).

Mechanisms of Reproductive Effects of DDT, DDD, or DDE. DDT and related compounds have been associated with altered reproductive outcomes in some epidemiological studies and laboratory animal studies. These effects are thought to involve anti-androgenic activities (e.g., androgen-receptor binding

and impaired male reproductive tissue development) of *p,p'*-DDE, and estrogenic activities (e.g., estrogen-receptor binding and promotion of female reproductive tissue development) of *o,p'*-DDT (see Harada et al. 2016; Kelce et al. 1995, 1997; Yamasaki et al. 2009; You et al. 1998, 1999a).

Numerous studies have shown that *o,p'*-DDT has estrogenic activities, albeit relatively weak properties, compared with 17β-estradiol. For example, *o,p'*-DDT showed significantly stronger estrogenic activity for initiating implantation and in increasing uterine weight in young rats than *p,p'*-DDT (Johnson et al. 1992; Singhal et al. 1970). Welch et al. (1969) reported an estrogenic activity ranking of *o,p'*-DDT > technical DDT > *p,p'*-DDT in immature female rats treated intraperitoneally. In various *in vitro* assays for estrogenicity, however, *o,p'*-DDT gave positive estrogenic responses, but with a potency that was several orders of magnitude weaker than 17β-estradiol and diethylstilbestrol (DES, a synthetic form of estrogen) (Soto et al. 1997). In one assay, *o,p'*-DDT, *o,p'*-DDD, and *p,p'*-DDT were full estrogenic agonists, *p,p'*-DDE and *p,p'*-DDD were partial agonists, and technical DDT was a full agonist. In another study, it took 10^7 times more *o,p'*-DDT, *o,p'*-DDD, *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, and technical DDT to produce an estrogenic response comparable to that of 17β-estradiol (Soto et al. 1998). Additional assays that used gene expression and transcription mediated by estrogen receptor activation showed *o,p'*-DDT's estrogenic activity to be at least 10^5 less potent than 17β-estradiol in inducing estrogen-regulated gene transcription (Balaguer et al. 1999; Gaido et al. 1997; Sohoni and Sumpter 1998; Tully et al. 2000). Results from *in vitro* studies also have shown that *o,p'*- isomers can compete with estradiol for binding to the estrogen receptor, although with a binding affinity significantly lower than 17β-estradiol (Danzo 1997; Kelce et al. 1995). Experiments also showed that *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD were relatively ineffective in binding to the estrogen receptor (Kelce et al. 1995).

Other studies have shown that the environmentally persistent metabolite, *p,p'*-DDE, has anti-androgenic activity (Kelce et al. 1995, 1997; You et al. 1998, 1999a). In competitive androgen receptor binding assays of *p,p'*-DDT, *p,p'*-DDE, *o,p'*- DDT, and *p,p'*-DDD, the four chemicals showed dose-dependent competitive inhibition, but *p,p'*-DDE was the greatest competitor with an inhibition constant similar to that of DES and about 30 times weaker than 17β-estradiol (Kelce et al. 1995). The other three isomers were 12–20-fold less effective than *p,p'*-DDE. Experiments also showed that *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 (Kelce et al. 1995). Maness et al. (1998) also showed that among DDT compounds, *p,p'*-DDE was the most potent for inhibiting androgen receptor regulated gene expression in a human cell line transiently transvected with the human androgen receptor and a reporter gene linked to an androgen responsive promoter. More recently, Tinwell et al. (2007) showed that the inhibitory action of *p,p'*-DDE

on the weight of the ventral prostate of immature male rats was associated with a 4.4-fold increase in activity of L-amino oxidase, a protein associated with apoptosis and suggested that this protein has the potential to be a biomarker for endocrine disruption.

Results from several studies suggest that Sertoli cells may be involved in DDT (DDT, DDE, DDD)-induced alterations in sperm parameters. Sertoli cells facilitate the progression of germ cells to spermatozoa, are activated by FSH, and produce the protein complex inhibin, which inhibits FSH synthesis and secretion. For example, *in vitro* incubation of Sertoli cells from immature rats with DDE resulted in decreased survival of the cells that appeared to be mediated by down-regulation of transferrin and up-regulation of androgen-binding protein (ABP) (Xiong et al. 2006). Both transferrin and ABP are glycoproteins produced and secreted by Sertoli cell into the lumen of the tubule and play an important role on differentiation and maturity of sperm. *In vitro* studies also have shown that DDT can reduce the number of FSH binding sites in Sertoli cells by triggering degradation of FSH receptors (Bernard et al. 2007), or by affecting intercellular junctions by altering the amount or inducing aberrant localizations of protein components of Sertoli cell tight junctions, specifically connexin 43 (Fiorini et al. 2004). Other studies have suggested that DDE can induce apoptotic Sertoli cell (and germ cell) death by mechanisms involving elevation of reactive oxygen species (ROS), reduction of mitochondria membrane potential, and induction of apoptotic activating factors, ultimately leading to altered spermatogenesis (Mota et al. 2011; Quan et al. 2016; Shi et al. 2009, 2013; Song et al. 2008, 2011). Results from a study of *in vivo* exposure of rats to DDE as well as exposure of Sertoli cells to DDE *in vitro* showed that DDE can alter mRNA and protein expression of vimentin, N-cadherin, and FSH receptors (Yan et al. 2013). Vimentin protein is an important component of the Sertoli cell cytoskeleton and plays a key role in anchoring germ cells to the seminiferous epithelium. N-Cadherin play an important role in cell-cell adhesions and has been found in spermatogonia, primary spermatocytes, and Sertoli cells. FSH receptor expression controls the magnitude of FSH stimulatory action on Sertoli cells.

Results from a study of *in vitro* incubation of human sperm with DDE in a medium simulating exposure in the female reproductive tract showed that DDE increased intracellular levels of calcium in sperm cells, prematurely triggering acrosomal loss through acrosomal reaction or by damaging sperm membranes (Tavares et al. 2013). Results from a more recent study from the same group of investigators suggested that DDE promoted mitochondrial calcium overload that in turn induced mitochondrial malfunction affecting sperm motility and, ultimately, male fertility (Tavares et al. 2015).

Leydig cells have also been shown to be potential targets for DDT via the adrenal toxicant metabolite, 3-methylsulphonyl-DDE. LH stimulates Leydig cells to produce testosterone; prolactin increases the response of Leydig cells to stimulation by LH. Castellanos et al. (2013) reported that incubation of unstimulated primary neonatal porcine cells with 3-methylsulphonyl-DDE resulted in a concentration-dependent increased secretion of testosterone and estradiol; however, in LH-stimulated cells treated with 3-methylsulphonyl-DDE, there was decreased secretion of testosterone, estradiol, and progesterone. In addition, the expression of important steroidogenesis genes was down-regulated in LH-stimulated cells. These results suggested that the endocrine-disruptive activity of 3-methylsulphonyl-DDE is determined by the physiological status of the Leydig cells. Proteonomic analysis of unstimulated and LH-stimulated cells showed that 3-methylsulphonyl-DDE was acting on several pathways, including mitochondrial dysfunction, oxidative phosphorylation, EIF2-signaling, and glutathione-mediated detoxification (Kalayou et al. 2016).

Studies have also assessed ovarian function in relation to DDT exposure; most of the research has been conducted using *in vitro* cell populations. *p,p'*-DDE increased proliferation of porcine granulosa cells, decreased FSH-stimulated cAMP in these cells and in cultured Chinese hamster ovary cells, and decreased progesterone synthesis in granulosa cells (Chedrese and Feyles 2001). The fact that estradiol could not mimic the DDE-induced decrease in progesterone suggests that DDE also possess non-estrogenic endocrine disrupting properties. *p,p'*-DDE was also shown to increase the concentration of calcium in human granulosa-lutein cells in culture by rapid mobilization calcium from extra- and intracellular sources, which could possibly affect the calcium response to FSH and human chorionic gonadotropin (Younglai et al. 2004a). Similar results were obtained when cells were incubated with *o,p'*-DDE; a mechanism involving a G-protein-coupled membrane receptor in the increase in cytoplasmic calcium was proposed (Wu et al. 2006). Further studies from the same group showed that *p,p'*-DDE can increase FSH stimulation of aromatase activity in human granulosa cells, which could result in overproduction of estradiol early in folliculogenesis and acceleration of oocyte maturation resulting ultimately in impaired fertilization (Younglai et al. 2004b). Incubation of human granulosa cells with *p,p'*-DDE also resulted in significant increases in the expression of the growth factors, vascular endothelial growth factor and insulin-like growth factor-1, both of which appear to play a key role in ovarian follicular development and corpus luteum function (Holloway et al. 2007). Similar results were obtained in ovarian tissue from young rats treated with a single dose of 100 µg *p,p'*-DDE/kg and sacrificed 20 days later (Holloway et al. 2007).

2.17 DEVELOPMENTAL

This section discusses human epidemiological evidence for effects on birth outcomes and subsequent postnatal growth patterns and evidence in laboratory animals exposed during gestation and/or early postnatal periods for fetotoxicity, birth weights and postnatal growth patterns, and developmental effects on neurological and reproductive systems.

Epidemiology Studies of Gestational or Early Life Exposures on Birth Outcomes and Subsequent Postnatal Growth Patterns

Birth outcomes. Inconsistent evidence is provided by 29 epidemiological studies examining possible associations between maternal serum or cord blood levels of DDT, DDD, or DDE and birth weight (Table 2-18). Eleven of these studies reported associations with decreased birth weight or birth weight status (Al-Saleh et al. 2012; De Cock et al. 2016; Govarts et al. 2012; Lenters et al. 2016; Longnecker et al. 2001; Lopez-Espinosa et al. 2011; Robledo et al. 2015; Sharma et al. 2012; Siddiqui et al. 2003; Weisskopf et al. 2005; Wojtyniak et al. 2010), three studies reported associations with increased birth weight (Arrebola et al. 2016; Tan et al. 2009; Weihe et al. 2003), one study reported both a decrease and an increase in birth weight (Kezios et al. 2013), and one study reported both a decrease and no association with body weight (Casas et al. 2015). The remaining 13 studies found no associations with birth weight or birth weight status (Bergonzi et al. 2011; Bjerregaard et al. 2000; Farhang et al. 2005; Fenster et al. 2006; Gladen et al. 2003; Guo et al. 2014; Jusko et al. 2006; Karmaus and Zhu 2004; Khanjani and Sim 2006; Ribas-Fito et al. 2002; Sagiv et al. 2007; Vafeiadi et al. 2014; Wolff et al. 2007). Two meta-analysis reports are included in Table 2-18 (Casas et al. 2015; Govarts et al. 2012). Both of these meta-analyses focused on birth weight data from study populations in European birth cohorts (comprising 8,825 and 7,530 mother-infant pairs, respectively), and found no association between maternal DDT-related biometrics and decreased infant weight at birth in the combined datasets using multiple linear regression techniques.

Some studies in Table 2-18 also included other birth measures such as birth length or length status [including crown-heel length (CHL) and small length for gestational age (SLGA)], gestational age or length, preterm birth, and head circumference. Evidence for associations of these birth parameters with maternal DDT-related biometrics also was inconsistent across studies. For example, 12 studies evaluated gestational age or length: 3 found associations with decreased duration of gestation (Al-Saleh et al. 2012;

Table 2-18. Summary of Studies of Associations Between Human Maternal DDT Exposure Metrics and Offspring Weight and Growth Endpoints at Birth[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Birth parameters			
Al-Saleh et al. 2012 1,571 consenting mother-infant pairs	Mean cord blood and maternal serum (ng/mL), placenta (ng/g) Serum / Placenta / Cord DDE 0.551 / 10.167 / 0.197 DDT 0.008 / 29.620 / 0.005 DDD 0.002 / 7.042 / 0.005	HC, CHL, BW, BL, PI, SGA	Adjusted betas for a 1-log_{10} unit change in DDT metrics Serum DDE / Placenta DDE / Placenta DDT / Placenta DDD HC **-0.112*↓** / NS / NS / NS CHL **-0.116*↓** / **-0.132*↓** / **-0.101*↓** / **-0.141*↓** BW **-0.109*↓** / **-0.065*↓** / NS / NS BL **-0.121*↓** / **-0.055*↓** / NS / NS PI NS / NS / NS / NS Adjusted ORs SGA **1.494*** / NS / NS / NS
Arrebola et al. 2016 200 mother-infant pairs	Cord blood DDE and *o,p'*-DDT (ng/mL) percentiles 25th / 50th / 75th DDE 0.26 / 1.01 / 2.52 *o,p'*-DDT 0.10 / 0.22 / 0.37	BW, GA, BH, PI, HC	Adjusted betas for *p,p'*-DDE / *o,p'*-DDT BW **0.008*↑** / NS GA **-0.004*↓** / NS BH NS / NS HC NS / NS PI NS / NS
Bergonzi et al. 2011 70 mother-infant pairs	Maternal serum, adipose, cord blood and placenta DDE and DDT Geometric mean (ng/g lipid) DDE / DDT Serum 130.0 / ND Cord blood 0.26 / ND Placenta 70.0 / ND Adipose 236.0 / 7.6	BW, BL, HC, SGA, SLGA	Adjusted ORs for SGA or SLGA with DDE or DDT in any tissue or fluid: NS Adjusted betas for BW, BL, or HC with DDE or DDT in any tissue or fluid: NS **Except increased BL with DDT in adipose (p<0.01)**
Bjerregaard et al. 2000 136 mother-infant pairs	Maternal serum, and cord blood DDE, DDT, and ΣDDT Geometric mean (ng/mL) DDE / DDT / ΣDDT Cord blood 3.7 / 0.1 / 3.8	BW, GL	Adjusted betas for BW or GL with DDE, DDT or ΣDDT: NS

Table 2-18. Summary of Studies of Associations Between Human Maternal DDT Exposure Metrics and Offspring Weight and Growth Endpoints at Birth[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Casas et al. 2015 8,825 mother-infant pairs from 11 European birth cohorts forming 14 study populations See **Govarts et al. 2012** for earlier analysis of the data from the same datasets.	Cord blood DDE across 14 study populations, ng/mL Mean: 0.846 25th percentile: 0.2 75th percentile: 1.0	BW	Adjusted betas for change in birth weight (g) per ng/mL DDE 13 study populations: each NS 1 study population: **-0.058***↓ Over all populations: NS
De Cock et al. 2016 91 mother-infant pairs 58 males, 31 females	Mean cord blood, milk and total DDE Cord blood: 0.133 Milk: 2.381 Tertile levels not provided	BW	Adjusted betas for T3 versus T1 DDE Boys: BW **-325.9***↓ Girls: BW 401.1 (p=0.086)
Farhang et al. 2005 420 mother-infant pairs Infants with hypospadias or cryptorchidism (n=155); control infants (n=265)	Maternal serum levels (ng/mL) Q1: DDE ≤31.5; DDT ≤8.1 Q2: DDE 31.7–42.5; DDT 8.2 – 11.0 Q3: DDE 42.6–54.7; DDT 11.1–16.2 Q4: DDE ≥57.5; DDT ≥16.3	Pre-term birth, SGA, BW, GA	Adjusted ORs or betas for Q4 versus Q1 Pre-term OR: **DDE** NS; **DDT** NS SGA OR: DDE NS; DDT NS BW beta: DDE NS; DDT NS GA beta: DDE NS; DDT NS
Fenster et al. 2006 385 mother-infant pairs	Maternal serum levels geometric mean (ng/g lipid) DDE: 1,363.0 DDT: 20.6 *o,p'*-DDT: 1.6	GL, BW, CHL	Adjusted beta per 10-fold change in metric DDE: GL NS; BW NS; CHL NS DDT: GL NS; BW NS; CHL NS *o,p'*-DDT: GL NS; BW NS; CHL NS

Table 2-18. Summary of Studies of Associations Between Human Maternal DDT Exposure Metrics and Offspring Weight and Growth Endpoints at Birth[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Gladen et al. 2003 197 mother-infant pairs	Milk, cord blood, and placenta DDE and DDT (ng/g lipid). Milk tertiles T1: DDE 1,900; DDT 257 Median: DDE 2,457; DDT 336 T3: DDE 3,250; DDT 425	BW, relative weight (ratio of observed weight to mean for gestational age)	Adjusted mean BW and relative weights by tertile (T3 versus T1) Birth weight: DDE NS; DDT NS Relative weight: DDE NS; DDT NS
Govarts et al. 2012 7,530 mother-infant pairs from 12 European birth cohorts (13 study populations)	Mean cord blood levels for study populations ranged from 0.075 to 1.708 ng/mL	BW	Adjusted betas for change in birth weight (g) per ng/mL DDE 12 study populations: each NS 1 study population: **-0.071***↓ Over all populations: NS
Guo et al. 2014 81 mother-infant pairs	Geometric mean maternal serum and cord blood DDT metrics (ng/g lipid) *p,p'*-DDE: Serum 203.54; Cord blood 116.14 *p,p'*-DDT: Serum 14.68; Cord blood 5.41 *o,p'*-DDE: Serum 0.62; Cord blood 0.85 *o,p'*-DDT: Serum 2.51; Cord blood 3.39 *p,p'*-DDD: Serum 1.07; Cord blood 0.66 ΣDDT: Serum 245.82; Cord blood 146.03	BW	Adjusted betas for change in BW per unit metric *p,p'*-DDE: Serum NS; Cord blood NS *p,p'*-DDT: Serum NS; Cord blood NS *p,p'*-DDD: Serum NS; Cord blood NS ΣDDT: Serum NS; Cord blood NS
Jusko et al. 2006 399 mother-infant pairs	Mean maternal serum DDT metrics (ng/g lipid) *p,p'*-DDE: 6,850 *p,p'*-DDT: 1,930 *o,p'*-DDT: 270 ΣDD: 9,050	BW, adjusted BW (z-score), GA, BL, HC	Adjusted betas comparing 75th to 25th percentiles DDT metrics BW: DDE NS; DDT NS; *o,p'*-DDT NS; ΣDDT NS BWz: DDE NS; DDT NS; *o,p'*-DDT NS; ΣDDT NS GA: DDE NS; DDT NS; *o,p'*-DDT NS; ΣDDT NS BL: DDE NS; DDT NS; *o,p'*-DDT NS; ΣDDT NS HC: DDE NS; DDT NS; *o,p'*-DDT NS; ΣDDT NS

Table 2-18. Summary of Studies of Associations Between Human Maternal DDT Exposure Metrics and Offspring Weight and Growth Endpoints at Birth[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Karmaus and Zhu 2004 168 mother-infant pairs	Maternal serum DDE ng/mL Q1: <5.0 Q2: 5.0–15.0 Q3: 15–<25 Q4: ≥25	BW, SGA	Adjusted BWs (g) (5–95 percentiles) Q1: 3,278 (2,979–3,577) Q4: 3,278 (3,065–3,815) OR for SGA: NS
Kezios et al**. 2013** 600 mother-infant pairs	Mean maternal serum DDT metrics (ng/mL) *p,p'*-DDE: 44.2 *p,p'*-DDT: 12.9 *o,p'*-DDT: 0.58	BW, GL, SGA	Adjusted betas for 1-ln unit increase in DDT metrics DDE DDT *o,p'*-DDT BW **-153*↓** **274*↑** NS GA **-0.49*↓** NS NS Adjusted ORs SGA NS NS NS
Khanjani and Sim 2006 815 mother-infant pairs	Milk DDE, DDT, DDD, and ΣDDT (ng/g lipid) DDE DDT Low 0–400 0–39 Medium >400–730 >39–66 High >730 >66	SGA, LBW, HC, BW	Adjusted ORs for high (DDT metrics) compared with low DDE DDT LBW NS NS SGA NS NS Adjusted means HC Low Medium High DDE ref NS NS DDT ref NS NS
Lenters et al. 2016 1,298 maternal-infant pairs Greenland (n=513), Poland (n=180), Ukraine (n=557).	Mean maternal serum DDE. Pooled median exposure level: 3.39 ng/mL	BW	Adjusted betas for change in BW per 2-SD increase in ln-transformed DDE Penalized method: **-47.02*↓** Unpenalized method: **-66.70*↓** Penalized linear regression model simultaneously adjusted for other measured exposure variables.

Table 2-18. Summary of Studies of Associations Between Human Maternal DDT Exposure Metrics and Offspring Weight and Growth Endpoints at Birth[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Longnecker et al. 2001 2,380 mother-infant pairs	Maternal serum DDE and DDT. DDE Exposure groups (ng/mL) Group 1: ≤15 Group 2: 15–29 Group 3: 30–44 Group 4: 45–59 Group 5: ≥60 DDT levels not shown	BW, BL, HC, SGA, Preterm birth	Adjusted ORs versus G1 Preterm birth <37 weeks: Group 1 ref; Group 2 **1.5***↑; Group 3 **1.6***↑; Group 4 **2.5***↑; Group 5 **3.1***↑ SGA <2,500 g: Group 1 ref; Group 2 **2.0***↑; Group 3 **2.3***↑; Group 4 **2.9***↑; Group 5 **4.1***↑
Lopez-Espinosa et al. 2011 494 mother-infant pairs	Geometric mean cord blood *p,p'*-DDE and *p,p'*-DDT (ng/mL) *p,p'*-DDE: 0.495 *p,p'*-DDT: 0.020	BW, BL, HC	Adjusted beta for change in growth per 10x ↑ in DDT metrics BW: *p,p'*-DDE **-107.39***↓; *p,p'*-DDT **-63.04***↓ BL: NS; NS HC: NS; NS
Ribas-Fito et al. 2002 70 mother-infant pairs	Maternal serum and cord blood DDE. Percentile levels in cord blood (ng/mL) 5th: 0.22 25th: 0.49 50th: 0.85 75th: 1.69 95th: 3.21	BW, CHL, preterm birth, SWGA, SLGA	Adjusted beta for change with doubling in DDE BW: NS CHL: NS Geometric means DDE (ng/mL) Preterm birth: Yes **2.40***↑; No 0.80 SWGA (adjusted): NS; NS SLGA (adjusted): NS; NS
Robledo et al. 2015 234 mother-infant pairs Boys (n=90–113), Girls (n=91–117)	Geometric mean maternal and paternal serum DDT metrics (ng/g) *p,p'*-DDE: Maternal 0.580; Paternal 0.752 *p,p'*-DDT: Maternal 0.012; Paternal 0.014 *o,p'*-DDT: Maternal 0.002; Paternal 0.003	BW, HC, PI	Adjusted beta for change per 1-SD increase maternal or paternal in DDT metric *o,p'*-DDT BW: Maternal boys NS, girls **-195.39***; Paternal boys NS, girls NS HC: Maternal boys NS, girls NS; Paternal boys NS, girls NS DDE PI: Maternal boys NS, girls NS; Paternal boys **0.12***↑, girls **0.01***↑

Table 2-18. Summary of Studies of Associations Between Human Maternal DDT Exposure Metrics and Offspring Weight and Growth Endpoints at Birth[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Sagiv et al. 2007 722 mother-infant pairs	Cord blood DDE (ng/g) Q1: 0–0.20 Q2: 0.20–0.30 Q3: 0.30–0.46 Q4: 0.47–14.93	BW, CHL, HC	Adjusted betas for change per quartile of DDE BW: NS CHL: NS HC: NS
Sharma et al. 2012 100 mother-infant pairs FGR cases (n=50) Normal control cases (n=50)	Mean maternal serum and cord blood DDE and DDT (ng/mL) Serum: Control / FGR; Cord blood: Control / FGR DDE: 2.58 / 2.68 / 1.31 / 1.95 DDT: 0.73 / 1.67 / 0.36 / 0.83	FGR	Adjusted ORs for FGR Serum: DDE NS; **DDT 1.82*↑** Cord blood: DDE NS; **DDT 3.07*↑**
Siddiqui et al. 2003 54 mother-infant pairs IUGR cases (n=30) Normal control cases (n=24)	Mean maternal serum, cord blood, placenta ng/mL DDE: Control / IUGR; ΣDDT: Control / IUGR Serum: 6.32 / 8.79 / 20.0 / 23.4 Placenta: 8.89 / 11.24 / 33.93 / 40.16 Cord: 5.33 / 7.81 / 25.7 / 33.2	IUGR	Adjusted ORs for IUGR Serum: **DDE 1.21*↑**; ΣDDT NS Placenta: DDE NS; ΣDDT NS Cord: DDE NS; ΣDDT NS ORs for IUGR with other DDT metrics were NS
Tan et al. 2009 41 mother-infant pairs	Mean cord blood levels ng/g lipid *p,p'*-DDE: 402 *p,p'*-DDT: 34.5 *p,p'*-DDD: 3.83	BL, BW, HC	Adjusted betas BL: DDE NS; **DDT ~0.1*↑**; **DDD ~0.1*↑** BW: DDE NS; **DDT ~0.1*↑**; **DDD ~0.1*↑** HC: DDE NS; **DDT ~0.1*↑**; **DDD ~0.1*↑** Betas are estimated from graphical presentations.

Table 2-18. Summary of Studies of Associations Between Human Maternal DDT Exposure Metrics and Offspring Weight and Growth Endpoints at Birth[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Vafeiadi et al. 2014 1,117 mother-infant pairs	Geometric mean maternal serum DDE and DDT (ng/mL) DDE: 2.07 DDT: 0.043	BW, GA, HC	Adjusted betas for change in measure per log10 unit increase DDE BW: NS GA: NS HC: NS Further adjustment for gestational weight gain did not change statistical significance
Weihe et al. 2003 500 mother-infant pairs 267 males, 233 females	Mean maternal serum, ng/mL *p,p'*-DDT: 0.175 *p,p'*-DDE: 5.534	BW, BL, HC	Adjusted partial correlation coefficients All: BW NS; BL **-0.115***↓; HC NS DDE: BW NS; BL **-0.115***↓; HC NS DDT: BW NS; BL **-0.097***↓; HC NS Boys DDE: BW NS; BL **-0.140***↓; HC NS DDT: BW **0.163***↑; BL NS; HC NS Girls DDE: BW NS; BL NS; HC NS DDT: BW NS; BL **-0.130***↓; HC NS
Weisskopf et al. 2005 143 mother-infant pairs Captains cohort (n=119) Control (n=24)	Mean maternal serum DDE (ng/mL) Captain cohort: 2.03 Control: 1.0	BW, GA	Adjusted beta for change per log10 unit DDE BW: **-146***↓ GA: NS
Wojtyniak et al. 2010 1,322 mother-infant pairs Greenland Inuit (n=572) Kharkiv (n=611) Warsaw (n=258)	Geometric mean maternal serum DDE (ng/g lipid) Inuit: 273.8 Kharkiv: 653.3 Warsaw: 356.8	BW, GA, preterm birth	Adjusted betas for changes per 1-unit increase lnDDE BW: Inuit **-56.0***↓; Kharkiv NS; Warsaw **-146.9***↓ GA: Inuit **-0.2***↓; Kharkiv NS; Warsaw **-0.6***↓ Adjusted ORs for 2x increase in DDE Preterm: Inuit NS; Kharkiv NS; Warsaw **2.44***↑

Table 2-18. Summary of Studies of Associations Between Human Maternal DDT Exposure Metrics and Offspring Weight and Growth Endpoints at Birth[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Wolff et al. 2007 404 mother-infant pairs	Median (range) maternal serum DDE (ng/mL): 0.64 (0–57.3)	BW, BL, PI, HC, GA	Adjusted betas for changes per a 1-unit increase log10 DDE BW: NS BL: NS PI: NS HC: **-0.69***↓ GA: NS

[a]See Table 11 in the *Supporting Document for Epidemiological Studies for DDT, DDE, and DDD Supporting Document for Epidemiological studies for DDT, DDE, and DDD.* Studies in this table were selected from those described in Table 11, because they: (1) measured DDT-related metrics in biological fluids or tissues in each maternal subject, and (2) evaluated associations using linear regression, logistical regression, or correlation techniques, adjusting for possible confounding factors.
[b]DDE = *p,p'*-DDE, unless otherwise specified; DDT = *p,p'*-DDT, unless otherwise specified.
[c]**Bold text,** "significant" and asterisk indicate statistically significant ($p<0.05$). Arrows "↑ or ↓" indicate direction of change observed for a given outcome (positive or inverse associations, respectively).

BH = birth height; BL = birth length; BW = birth weight; BWz = birth weight z-score; CHL = crown heal length; FGR = fetal growth restriction; GA = gestational age; GL = gestational length; HC = head circumference; IUGR = intrauterine growth retardation; LBW = low birth weight; ND = not detected; NS = not statistically significant; OR = odds ratio; PI = Ponderal Index; Q = quartile; SD = standard deviation; SGA = small for gestational age; SLGA = small length for gestational age; SWGA = Small weight for gestational age; T = tertile

1

Arrebola et al. 2016; Kezios et al. 2013) and the remaining 9 found no association with duration of gestation (Bergonzi et al. 2011; Bjerregaard et al. 2000; Fenster et al. 2006; Jusko et al. 2006; Ribas-Fito et al. 2002; Sagiv et al. 2007). Thirteen studies evaluated infant head circumference at birth: 2 reported associations with decreased head circumference (Al-Saleh et al. 2012; Wolff et al. 2007); 1 reported an association with increased head circumference (Tan et al. 2009); and 10 reported no associations with infant head circumference (Bergonzi et al. 2011; Jusko et al. 2006; Khanjani and Sim 2006; Longnecker et al. 2001; Lopez-Espinosa et al. 2011; Robledo et al. 2015; Sagiv et al. 2007; Vafeiadi et al. 2014; Weihe et al. 2003). Similarly, 13 studies evaluated infant body length measures: 2 reported associations with decreased length (Al-Saleh et al. 2012; Weihe et al. 2003); 1 reported an association with increased length (Tan et al. 2009); and 10 reported no association with body length (Fenster et al. 2006; Jusko et al. 2006; Longnecker et al. 2001; Lopez-Espinosa et al. 2011; Ribas-Fito et al. 2002; Sagiv et al. 2007; Wolff et al. 2007).

Subsequent growth patterns. Inconsistent evidence is provided by 24 epidemiological studies examining possible associations between maternal serum, cord blood, or breast milk levels of DDT, DDD, or DDE and changes in growth patterns of offspring (Table 2-19). Among the 22 studies examining body weight endpoints in offspring (e.g., BMI, BMI z-score, overweight or obese status, rapid infant growth), 11/22 found associations of maternal DDT biometrics with increased weight or weight status (Agay-Shay et al. 2015; Delvaux et al. 2014; Gladen et al. 2000; Iszatt et al. 2015; Karmaus et al. 2002, 2009; Mendez et al. 2011; Ribas-Fito et al. 2006; Tang-Peronard et al. 2014; Vafeiadi et al. 2015; Valvi et al. 2012, 2014; Warner et al. 2014; Table 2-19). Eleven of these 22 studies found no associations of maternal biometrics and offspring weight or weight status (Cupul-Uicab et al. 2010, 2013; Garced et al. 2012; Gladen et al. 2004; Heggeseth et al. 2015; Hoyer et al. 2014; Jusko et al. 2006; Karlsen et al. 2016; Pan et al. 2010; Verhulst et al. 2009; Warner et al. 2013; Table 2-19).

Offspring height status also was assessed in 12/24 of the studies in Table 2-19; 2/12 found associations with decreased height in offspring (Karmaus et al. 2002; Ribas-Fito et al. 2006), but no associations were found in the other 10 studies assessing offspring height (Cupul-Uicab et al. 2010; Delvaux et al. 2014; Garced et al. 2012; Gladen et al. 2000, 2004; Jusko et al. 2006; Karmaus et al. 2009; Pan et al. 2010; Verhulst et al. 2009; Warner et al. 2014; Table 2-19). More details for these studies and other related studies are provided in Table 12 in the *Supporting Document for Epidemiological Studies for DDT, DDE, and DDD*.

Table 2-19. Summary of Studies of Associations between Human Maternal DDT Exposure Metrics and Post-Birth Offspring Body Weight and Growth[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Studies finding at least one statistically significant association between any DDT metric and any child growth parameter			
Agay-Shay et al. 2015 470 consenting mother-child pairs	Mean (range) maternal serum DDE ng/g lipid: 236.4 (7.7–17,263.4) (tertiles not reported) Geometric mean: 126.3	Overweight, BMIz at 7 years	RRs for overweight: T1 referent; T2 **2.31***↑; T3 **2.21***↑ β for BMIz: T1 referent; T2 NS; T3 NS
Delvaux et al. 2014 114 children 57 males, 57 females	Median (range) cord blood and maternal serum (ng/mL) DDE Boys: 0.24 (0.14, 0.44) Girls: 0.23 (0.12, 0.44)	Height, weight, sum of 4 skinfolds, W/H, BMIz, WC at 7–9 years	Adjusted beta for IQR increase DDE: All: Height NS; BMIz NS; Weight NS; WC NS; Skinfolds NS Boys: BMIz NS; WC NS; W/H NS Girls: BMIz NS; WC **1.018***↑; W/H **1.037***↑
Gladen et al. 2000 594 mother-child pairs 316 females, 278 males	Maternal serum, milk, cord blood, and placenta DDE (ng/g fat) Transplacental Group 2: 1,000–2,000 Group 3: 2,000–3,000 Group 4: 3,000–4,000 Group 5: ≥4,000	Height, weight	p-values for increasing adjusted weight or height at 14 years with DDE transplacental exposure groups: Boys: Height NS; Weight **0.025***↑ Girls: Height NS; Weight 0.51 No significant associations found for either height or weight with lactational DDE exposure groups

Table 2-19. Summary of Studies of Associations between Human Maternal DDT Exposure Metrics and Post-Birth Offspring Body Weight and Growth[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Iszatt et al. 2015 Consenting mother-child cohorts: Duisburg (n=222); FLEHS (n=134) HUMIS (n=399); Michalovce (n=938); PELAGIE (n=171). Pooled (n=2,487)	Cord blood and milk DDE (ng/g lipid) Estimated concentrations Prenatal / Postnatal Duisburg 141.4 / 255.3 FLEHS 214.7 / 272.6 HUMIS 63.4 / 177.3 Michalovce 540.5 / 954.3 PELAGIE 73.5 / 75.7	Weight for age z-scores, based on measurements at birth and 24 months	Adjusted Betas for weight for age z-score per total exposure IQR increase in cohorts Duisburg: NS FLEHS: NS HUMIS: NS Michalovce: NS PELAGIE: NS **Pooled prenatal: 0.12*↑** Pooled postnatal: NS
Karmaus et al. 2002 343 mother-child pairs	Child serum DDE (ng/mL) Q2: 210–290 Q3: 300–430 Q4: >440	Height at birth through 10 years	Trend test for decreased height across exposure quartiles at different ages (p-value) Girls / Boys **4–6 weeks** **0.002*↓** / NS **3–4 months** **0.0049*↓** / NS **6–7 months** **0.012*↓** / NS **8 years** **0.009*↓** / NS 9 years NS / **0.0489*↓** No significant trend at birth, 10–12, 12–24, 43–48 months or at 10 years
Karmaus et al. 2009 259 consenting mothers 151 consenting adult daughters (2001–2002) 129 consenting adult daughters (2006–2007)	Maternal serum DDE (ng/mL) Q2: 1.503–2.9 Q3: 2.9–6.1 Q4: 6.1–9.4 Q5: >9.4	Height, weight, BMI in adult offspring (20–50 years old) at 2001–2002 and 2006–2007	Adjusted betas for measures in Q2 and Q3–Q5, versus Q1 Q2 / Q3–Q5 Height NS / NS Weight **5.93*↑** / **9.22*↑** BMI **1.65*↑** / **2.88*↑**

Table 2-19. Summary of Studies of Associations between Human Maternal DDT Exposure Metrics and Post-Birth Offspring Body Weight and Growth[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Mendez et al. 2011 Consenting mother-child pairs 374 normal weight mothers 144 overweight mothers 125 rapid growth children 393 average/slow growth children	Maternal serum DDE (ng/g lipid) Q2: 71.71–116.92 Q3: 116.92–186.17 Q4: >186.17	Rapid infant growth, BMIz at birth, 6, 14 months	Adjusted RRs of infant rapid growth for Q2–Q4, versus Q1 Maternal status Normal weight / Overweight **Q2** **2.42*↑** / NS **Q3** **2.47*↑** / NS **Q4** **2.47*↑** / NS Adjusted RRs for unit ↑ in DDE **BMIz** **1.64*↑** / NS
Ribas-Fito et al. 2006 Consenting mother-child pairs Follow-up visits at 1 year (n=1,540); 4 years (n=1,371); and 7 years (n=1,371)	Maternal serum levels of DDE (ng/mL) Q1: <15 Q2: 15–29 Q3: 30–44 Q4: 45–59 Q5: ≥60	Height at 1, 4, and 7 years	Adjusted betas for change in height, Q5 versus Q1 1 year / 4 years / 7 years Q5 **-0.76*↓** / **-1.24*↓** / **-2.43*↓** Adjusted betas for lower quintiles were NS

Table 2-19. Summary of Studies of Associations between Human Maternal DDT Exposure Metrics and Post-Birth Offspring Body Weight and Growth[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Tang-Peronard et al. 2014 539 mother-child pairs 290 males, 271 females Normal weight mothers (n=390) Overweight mothers (n=195)	Maternal serum and milk DDE Serum levels (ng/g lipid): Q1: <340 Q2: 340–560 Q3: 570–920 Q4: ≥920	BMI, waist circumference, change in BMI and WC from 5 to 7 years old	Adjusted betas for Q3–Q4 versus Q1 or a 10x↑ DDE in children of OW mothers Girls Boys Δ 5–7 BMI WC BMI WC Q3 NS – NS – Q4 **1.11***↑ – NS – 10x **0.46***↑ – NS – At 7 years old Q3 NS **2.20***↑ NS NS Q4 NS **2.21***↑ NS NS 10x NS **0.93***↑ NS NS No significant associations at 5 years old No significant associations with BMI or WC in offspring of normal weight mothers
Vafeiadi et al. 2015 698 mother-child pairs	Maternal serum DDE Geometric mean: 2.036 ng/mL	Early rapid growth (0–6 months), BMIz, obesity status, WC, sum of four skinfolds at 4 years	Adjusted betas or RR (95% CI) for a 10-fold increase in DDE Rapid growth (RR) 1.33 (0.89–1.99) 4-year measures BMIz (beta): **0.27 (0.04–0.51)***↑ Obesity (RR): **3.80 (1.19–12.14)***↑ WC ≥58.6 cm (RR): **3.76 (1.70–8.3)***↑ Skinfold sum (beta): 2.75 (-0.86–6.35)

Table 2-19. Summary of Studies of Associations between Human Maternal DDT Exposure Metrics and Post-Birth Offspring Body Weight and Growth[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Valvi et al. 2012 344 mother-child pairs 178 females, 166 males Child serum (n=216)	Cord blood and child serum DDE and DDT Cord blood (ng/mL) DDE / DDT T1 <0.7 / <0.06 T2 0.7–1.5 / 0.06–0.18 T3 >1.5 / >0.18	Overweight status at 6 years of age	Adjusted RRs for overweight status, T2 or T3 versus T1 DDE: Girls / Boys / Total T2 **2.64*↑** / NS / **1.67*↑** T3 NS / NS / NS DDT T3 NS / **1.96*↑** / NS Associations lost after adjustments for other persistent organochlorine analytes
Valvi et al. 2014 1,285 mother-child pairs Valencia and Gipuzkoa subcohort (n=790)	Maternal serum DDE (ng/g lipid) Q2: >73.6–118.8 Q3: >118.8–203.1 Q4: >203.1	OW status at 14 months old, ERG status (0–6 months)	Adjusted RRs for Q4 versus Q1 or a 10x↑ DDE Total: OW / ERG; Subcohort: OW / ERG Q4 **1.39*↑** / NS / **1.38*↑** / NS 10x **1.15*↑** / **1.13*↑** / **1.16*↑** / NS
Warner et al. 2014 261 mother-child pairs 143 females, 118 males	Maternal serum *p,p'*-DDT, DDE, and *o,p'*-DDT Mean (ng/g serum) *p,p'*-DDT: 1.3 DDE: 1.5 *o,p'*-DDT: 2.9	OW/obese status, increased WC status, BMIz, WCz, percent body fat; all based on data from 9-year-old children	Adjusted betas or ORs for measures in boys per 10x↑ DDT metrics BMIz Beta / WCz beta / OW/obese OR / WC OR *o,p'*-DDT **0.34*↑** / **0.30*↑** / **2.51*↑** / NS *p,p'*-DDT **0.23*↑** / **0.23*↑** / **2.11*↑** / **2.05*↑** *p,p'*-DDE NS / NS / NS / NS No significant associations were observed in girls No associations with percent body fat

Table 2-19. Summary of Studies of Associations between Human Maternal DDT Exposure Metrics and Post-Birth Offspring Body Weight and Growth[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
	Studies finding no associations between any DDT metric and any child growth parameters		
Cupul-Uicab et al. 2010 788 consenting mother-child pairs	Maternal serum DDE and DDT exposure quartiles, ng/g lipid DDE DDT Q2 3,001–6,000 270–750 Q3 6,001–9,000 760–1,990 Q4 ≥9,000 ≥2,000	Overweight, obese, BMI, height in boys between 14 and 22 months	Adjusted betas for Q2–Q4 DDE versus Q1 Height BMI Q2 NS NS Q3 NS NS Q4 NS NS Nonsignificant trend test for height, weight and BMI
Cupul-Uicab et al. 2013 1,833 (for DDE) and 1,902 (for DDT) mother-child pairs	Maternal serum DDE and DDT Percentiles (ng/mL) 25th 95th DDE 16.93 69.63 DDT 6.46 26.57	Overweight, obese, BMI at 7 years old	Adjusted ORs for IQR increase or β DDE DDT OW (OR) NS NS Obese (OR) NS NS BMI (β) NS NS
Garced et al. 2012 253 mother-child pairs	Geometric mean maternal serum DDT and DDE by trimester (ng/mL) DDE DDT 1st 6.3 0.00697 2nd 6.6 0.00581 3rd 7.6 0.00591	Weight, length, BMI, and HC for age, weight for length from birth to 1 year of age	Adjusted betas for doubling of DDT metrics 1st 2nd 3rd Weight NS NS NS Length NS NS NS BMI NS NS NS HC NS NS NS W/L NS NS NS
Gladen et al. 2004 304 mother-child pairs	Maternal serum *p,p'*-DDT, DDE, *o,p'*-DDT, and ΣDDT (ng/g lipid) DDE Q2: 3,000–5,900 Q3: 6,000–8,900 Q4: 9,000–11,900 Q5: ≥12,000	Height, height ratio (%), BMI, triceps, central adiposity (%), skeletal age from birth through 20 years	Adjusted betas for Q4 DDE versus Q1 Height: NS Height ratio: NS BMI: NS Triceps: NS Central adiposity: NS Skeletal age: NS

Table 2-19. Summary of Studies of Associations between Human Maternal DDT Exposure Metrics and Post-Birth Offspring Body Weight and Growth[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Heggeseth et al. 2015 249 mother-child pairs 113 females, 136 males	Maternal serum *p,p'*-DDT, DDE *o,p'*-DDT Geometric mean (ng/g lipid) *p,p'*-DDT: 21.2 *p,p'*-DDE: 1,428 *o,p'*-DDT: 1.7	Probability of being in increasing BMI growth pattern groups, compared with Group 4 showing flat and stable BMI during 2–9 years: Group 1 linear increase through 2–9 years; Group 2 stable with increasing BMI at age 4–5 years; Group 3 stable with increasing BMI at age 6–7 years	Boys showed high RRRs (>1.0) for being in BMI increasing groups (Group 1, Group 2, Group 3) with a 10-fold increase in maternal DDT metrics; after adjustment for baseline risk factors, however, the RRRs were NS For girls, RRRs were <1.0 for being in BMI-increasing groups with or without adjustment
Hoyer et al. 2014 Consenting mother-child pairs in: Greenland (n=525); Poland (n=92); Ukraine (n=492)	Maternal serum and estimated postnatal exposure of DDE Median (ng/g lipid) Greenland: Maternal 300, Postnatal 7,075 Poland: Maternal 385, Postnatal 11,627 Ukraine: Maternal 639, Postnatal 12,535	BMIz, overweight status at 5–9 years old	Adjusted betas for increased BMIz, T3 versus T1 Greenland: Maternal NS, Postnatal NS Poland: Maternal NS, Postnatal NS Ukraine: Maternal NS, Postnatal NS Pooled: Maternal NS, Postnatal NS Test for trend across exposure levels was NS Adjusted ORs for overweight status and maternal or postnatal DDE were reported to be NS

Table 2-19. Summary of Studies of Associations between Human Maternal DDT Exposure Metrics and Post-Birth Offspring Body Weight and Growth[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Jusko et al. 2006 399 mother-child pairs	Maternal serum of *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDT, *o,p'*-DDT and ΣDDT Mean (ng/g lipid) *p,p'*-DDE: 6,850 *p,p'*-DDT: 1,930 *o,p'*-DDT: 270 ΣDDT: 9,050	Sitting height, standing height, standardized height, standardized weight, HC, biacromial distance, chest breadth, and depth, and bi-iliac distance at birth and 5 years old	Adjusted differences between 25th and 75th percentile DDE values for all growth measures at 5 years were NS, except for a small difference in HC (2 mm [95% CI 0–4])
Karlsen et al. 2016 371 mother-child pairs	Maternal serum, cord blood, and child serum DDE, ng/g lipid Maternal / Child T2: 90–220 / 130–310 T3: >220 / >310	Overweight status, BMIz at 5 years old	Adjusted betas BMIz increase per log10-unit increase in maternal DDE 18 months: NS 5 years: NS RR <1.0 for overweight status in two highest tertiles, compared with T1
Pan et al. 2010 210 mother-child pairs	Milk DDT and DDE and calculated LEM over 12 months Range (ng/g lipid) DDE / DDT Milk: 15–2,140 / <LOD–36 LEM: 134–19,260 / 1–326	Weight, length, weight length and weight for length z-scores for measures from birth to 12 months	Adjusted betas for 10 ng/g lipid ↑ in DDT metrics in milk DDE / DDT Weight: NS / NS Length: NS / NS Adjusted betas were NS for all z-score values
Verhulst et al. 2009 138 mother-child pairs	Mean (range) cord blood DDE: 212 (24–1,816) ng/g lipid	BMI standard deviation scores (BMI-SDS), weight-SDS, length-SDS for 1–3 years of age	Adjusted betas for growth-SDS with DDE, 1–3 years old Weight-SDS: NS Length-SDS: NS BMI-SDS: NS

Table 2-19. Summary of Studies of Associations between Human Maternal DDT Exposure Metrics and Post-Birth Offspring Body Weight and Growth[a]

Reference and study population	DDT exposure metric[b]	Outcome evaluated	Results[c]
Warner et al. 2013 270 mother-child pairs	Maternal serum *p,p'*-DDT, DDE, and *o,p'*-DDT Geometric mean (ng/g lipid) *p,p'*-DDT: 20.45 DDE: 1,422.0 *o,p'*-DDT: 1.66	BMIz, obesity status, overweight or obesity status, increased WC status in children at 7 years old	Adjusted ORs for 10x↑ maternal DDT metrics Obese / OW or obese / WC *p,p'*-DDT: NS / NS / NS *p,p'*-DDE: NS / NS / NS *o,p'*-DDT: NS / NS / NS Adjusted BMIz betas were NS for 10-fold increase in each DDT metric

[a]See Table 12 in the *Supporting Document for Epidemiological Studies for DDT, DDE, and DDD.* Studies in this table were selected from those described in Table 12, because they: (1) measured DDT-related metrics in biological fluids or tissues in each maternal subject, and (2) evaluated associations using linear regression, logistical regression, or correlation techniques, adjusting for possible confounding factors.
[b]DDE = *p,p'*-DDE, unless otherwise specified; DDT = *p,p'*-DDT, unless otherwise specified.
[c]**Bold text,** "significant" and asterisk indicate statistically significant (p<0.05). Arrows "↑ or ↓" indicate direction of change observed for a given outcome (positive or inverse associations, respectively).

BMI = body mass index; BMIz = BMI z-score; CI = confidence interval; ERG = early rapid growth; FLEHS = Flemish Environment and Health Studies; HC = head circumference; HUMIS = Norwegian Human Milk Study; IQR = interquartile range; LEM = lactational exposure metric; LOD = limit of detection; NS = not statistically significant; OR = odds ratio; OW = over weight; PELAGIE = Perturbateurs endocriniens, Étude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance; Q = quartile or quintile; RR = relative risk; RRR = relative risk ratio; SDS = standard deviation score; T = tertile; WC = waist circumference; WCz = waist circumference z-score; W/H = waist:height

Developmental Toxicity in Laboratory Animals

Numerous adverse developmental outcomes have been reported in the offspring of rodents exposed to DDT/DDE during gestation and/or lactation. Effects include fetotoxicity, alterations in growth, neurodevelopmental toxicity, and impaired development of the reproductive system; these effects are discussed in greater detail below. Some developmental studies also evaluated cardiovascular (La Merrill et al. 2016), renal (La Merrill et al. 2016), and diabetic (La Merrill et al. 2014a, 2014b) outcomes in the offspring of rats perinatally exposed and evaluated as adults; these effects are considered developmental toxicity and are discussed in Sections 2.5, 2.10, and 2.18, respectively.

Fetotoxicity. Fetotoxicity was observed in mice and rats following gestational and early postnatal exposure to technical DDT or *p,p'*-DDE at dose levels >30 mg/kg/day (Clement and Okey 1974; Yamasaki et al. 2009). Exposure of pregnant mice to 34.3 mg technical DDT/kg on GDs 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). All F1 offspring died within 10 days after birth when exposed *in utero* and through lactation when dams were treated with 41.1 mg/kg/day *p,p;*-DDT (Clement and Okey 1974). No deaths occurred in pups exposed *in utero* only (Craig and Ogilvie 1974). Reduced weaning index and decreased number of PND-21 live pups were observed in female Sprague-Dawley rats given gavage doses of 50 mg *p,p'*-DDE/kg/day, but not 15 mg/kg/day, on GD 6 through PND 20 (Yamasaki et al. 2009).

Birth outcomes and subsequent growth patterns. Results from developmental toxicity studies in laboratory animals show no consistent effects of gestational or lactational exposure to DDT and related compounds on birth weight or early growth parameters. The presence of an effect appears to be dependent on the isomeric form, the dose, and the timing of exposure.

Acute-duration exposures during gestation showed small, but significant increases (9–13%) in body weights in adult offspring from pregnant Sprague-Dawley rat dams orally exposed during GDs 15–19 to 28 mg *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE/kg/day (Gellert and Heinrichs 1975), but not in Long-Evans or Sprague-Dawley male offspring (at 2 days of age, or as adults) of rat dams exposed during GDs 14–18 of gestation up to 100 mg *p,p'*-DDE/kg/day (Gray et al. 1999; You et al. 1999a, 1998). No clear effects on early life body weights were observed in offspring of CF-1 mice gavaged with doses ranging from 0.02 to 100 mg *o,p'*-DDT/kg/day: small decreases at 20 mg/kg/day and small increases at 100 mg/kg/day were observed on PNDs 2 and 5, but no exposure-related differences from control values were observed on

PND 10 (Palanza et al. 2001). In rabbits, acute exposure to doses of 1 mg DDT(NS)/kg/day on GDs 4–7 (Fabro et al. 1984) or to dose levels ≥10 mg *p,p'*-DDT/kg/day by gavage on GDs 7–9 (Hart et al. 1971), resulted in significant reductions in fetal body weights relative to controls (up to 25%). However, treatment late in gestation (GDs 21–23) did not induce such an effect (Hart et al. 1972).

After intermediate-duration exposure studies, a decrease in growth was observed in Wistar rat pups exposed via nursing from dams receiving 16.8 or 42.1 mg *p,p'*-DDT/kg/day or 84 mg *o,p'*-DDT/kg/day, but the effect was reversible once they were switched to a standard diet (Clement and Okey 1974). No abnormal body weights were reported in rat F1 offspring at birth, or any time point up through sacrifice at 10 weeks of age in Sprague-Dawley rats exposed *in utero* through lactation with dam exposure doses up to 50 mg *p,p'*-DDE /kg/day (Yamasaki et al. 2009). Similarly, no exposure-related birth weight changes or changes in growth were observed in F1 and F2 rat pups from a 2-generation study; F1 offspring were exposed from gestation through weaning from dams fed doses as high as 27.7 mg *p,p'*-DDT/kg/day, and then in their diets through mating, gestation, and lactation (Hojo et al. 2006). In contrast, both CD-1 mouse male and female F1 offspring in a 2-generation study, exposed from gestation through 18 months of age to doses of technical DDT as low as 0.4 mg/kg/day showed significant increases in body weights, relative to controls, beginning from 5–9 months of age (Tomatis et al. 1972).

Neurodevelopmental effects. Acute oral administration of DDT isomers *in utero* or to neonates during sensitive periods in nervous system development has caused behavioral and neurochemical changes in mice. Observations include impaired maze learning and memory functions in surviving 1–2-month-old mice whose dams were exposed to 34.3 mg/kg/day technical-DDT during gestation and lactation (Craig and Ogilvie 1974), and increased spontaneous motor activity (reduced habituation) and decreased cerebral cortex muscarinic receptors in 4–7-month-old mice exposed to 0.5 mg/kg/day technical-grade DDT on PND 10, but not on PND 3 or 18 (Eriksson and Nordberg 1986; Eriksson et al. 1990a, 1990b, 1992, 1993; Johansson et al. 1995, 1996 supported by Talts et al. 1998), and increased urine marking behavior in 70-day-old male mice whose dams were exposed to *o,p'*-DDT doses ≥0.018 mg/kg/day during GDs 11–17; however, because of the lack of statistical analysis or description of the number of males observed, it is unclear at what doses increased urine marking behavior became significant (vom Saal et al. 1995). No statistically significant differences in signs of aggression (latency to attack, number of bites, total attack time, and tail rattling) were observed in 30-day-old male mice from dams exposed from GDs 11–17 to 0.018 or 0.18 mg/kg/day *o,p'*-DDT compared to controls (Palanza et al. 1999); however, the percent of attacking males approached significance, and when subgroups of attacking animals only were evaluated, exposed males showed lower bite frequencies, total attack times, and reduced tail rattling. Exposure

during GDs 11–17 to up to 100 mg *o,p'*-DDT/kg/day had no effect on righting reflex or cliff avoidance in pups on PND 2 or 5 (Palanza et al. 2001).

Fetuses (28-day-old) of pregnant rabbits given gavage doses of 1 mg DDT (NS)/kg/day on GDs 4–7 were reported to have decreased brain weight of unspecified magnitude (Fabro et al. 1984); however, male Sprague-Dawley offspring, or the dams, exposed during GD 6 to PND 20 to 5 or 15 mg *p,p'*-DDE/kg/day had no significant changes in relative brain weight (Yamasaki et al. 2009).

Developmental reproductive effects. Findings for effects on developing male reproductive tissues after gestational exposure to *p,p'*-DDT or *p,p'*-DDE include significantly decreased ventral prostate weights in PND-21 male offspring of pregnant Holtzman, Long-Evans, or Sprague-Dawley rats exposed to 50–200 mg *p,p'*-DDE/kg/day on GDs 14–18 (Loeffler and Peterson 1999; Gray 1999); significantly decreased AGD at PND 1 or 2 at ≥100 mg *p,p'*-DDE/kg/day or retained thoracic nipples at PND 13 in male rats gestationally exposed to ≥10 mg *p,p'*-DDE/kg/day (Gray 1999; Kelce et al. 1995; Loeffler and Peterson 1999; You et al. 1998); and decreased number of lipid droplets in Leydig cells, with no changes in testicular testosterone levels, in GD-19.5 male fetuses of Sprague-Dawley rats given 100 mg *p,p'*-DDE/kg/day on GDs 13.5–17.5 (Adamsson et al. 2009). No significant effects on weights of testes, epididymides, seminal vesicles, or ventral prostate were observed in PND 21 male offspring of Sprague-Dawley or Long-Evans rats given gavage doses up to 100 mg *p,p'*-DDE/kg/day on GDs 14–18 (You et al. 1998).

Other reproductive effects associated with gestational exposure to *p,p'*-DDT, DDT(NS), *p,p'*-DDE, or DDE(NS) include decreased fertility index in F1 male and female Sprague-Dawley rats exposed *in utero* to 50 mg *p,p'*-DDE/kg/day on GD 6 to PND 20 (no significant effect on fertility was reported at doses ≤15 mg/kg/day) (Yamasaki et al. 2009); increased resorptions in pregnant New Zealand rabbits exposed to 10 or 50 mg *p,p'*-DDT/kg/day on GDs 7–9, but not when exposure occurred on GDs 21–23 (Hart et al. 1971, 1972); and qualitatively reported histological changes to reproductive organs from adult male Sprague-Dawley rats exposed to gavage doses of 35 mg DDT(NS) or DDE(NS)/kg/day during gestation, lactation, and through PND 90 (Patrick et al. 2016).

Acute, gestational exposure has been associated with delayed vaginal opening and increased ovary weight in female offspring of Sprague-Dawley rat dams given gavage doses of 28 mg/kg/day *o,p'*-DDD or *p,p'*-DDT on GDs 15–19, but these effects were not observed after GD 15–19 exposure to *o,p'*-DDT or

o,p'-DDE at the same dose level (Gellert and Heinrichs 1975). Earlier vaginal opening was observed in female offspring exposed to 50 mg *p,p'*-DDE/kg/day on GD 6 to PND 20 (Yamasaki et al. 2009).

Decreased fertility was reported in F1 female Wistar rat progeny exposed to 128 mg *o,p'*-DDT/kg/day in food during gestation and lactation and bred to nonexposed males at PND 105, but not in F1 female Wistar rat progeny similarly exposed to up to 26 mg *p,p'*-DDT/kg/day (Clement and Okey 1974).

Three reports from the same group of investigators have specified several reproduction-related effects in adult mice after gestational exposure to very low oral doses of *o,p'*-DDT; significantly decreased testes weight in adult male CD-1 mice exposed to 0.018 mg *o,p'*-DDT/kg/day on GDs 11–17 (~12% decreased compared with control values), but not 0.18 mg/kg/day (Palanza et al. 1999); and significantly increased AGD at birth in female offspring of CF-1 mouse dams exposed to gavage doses ~100 mg *o,p'*-DDT/kg/day on GDs 11–17 (the highest dose tested) and in male offspring at doses of ~0.2 and ~100 mg/kg/day, but not at ~0.02, ~2, or ~20 mg/kg/day (Palanza et al. 2001). These observations in adult mice after gestational exposure to *o,p'*-DDT were not included in Table 2-1 and Figure 2-2 due to the lack of supporting evidence for reproductive or developmental effects in laboratory animals at gestational dose levels <10 mg *o,p'*- isomers/kg/day in studies conducted by other laboratories.

Mechanisms of Developmental Effects of DDT, DDD, or DDE. Effects on growth patterns from prenatal or early-life exposure are not a well-established target of exposure to DDT, DDD, or DDE and relevant mechanistic studies were not located, except for those related to associations between obesity, diabetes, and exposure to persistent organochlorine compounds, like DDT, as discussed in Section 2.18.

As discussed in Section 2.15, DDT can disrupt nerve membrane ion fluxes through induced closure of sodium channels, inhibition of potassium transport, and by targeting Na^+/K^+ and Ca^{2+}/Mg^{2+} ATPases, potentiate neurotransmitter release through interference with calcium calmodulin binding, and inhibit the plasma membrane dopamine transporter (DAT) and the vesicular monoamine transporter (VMAT2) (Harada et al. 2016; Hatcher et al. 2008). It is uncertain if these actions may be involved in the increased spontaneous motor activity and decreased cerebral cortex muscarinic receptors observed in 4–7-month-old mice exposed to 0.5 mg/kg/day technical-grade DDT on PND 10 (Eriksson and Nordberg 1986; Eriksson et al. 1990a, 1990b, 1992, 1993; Johansson et al. 1995, 1996 supported by Talts et al. 1998).

Mechanistic studies were not located that explained details of male and female reproduction effects observed in laboratory animals following prenatal and early postnatal exposure to DDT and related

compounds, with the exception of studies showing that *p,p'*- isomers have anti-androgenic effects and *o,p'*- isomers have estrogenic effects (see Section 2.16).

2.18 OTHER NONCANCER

Epidemiology Studies of Diabetic Outcomes in Humans

Overall summary. Table 2-20 describes results from 35 epidemiological studies that examined possible associations between human DDT exposure biometrics (e.g., serum levels of DDT, DDE, or DDD) and prevalence of DMT2 or biomarkers indicative of DMT2. Table 2-20 also describes single studies of possible associations between DDT exposure biometrics and gestational diabetes (Vafeiadi et al. 2017) and Type 1 diabetes (Rignell-Hydbom et al. 2010). All located human studies examining possible associations between DDT exposure biometrics and diabetes outcomes are described in more detail in Table 13 in the *Supporting Document for Epidemiological Studies for DDT, DDE, and DDD.*

A clear majority of studies, including several meta-analyses, provide evidence for an association between DDT exposure biometrics in groups of humans and increased prevalence of DMT2 (Table 2-20). Among the studies evaluating associations between DDT exposure biometrics and DMT2 prevalence, 18 found associations, 2 found marginal associations, and 5 found no associations between DDT exposure biometrics and DMT2 (see Table 2-20). There is inconsistency across eight studies evaluating associations with other indicators of diabetes (e.g., fasting blood glucose [FBG], insulin, HbA1C, homeostatic model assessment insulin resistance [HOMA-IR], insulin resistance [IR], leptin, or adiponectin). Clear explanations of the inconsistency across the studies are not available, but possible contributors could be small case sample sizes and different distributions of age, BMI, gender, and exposure levels; co-exposure to varying levels of other persistent organic pollutants (e.g., PCBs, CDDs, etc.) may also influence the study results.

Associations with DMT2. The 18 studies reporting positive evidence for a statistically significant association between DDT exposure biometrics and DMT2 include five nationwide studies in the United States (Everett and Matheson 2010; Everett and Thompson 2015; Everett et al. 2007, 2017a; Lee et al. 2006). Associations were reported across a wide range of age groups starting from 18 years of age (Aminov et al. 2016). Four U.S. studies examined whether or not adjustments for other organochlorine analytes would influence the statistical significance of the association (Table 2-20). With adjustments for

Table 2-20. Summary of Diabetes Outcomes in Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric	Outcome evaluated	Result[b]
DMT2 prevalence			
Studies providing statistically significant evidence for an association between DDT and DMT2 prevalence (n=18)			
Al-Othman et al. 2015 136 DMT2 cases 144 controls	ΣDDT arithmetic means, serum Cases: 18.3 ng/mL Controls: 11.8 ng/mL	DMT2 based on FBG	Adjusted ORs (95% CI): **M 4.8 (1.3–9.6)*↑** **F 5.7 (1.4–10.3)*↑**
Aminov et al. 2016 111 DMT2 cases 144 controls	DDE 4th quartile range serum: 4.02–22.51 ng/mL	DMT2, based on self report or FBG	Adjusted OR, Q4 versus Q1 (95% CI): **2.69 (1.00–7.16, p trend 0.004)*↑** Significance lost after further adjustment for PCB serum levels
Arrebola et al. 2013 34 DMT2 cases 352 controls	DDE geometric means, ng/g lipid Cases Controls Serum 243.2 152.5 Adipose 199.5 73.7	DMT2, based on self report and FBG	Adjusted ORs, T3 versus T1 (95% CI): Adipose: **4.44 (1.03–21.02*↑, p trend 0.07)** Serum: adjusted ORs (T3 or T2 versus T1) not significantly elevated
Codru et al. 2007 71 DMT2 cases 281 controls	DDE arithmetic mean, serum 537 ng/g lipid	DMT2, based on self report or FBG	Adjusted ORs, T3 versus T1 (95% CI): **6.2 (1.8–21.9)*↑** Significance lost after further adjustment for serum levels of PCBs, HCB, and mirex: 2.4 (0.7–8.3)
Cox et al. 2007 89 DMT2 cases 1,214 controls	DDT 2nd tertile range serum: 2.00–3.70 ng/mL DDE 3rd quartile range serum: 39.1–58.60 ng/mL	DMT2 self reported	DDT Adjusted ORs T3 versus T1 (95% CI): **2.9 (1.2–6.8)*↑** DDE Adjusted ORs, 4th quartile versus 1st quartile (95% CI)**: 2.63 (1.2–5.8)*↑**

Table 2-20. Summary of Diabetes Outcomes in Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric	Outcome evaluated	Result[b]
Eden et al. 2016 114 DMT2 cases 149 controls	DDE arithmetic means, serum Cases: 448.2 ng/g lipid Controls: 197.9 ng/lipid	DMT2 based on self report or medical records	ORs (95% CI) by age and BMI ***Normal BMI <23.5 kg/m²*** **55 years: 2.01 (1.11–3.65)*↑** **65 years: 2.85 (1.39–5.86)*↑** **ORs decreased with increasing BMI groups (27.5 and 31.5 kg/m²), but remained significantly elevated (i.e., >1.0) in the ≥65-year groups**
Everett et al. 2007 2,163 participants with DMT2 diagnosis or undiagnosed	DDT tertile ranges, serum T1: <20.7 ng/g lipid T2: 20.8–26.6 ng/g lipid T3: >26.6 ng/g lipid	DMT2 based on self report of physician diagnosis and HbA1c >6.1% (total diabetes)	Adjusted ORs versus T1 (95% CI) **T2: 2.69 (1.35–5.36)*↑** **T3: 2.46 (1.45–4.15)*↑** **T2 and T3 ORs remained significantly elevated with further adjustment for other chlorinated serum analytes (a dioxin and a PCB)**
Everett et al. 2017a 76 DMT2 participants with total diabetes (self reported or with HbA1c ≥6.5%) without nephropathy and 52 with total diabetes with nephropathy	Non-fasting serum levels *p,p'*-DDT: >14.50 ng/g lipid *p,p'*-DDE: ≥1,195.1 ng/g lipid	Diabetes (with or without nephropathy)	Adjusted OR for total diabetes DDT: **2.46 (1.59–3.79)*↑** DDE: **3.35 (1.40–8.02)*↑** Without nephropathy / With nephropathy DDT **2.14 (1.46-3.15)*↑** / **3.18 (1.59-6.36)*↑** DDE 2.12 / 14.69 (2.94-73.29)**↑**
Everett and Mathescon 2010 334 DMT2 cases (self reported or with HbA1c ≥6.5%) 462 cases with prediabetes (HbA1c 5.7-6.4%)	DDT group range serum T1: <20.7 ng/g lipid T2: >20.7 ng/g lipid DDE tertiles serum T1: <168.6 ng/g lipid T2: >168.6 ng/g lipid	DMT2 based on self report of physician diagnosis and HbA1c ≥6.5% (total diabetes)	Adjusted ORs versus T1 (95% CI**)** **DDT T2: 1.96 (1.29–2.98)*↑** **DDE T2: 1.90 (1.13–3.18)*↑** ORs were *not* significantly elevated with further adjustment for serum levels of six other pesticides

Table 2-20. Summary of Diabetes Outcomes in Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric	Outcome evaluated	Result[b]
Everett and Thompson 2015 341 diabetes cases 2,204 controls	Serum DDE and DDT exposure categories (ng/g blood) DDE / DDT T1 <0.8340 / <0.0860 T2 0.8340–3.8410 / ≥0.0860 T3 ≥3.8410 / –	Diabetes (with or without nephropathy)	Adjusted ORs for T2 or T3 versus T1 Without nephropathy / With nephropathy T2 (DDE) 2.32 / 0.60 T3 (DDE) 2.66 / 0.78 T3 (DDT) 1.82 / **2.08 (1.03–4.11)*↑**
Lee et al. 2006 217 DMT2 cases 1,529 controls	DDE 4th and 5th quintile means serum Q4: 1,560 ng/g lipid Q5: 3,700 ng/g lipid	DMT2 based on self report of physician diagnosis or FBG	Adjusted ORs (95% CI) versus Q1 **Q4: 2.3 (1.0–5.5)*↑** **Q5: 4.3 (1.8–10.2) p trend <0.05*↑**
Philibert et al. 2009 25 DMT2 cases 76 controls	DDE serum percentiles 75th: 10.65 ng/mL 75th: 1,617 ng/g lipid	DMT2 self report of physician diagnosis	Adjusted OR (95% CI) >75th percentile versus <75th percentile **ng/mL: 6.11 (1.37–27.30)*↑** ng/g lipid: 3.56 (0.97–13.08)
Rignell-Hydbom et al. 2007 15 DMT2 cases 528 controls	DDE 3rd and 4th quartile ranges serum: Q3: >144–240 ng/g lipid Q4: >240 ng/g lipid	DMT2 self report of physician diagnosis	**Significant linear trend (p=0.002) for increasing adjusted prevalence across quartiles*** OR (95% CI) for a 100 DDE ng/g lipid change: **1.3 (1.2–1.5)*↑**
Rignell-Hydbom et al. 2009 371 DMT2 cases 371 controls	DDE arithmetic mean serum Cases: 5.68 ng/mL Controls: 3.89 ng/mL (for diagnosis >7 years after baseline)	DMT2 based on glucose tolerance test	Adjusted ORs (95% CI) for diagnoses >7 years after baseline examination **(Q4 versus Q1): 5.5 (1.2–25)*↑**
Rylander et al. 2005 22 DMT2 cases 358 controls	DDE exposure group ranges serum, ng/g lipid M: <410; >410–850; >850 F: <180; >180–290; >290	DMT2 self report	Linear trend for increasing prevalence across groups. **(M: p=0.04*↑**, F = 0.07**)** OR (95% CI) for a 100 DDE ng/g lipid change: **M+F 1.05 (1.01–1.09)*↑**

Table 2-20. Summary of Diabetes Outcomes in Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric	Outcome evaluated	Result[b]
Son et al. 2010 40 DMT2 cases 40 controls	Mean serum ng/g lipid Cases Controls DDE 652 376 DDD 6.6 5.7 *p,p'*-DDT 34.2 23.8 *o,p'*-DDT 4.5 3.2	DMT2 based on self report or FBG	Adjusted ORs (95%), p trend (T3 versus T1) **DDE 26.6 (2.0–349.1)*↑, 0.01*** **DDD 10.8 (1.2–101.1)*↑, 0.03*** ***p,p'-DDT* 7.6 (1.0–57.3)*↑, 0.04*** *o,p'*-DDT 10.1 (0.9–119), 0.07 Above results with ng/mL serum **Similar significant results with ng/g lipid serum**
Turyk et al. 2009 61 DMT2 cases 589 controls	DDE 4th quartile range serum 4.1–24.0 ng/mL	DMT2 self report or HbA1c>6.3%	Adjusted ORs (95% CI) for DMT2, Q4 versus Q1**: 3.6 (NR), trend (<0.05)**↑
Ukropec et al. 2010 296 DMT2 cases 1,751 DMT2 controls 1,269 prediabetic cases 278 prediabetic controls	5th quintile ranges serum DDE: 3,605–22,328 ng/g lipid DDT: 103–940 ng/g lipid	DMT2 based on FBG and 2hBG PD based on FBG and 2hBG	Adjusted ORs (95% CI) for DMT2 versus Q1 **DDE** **DDT** **Q3 1.85 (1.06–3.21)* 1.84 (1.03–2.27)*** **Q4** 1.34 (0.76–2.35) **2.51 (1.43–4.38)*** **Q5 1.94 (1.11–3.78)* 2.49 (1.42–4.35)*** **Similarly significant results reported for analysis of pre-diabetes prevalence**
Studies finding marginally statistically significant associations between DDT biometrics and DMT2 prevalence (n=2)			
Airaksinen et al. 2011 308 DMT2 cases 1,680 controls	DDE quartile ranges serum, ng/g lipid Q1: 9.1–170; Q2: 170–470 Q3: 470–1,200 Q4: 1,200–10,000	DMT2 based on FBG or 2hBG	Adjusted ORs (95% CI) versus Q1 All subjects BMI ≥30 kg/m^2 Q2 1.00 (0.59–1.69) 0.89 (0.37–2.11) Q3 1.62 (0.97–2.69) 1.04 (0.46–2.36) Q4 1.75 (0.96–3.19) 1.82 (0.71–4.65) **p-trend 0.02*** 0.087 p-trends BMI <25 BMI 25–30 0.99 (NS) 0.15 (NS)

Table 2-20. Summary of Diabetes Outcomes in Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric	Outcome evaluated	Result[b]
Kim et al. 2014 25 DMT2 cases 25 controls	Subcutaneous (SAT) or visceral (VAT) adipose tissue ~Median ng/g lipid DDD DDE DDT SAT 4 150 22 VAT 21 120 3	DMT2 based on self report or FBG	Adjusted ORs for ∑DDT in SAT or VAT in 2nd or 3rd tertiles (versus T1) were *not* significantly elevated **Adjusted ORs (95% CI) for DDE in VAT: T2: 2.3 (NR)*** **T3: 7.8 (1.2–49.3, p trend 0.03)*** Adjusted ORs for other single DDT metrics in either tissue were not significantly elevated
Studies finding no statistically significant association between DDT biometrics and DMT2 prevalence (n=5)			
Grandjean et al. 2011 168 DMT2 cases 78 IFG cases 466 controls	DDE geometric means serum DMT2: 3,200 ng/g lipid IFG: 3,100 ng/g lipid Controls: 2,800 ng/g lipid	DMT2 based on physician diagnosis, FBG or HbA1c >6.4%; IFG based on FBG	Adjusted ORs (95% CI) for DMT2 or IFG were not significantly elevated DMT2: 1.01 (0.87–1.16) IFG: 1.13 (0.92–1.37)
Gasull et al. 2012 143 DMT2 cases 202 Prediabetic cases 541 controls	Quartile ranges serum, ng/mL DDT: Q3 0.179–0.349 DDE: Q3 2.64–5.56	DMT2 based on FBG or self report Prediabetic based on FBG	Fully adjusted ORs (95% CI) versus Q1 DMT2 DDT Q4 0.6 (0.3–1.2) DDE Q4 1.0 (0.5–2.1) Prediabetic DDT Q4 0.6 (0,.4–1.1) DDE Q4 1.0 (0.5–1.9) All p-trends >0.05
Lee et al. 2011a 112 DMT2 cases at age 70 years 877 controls at age 70 years 36 DMT2 cases at age 75 years 689 controls at age 75 years	DDE 5th quintile range serum At 70 years old: 4.04–23.27 ng/mL	DMT2 based on self report of FBG	At age 70: adjusted ORs for Q2–Q5 were not significantly elevated versus Q1; p trend 0.11 At age 75: no significant trend across quintile adjusted ORs
Lee et al. 2010 90 DMT2 cases 90 controls	DDE 3rd and 4th quartile range serum Q3: 3.313–5.731 ng/mL Q4: >5.731 ng/mL	DMT2 based on self report or FBG	Adjusted ORs for DDE Q2–Q4 or DDT Q2–Q4 (versus Q1) were not significantly elevated

Table 2-20. Summary of Diabetes Outcomes in Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric	Outcome evaluated	Result[b]
Wu et al. 2013 Study 1: 24 DMT2 cases, 398 controls Study 2: 24 DMT2 cases; 649 controls	Median 3rd tertile serum, ng/g lipid DDE DDT Study 1 2,099.5 83.3 Study 2 1,535.3 120.9	DMT2 self report of physician diagnosis	Adjusted ORs (95% CI) from individual study or pooled analysis (T3 versus T1) were not significantly elevated Pooled results DDE: 1.53 (0.66–3.53), p trend 0.31 DDT: 1.05 (0.48–2.28), p trend 0.90
Diabetes indicators (n=9)			
Arrebola et al. 2015c 109 women with gestational diabetes	DDE 1.54 ng/mL serum (arithmetic mean of 50th percentile)		Adjusted β (95% CI)
		2h IRI	**7.90 (4.67–11.14)***
		ISI-gly	**-0.05 (-0.08 to -0.02)***
		HOMA-IR	NS
		Fasting IRI	NS
		FBG	NS
		2hBG	NS
		HbA1C	NS
Burns et al. 2014 ~300 boys examined at 8–9, 10–11, and 12–13 years old	DDE 1st and 5th quintile ranges, ng/mL Q1: 0.26–0.52 Q5: 2.7–41.3		Adjusted% change (95% CI) p-trend
		Leptin Q2 versus Q1	**-39.2 (-55.3 to -17.3)**
		Leptin Q3 versus Q1	**-43.8 (-57.7 to -25.4)**
		Leptin Q4 versus Q1	**-49.7 (-65.5 to -32.4)**
		Leptin Q5 versus Q1	**-68.8 (71.7 to -48.4), <0.001**
		Insulin Q2–Q4	NS
		Insulin Q5 versus Q1	**-23.1 (-36.4 to -7.0), 0.02**
		HOMA-IR Q2–Q4	NS
		HOMA-IR Q5 versus Q1	**-22.3 (-36.2 to -5.4), 0.04**
			Insulin and HOMA-IR not significant with further adjustment for BMI z-score
		IR	Adjusted ORs for T2–T3 versus T1 were NS

Table 2-20. Summary of Diabetes Outcomes in Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric	Outcome evaluated	Result[b]
Debost-Legrand et al. 2016a, 2016b 268 mother-child pairs	DDE quartile ranges in cord blood Q1: ≤0.100 Q2: 0.100–0.180 Q3: 0.180–0.290 Q4: >0.290	Log-insulin and adiponectin in cord blood	Adjusted betas for Q2–Q4 versus Q1 (boys, girls, and total) Insulin / Adiponectin Q2: NS / NS Q3: NS / NS Q4: NS / NS p-trend: NS / (girls only) **0.03***↓
Dirinck et al. 2014 195 participants	DDE arithmetic means serum Obese: 120.3 ng/g lipid Lean: 99.4 ng/g lipid	Abnormal glucose tolerance	Adjusted ORs (95% CI) **Serum: 3.47 (1.08–11.15, p trend 0.037)***↑ **Adipose: 81.69 (1.18–5,665.89, p trend 0.04)***↑
Langer et al. 2014 Adults general population Young (21–40 years old): 248 males, 330 females Old (41–75 years old): 568 males, 889 females	DDE 50th percentile serum, ng/g lipid Young: 872 males, 926 females Old: 2,340 males, 2,173 females		p for correlation
		FBG	**Young males: <0.01** **Young females: <0.01** **Old males: <0.01** **Old females: <0.01**
		Insulin	**Old males: <0.01** Other groups: NS
Lee et al. 2007b Adults, general population (n=721) Metabolic syndrome cases (n=175)	Serum levels of DDE Exposure percentiles: <25th 25th–50th 50th–75th ≥75th		Adjusted ORs for metabolic factors per DDE percentiles versus <25th 25th–50th / 50th–75th / >75th / p-trend
		Metabolic syndrome	NS / NS / NS / NS
		Waist circumference	NS / NS / NS / NS
		Triacylglycerol	NS / NS / NS / NS
		HDL	NS / NS / NS / NS
		Blood pressure	NS / NS / NS / NS
		FBG	**4.4***↑ / **3.7***↑ / **6.6***↑ / **<0.01***
Lee et al. 2011b 5,115 U.S. adults at study initiation (18–30 years old at initiation)	DDE and DDT, serum; no exposure levels were provided	HOMA-IR at 20-year follow-up (ages 38–50 years)	DDT / DDE Q2: NS / NS Q3: NS / NS Q4: NS / NS p-trend: NS / NS

Table 2-20. Summary of Diabetes Outcomes in Humans with DDT Exposure Biometrics[a]

Reference and study population	DDT exposure metric	Outcome evaluated	Result[b]
Tang-Peronard et al. 2015b 520 mother-child pairs 273 males, 247 females	Median maternal serum DDE (ng/g lipid) Boys: 600 Girls: 600	Insulin >75th % Leptin >75th %	Adjusted ORs for Q2–Q4 versus Q1 Girls: Q2 NS, Q3 NS, Q4 **2.74***↑ Boys: Q2 NS, Q3 NS, Q4 NS Girls: Q2 NS, Q3 NS, Q4 NS Boys: Q2 NS, Q3 NS, Q4 NS p-trends in all cases were NS
Teeyapant et al. 2014 1,137 adults, general population	Means serum ng/g lipid Males / Females DDE 1,539 / 1,547 DDT 135 / 133	FBG	**Significant positive correlation between DDE and FBG** DDT and FBG not significantly correlated
Type 1 diabetes or gestational diabetes prevalence (n=2)			
Rignell-Hydbom et al. 2010 150 Type 1 children 150 controls	DDE quartile ranges in maternal serum, ng/mL Q1: <5.8 Q2: 5.8–9.6 Q3: 9.7–16.8 Q4: >16.8	Type 1 diabetes in children based on parental report of physician diagnosis	Adjusted ORs (95% CI) versus Q1 Q2: 0.58 (0.28–1.20) Q3: 0.51 (0.24–1.07) Q4: 0.64 (0.28–1.46)
Vafeiadi et al. 2017 68 gestational diabetes cases 871 controls	DDE tertile ranges serum, ng/mL T1: 0.15–1.40; T2: >1.40–2.85 T3: >2.85–32.47	Gestational diabetes based on glucose tolerance test	Adjusted ORs (95% CI) versus T1 T2: 0.83 (0.38–1.82) T3: 0.65 (0.28–1.47) p-trend: 0.297

[a]See Table 13 in the *Supporting Document for Epidemiological Studies for DDT, DDE, and DDD* for more detailed descriptions of studies.
[b]**Bold text,** "significant" and asterisk indicate statistically significant (p<0.05). Arrows "↑ or ↓" indicate direction of change observed for a given outcome (positive or inverse associations, respectively). Adjusted OR or adjusted β indicates adjustment for standard confounding variables for diabetes, such as BMI, serum triglycerides and cholesterol, and age. Further adjustments for other analyzed chlorinated compounds or pesticides in biological fluid samples are expressly noted in the table.

2hBG = 2-h blood glucose (in glucose tolerance test); BMI = body mass index; CI = confidence interval; DMT2 = diabetes mellitus type 2; F = female(s); FBG = fasting blood glucose; HbA1c = hemoglobin A1C; HDL = high-density lipoprotein; HOMA-IR = Homeostasis Model Assessment for Insulin Resistance; IFG = impaired fasting glycemia; IR = insulin resistance; IRI = immunoreactive insulin; ISI-gly = Insulin Sensitivity Index; M = male(s); NR = not reported; NS = not statistically significant; OR = odds ratio; Q = quartile or quintile; SAT = subcutaneous adipose tissue; T = tertile; VAT = visceral adipose tissue

other analytes in the biological samples, statistical significance was lost in three of these studies (Aminov et al. 2016; Codru et al. 2007; Everett and Matheson 2010) and not influenced in the fourth (Everett et al. 2007). Two studies with marginal associations with DMT2 include a study by Airaksinen et al. (2011) that reports a significant trend for increasing ORs across DDE exposure quartiles, but individual ORs were not elevated for quartiles 2–4, compared with quartile 1 (Table 2-20). Kim et al. (2014) reported no significant elevation in ORs for DMT2 prevalence for the sum of measured DDT analytes (∑DDT) in subcutaneous or visceral adipose tissues in the two highest exposure tertiles, compared with the first tertile, but found elevated ORs for DDE in visceral adipose tissue in the highest exposure groups (Table 2-20).

Several published meta-analyses provide support for the association between DDT exposure biometrics and DMT2 prevalence. From a consideration of 22 ORs (from 18 studies), Tang et al. (2014) calculated a total OR of 1.33 (95% CI 1.15–1.54) indicative of an association between DDE serum levels and DMT2 prevalence. This analysis also reported significantly elevated ORs for other analytes in these studies: PCB-153 and PCBs (Tang et al. 2014). Evangelou et al. (2016) considered ORs from 14 studies of DMT2 prevalence and calculated a total OR of 1.95 (95% CI 1.44–2.66) for DDE and statistically significant ORs for other analytes included in the evaluated studies (e.g., DDT, dieldrin, heptachlor, hexachlorobenzene). Another analysis of 72 epidemiological studies evaluating associations between persistent organochlorine compounds and DMT2 concluded that heterogeneity of the studies precluded a meta-analysis, but noted that the overall evidence was sufficient to demonstrate an association (but not causality) between several persistent organochlorines (including DDE, PCBs, and dioxins) and DMT2 prevalence (Taylor et al. 2013). Wu et al. (2013) pooled results from their U.S. Nurse's Health study of DMT2 prevalence with data from four other studies (Lee et al. 2010, 2011b; Rignell-Hydbom et al. 2009; Turyk et al. 2009) and reported a marginally elevated total ORs for DDE (OR 1.25 [95% CI 0.94–1.66]). Fakhri et al. (2017) evaluated ORs from six prospective and seven cross-sectional studies; a total OR of 1.52 (1.26–1.84; $p<0.001$) indicates an association between increasing concentrations of *p,p'*-DDE in serum and adipose tissue with increased risk of DMT2.

Associations with diabetes indicators. Eight studies that examined associations between DDT biometrics and biomarkers of diabetes preclude definitive conclusions about possible associations with any specific diabetes biomarker due to the small number of studies evaluating each indicator and marked differences in study populations (Table 2-20; Arrebola et al. 2015c; Burns et al. 2014; Debost-Legrand et al. 2016a, 2016b; Langer et al. 2014; Lee et al. 2007b; Tang-Peronard et al. 2015b; Teeyapant et al. 2014). In the only three studies evaluating adults from the general population, correlation analysis found consistent

associations between DDE serum levels and elevated fasting blood glucose (FBG) levels (Langer et al. 2014; Lee et al. 2007b; Teeyapant et al. 2014). In a study of women with gestational diabetes, linear regression found no evidence of an association with FBG, HOMA-IR, or HbA1C, but significant associations were reported with 2-hour immunoreactive insulin levels (2-hIRI) (Arrebola et al. 2015c). Two studies involved mother-child pairs: Debost-Legrand et al. (2016a, 2016b) found no correlation between levels of DDE and insulin or adiponectin in cord blood and Tang-Peronard et al. (2015b) found that elevated levels of insulin in girls, but not boys, were associated with maternal serum DDE, but only in the highest quartile. In a single study in children, in boys examined at 8 through 13 years of age, DDE serum levels were associated with leptin levels, but not with insulin serum levels or HOMA-IR, when further adjustments for BMI z-scores were taken into account (Burns et al. 2014).

Associations with gestational or Type-1 diabetes. Maternal levels of DDT biomarkers were not associated with prevalence of gestational diabetes (Vafaiadi et al. 2017) or development of Type 1 diabetes in offspring (Rignell-Hydbom et al. 2010). The sparse data for these types of diabetes preclude making definitive conclusions about possible association of gestational diabetes or Type 1 diabetes with levels of DDT exposure biometrics.

Studies of Diabetic Outcomes in Laboratory Animals. There are a limited number of animal studies that directly evaluate associations between exposures to DDT, DDE, or DDD and DMT2. In an acute exposure study, mice exposed orally to 2.0 mg/kg/day *p,p'*-DDE, but not 0.4 mg/kg/day, for 5 days, had significant elevations in FBG levels that persisted for up to 21 days (Howell et al. 2014). Glucose tolerance and levels of Akt phosphorylation (an indicator of insulin-induced glucose disposal) in liver and skeletal muscle were comparable to untreated controls. Hyperglycemia was not associated with changes in measured metabolic hormones or adipokines including insulin, glucagon, leptin, resistin, IL-6, TNFα, or MCP-1 (Howell et al. 2014).

In an intermediate-exposure duration follow-up study, Howell et al. (2015) investigated whether exposure to *p,p'*-DDE would influence the development of obesity and DMT2 using a rodent model of DMT2. Male mice were similarly treated orally with 2.0 mg/kg/day *p,p'*-DDE for 5 days; following 7 days of rest, animals then received 2.0 mg/kg *p,p'*-DDE weekly for 13 weeks, in combination with either a low-fat (LFD) or high-fat diet (HFD) (Howell et al. 2015). Hyperglycemia was observed at 4- and 8-week timepoints in HFD and DDE-HFD animals; by 13 weeks, however, all DDE-HFD exposed animals returned to normoglycemia. This could partially be explained by an observed increase in *Glut4* expression in skeletal muscle of DDE-HFD mice, which facilitates insulin-stimulated glucose uptake,

increased insulin sensitivity, and decreased hepatic glucose production (Howell et al. 2015). FBG levels of the DDE-LFD group were comparable to controls at all time points indicating the complexities of diet and weight influences on DDE activity. In contrast to the hypothesis that DDE exposure may enhance the effects of HFDs on diabetic endpoints, prolonged DDE exposure exhibited protective effects. Mice with prolonged exposure to DDE and HFD had values for these endpoints similar to values for LFD-vehicle controls. Only fasting insulin levels and insulin resistance in DDE-HFD mice were slightly, but significantly, elevated, compared to LFD animals; the values were lower than the HDF-vehicle controls. No metabolic effects or other effects relating to DMT2 were observed in DDE-exposed animals on a LFD (Howell et al. 2015).

In a gestational exposure study, mice exposed perinatally from GD 11.5 to PND 5 to a 1.7 mg/kg/day mixture of *p,p'*-DDT and *o,p'*-DDT had normal glucose tolerance, FBG, insulin, and lipid levels throughout their first 6 months of life (La Merrill et al. 2014a, 2014b). Female mice, but not male mice, however, exhibited signs of compromised thermogenesis including reduced core temperature, oxygen consumption, and energy expenditure, and increased cold intolerance (La Merrill et al. 2014a, 2014b). At 6 months of age, when challenged with a low- or high-fat diet for 12 weeks, DDT gestationally-exposed-only females on HFDs displayed significant glucose intolerance, insulin resistance, and mild dyslipidemia (La Merrill et al. 2014a, 2014b).

An increased incidence and earlier development of diabetes occurred in pre-diabetic female NOD mice administered via intraperitoneal injection 50 mg/kg p,p′-DDE twice weekly for 16 weeks (Cetkovic-Cvrlje et al. 2016). Elevated blood glucose levels were also observed in these mice. Exposure to a lower dose (25 mg/kg) did not result in significant alterations.

Mechanistic Information on DDT Influence on Diabetic Outcomes. DMT2 is a complex disease of metabolic dysfunction that can take years to develop. The underlying etiologic agents include a multitude of both genetic and environmental factors. Emerging evidence suggests that EDCs, including DDT, are capable of disrupting metabolism and inducing obesity, which then can contribute to the development of obesity-related diseases including DMT2 and cardiovascular disease (Lee et al. 2014; Legler et al. 2015; Tang-Peronard et al. 2011).

There is a vast amount of mechanistic information on organochlorines, including DDT, their hormonal influences, and their ability to disrupt lipid and glucose homeostasis, mitochondrial function, energy expenditure, and insulin signaling (for reviews, see Heindel et al. 2017; Ishikawa et al. 2015; Karami-

Mohajeri et al. 2010; Lee et al. 2014; Mrema et al. 2013). Adipose tissue dysfunction and metabolic changes that contribute to obesity are believed to play a major role in the development of insulin resistance, leading to DMT2. Several studies demonstrate the ability of DDT to disrupt lipid homeostasis. *In vitro* studies with *p,p'*-DDT or *p,p'*-DDE suggest there may be AhR-independent effects causing increased adipogenesis (Kim et al. 2016; Mangum et al. 2015; Moreno-Aliaga and Matsumuru 2002), adipocyte fatty acid uptake (Howell and Mangum 2011), and dipokine (adiponectin, leptin, resistin) levels (Howell and Mangum 2011). In HepG2 cells treated with 1 or 10 ng/mL *p,p'*-DDE, Liu et al. (2017a, 2017b) observed acceleration of lipid accumulation, a reduction in mRNA and protein levels of enzymes involved in hepatic fatty acid β-oxidation (Cpt1α, MCAD, SCAD), and reduced ATP turnover in the mitochondria. Other *in vitro* studies further support the hypothesis that *p,p'*-DDE exposure is associated with effects on lipid synthesis, accumulation, degradation, and transport or secretion (Ward et al. 2016). Many of these observations translate to *in vivo* studies in mice. Following an 8-week exposure to 1 mg *p,p'*-DDE/kg/day orally, significant increases in protein levels, but not transcripts, of key enzymes involved in fatty acid synthesis (Fas, Acc, Scd1) in the liver were observed, and alterations in metabolomic profiles relevant to fatty acid metabolism and phospholipids were also noted (Liu et al. 2017a, 2017b). Another *in vivo* study in adult female mice perinatally exposed to DDT observed changed gene expression in transcripts involved in lipolysis (*Pnpla*) and thermogenesis (*Ppargc1a*) (La Merrill et al. 2014a, 2014b).

A human study, evaluating the metabolomes of 1,016 adults 70 years of age from Uppsala, Sweden with known serum DDE levels found evidence consistent with animal studies linking DDE exposures to altered metabolic effects (Salihovic et al. 2016). DDE was significantly inversely associated with seven metabolites, including several lysophosphatidylcholine congeners, which have been linked to diabetes in other studies; an increase in monoacylglycerol (18:2), which has been associated with insulin secretion and obesity in mice; and increased levels of three fatty acid metabolites that play a role in lipid metabolism (Salihovic et al. 2016). It is unclear, however, whether these changes in metabolite levels translate to functional changes that could trigger, or contribute to, obesity and DMT2.

Timing of exposure may be a crucial factor in the development of DDT-related metabolic pathologies. It has been hypothesized that exposure to obesogens, including DDT, during critical phases of development may lead to metabolic-related consequences later in life (Russ et al. 2016). This hypothesis is supported by a study in mice (La Merrill et al. 2014a, 2014b), and in a limited number of epidemiological studies relating early exposure to obesity (see Section 2.3) and hyperinsulinemia (Tang-Peronard et al. 2015b). Because of the crucial roles hormones play during early development, it is hypothesized that DDT

disruption of hormonal activities, including its estrogenic and anti-androgenic effects, during vulnerable windows, could contribute to the disruption in multiple systems involved in metabolism and adipocyte function that contribute to diseases such as DMT2 later in life. Additional long-term mechanistic studies evaluating early-life exposures that monitor effects later in life will help to further test this hypothesis.

2.19 CANCER

Evidence for Cancer in Humans

Scope of the analysis. Numerous studies have examined possible association between levels of DDT, DDD, or DDE in serum or adipose tissues and risks of several types of cancer in groups of humans from many regions throughout the world, including the United States. Multiple case-control epidemiological studies of this type have been published for the following types of cancer, listed in order of decreasing number of studies: breast cancer in women, NHL, prostate cancer, testicular cancer, pancreatic cancer, liver cancer, and endometrial cancer. In addition, there are single studies that examined associations with risks for bladder cancer and colorectal cancer or mortality rates for multiple myeloma or all cancers. The oral route of exposure is the presumed principal route of exposure for the subjects in all of these studied groups, although small contributions from dermal or inhalation exposure cannot be excluded. This section provides overviews of the evidence provided by these specific types of case-control epidemiological studies. The ensuing discussion does not include published studies that examined possible associations between reports of DDT use and cancer risk, because reported-use exposure data are less reliable than internal biometric data. Also not included are studies that compared serum or adipose levels of DDT, DDD, or DDE in cancer cases and controls, but did not examine associations with cancer risk.

Overview of epidemiological results. Consistent evidence from up to 46 case-control studies does not support the hypothesis that serum or adipose tissue levels of DDT, DDE, or DDE in adult women is associated with increased risk of breast cancer (Ingber et al. 2013; Lopez-Cervantes et al. 2004; Park et al. 2014). The lack of an association may be due to the paucity of exposure information during postulated early life periods of breast vulnerability (Cohn 2011; Cohn et al. 2007, 2015). Other case-control studies provide inconsistent evidence for associations with NHL, prostate cancer, or testicular cancer; and no evidence for associations with pancreatic cancer or endometrial cancer (see sections below for references). No evidence for associations was found in single case-control studies for bladder cancer and

colorectal cancer and single studies of mortality rates from multiple myeloma or all cancers. Consistent evidence for associations with liver cancer was found.

Breast cancer. Many epidemiological studies have investigated the association between breast cancer risk in groups of women and levels of DDT or DDE in blood or adipose tissue from the subjects, mostly mature adult women. Wolff et al. (1993) were the first to report a positive association between DDE levels (in serum) and breast cancer prevalence, but many subsequent studies did not find evidence for associations. Bottom-line conclusions from the three most recent meta-analyses of case-control studies examining associations between DDT or DDE levels in serum or adipose tissue and breast cancer were similar: the available evidence does not support the hypothesized association between DDT/DDE levels and risk of breast cancer (Ingber et al. 2013; Lopez-Cervantes et al. 2004; Park et al. 2014). The meta-analyses were based on 22 (Lopez-Cervantes et al. 2004), 46 (Ingber et al. 2013), and 35 studies (Park et al. 2014).

The literature searches for this document identified two new breast cancer case-control studies not included in the meta-analyses, but the results are not expected to affect the overall meta-analytic conclusions (Arrebola et al. 2015a; Boada et al. 2012). Clear associations with DDT biometrics were not found in a study of 69 cases and 56 controls from Tunisia (Arrebola et al. 2015a) or in a study of 121 cases and 103 controls from the Spanish Canary Islands (Boada et al. 2012).

Each of the meta-analyses noted that exposure metrics in most of the case-control studies were measured in mature adult women and may not reflect exposure during early life periods when the breast may be vulnerable (Ingber et al. 2013; Lopez-Cervantes et al. 2004; Park et al. 2014). Cohn (2011) postulated that the lack of an association might be due to the lack exposure metrics during a critical early period of life. Two studies provide support for this hypothesis (Cohn et al. 2007, 2015). In a study of 129 breast cancer cases and 129 controls who provided blood samples shortly after giving birth in 1959–1967, Cohn et al. (2007) reported that the highest category of serum *p,p'*-DDT levels was associated with increased breast cancer risk in a subgroup of women exposed to DDT before 14 years of age (after 1931 when DDT use became widespread), but no association was found in women born before 1931 who were not expected to have been exposed to DDT in early life periods. In a study of 118 breast cancer cases and 118 controls whose mothers provided perinatal blood samples between 1959 and 1967, Cohn et al. (2015) reported that daughters of mothers in the highest category of serum *o,p'*-DDT had higher risk of breast cancer (adjusted OR 3.7, 95% CI 1.5–9.0, p-value 0.0004 for 4th versus 1st quartile).

A single study examined possible associations between DDT or DDE serum levels and increased risk of mortality within 5 or 15 years of breast cancer diagnosis. In a sample of 622 breast cancer cases, Parada et al. (2016) reported that women with DDT levels in the highest tertile of DDT serum levels had an increased risk of dying within the first 5 years of diagnosis from all causes of mortality (adjusted HR 2.9, 95% CI 1.02–4.67) and breast cancer mortality (adjusted HR 2.72, 95% CI 1.04–7.1). At 15 years, the adjusted HRs (95% CI) were 1.42 (0.99–2.06) for all causes and 1.59 (0.90–2.83) for breast cancer mortality.

Non-Hodgkin Lymphoma. Inconsistent evidence comes from a number of case-control studies that examined possible associations between risk for NHL and serum or adipose levels of DDT (Brauner et al. 2012; De Roos et al. 2005; Quintana et al. 2004; Rothman et al. 1997; Spinelli et al. 2007; Viel et al. 2011) or DDE (Bertrand et al. 2010; Brauner et al. 2012; Cocco et al. 2008; De Roos et al. 2005; Engel et al. 2007; Hardell et al. 2001, 2009; Laden et al. 2010; Quintana et al. 2004; Spinelli et al. 2007; Viel et al. 2011). Only a few of these studies reported statistically significant associations between NHL risk and levels of DDE or DDT. For example, Quintana et al. (2004) reported elevated adjusted ORs for NHL in the highest exposure category (compared with the lowest category) with adipose levels of DDT (1.39, not statistically significant) and DDE (1.99 [95% CI 1.14–3.47]), but in logistic models that included adipose levels of another organochlorine pesticide (heptachlor epoxide or beta-benzene hexachloride), the significance of the association between DDE levels and increased risk for NHL was not apparent. In the only other report of an association, Viel et al. (2011) reported ORs for a 10-ng/g lipid increase of 1.03 (95% CI 0.99–1.08) for DDE and 1.20 (95% CI 1.01–1.45) for DDT, indicative of weak associations.

In a meta-analysis of 5 DDT datasets and 11 DDE data sets from all of the references cited above (except Viel et al. 2011), Luo et al. (2016) reported overall adjusted ORs of 1.02 (95% CI 0.81–1.28) for DDT and 1.38 (1.14–1.66) for DDE.

Prostate cancer. Inconsistent evidence is provided by seven case-control studies examining possible associations between serum or adipose levels of DDT, DDD, or DDE and increased risk for prostate cancer (Aronson et al. 2010; Emeville et al. 2015; Hardell et al. 2006a; Pi et al. 2016; Ritchie et al. 2003; Sawada et al. 2010; Xu et al. 2010). No associations with increased risk of prostate cancer were found in: (1) a study that measured DDE in serum from 58 cases and 99 controls from Iowa (Ritchie et al. 2003); (2) a Japanese study that measured *o,p'*-DDT, *p,p'*-DDT, and *p,p'*-DDE in 201 cases and 402 controls (Sawada et al. 2010); (3) a Canadian study that measured DDE and DDT in serum from 79 cases and

135 controls (Aronson et al. 2010); (4) a Swedish study that measured adipose tissue DDE levels in 42 cases and 20 controls (Hardell et al. 2006a); and (5) a U.S. study that used serum DDE measurements in 65 prostate cancer cases and 1,492 noncases from 1999–2004 NHANES data (Xu et al. 2010). Associations were reported in two studies (Emeville et al. 2015; Pi et al. 2016). Pi et al. (2016) reported elevated adjusted ORs (95% CI) for prostate cancer in high versus low serum concentration categories in a study of 60 cases and 60 controls from Singapore for DDE: 5.67 (1.07–5.16), DDD: 2.35 (1.07–5,16), and DDT: 2.35 (1.07–5.16). Emeville et al. (2015) reported an adjusted OR (95% CI) of 1.53 (1.02–2.30) for the highest versus lowest serum DDE quintiles in a study of 567 prostate cancer cases and 655 controls from Guadaloupe in the French West Indies (Emeville et al. 2015).

Published meta-analyses suggest that evidence is not strong for an association between DDT, DDD, or DDE concentrations in serum or adipose tissue and risk for prostate cancer. In a meta-analysis of six of these seven studies (Pi et al. 2016 was not included), plus another report (Kumar et al. 2010) from which the meta-analysis authors calculated an ORs of 2.27 (95% CI 1.21–4.27), Lim et al. (2015) reported an overall OR of 1.41 (95% CI 1.12–1.78). In a separate analysis of data from four studies (Aronson et al. 2010; Ritchie et al. 2003; Sawada et al. 2010; Xu et al. 2010), an overall OR of 1.25 (95% CI 0.86–1.84) was reported for a 10 ng/g lipid increase in DDE serum concentration (Lim et al. 2015). Another meta-analysis reported overall ORs of 1.02 (95% CI 0.69–1.35) for DDE based on data from five studies (Aronson et al. 2010; Emeville et al. 2015; Hardell et al. 2006a; Ritchie et al. 2003; Sawada et al. 2010) and 0.81 (95% CI 0.35–1.26) for DDT based on data from Aronson et al. (2010) and Sawada et al. (2010) (Lewis-Mikhael 2015).

Testicular cancer. Inconsistent evidence is provided by five case-control studies examining possible associations between serum or adipose levels of DDT, DDE, or DDE and increased risk for testicular cancer (Biggs et al. 2008; Giannandrea et al. 2011; Hardell et al. 2006b; McGlynn et al. 2008; Purdue et al. 2009). McGlynn et al. (2008) reported adjusted ORs of 1.71 (95% CI 1.23–2.38) for DDE and 1.13 (0.71–1.82) for DDT, comparing risk for testicular germ cell tumors in the highest versus lowest quartile categories of serum concentrations, in a study of 734 cases and 912 controls who were U.S. military men. No associations between maternal serum DDE levels and risks for testicular cancer were found in a Swedish study of 44 testicular cancer cases (diagnosed between 1997 and 2000) and 45 controls: OR 1.3 (95% CI 0.5–3.0), comparing mother's serum levels above the median versus below the median (Hardell et al. 2006b). Likewise, no associations with concentrations in blood collected from each male subject were reported in studies of: (1) 246 cases and 630 controls diagnosed between 1993 to 2003 in the U.S. state of Washington, comparing adjusted risks in the highest versus lowest tertiles of

serum levels of *o,p'*-DDT, *p,p'*-DDT, or *p,p'*-DDE (Biggs et al. 2008); (2) 49 cases and 51 controls diagnosed through 1999 in Norway, comparing adjusted risks in the highest versus lowest tertiles of serum levels of *o,p'*-DDT, *p,p'*-DDT, or *p,p'*-DDE (Purdue et al. 2009); and (3) 50 cases and 48 controls diagnosed between 2006 and 2008 in Rome, Italy, comparing adjusted risks in groups with levels above and below 0.2 ng DDE/mL serum (Giannandrea et al. 2011).

Pancreatic cancer. No evidence was found in three case-control studies for associations between serum DDE levels and risks for pancreatic cancer (Gasull et al. 2010; Hardell et al. 2007; Hoppin et al. 2000). In a study of 21 Swedish cases and 59 controls, Hardell et al. (2007) reported an adjusted OR of 2.39 (95% CI 0.73–7.78) for pancreatic cancer in subjects with serum DDE levels greater than the median value, compared with subjects with serum DDE levels less than the median. Hardell et al. (2007) noted shorter mean survival time after diagnosis, in cases with serum DDE levels above the median, compared with cases with serum levels below the median (208 versus 427 days). In a study of 108 pancreatic cancer cases and 82 controls from the San Francisco area, an adjusted OR of 2.1 (95% CI 0.9–4.7) was reported for the highest serum DDE concentration tertile, compared with the lowest tertile (Hoppin et al. 2000). In a study of 97 pancreatic cancer cases with information about K-ras mutation in tumor tissue (75 with K-ras mutation and 22 with normal K-ras), no association was found between DDE serum levels and risk for K-ras-mutated pancreatic cancer (Gasull et al. 2010). Likewise, Cocco et al. (2000) found no association between DDE levels in adipose samples collected in 1968 from subjects in 22 U.S. states and mortality rates from pancreatic cancer between 1974 and 1994. In contrast, there were associations between serum DDT and DDD levels and pancreatic cancer deaths in workers exposed at least 10 years prior to death (Garabrant et al. 1992). The RRs were 4.8 (95% CI 1.3–17.6) for DDT (6 cases, 7 controls) and 4.3 (95% CI 1.5–12.4) for DDD (9 cases, 12 controls).

Liver cancer. Three case-control studies of Chinese populations provide consistent evidence of associations between serum DDT levels and increased risk of liver cancer (McGlynn et al. 2006; Persson et al. 2012; Zhao et al. 2012). Adjusted ORs (95% CI) of 3.8 (1.7–8.6) for DDT and 0.8 (0.3–1.7) for DDE, comparing risks in the highest and lowest serum level quintiles, were reported in a study of 168 liver cancer cases and 385 controls from Linxian, China (McGlynn et al. 2006). Adjusted ORs (95% CI) of 2.96 (1.19–7.40) for DDT and 0.81 (0.33–2.03) for DDE, comparing risks in the highest and lowest serum level quintiles, were reported in a study of 473 hepatocellular carcinoma cases and 492 controls from Haimen City, China (Persson et al. 2012). Adjusted ORs (95% CI) of 4.07 (2.72–6.10) for DDT and 1.96 (1.39–2.76) for DDE, comparing risks in the highest and lowest serum level quartiles, were reported in a study of 346 hepatocellular carcinoma cases and 961 controls from Xiamen China (Zhao et al. 2012).

Liver cancer mortality rate increased with adipose DDE levels in U.S. white males and females, but not among African Americans, in a study that examined associations between DDE levels in 1,968 adipose samples and age-adjusted mortality rates between 1974 and 1994 (Cocco et al. 2000).

Endometrial cancer. No associations between serum or adipose tissue levels of DDT, DDE, or *o,p'*-DDT and increased risk of endometrial cancer were found in a study of 90 endometrial cancer cases and 90 controls from five regions of the United States that measured serum levels of DDT, DDE, and *o,p'*-DDT (Sturgeon et al. 1998) and a study of 76 endometrial cancer and 39 controls from two hospitals in Sweden that measured DDE levels in adipose tissue samples (Hardell et al. 2004, also reported in Lindstrom et al. 2004).

Multiple myeloma. Cocco et al. (2000) found no association between DDE levels in adipose samples collected in 1968 from subjects in 22 U.S. states and mortality rates from multiple myeloma between 1974 and 1994.

Bladder cancer. No associations between serum levels of DDT, DDD, or DDE and increased risk of bladder cancer were found in a study of 140 bladder cancer cases and 206 controls from the Spanish Canary Islands (Boada et al. 2016).

Colorectal cancer. No associations between serum level of DDT or DDE and increased risk of colorectal cancer were found in a study of 132 colorectal cancer cases and 72 controls from a hospital in Barcelona Spain (Howsam et al. 2004).

All cancer. No association was found between serum levels of DDT + DDE (collected in 1974–1975) and adjusted mortality rates for any cancer between 1975 and 1985 in a study of 919 adults from Charleston, South Carolina (Austin et al. 1989).

Animal Studies. DDT is one of the most widely studied pesticides in laboratory animals, and data are available from many 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 (*p,p'*-DDT: Kimbrough et al. 1964; technical DDT: Laug et al. 1950; DDT(NS): Numoto et al. 1985) or in hamsters fed 50 mg *p,p'*-DDT/kg/day for 30 weeks (Tanaka et al. 1987).

Chronic exposure (>1 year) to technical DDT, *p,p'*-DDT, or DDT(NS) caused cancer in multiple strains of mice and in some rat studies, but not in dogs; most studies in nonhuman primates have also been negative.

Chronic exposure to DDT (technical DDT, *p,p'*-DDT, or DDT(NS)) produced predominantly liver tumors in several mouse strains ([C57BL/6 x C3H/Anf]F_1, [C57BL/6 x AKR]F_1, BALB/c, Swiss inbred, and CF1) fed DDT at dietary doses as low as 0.33 mg/kg/day for a minimum of 78 weeks (*p,p'*-DDT: Innes et al. 1969; Thorpe and Walker 1973; Tomatis et al. 1972, 1974a; technical DDT: Kashyap et al. 1977; Turusov et al. 1973; DDT(NS): Terracini et al. 1973). An increased incidence of pulmonary adenomas was observed in strain A mice (a susceptible strain for lung tumors) after chronic gavage administration of doses ≥1.7 mg technical DDT/kg/day (Shabad et al. 1973). Malignant lymphomas and lung and liver tumors were also observed in Swiss inbred mice fed 16.5 mg technical DDT/kg/day DDT in food for 80 weeks (Kashyap et al. 1977). No significant increases in tumor incidence were observed in ICR mice administered 16.5 technical DDT/kg/day for 55 weeks in several generations (Del Pup et al. 1978), consistent with the hypothesis that DDT-induced tumors develop in later stages of life with continued exposure. No significantly increased incidences of any type of tumors were observed in B6C3F1 mice fed up to 30.2 mg technical DDT/kg/day for 78 weeks (NCI 1978).

Several multigeneration studies have been conducted in mice. In these studies, exposure of the F1 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 F2 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 (1.7 mg/kg/day) (Shabad et al. 1973) in the F1 generation had a shorter latency period than in the parental generation, but the tumor incidence was comparable and did not increase with consecutive generations.

Liver tumors also have been observed in rats chronically exposed to DDT. Rats maintained on diets containing DDT for >2 years or at doses >25 mg technical DDT/kg/day developed liver tumors, primarily

in female rats (Cabral et al. 1982b; Fitzhugh and Nelson 1947; Rossi et al. 1977). Increased incidences of liver tumors also occurred in rats at doses of 12 mg technical DDT/kg/day for 2 years (Cabral 1982b) and in F334 rats receiving doses ≥1.7 mg *p,p'*-DDT/kg/day for 2 years (Harada et al. 2003, 2006). In contrast, no evidence of carcinogenicity was seen in Osborne-Mendel rats receiving up to 45 mg technical DDT/kg/day for 78 weeks in the NCI (1978) bioassay.

Long-term exposure failed to induce significant increases in tumors in monkeys at doses of 3.9–20 mg DDT(NS)/kg/day for up to 5 years (Adamson and Sieber 1979, 1983; Durham et al. 1963) or in dogs at 80 mg technical DDT/kg/day for 49 months (Lehman 1965). A 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 technical 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 technical 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).

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 *p,p'*-DDE/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 at doses ranging from 12 to 42 mg, *p,p'*-DDE/kg/day for 78 weeks (NCI 1978), but doses of approximately 43 mg *p,p'*-DDE/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 *p,p'*-DDD/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 technical DDD/kg/day (NCI 1978). In rats, 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 exposed to technical DDD, respectively (NCI 1978). 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.

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.

The HHS determined that DDT is "reasonably anticipated to be a human carcinogen", based on sufficient evidence of carcinogenicity in experimental animals (NTP 2016). EPA IRIS last revised carcinogenicity assessments for DDT, DDD, and DDE in 1988, classifying each as a "probable human carcinogen" (Group B2), based on sufficient evidence of carcinogenicity in animals (2002a, 2002b, 2003). IARC determined that DDT is "probably carcinogenic to humans", based on limited evidence in humans and sufficient evidence in experimental animals (IARC 2017).

Mechanisms of Carcinogenicity of DDT, DDE, or DDD. Many epidemiological studies have looked for associations between concentrations of DDT, DDE, or DDD in biological fluids and risks for various types of cancer in human populations. Consistent evidence for positive associations has been presented only for liver cancer; consistent evidence for positive associations is not currently available for any other type of cancer. In contrast, fairly consistent evidence is available for increased incidence of liver tumors in rodents exposed chronically to DDT, DDE, or DDD in food. Harada et al. (2016) recently reviewed evidence that DDT and its metabolites may produce liver tumors in rodents via non-genotoxic mechanisms involving mitogenicity in the liver through activation of the *CAR* and induction of eosinophilic foci in liver cells as a result of oxidative DNA damage, in combination with inhibitory effects on GJIC. Evidence presented included concordance between doses producing liver tumors in F344 rats fed *p,p'*-DDT for 2 years and doses producing: (1) early hepatic induction of *CAR*-mediated CYP isozymes (e.g., CYP2B1, CYP3A2); (2) persistently increased hepatic levels of markers of oxidative stress (lipid peroxide and 8-OHdG); (3) transiently enhanced cell proliferation in the liver; and (4) persistently decreased hepatic levels of GJIC protein px32.

2.20 GENOTOXICITY

The genotoxicity of DDT and related compounds has been examined in humans and animals, and in isolated cell systems. Tables 2-21 and 2-22 summarize pertinent results.

Table 2-21. Genotoxicity of DDT, DDE, and DDD *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Epidemiological evidence			
Human (serum; semen)	Chromosomal aberrations (sex-chromosome aneuploidy, total disomy)	+	McAuliffe et al. 2012
Human (serum; semen)	Chromosome aberrations (sperm aneuploidy)	+	Perry et al. 2016
Human (serum; semen)	Chromosomal aberrations (sex ratio changes)	–	Tiido et al. 2005, 2006
Human (serum; semen)	Chromosomal aberrations (sperm chromatin integrity)	–	Rignell-Hydbom et al. 2005b
Human (serum; semen)	Chromosomal aberrations (sperm chromatin integrity)	–	Spanò et al. 2005
Human (serum; semen)	Chromosomal aberrations (sperm chromatin integrity)	(+)	de Jager et al. 2009
Human (lymphocytes)	Chromosome aberrations (sister chromatid exchanges)	+	Nagayama et al. 2003
Human (lymphocytes)	Micronuclei	–	Alvarado-Hernandez et al. 2013
Human (lymphocytes)	Micronuclei	–	Vine et al. 2001
Human (lymphocytes)	DNA damage (comet assay)	–	Alvarado-Hernandez et al. 2013
Human (lymphocytes)	DNA damage (Fpg-modified comet assay)	+	Franken et al. 2017
Human (lymphocytes)	DNA damage (alkaline-modified comet assay)	–	Franken et al. 2017
Human (lymphocytes)	DNA damage (biomarkers in urine, 8-OHdG)	–	Franken et al. 2017
Human (lymphocytes)	DNA damage (comet assay)	+	Jasso-Pineda et al. 2015
Human (lymphocytes)	DNA damage (comet assay)	(+)	Yáñez et al. 2004
Human (lymphocytes)	DNA damage (methylation)	+	Itoh et al. 2014
Human (lymphocytes)	DNA damage (hypomethylation)	(+)	Kim et al. 2010
Human (lymphocytes)	DNA damage (methylation)	(+)	Rusiecki et al. 2008
Human (serum; semen)	DNA damage (sperm DNA methylation)	–	Consales et al. 2016
Human (serum, semen)	DNA damage (comet assay)	–	Hauser et al. 2003
Human (serum, semen	Sperm DNA fragmentation (TUNEL assay)	–	Stronati et al. 2006

Table 2-21. Genotoxicity of DDT, DDE, and DDD *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Human (peripheral leukocytes)	DNA damage (telomere length)	–	Guzzardi et al. 2016
Human (peripheral leukocytes)	DNA damage (telomere length)	(+)	Shin et al. 2010
Human (buccal cells)	DNA damage (telomere length)	±	Hou et al. 2013
Laboratory animal evidence			
Mouse (spermatocytes)	Chromosomal aberrations	+	Clark 1974
Rat	Chromosomal aberrations	–	Legator et al. 1973
Rabbit (fetus' liver)	Chromosomal aberrations	–	Hart et al. 1972
Mouse (bone marrow)	Chromosomal aberrations	(+)	Larsen and Jalal 1974
Rat (mammary glands)	Chromosomal aberrations	+	Uppala et al. 2005
Rat (mammary glands)	Micronuclei	–	Uppala et al. 2005
Rat (buccal cells)	Micronuclei	+	Canales-Aguirre et al. 2011
Mouse	Dominant lethal	+	Clark 1974
Rat	Dominant lethal	(+)	Palmer et al. 1973
Rat (peripheral blood lymphocytes)	DNA damage	+	Canales-Aguirre et al. 2011
Rat (mammary epithelial cells)	DNA damage	+	Canales-Aguirre et al. 2011
Mouse (inhibition of testicular synthesis)	DNA synthesis	– (DDE)	Seiler 1977
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 results; ± = equivocal; 8-OHdG = 8-hydroxydeoxy-guanosine; DNA = deoxyribonucleic acid

Overview of Genotoxicity Results. For the most part, the evidence for DDT-induced genotoxic effects at the blood levels of DDT (including different isomers and metabolites) currently found in the U.S. population is weak. Genotoxicity has been reported in populations with the highest exposures, usually in foreign countries, and even then, the associations between DDT biomarkers and the outcomes measured have not been strong. Studies in animals *in vivo* have not provided a clear picture, possibly due to differences in the studies' protocols, such as differences in routes of exposure (inhalation, gavage, intraperitoneal injection) or in duration of exposure (single versus repeated doses). Results from *in vitro*

studies in mammalian cells were also mixed, whereas *in vitro* studies in prokaryotic organisms were negative for DDT compounds.

Epidemiological Evidence for Effects on Chromosomes and DNA. Studies of humans exposed to DDT have provided information on effects on chromosomes and DNA using a wide variety of tests (Table 2-21). For example, a study by Nagayama et al. (2003) revealed a positive association (r= -0.247, p=0.05) between the frequency of sister chromatid exchanges (SCEs) in cultured lymphocytes from 10-month-old infants (n=105; 60 males, 45 females) and lactational exposure to DDT (estimated median exposure via maternal milk during the 2nd and 4th months postpartum was 272 mg DDT/kg/day). The negative correlation coefficient reflects the fact that the authors measured the difference in response between negative and positive controls; therefore, as DDT in milk increased, the difference between the two responses became smaller. In another study, an Fpg-modified comet assay in peripheral blood lymphocytes from 606 Belgian adolescents revealed a positive association (28.9% [95% CI: 0.01–66.1]) between increased blood concentrations of DDT (mean in serum was not reported, DDT was detected in only 40% of the blood samples) and DNA damage (Franken et al. 2017). Results from two additional tests, an alkaline-modified comet assay and analysis of 8-OHdG levels in urine (biomarker of DNA damage/repair) produced no associations. In a study of 276 Mexican children living in areas of high risk contamination, high levels of total DDT in blood (1,400–32,000 ng/g lipid) were positively correlated (r=0.59, p<0.01) with DNA damage in peripheral lymphocytes (Jasso-Pineda et al. 2015). Yáñez et al. (2004) reported a correlation between DNA damage in peripheral blood mononuclear cells from 54 healthy women who were residents in malarious communities with previous DDT spraying. Mean serum concentrations of *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE were 4.57, 1.15, and 6.21 ng/mL, respectively; the associations remained significant after accounting for confounding factors (smoking habits, nutrition, alcohol consumption). It should be noted, however, that although the associations were significant (p<0.05), the correlations were weak based on r^2 values of 0.19, 0.11, and 0.21 for DDT, DDE, and DDD, respectively. In a study by Alvarado-Hernandez et al. (2013), no significant correlation was found between frequency of micronuclei or DNA damage and plasma levels of *p,p'*-DDT and *p,p'*-DDE in maternal and umbilical cord blood collected from 50 mother-infant pairs (median levels for DDE and DDT in umbilical cord were 192 and 421 ng/g lipid, respectively; median maternal levels of 472 and 204 ng/g lipid, respectively). Similarly, a study of 302 individuals residing near a waste site in North Carolina found that plasma DDE levels (median 2 ng/mL) were not associated with frequency of micronuclei (Vine et al. 2001).

Epidemiological Evidence for Change in Telomere Length. Three studies provide information regarding DDT and telomere length (Table 2-21). The telomere is a region of repetitive nucleotides at the end of linear eukaryotic chromosomes that is essential for maintaining stability and integrity of the genome; it has been shown that loss (shortening) of the telomere can lead to genomic instability. Evaluation of participants in the Agricultural Health Study (AHS) (a prospective cohort study of nearly 90,000 private pesticide applicators [mostly farmers], their spouses, and commercial pesticide applicators in Iowa and North Carolina) showed an association between shortening of relative telomere length (RTL) and lifetime intensity-weighted days of exposure to DDT (as well as other pesticides) in buccal cell DNA from pesticide sprayers (β -0.006; p for trend=0.03). The association, however, was not significant for lifetime days of use of DDT (β -0.02; p for trend= 0.08) (Hou et al. 2013). No quantitative assessment of exposure was conducted in the AHS. In a cross-sectional study of 84 healthy adult males and females from South Korea, blood levels of *p,p'*-DDE were significantly correlated with increased telomere length in peripheral blood leukocytes ($r=0.31$, $p<0.01$ in models adjusted for age, sex, BMI, cigarette smoking, and alcohol consumption) (Shin et al. 2010). However, the relatively low values for r indicate that the contribution of *p,p'*-DDE to the change in telomere length was minimal. No significant associations were found with *p,p'*-DDD or *p,p'*-DDT. Further analyses categorizing serum *p,p'*-DDE into quintiles showed that telomere length was increased at the lower concentrations of *p,p'*-DDE (<400 ng/g lipid) and decreased at higher concentrations (≥500 ng/g lipid). Shin et al. (2010) had no explanation for the increase in telomere length across low *p,p'*-DDE concentrations, but suggested that *p,p'*-DDE may act as a tumor promoter at low doses. In a study of an elderly population of 1,082 Finish subjects from the Helsinki Birth Cohort Study, there were no significant associations between circulating *p,p'*-DDE (mean concentration of *p,p'*-DDE in serum for all participants was 2.08 ng/mL) and telomere length (Guzzardi et al. 2016).

Epidemiological Evidence for DNA Methylation Effects. Several studies have examined associations between DDT and DNA methylation (Table 2-21). Decreases in global methylation (hypomethylation) are believed to be a product of chromosomal instability and/or increased mutation events and are associated with an increased risk of cancer. In a cross-sectional study, Itoh et al. (2014) reported significant decreases in mean global methylation levels (0.41–0.83% per quartile category, $p\leq0.034$) in leukocyte DNA of 403 Japanese women (aged 20–74 years) who were previously used as controls for a breast cancer case-control study. Mean blood concentrations of *o,p'*-DDT, *p,p'*-DDT and *p,p'*-DDE were 1.6, 9.9, and 370 ng/g lipid, respectively. Kim et al. (2010) reported inverse linear relationships between *p,p'*-DDT and *p,p'*-DDE in serum (medians: 20.2 and 393.0 ng/g lipid, respectively) and global DNA methylation in peripheral blood leukocytes of a population of 86 healthy South Koreans assessed by the

Alu assay, but not when the LINE-1 (long interspersed nucleotide element) assay was used. Yet, Pearson correlation coefficients showed that the associations were weak (r values of -0.29 for *p,p'*-DDE and -0.22 for *p,p'*-DDT). No association was found for *p,p'*-DDD in either assay. Inverse linear relationships also were reported between *p,p'*-DDT and *p,p'*-DDE in plasma (means of 44.03 and 1,624.1 ng/g lipid, respectively) and global DNA methylation in peripheral blood leukocytes in a study of 70 Greenlandic Inuit subjects in adjusted models using the *Alu* assay, but not when using the LINE-1 assay (Rusiecki et al. 2008). As in the Kim et al. (2010) study, associations were weak (r values -0.26 for *p,p'*-DDT and -0.38 for *p,p'*-DDE) even though concentrations of *p,p'*-DDT and *p,p'*-DDE were considerably higher. Consales et al. (2016) did not find consistent associations between plasma *p,p'*-DDE and DNA methylation changes in sperm from 607 fertile men from Greenland, Poland and the Ukraine using four different assays or in separate analyses of the three cohorts (the mean *p,p'*-DDE concentration for the combined cohort was 888.2 ng/g lipid). The most notable finding was an inverse association between *p,p'*-DDE and DNA methylation for the combined cohort in an assay for DNA global methylation (β= -29.20 [95% CI -52.11 to -6.29), but not in tests that measured methylation at specific repetitive DNA sequences.

Epidemiological Evidence for Effects on Sperm Genetic Material. Results of studies concerning DDT and alterations in sperm genetic material were mixed (Table 2-21). No associations were found between *p,p'*-DDE (geometric mean 254 ng/g lipid) and sperm chromatin integrity of 212 male partners of sub-fertile couples who were previously evaluated for fertility at the Massachusetts General Hospital between January 2000 and April 2002 (Hauser et al. 2003). Similarly, there were no associations between *p,p'*-DDE in serum (mean 233 ng/g lipid) and sperm chromatin integrity in a population of 176 Swedish fishermen with low and high consumption of fatty fish (Rignell-Hydbom et al. 2005a) or in a cross sectional study involving 707 adult males from Greenland, Sweden, Poland and the Ukraine (serum *p,p'*-DDE means ranged from 340 to 1,300 ng/g lipid) (Spanò et al. 2005). There was a weak association (only marginally statistically significant) between lipid adjusted *p,p'*-DDT (mean: 109,200 ng/g; median: 83,900 ng/g) and *p,p'*-DDE (mean: 246,200 ng/g; median: 177,800 ng/g) and the incidence of sperm with chromatin defects in a population of 209 men (aged 18–44 years) living in a malaria area in the Limpopo Province, South Africa where DDT is sprayed annually resulting in very high exposure, as evidenced by the measured levels of DDT and DDE in blood (de Jager et al. 2009). A study conducted by Stronati et al. (2006) revealed no correlation between exposure to DDE and sperm DNA fragmentation or apoptotic markers in a group of 652 men (n=200 Inuits from Greenland, 166 from Sweden, 124 from Poland, and 153 from the Ukraine). Similar results were reported when the European populations were taken together and analyzed separately from the Inuit group. McAuliffe et al. (2012) reported associations between

serum *p,p'*-DDE and increased rates of XX, XY, and total sex-chromosome disomy, but not YY disomy in sperm nuclei of 192 adult men (aged 20–54 years). Analysis by *p,p'*-DDE quartiles showed that increases in disomy occurred between the 1st and 2nd quartile with no further increases in the 3rd or 4th quartiles. The adjusted incidence rate ratio (IRR) for the comparison of the 4th quartile (≥1.68 ng/g) with the 1st quartile (≤0.61 ng/g) was 1.27 (95% CI 1.22–1.33). Men were from sub-fertile couples who had previously been evaluated at the Massachusetts General Hospital Fertility Center between January 2000 and May 2003, the geometric mean serum *p,p'*-DDE concentration for the group was 1.11 ng/g serum (McAuliffe et al. 2012). Perry et al. (2016) examined the association between serum *p,p'*-DDE and sperm aneuploidy in a group of 90 adult Faroese men who participated in Faroe Island health studies; cord blood and age-14 serum were also available for a subgroup (n=40). Geometric mean concentrations of *p,p'*-DDE were 280 ng/g lipid, 790 ng/g lipid, and 0.45 ng/mL blood, in adults, adolescents, and cord blood, respectively. Associations were found between *p,p'*-DDE and total disomy in adults (IRR 1.32 [95% CI 1.25–1.35] for 3rd tertile [>390 ng/g lipid] versus referents) and in adolescents (IRR 1.19 [95% CI 1.08–1.32) for 3rd tertile [1,080 ng/g lipid] versus referents), but not for cord blood (IRR 0.94 [95% CI 0.86–1.03] for 3rd tertile [0.54 ng/mL blood] versus referents). Tiido et al. (2006) examined the association between serum *p,p'*-DDE and Y/X chromosome distributions in the same populations studied by Rignell-Hydbom et al. (2005b) and Spanò et al. (2005) (see above). Mean serum concentrations of *p,p'*-DDE ranged from 350 ng/lipid in Swedish fishermen to 1,300 ng/g lipid in men from the Ukraine. Tiido et al. (2006) reported a positive association between Y-chromosome fractions in sperm of Swedish fisherman (β=0.66; 95% CI 0.30–1.02) and natural log-transformed *p,p´*-DDE. However, when *p,p'*-DDE was categorized into quintiles, there was no association for the comparison of the highest quintile (>1,500 ng/g lipid) with the lowest (≤250 ng/g lipid) (β=0.03 [95% CI -2.31–2.36]). No significant associations were found between *p,p'*-DDE and Y-chromosome fractions in populations of men from Greenland (n=157), Poland (n=121), or the Ukraine (n=120).

Evidence for Dominant Lethal Mutations in Laboratory Animals. Consistent evidence for dominant lethal mutations comes from studies in rats, mice, and *Drosophila melanogaster* (Table 2-21). In a dominant lethal assay study, 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). No such effect was observed in animals given intraperitoneal doses of ≤80 mg/kg for 5 consecutive days. In another dominant lethal assay, 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 aberrations (breakage, univalents, and stickiness) in spermatocytes. Clark (1974) also investigated dominant lethal effects in *D. melanogaster*. Male Canton-S *D. melanogaster* were treated with a drop containing 1 µg DDT to the surface of a treacle-meal-agar medium and were then mated sequentially with a brood interval of 3 days. There was a significant increase in the proportion of unhatched eggs in broods 3 and 4, which was attributed to dominant lethal mutations. When DDE was administered in a single oral dose to male mice at the rate of 50 mg/kg, it did not inhibit testicular DNA synthesis (Seiler 1977).

Evidence for Effects on Chromosomes and Micronuclei Induction in Laboratory Animals. Studies evaluating chromosomal effects *in vivo* were mixed (Table 2-21). In a study by Uppala et al. (2005), juvenile rats were exposed to *o,p'*-DDT via subcutaneous injection (50 mg/kg) on days 21, 23, 25, 27, 29, 31, 32, and 34 postpartum; selected rats were also gavaged with 40 mg/kg 7,12-dimethylbenz-[a]anthracene (DMBA, a prototype chemical carcinogen) on day 28. Exposure with or without DMBA resulted in significant increases in the frequency of chromosomal aberrations in mammary cells ($p \leq 0.01$), but did not induce significant increases in micronuclei. Cell proliferation (as measured by BrdU) in mammary cells was also significantly increased in rats treated with DDT and DMBA ($p=0.0005$); however, there was no significance with DDT alone. Legator et al. (1973) reported that rats treated orally (by gavage) with *p,p'*-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 the percent of chromosomal aberrations over the solvent control. BALB/C mice injected intraperitoneally with 25–250 mg/kg DDT in peanut oil exhibited a significantly higher proportion of deletions in bone marrow cells than controls, but gaps, stickiness, and the mitotic index were not significantly affected (Larsen and Jalal 1974). Administration of up to 50 mg *p,p'*-DDT/kg by gavage to rabbits on GDs 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. In a repeated inhalation exposure study, female rats were exposed to approximately 7 mg/m^3 DDT for 8 hours/day, 6 days/week for 5 months (Canales-Aguirre et al. 2011). Repeated exposure caused statistically significant increases in micronuclei of buccal cells, DNA damage in peripheral lymphocytes and mammary epithelial cells (measured by comet tail

length and tail moment), and an increase in lipid peroxidation in mammary tissue (measured by free radical production in tissue).

Host-mediated Assays. Host-mediated assays have also provided mixed results (Table 2-21). Buselmaier et al. (1973) reported positive results for gene mutation in a mouse host-mediated assay in *Serratia marcescens* following injection of DDD; no mutation was observed after exposures to DDT or DDE (additional details were not provided). Clark (1974) reported negative results in host-mediated assay to detect mutations in *Neurospora crassa*. Mice received an initial oral dose of 150 mg/kg DDT in olive oil 3 hours before injection with conidia of *N. crassa*; a second dose of 150 mg/kg was administered 10 hours after injection of conidia. Results indicated that the host did not potentiate mutagenicity in this assay.

Assays with Prokaryotic Cells. As shown in Table 2-22, DDT and related compounds were non-mutagenic and did not induce DNA damage in prokaryotic organisms under the conditions tested. No evidence of gene mutation was found in *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, G46, C3076, or D3052 or *E. coli* strains *Pol-A, WP2 and WP2 uvrA*$^-$ with or without metabolic activation (Fahrig 1974; Fluck et al. 1976; McCann et al. 1975; Probst et al. 1981). Results were negative in recessive lethal test in *Neurospora crassa* (Clark 1974) and in a mitotic gene conversion test in *Saccharomyces cerevisiae* (Fahrig 1974) in the absence of metabolic activation. In addition, tests assessing DNA damage in *Bacillus subtilis* (rec assay) and *E. coli* (col E1 plasmid DNA and DNA cell binding) in the absence of metabolic activation yielded negative results (Griffin and Hill 1978; Kubinski et al. 1981; Shirasu et al. 1976).

Table 2-22. Genotoxicity of DDT, DDE, and DDD *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
Prokaryotic organisms				
Salmonella typhimurium (TA1535, TA1537, TA98, TA100)	Gene mutation	–	–	McCann et al. 1975
S. typhimurium (histidine auxotrophs G46, TA1535, TA1000, C3076, TA1537, D3052, TA1538, TA98)	Gene mutation	–	–	Probst et al. 1981
Escherichia coli (WP2 and WP2 uvrA$^-$*)*	Gene mutation	–	–	Probst et al. 1981

Table 2-22. Genotoxicity of DDT, DDE, and DDD *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
E. 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				
Human (hepatocyte-mediated cell)	Gene mutation	–	–	Tong et al. 1981
Chinese hamster (V79 cells [6-thioguanine resistant mutation])	Gene mutation	–	No data	Tsushimoto et al. 1983
Rat (liver epithelial cell)	Gene mutation	–	No data	Telang et al. 1981
Mouse (L51784 lymphoma cells)	Gene mutation	+	No data	Amacher and Zelljadt 1984
Chinese hamster ovary (CHO) cells	Chromosomal aberrations	+	No data	Amacher and Zelljadt 1984
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
Cultured human lymphocytes	Micronuclei	No data	+	Ennaceur et al. 2008
Cultured human lymphocytes	Micronuclei	No data	+	Gerić et al. 2012
Cultured human lymphocytes	DNA damage	No data	+	Gerić et al. 2012
Cultured human lymphocytes	DNA damage	No data	+	Yáñez et al. 2004
Rat (hepatocytes-UDS)	DNA damage	–	–	Probst et al. 1981
Rat (hepatocytes-UDS)	DNA damage	No data	–	Probst and Hill 1980
Mouse, rat, hamster (hepatocytes-UDS)	DNA damage	No data	–	Maslansky and Williams 1981

+ = positive results; – = negative results; DNA = deoxyribonucleic acid; UDS = unscheduled DNA synthesis

Mutation Assays in Mammalian Cells. The majority of *in vitro* gene mutation studies with mammalian cells were negative (Table 2-22). DDT did not induce gene mutations in human hepatocyte-mediated cells in the presence or absence of metabolic activation (Tong et al. 1981), in Chinese hamster V79 cells (Tsushimoto et al. 1983), or in rat liver epithelial cells in the absence of activation (Telang et al. 1981). Conversely, a study by Amacher and Zelljadt (1984) reported positive results for gene mutations in mouse L51784 lymphoma cells exposed to *p,p'*-DDE at concentrations between 25 and 35 µg/mL in the absence of metabolic activation. Exposure to 16–24 µg/mL was sufficient to produce a dose-related increase in 6TG-resistant colonies.

Chromosomal Effects in Mammalian Cells. Studies assessing chromosomal aberrations in mammalian cells yielded positive results (Table 2-22). Amacher and Zelljadt (1984) reported a significant increase in chromosome aberrations in Chinese hamster ovary cells exposed to 35–40 µg/mL *p,p'*-DDE for 24 hours. Mahr and Miltenburger (1976) reported chromosomal damage in the B14F28 Chinese hamster cell line after exposure to 44–88 ppm *p,p'*-DDT, DDE, or DDD; no effects were observed for DDA. Palmer et al. (1972) also observed similar results in kangaroo rat cells (*Potorus tridactylis*) after exposure to 20–50 µg/mL *p,p'*- and *o,p'*-DDT, DDE, or DDD; *p,p'*-DDA was toxic at 200 µg/mL. Kelly-Garvert and Legator (1973) reported a significant increase in chromosomal aberrations in Chinese hamster V79 cells after exposure to 33–40 µg/mL DDE; no significant increases in aberrations were observed following exposure to similar concentrations of DDT. Ennaceaur et al. (2008) reported a reduction in cell proliferation and an increase in the frequency of micronuclei in cultured human peripheral blood lymphocytes following exposure to 10–80 mM *p,p'*-DDE; however, effects were only significant at the highest tested concentration (80 mM).

DNA Damage Assays in Mammalian Cells. Results were mixed for DNA damage in mammalian cells (Table 2-22). Gerić et al. (2012) observed significant increases in the number of micronucleated cells and in the frequency of DNA damage (measured in a comet assay) in cultured human peripheral blood lymphocytes following exposure to *p,p'*-DDT (0.1 µg/mL), *p,p'*-DDE (4.1 µg/mL), and *p,p'*-DDD (3.9 µg/mL). Yáňez et al. (2004) also reported significant DNA damage in peripheral blood mononuclear cells (measured in a DNA content assay and a comet assay) from healthy human donors following exposure (24–72 hours) to 40, 80, or 100 µg/mL *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD. Conversely, negative results were obtained in three studies evaluating unscheduled DNA synthesis (UDS) in rodents (Maslansky and Williams 1981; Probst and Hill 1980; Probst et al. 1981). Probst et al. (1981) and Probst and Hill (1980) reported negative results for UDS in rat hepatocytes exposed to DDT at concentrations up to 1,000 nmoles/mL. Similarly, Maslansky and Williams (1984) reported negative results for UDS in

primary cultures of mouse, rat, and hamster hepatocytes exposed to DDT, DDD, and DDE (tested up to 10^{-4} M) for 18 hours.