CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

Toxicokinetic data for DDT, DDE, and DDD are summarized below.

- DDT, DDD, and DDE are absorbed following inhalation, oral or dermal exposure, but humans are predominately exposed via the oral route.
- DDT, DDE, and DDD are readily distributed in the lymph and blood to all body tissues and ultimately stored in proportion to the lipid content of the tissue, regardless of the route of exposure.
- Metabolism of DDT is similar in humans, rats, mice, and hamsters. The stable metabolite, *p,p'*-DDE, is found at higher tissue concentrations than DDT and DDD isomers, and DDA [2,2-bis(4-chlorophenyl)acetic acid] is the major urinary metabolite.
- Excretion of DDT in the form of its metabolites is largely via the urine, but DDT excretion also may occur via feces and breast milk. The excretion of DDT is slow, and DDT and DDE may persist in the human body for decades after exposure.

3.1.1 Absorption

Absorption of DDT by the lung is considered to be a minor route of entry, although evidence of DDT absorption after inhalation exposure was indicated by the appearance of DDA (a DDT metabolite) in the urine (Laws et al. 1967; Ortelee 1958), the presence of DDT in adipose tissue (Laws et al. 1967) and the presence of DDT and/or DDE in plasma or serum (Morgan and Lin 1978; Rabello et al. 1975). However, no studies were located that quantified the rate or extent of absorption of DDT, DDE, or DDD in humans after inhalation exposure. No studies were located regarding the absorption of DDT, DDE, or DDD after inhalation exposure in animals.

Absorption following ingestion of DDT, DDE, or DDD is evident in humans both from measurements of serum and adipose tissue concentrations of these chemicals and from measurements of DDA in the urine (Hayes et al. 1956, 1971; Morgan and Roan 1971, 1974). In subjects chronically exposed to oral doses of DDT up to 20 mg/day (approximately 0.3 mg/kg/day), DDT appeared in the serum and reached peak serum concentrations 3 hours after ingestion (Morgan and Roan 1971). Serum levels remained elevated, but returned to near pre-dose values 24 hours after each dose.

The presence of urinary metabolites in mice, rats, and hamsters (Fawcett et al. 1987; Gold and Brunk 1982, 1983, 1984), and the presence of DDT and its metabolites in bile collections (Jensen et al. 1957), provide evidence of gastrointestinal absorption. In animals, absorption of orally administered DDT was enhanced when it is dissolved in digestible oils (Keller and Yeary 1980). Approximately 70–90% of the administered dose was absorbed by rats after oral exposure to DDT in vegetable oils (Keller and Yeary 1980; Rothe et al. 1957). DDT was absorbed 1.5–10 times more effectively in laboratory animals when given in digestible oils than when dissolved in nonabsorbable solvents (Hayes 1982).

Gastrointestinal absorption by way of the intestinal lymphatic system plays a major role in the uptake of DDT in animals (Jandacek et al. 2009; Noguchi et al. 1985; Palin et al 1982; Pocock and Vost 1974; Sieber 1976; Turner and Shanks 1980). For example, Sieber (1976) showed that 12–24% of the administered dose was recovered in the 24-hour lymph after intraduodenal administration of ^{14}C-isomers to thoracic duct-cannulated rats, and most of the radioactivity was attributed to parent compounds. Other studies indicate that relatively little DDT is absorbed from the gastrointestinal tract directly into the blood (Jandacek et al. 2009; Palin et al. 1982; Rothe et al. 1957). In studies of rats with cannulated mesenteric lymph ducts and portal veins, radioactivity collected in 4 hours from lymph ducts and portal veins accounted for 29.4 and 4.6%, respectively, of administered radioactivity delivered intraduodenally as ^{14}C-*p,p'*-DDT in olive oil (Jandacek et al. 2009). Similar results were reported after administration of ^{14}C-*p,p'*-DDE (Jandacek et al. 2009).

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

3.1.2 Distribution

The distribution and storage of DDT in humans and animals has been extensively studied. DDT and its metabolites, DDE and DDD, are lipid-soluble compounds. Once absorbed, they are readily distributed

via the lymph and blood to all body tissues and are stored in these tissues generally in proportion to organ tissue lipid content (Morgan and Roan 1971).

Hayes et al. (1971) and Morgan and Roan (1971, 1974) evaluated the distribution of orally administered DDT, DDE, or DDD in volunteers. Morgan and Roan (1971, 1974) and Roan et al. (1971) measured the concentration of DDT, DDE, DDD, and DDA in blood, fat, and urine after oral dosing. The administered doses ranged from 5 to 20 mg DDT/kg/day for up to 6 months; the ratio of concentration of DDT stored in adipose tissue to that present in blood was estimated to be 280:1. DDT uptake into tissues is a function of the blood flow, the lipid content of that tissue, and the partition coefficient for DDT between the blood and lipids in specific organs. The ratio of DDT concentrations in adipose tissue to blood may remain relatively constant; however, the amount of DDT from past exposure cannot be determined from present blood levels only. DDT, DDE, and DDD have been reported to be distributed to, and retained in, the adipose tissue of humans (Morgan and Roan 1971). The affinity for storage in adipose tissue is related to each chemical's lipophilicity and increases in the order *p,p'*-DDD ≤ *o,p,'*-DDT < *p,p'*-DDT < *p,p'*-DDE (Morgan and Roan 1971).

DDT and DDE selectively partition into fatty tissue and into human breast milk, which has a higher fat content than cow's milk. In a 1969–1970 U.S. national human milk study, the *p,p'*- isomers of DDT and DDE were found in 100% of the samples tested, with mean concentrations of 0.19 and 1.9 ppm (lipid-basis), respectively (Takei et al. 1983). Variance in levels of DDT and its metabolites in breast milk may be influenced by such factors as number of parity, children nursed, diet, and cigarette smoking (Bouwman et al. 1990; Bradt and Herrenkohl 1976; Rogan et al. 1986). A steady decrease in the levels of DDT and its metabolites in human milk has been reported as a result of decreased intake of DDT in many regions throughout the world (Needham et al. 2011; Smith 1999; Wickstrom et al. 1983). In recent global surveys of human breast milk samples between 2000 and 2010, ΣDDT concentrations ranged from <100 ng/g lipid in several northern European nations, 100–1,000 ng/g lipid in the United States, Brazil, Chile, Australia, Russia, Spain, and other countries, to >1,000 ng/g lipid in India, Haiti, Mauritius, Mali, the Philippines, Hong Kong, and other countries (Van den Berg et al. 2016).

DDT and metabolites are known to cross the placenta from their detection in samples of maternal blood levels, umbilical cord blood, placenta, and newborn blood from numerous studies of mother/infant pairs. For example, a study of 90 mother/infant pairs from Mexico found that: (1) all 90 cord blood samples had detectable levels of *p,p'*-DDE, 9 had detectable levels of *o,p'*-DDT, and 44 had measurable levels of *p,p'*-DDT; (2) concentrations in maternal blood were similar to those in cord blood; and

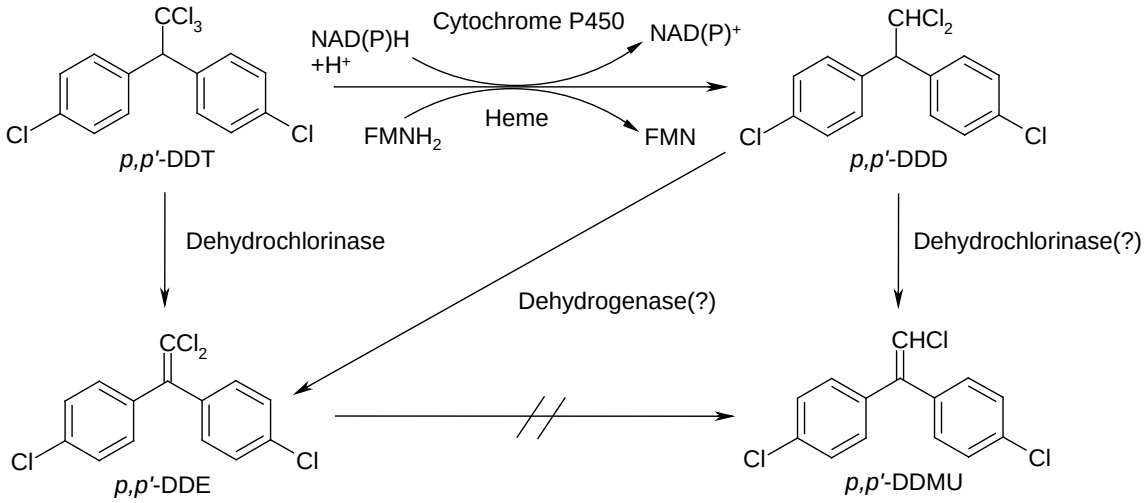
(3) concentrations showed the following order: *p,p'*-DDE > *p,p'*-DDT > *o,p'*-DDT (Waliszewki et al. 2000). In a 2000–2002 study of placentas from 150 mother-infant pairs in Spain, median concentrations in placentas were 2.37 ng/g placenta for *p,p'*-DDE, 1.42 ng/g for *o,p'*-DDD, 1.02 ng/g for *p,p'*-DDT, and 0.60 ng/g for *o,p'*-DDT (Lopez-Espinosa et al. 2007). In a 2014 report on 42 placenta specimens collected in three regions of the United States, DDE concentrations ranged from 10 to 1,968 ng/g tissue, with a median of 74 ng/g (Nanes et al. 2014). A recent review of global monitoring studies of DDE placental concentrations indicated a wide range from 58 pg/g lipid to $5x10^6$ pg/g lipid, with a declining trend over time and high variability in recent years (Nanes et al. 2014).

Results from studies of laboratory animals have demonstrated the preferential distribution of DDT and metabolites to fatty tissue, as well as the transplacental and lactational transfer. For example, in rats after a single intravenous dose of radiolabeled 5 mg *p,p'*-DDE/kg, peak concentrations of DDE were observed before 1 hour in the liver and muscle, at 3 hours in the skin, and between 1 and 4 days in adipose tissue (Mühlebach et al. 1991). Between 4 and 14 days after exposure, the tissue/blood concentration ratio was about 6 for liver and muscle, 35 for skin, and 400 for adipose tissue (Mühlebach et al. 1991). Similar results were found in a study designed to induce diabetes in high saturated fat-fed mice, administered DDE for 5 days followed by weekly gavage doses of DDE for 13 weeks; the adipose/serum and liver/serum concentration ratios were approximately 950 and 70, respectively (Howell et al. 2015). Another study of rats administered DDE for 5 days showed that serum and liver DDE levels significantly decreased between 7 and 21 days post-exposure; in contrast, adipose levels increased (although the change between days 7 and 21 was not statistically significant) (Howell et al. 2014). Evidence for transplacental and lactational transfer include observations that newborn rats of dams given *p,p'*-DDT in the diet before mating and throughout gestation had detectable levels of *p,p'*-DDT in the brain, liver, kidneys, and stomach, which were lower than levels in offspring sacrificed after suckling (Woolley and Talens 1971). In rats dams given gavage doses of *p,p'*-DDE before mating and during gestation, tissue concentrations in dams after suckling were only about 1/3 of values immediately after exposure, indicating substantial transfer of stored *p,p'*-DDE in rat dam tissue to the milk (You et al. 1999b). Other observation indicate that tissue burdens of rat offspring are influenced more by lactational exposure than gestational exposure. Rat offspring of dams given gavage doses of *p,p'*-DDE only before mating and during gestation had lower tissue concentrations than tissue concentrations in offspring of dams exposed only during lactation (You et al. 1999b).

3.1.3 Metabolism

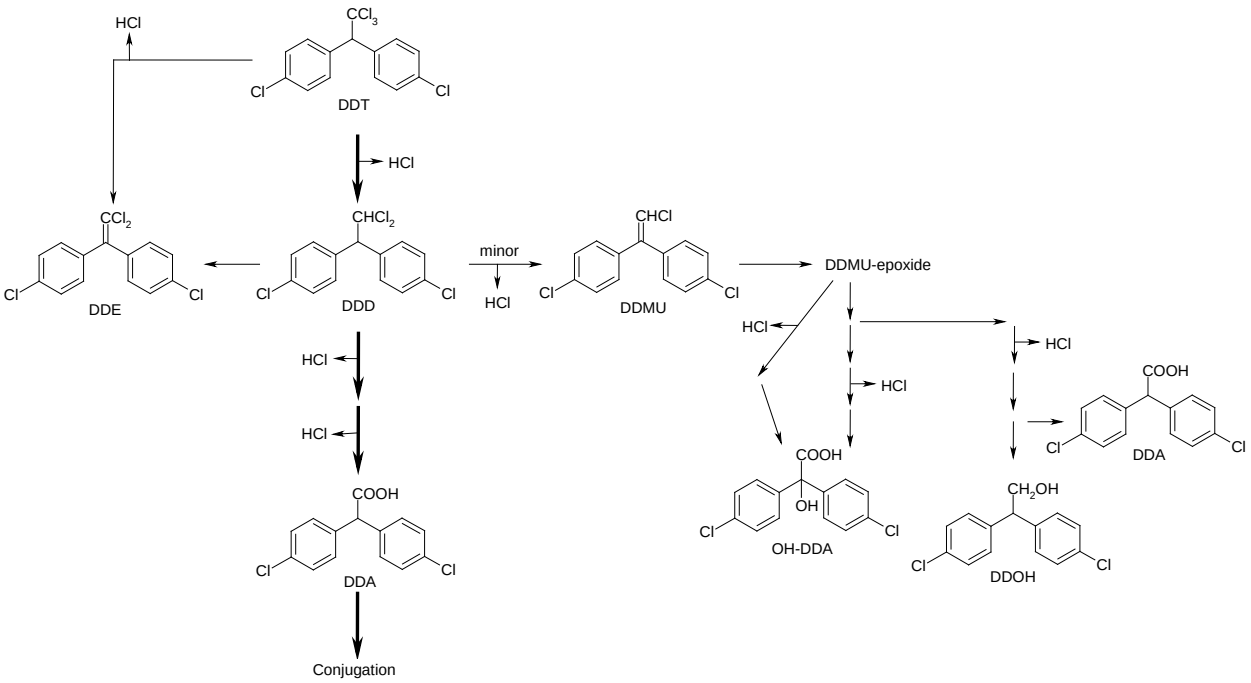
The metabolism of DDT, DDE, and DDD has been studied in humans and a variety of other mammalian species. Observations of higher levels of *p,p'*-DDE in human and animal tissues than levels of *p,p'*-DDT have identified *p,p'*-DDE as a principal stable metabolite (Morgan and Roan 1971; You et al. 1999c). Other studies with liver tissue from laboratory animals established that *p,p'*-DDD is a principal intermediate in the pathway to *p,p'*-DDE involving reductive dechlorination of *p,p'*-DDT to *p,p'*-DDD and a dehydrogenase conversion of *p,p'*-DDD to *p,p'*-DDE (Kitamura et al. 2002). Figure 3-1 describes an initial metabolic pathway that proposes the formation of *p,p'*-DDE directly from *p,p'*-DDT and through *p,p'*-DDD, as well as a dehydrochlorinase step converting *p,p'*-DDD to *p,p'*-DDMU [1-chloro-2,2-bis(4-chlorophenyl)ethylene], another principal metabolite identified in studies with rat liver microsomes (Kitamura et al. 2002). After Phase I metabolism (reactions involving oxidation, reduction, and hydrolysis), many of the DDT metabolites ultimately are excreted in the conjugated form. Conjugates have been reported to include glycine, bile acid conjugates, serine, aspartic acid, and glucuronic acid (Gingell 1975; Pinto et al. 1965; Reif and Sinsheimer 1975). The principal metabolite excreted in urine of animals is *p,p'*-DDA, which has been proposed to be oxidized from *p,p'*-DDT and *p,p'*-DDD in a postulated scheme described in Figure 3-2 (Gold and Brunk 1982).

Figure 3-1. Proposed Metabolic Pathway of *p,p'*-DDT by Rat Liver Microsomes



Source: Kitamura et al. 2002

Figure 3-2. Proposed Metabolic Scheme Converting DDT to DDA, the Principal Metabolite Excreted in Rat Urine



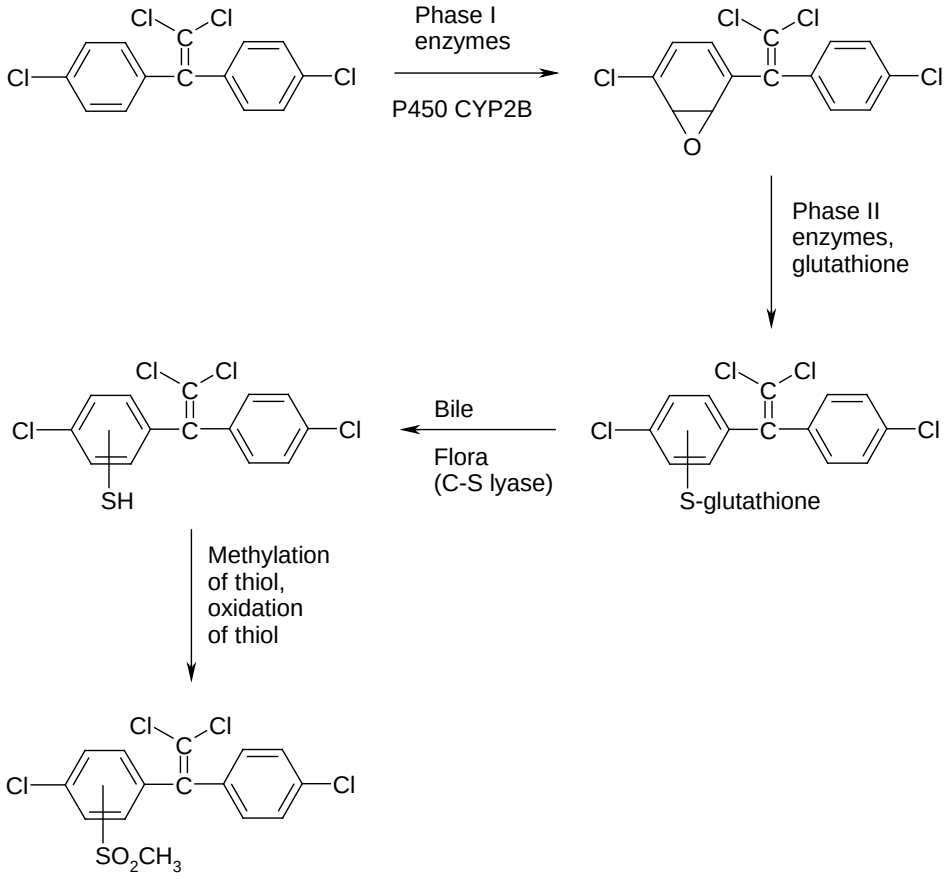
Sources: Gold and Brunk 1982; Kitamura et al. 2002

p,p'-DDT, *p,p'*-DDE, and *p,p'*-DDD are phenobarbital-type cytochrome P-450 (CYP) inducers in rats, causing induction of hepatic CYP2B and CYP3A proteins and CYP1A protein induction to a lesser extent (Nims et al. 1998). Activities for the dechlorinating formation of *p,p'*-DDD from *p,p'*-DDT were much higher with human recombinant CYP3A4 and 2B6 (expressed in human B lymphoblast cells) than with CYP2D6, CYP 2A6, CYP1A1, CYP1A2, and 4A11 (Kitamura et al. 2002). Together, these observations indicate that the metabolic dechlorination of *p,p'*-DDT may be enhanced by previous exposure to DDT and its metabolites (Kitamura et al. 2002; Nims et al. 1998).

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

oxidized to the corresponding methylsulfones, which are distributed by the blood (Haraguchi et al. 1989). Figure 3-3 shows a proposed pathway for sulfonyl metabolites.

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



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

3.1.4 Excretion

Excretion of DDT has been studied in humans and a variety of animals. The major route of excretion of absorbed DDT in humans appears to be as DDA conjugates in the urine (Hayes et al. 1956, 1971; Roan et al. 1971), but some excretion also occurs by way of feces (via biliary excretion) (Jensen et al. 1957) and breast milk (Takei et al. 1983). Results of studies with mice, rats, and hamsters indicate that the metabolites of DDT and small amounts of unmetabolized DDT are excreted primarily in the urine and to a lesser degree in feces (Gold and Brunk 1982, 1983, 1984).

The biological half-lives for the elimination of these compounds are ranked as follows: DDE > DDT > DDD. This relationship, and the observation that DDT and DDE can persist for decades in the human body, has been explained to be collectively due to the chemical stability of each compound in the body (i.e., relatively low metabolic efficiencies), the relative efficiencies of excretory mechanisms, and transport in and out of fat depots (Morgan and Roan 1971, 1974). As mentioned in Section 3.1.3, observations of higher levels of *p,p'*-DDE in human and animal tissues than levels of *p,p'*-DDT identify *p,p'*-DDE as a principal stable metabolite (Morgan and Roan 1971; You et al. 1999c).

In volunteers receiving 35 mg DDT/day (approximately 0.5 mg/kg) for up to 18 months, urinary excretion of DDA increased rapidly for the first few days and a steady-state excretion of approximately 13–16% of the daily dose was reached and remained stable for 56 weeks (Hayes et al. 1971). No DDT metabolites were detected in feces. An earlier study by this group (Hayes et al. 1956) reported DDT and DDE levels in the feces of one volunteer receiving approximately 35 mg DDT/day; although DDA was not detected, the investigators did not exclude that it was present in the sample. Another study reported that elevated rates of urinary excretion of DDA occurred within 24 hours of administering single oral doses of DDT (5, 10, or 20 mg), or DDD (5 mg) to volunteers and did not return to pre-exposure rates until >4 months after ingestion (Roan et al. 1971)

Studies with bile-cannulated laboratory animals have demonstrated that some fecal elimination of DDT metabolites can occur through enterohepatic circulation of conjugated DDA (Gingell 1975; Jensen et al. 1957; Pinto 1965). Other studies with rats given single intravenous doses of radiolabeled DDE indicated a body burden half-life of 120 days with 34 and 1% of the administered dose excreted in feces and urine, respectively, collected for 14 days after dose administration (Mühlebach et al. 1991).

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

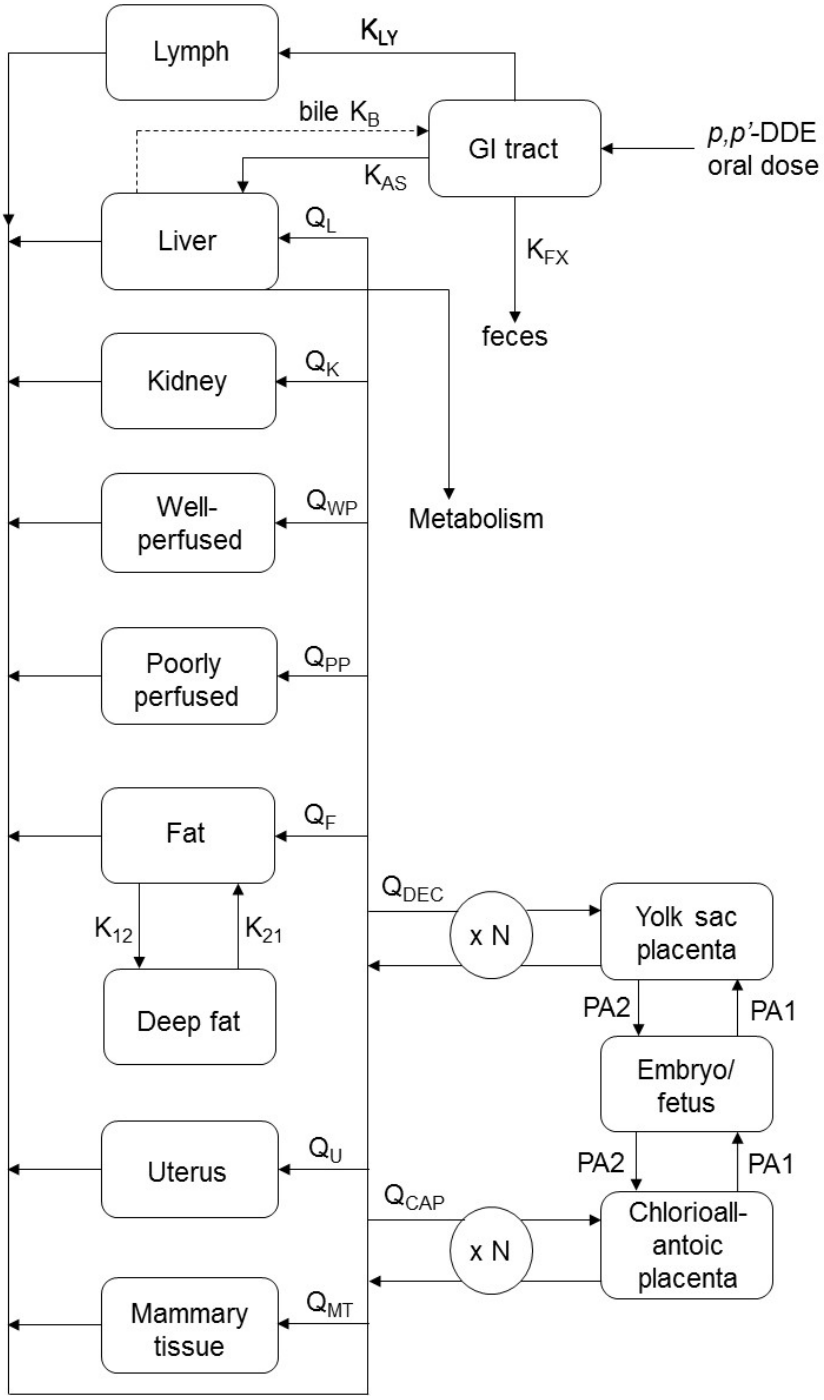
PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly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

PBPK models of the pharmacokinetics of *p,p'*-DDE, a principal metabolite of DDT, in pregnant and lactating rat dams and nursing pups have been developed by You et al. (1999b). The models were based on experimental studies in which pregnant Sprague-Dawley rats were administered gavage doses of *p,p'*-DDE, and the kinetics of *p,p'*-DDE tissue and blood levels in the dams, fetuses, and pups were measured. The models provide an approach to estimating tissue doses in fetuses and pups associated with maternal oral exposure to *p,p'*-DDE and can be used to explore dose-response relationships for the developmental effects of *p,p'*-DDE in the Sprague-Dawley rat, but are inadequately developed or calibrated to extrapolate to other physiological states, other species (most importantly humans), or other routes of exposure, such as the inhalation or dermal routes.

Figures 3-4 and 3-5 show conceptualized representations of the gestation model and the lactation/nursing models, respectively. Parameters used in the models are shown in Tables 3-1 and 3-2. The gestation model simulates the kinetics of transfer of an oral dose of *p,p'*-DDE from the dam to the developing fetus and the lactation/nursing model simulates the transfer of *p,p'*-DDE from the dam to the nursing pup via mammary milk, followed by exchanges with pup fat, kidney, and other richly- and poorly-perfused tissues.

All exchanges with blood plasma, in both models, were simulated as flow-limited processes, with the exception of the following. Exchanges between maternal fat and a *deep fat* compartment were assumed to be diffusion-limited and were represented with first-order rate constants. Exchanges between the embryo/fetus and placenta were modeled as diffusion-limited processes and were represented with diffusion coefficients (L/day). Parameters used in the model were either taken from the literature, estimated by using the SIMUSOLV simulation program, or optimized by visually inspecting the fit of the collected pharmacokinetic data. Elimination pathways in the maternal model included transfer from mammary tissue to maternal milk (in the lactation model), and fecal excretion, including transfer from the liver via bile to the gastrointestinal tract. A fecal pathway from liver (through bile to the gastrointestinal tract) was included in the pup model. In models for dams and pups, slow metabolism of *p,p'*-DDE in the liver was assumed to be accounted for by the rate constant for biliary excretion.

Figure 3-4. Diagrammatic Representation of the Physiologically Based Pharmacokinetic Model for Gestation

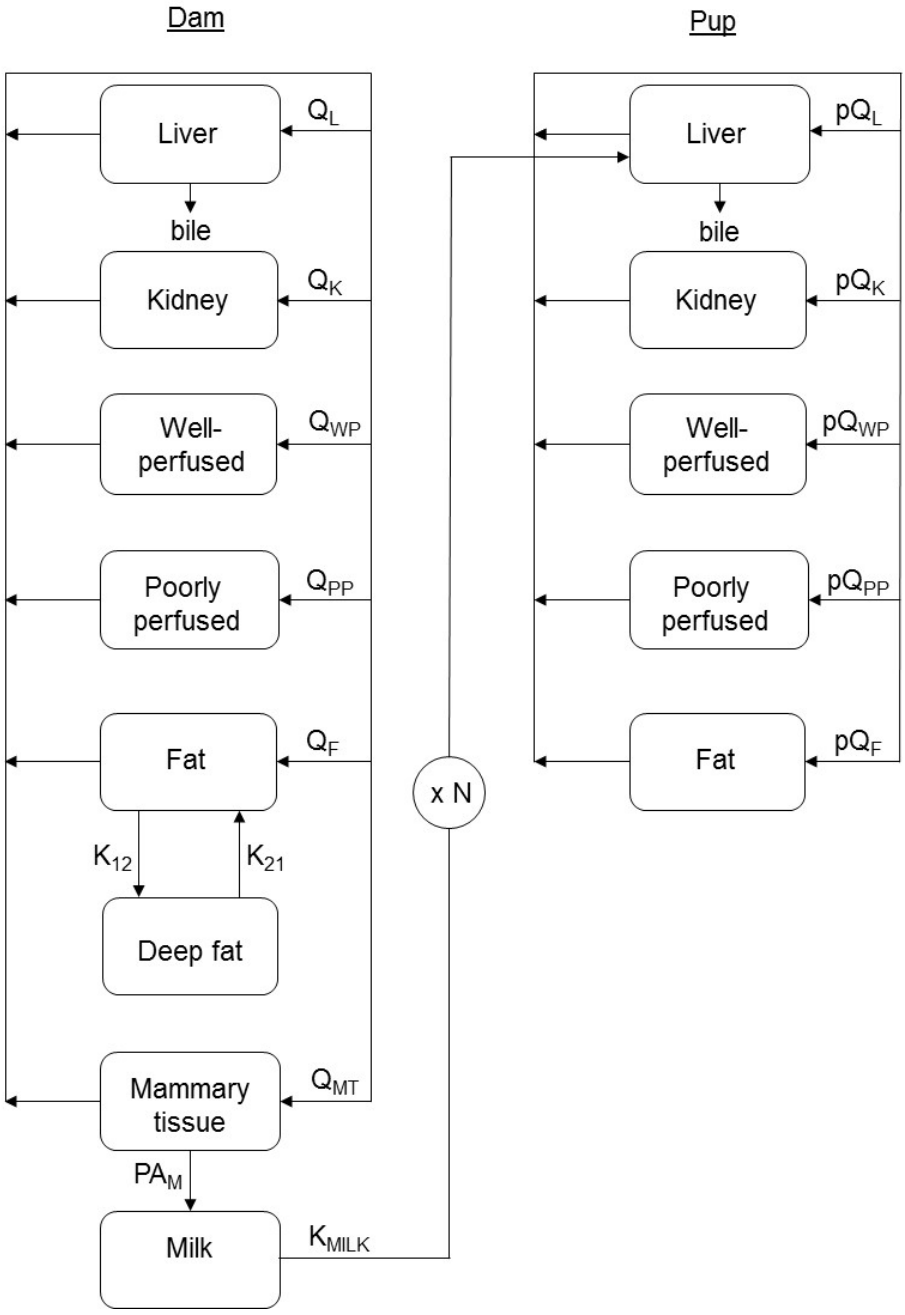


GI = gastrointestinal; N = number of concepti; PBPK = physiologically based pharmacokinetic

Terms are defined in Tables 3-1 and 3-2.

Source: You et al. 1999b

Figure 3-5. Diagrammatic Representation of the Physiologically Based Pharmacokinetic Model for the Lactating Dam and Nursing Pup



N = number of pups; PBPK = physiologically based pharmacokinetic

Portal and lymphatic absorption routes for dams are not shown (see Figure 3-4); terms are defined in Tables 3-1 and 3-2.

Source: You et al. 1999b

Table 3-1. Tissue:Blood Partition Coefficients and Pharmacokinetic Constants for Modeling DDE Disposition in the Pregnant Rat

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

Source: You et al. 1999b

Table 3-2. Physiological Constants Used in the PBPK Model for the Lactating Dam and the Nursing Pup

	Dam	Pup
Body weight (kg) (BW)	0.290–0.340	0.0061–0.58
Tissue volumes (% of body weight)		
Liver, V_L	4	4
Well-perfused tissues, V_{WP}	8	8
Poorly-perfused tissues, V_{PP}	76-V_{MT}	76
Fat, V_F	7	0.0199*pBW+1.664
Mammary tissue, V_{MT}	4.4–9.6	
Milk, V_{milk}	0.002L	

Table 3-2. Physiological Constants Used in the PBPK Model for the Lactating Dam and the Nursing Pup

	Dam	Pup
Cardiac output (L/hour)	$14*pBW^{0.75}$	$18*pBW^{0.74}$
Blood flows (% of cardiac output)		
Liver, Q_L	25	25
Well-perfused tissues, Q_{WP}	41-Q_{MT}	49
Poorly perfused tissues, Q_{PP}	25	25
Fat, Q_F	7	1
Mammary tissue, Q_{MT}	9–15	

Source: You et al. 1999b

The models were calibrated with data from experimental studies in which pregnant Sprague-Dawley rats were administered gavage doses of 0, 10, or 100 mg *p,p'*-DDE on GDs 14–18 (You et al. 1999b). A subset of the dams was sacrificed 4 hours after each dosing, and tissue levels of *p,p'*-DDE were measured in the dams, placenta, and fetuses. A subset of pups in each dose group was cross-fostered to assess *p,p'*-DDE transfer to tissues from maternal milk. Consistent with the collected data, the models predicted that lactational exposure was more important in determining pup body burden than *in utero* exposure.

Verner et al. (2009, 2008) developed a generic human mother-infant PBPK model to estimate infant exposure to chlorinated persistent organic pollutants (POPs) including *p,p'*-DDT and *p,p'*-DDE via transplacental exposure during gestation and breast milk during 12 months of lactation, based on mothers' exposure during gestation and lactation. Figure 3-6 presents a conceptual representation of the model showing the mother model as a tissue network of nine compartments with ingested POCs assumed to be completely absorbed from contaminated food and directly transferred to the liver. Excretion in milk was modeled as output from the mammary tissue. The infant model consisted of five compartments and was integrated with the mother model via breast milk, which was assumed to be the only source of POC exposure of the infants during the first 12 months of life, and via transplacental transfer from the mother to the developing fetus (see Figure 3-6). The mother and infant models described rates of metabolism in the liver compartment as the product of the hepatic extraction ratio, the liver blood flow and the arterial blood concentration of the pertinent POC; POC-specific hepatic extraction ratios were calculated from hepatic intrinsic clearance values, which were calculated from published half-life values. The POC concentrations in model compartments were modeled with mass balance differential equations that included blood flow, and POC-specific tissue:blood partition coefficients estimated from ratios of lipid fractions in tissues and blood (Verner et al. 2008, 2009). Predictions from the models for cord blood,

breast milk, and infant blood concentrations of *p,p'*-DDE (based on maternal inputs) were significantly correlated (r>0.9) with measured values from a group of Inuit mothers and infants from Northern Quebec, Canada, whereas correlations between predicted and observed values of *p,p'*-DDT concentrations were less strongly correlated (r=0.75–0.78). Verner et al. (2009) proposed that use of the mother-infant model to predict infant exposures from maternal blood levels could reduce sampling efforts in future epidemiological studies of potential effects of POCs on child development (Verner et al. 2009). Verner et al. (2013, 2015) used a similar generic POC human mother-infant PBPK model to predict prenatal exposure to *p,p'*-DDE or *p,p'*-DDT from maternal or children's blood levels collected 9 years after delivery, noting that predictive tools that could back-extrapolate prenatal levels could lead to increased sample sizes in epidemiology studies of associations between POCs and child development endpoints.

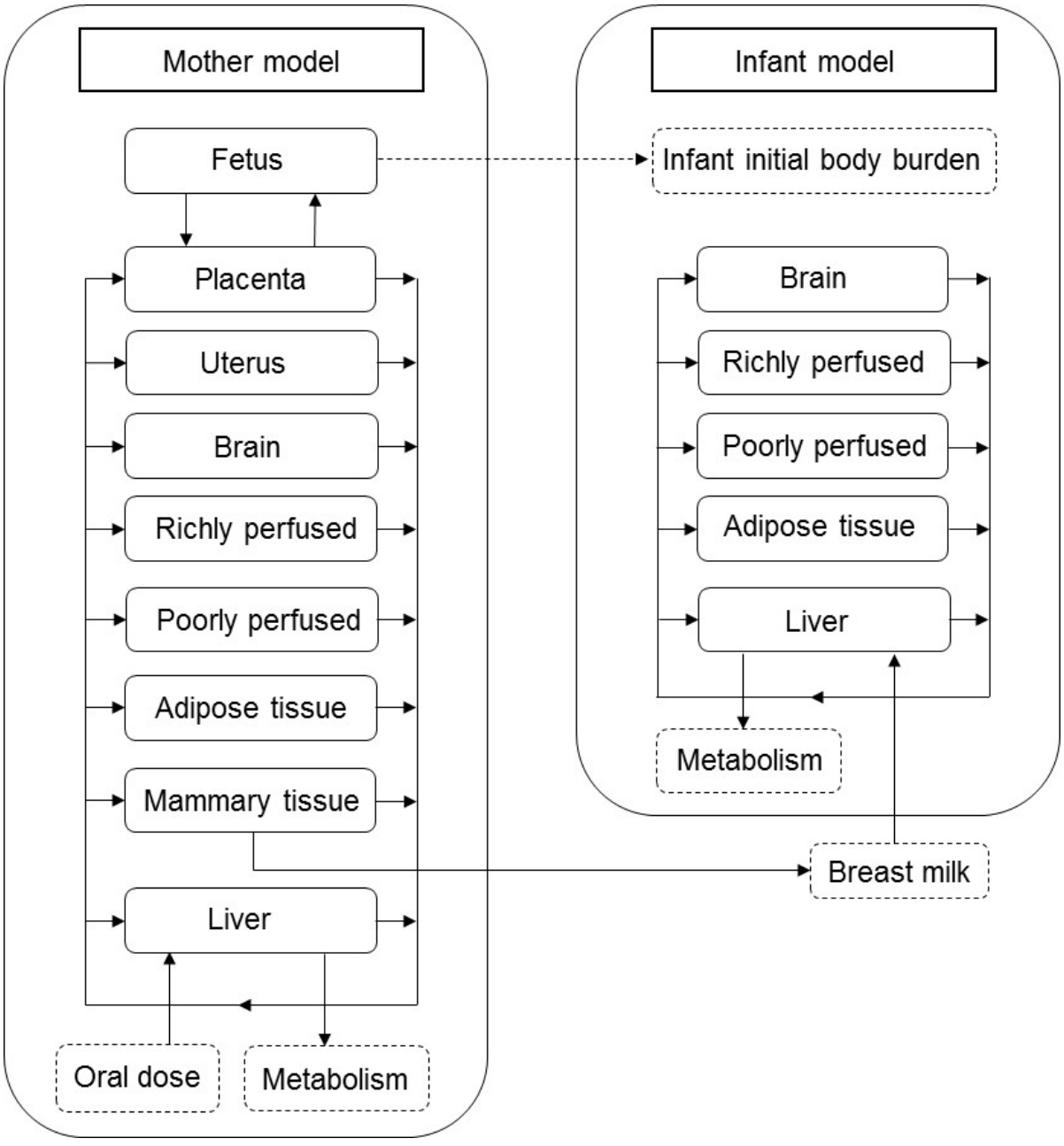
Using a generalized human PBPK model for persistent chlorinated organic chemicals developed by Cahill et al. (2003), Sonne et al. (2014) found that model-predicted blood levels of DDE (and other chemicals studied like hexachlorobenzene) based on estimated intakes from dietary sources were within a 2–3-fold factor of measured blood levels in members of Greenland Inuit communities with a traditional diet high in fish, whale, polar bear, reindeer, and musk oxen.

3.1.6 Animal-to-Human Extrapolations

The metabolism of DDT, DDE, or DDD in animals is similar to that in humans, but observed interspecies metabolic differences suggest that interspecies differences in susceptibility to the neurotoxicity or hepatotoxicity of these chemicals may exist. Comparisons of elimination rates of DDT from fat showed that the process is faster in rats, followed by dogs and monkeys, and is slowest in humans (Morgan and Roan 1974). Rats eliminated DDT 10–100 times faster than humans. Morgan and Roan (1974) suggested that the differences in elimination rates could be due to differences in liver metabolism, gut bacterial metabolism, enterohepatic recirculation, or factors related to the accessibility of plasma-transported pesticide to the excretory cells of the liver.

Development of a human PBPK model similar to the rat dam-infant model developed by You et al. (1999b) may be useful to improve extrapolation from rats to humans in the development of acceptable exposure levels (e.g., MRLs) for DDT, DDE, and DDD. The development of such a model or a model for nonpregnant humans, however, is limited by the lack of suitable kinetics data for adult humans, human mother-fetuses pairs, or human mother-infant pairs to calibrate the model.

Figure 3-6. Conceptual Representation of the Mother-Infant Physiologically Based Pharmacokinetic Model



Source: Verner et al. 2009

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to DDT, DDE, and DDD are discussed in Section 5.7, Populations with Potentially High Exposures.

Available epidemiological studies provide some evidence for the potential susceptibility of developing fetuses, infants, or children to toxic actions of DDT, DDE, or DDD, depending on the endpoint. Seven case-control studies provided consistent evidence for associations between very high maternal serum levels of DDT, DDE, or DDD with abortion or preterm births (see Section 2.16), and five case-control studies provided consistent evidence for associations between maternal levels of DDE during pregnancy and prevalence of wheeze in infant or child offspring (see Section 2.14). However, inconsistent evidence has been provided by studies looking for associations between maternal levels of DDT, DDE, or DDD in biological fluids or tissues and other immune conditions in infant or child offspring, such as prevalence of asthma or infections (Section 2.14); adverse early neurodevelopmental effects in offspring (see Section 2.15); and changes in birth weight or early growth patterns in offspring (see Section 2.17). Six case-control studies provided consistent evidence for no significant associations between levels of DDT, DDE, or DDD in maternal fluids or tissues and risk for the male birth defects, cryptorchidism, and hypospadias (see Section 2.16).

Other case-control studies suggest that chronic exposure of older adults to DDT, DDE, or DDD may be associated with increased risks for elevated body mass index (BMI) or development of DMT2. Consistent evidence for significant positive associations between serum DDE levels and BMI was found

in most studies examining this endpoint in adults ≥50 years of age (see Section 2.3). A clear majority of studies, including several meta-analysis, provided evidence for an association between serum levels of DDT, DDE, or DDD in adults and increased prevalence of DMT2 (see Section 2.18).

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

In animals, DDT can cause abnormal development of sex organs, embryotoxicity, and fetotoxicity in the absence of maternal toxicity (Clement and Okey 1974; Fabro et al. 1984; Hart et al. 1971, 1972). Developmental effects, including preweanling mortality and premature puberty, have been reported in animals in multigeneration studies (Del Pup et al. 1978; Green 1969; Ottoboni 1969; Ottoboni et al. 1977; Tomatis et al. 1972; Turusov et al. 1973). DDT has shown estrogenic properties in animals administered the pesticide orally or parenterally (Bitman and Cecil 1970; Clement and Okey 1972; Fabro et al. 1984; Gellert et al. 1972, 1974; Singhal et al. 1970). In female neonates injected subcutaneously with *o,p'*-DDT or *o,p'*-DDD, there were significant alterations in the estrous cycle, decreases in ovary weight, and decreases in corpora lutea when the animals were evaluated as adults (Gellert et al. 1972, 1974). In general, the estrogenic potency of DDT is orders of magnitude lower than that of estradiol.

p,p'-DDE, a persistent metabolite of DDT, was an androgen receptor antagonist in male rats exposed *in utero*, and also as juveniles (Gray et al. 1999; Kelce et al. 1995, 1997; Krause et al. 1975; Loeffler and Peterson 1999; You et al. 1998, 1999a). Rat pups from dams exposed during GDs 14–18 to 100 mg *p,p'*-DDE/kg/day and then exposed indirectly to maternally stored *p,p'*-DDE via breast milk had

significantly reduced AGD at birth and retained thoracic nipples on PND 13 (Kelce et al. 1995, 1997). Treatment of weanling male rats until day 57 of age with 100 mg *p,p'*-DDE/kg/day resulted in a statistically significant delayed onset of puberty by 5 days (Kelce et al. 1995, 1997). Gray et al. (1999) and You et al. (1998) reported that AGD was not affected in male Sprague-Dawley rats on PND 2 after treating the dams with up to 100 mg *p,p'*-DDE/kg on GDs 14–18, but was significantly reduced in similarly exposed Long-Evans pups. A 10 mg/kg dose to the dams was without effect in the Long-Evans pups. AGD was not affected in female pups from either strain. Treatment of the dams with 10 mg *p,p'*-DDE/kg resulted in retention of thoracic nipples in Sprague-Dawley pups, but only the higher dose (100 mg/kg) had this effect in Long-Evans pups. An additional study from the same group showed that prenatal exposure to *p,p'*-DDE was associated with expression of TRPM-2, an androgen-repressed gene (You et al. 1999a). A similar study in Holtzman rats exposed during GDs 14–18 to doses between 1 and 200 mg *p,p'*-DDE/kg (offspring were exposed to *p,p'*-DDE *in utero* and via breast milk) found reduced AGD in males on PND 1 and reduced relative ventral prostate weight on PND 21 at 50 mg *p,p'*-DDE/kg, but not at 10 mg *p,p'*-DDE/kg (Loeffler and Peterson 1999). Doses up to 100 mg/kg/day to the dams had no effect on onset of puberty, but 200 mg/kg/day did significantly delay puberty in males by <2 days. Androgen receptor staining in the ventral prostate was also reduced on PND 21. Serum levels of testosterone or 3α-diol androgens were not significantly altered at any time. This study also reported that at the 100 mg/kg dose level, cauda epididymal sperm number was reduced by 17% on PND 63 relative to controls.

Alterations in learning processes and in other behavioral patterns have also been described in adult mice exposed to DDT perinatally (Craig and Ogilvie 1974; Palanza et al. 1999; vom Saal et al. 1995) or as neonates (Eriksson et al. 1990a, 1990b, 1992, 1993; Johansson et al. 1995, 1996); this endpoint is the basis of an acute oral MRL, which is discussed in detail in Section 1.3 and Appendix A. These studies suggest that exposure of the developing fetus or newborn to DDT during critical stages in nervous system development can cause developmental toxicity manifested later in life. Eriksson et al. (1990a, 1990b) pointed out that the dose levels that caused behavioral alterations in mice are comparable to those levels to which human neonates might be exposed in areas where DDT is still being used. Behavioral neurotoxicity has been described in rats treated with DDT as adults (Sobotka 1971), but only at doses at least 50 times those that produced learning deficits in neonates.

Studies in animals have demonstrated placental transfer of DDT and DDE to fetuses and also to newborns via mother's milk (Fang et al. 1977; Seiler et al. 1994; Wooley and Talens 1971; You et al. 1999b). The

results of these studies indicate that the amounts of chemical transferred via mother's milk are much greater than the amounts that reach the fetus through the placenta.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

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

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to DDT, DDE, and DDD are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for DDT, DDE, and DDD from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by DDT, DDE, and DDD are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Levels of DDT, DDE, or DDD in serum, blood, or breast milk (expressed on a lipid basis, ng/g lipid) are the most widely used biomarkers of exposure in modern biomonitoring and epidemiological studies (e.g., Axmon and Rignell-Hydbom 2006; Bonde et al. 2017; Chen et al. 2005; Everett and Matheson 2010; Jaga and Dharmani 2003; Kim et al. 2015a; Patterson et al. 2009; Roberts and Karr 2012; Sexton et al. 2006; van den Berg et al. 2016). Some recent efforts have assessed that determination of concentrations of pesticides, including DDT and metabolites, in multiple sources (e.g., blood, hair, placenta) may increase rates of exposure detection, compared with single source determinations (Ostrea et al. 2008).

For biomonitoring of DDT, DDE, and DDD, as well as other persistent halogenated organic chemicals, levels in breast milk are popular biomarkers of exposure, because breast milk is easily obtained through non-invasive techniques, extraction from the medium is not difficult due to the high lipid content, and levels are thought to be reflective of whole body burdens (van den Berg et al. 2016). Recent global surveys of concentrations of DDT, DDE, and DDD (ΣDDT) in human breast milk samples for numerous countries collected from 2000 to 2010 indicate levels ranging from about 20 ng/g lipid in Finland to about 1,400 ng/g lipid in India, with tropical countries (where DDT is still used for malaria control) representing the majority of the upper half of the distribution of concentrations (van den Berg et al. 2016).

There are no quantitative data available that allow correlation of DDT/DDD/DDE levels in human tissue or fluids and exposure to specific levels of environmental contamination. Studies of pesticide production workers reported that blood levels of these compounds are generally higher in persons exposed in the workplace. Since the biological half-lives for elimination of these compounds are ranked as follows: DDE > DDT > DDD, detection of higher ratios of DDD or DDT to DDE has been proposed to indicate more recent exposure, while lower ratios are believed to correlate with long-term exposure and storage capacity (Morgan and Roan 1971). There is a direct correlation between DDT and DDE levels in blood and adipose tissue when concentrations are expressed on a lipid basis (Hayes et al. 1971; Morgan and Roan 1971; Mussalo-Rauhamaa 1991). On a wet tissue basis, concentrations of DDT in adipose tissue are approximately 280 times higher than those of blood (Anderson 1985). However, because DDT and DDE are extensively stored in fatty tissue and slowly released from storage sites, there is no correlation between levels in tissues and the time course of exposure in short time spans.

3.3.2 Biomarkers of Effect

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

3.4 INTERACTIONS WITH OTHER CHEMICALS

DDT may have broad effects by changing the metabolism of other chemicals, both xenobiotics and endogenous macromolecules. As discussed in Section 3.1.3, *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD are phenobarbital-type cytochrome P-450 (CYP) inducers in rats, causing induction of hepatic CYP2B and CYP3A proteins and CYP1A protein induction to a lesser extent (Nims et al. 1998). For some chemicals, this enhancement of biotransformation produces less toxic metabolites and may inhibit toxic effects, whereas, for other chemicals with toxic metabolites, the metabolic enhancement could lead to enhancement of toxic effects.

One interaction of concern is the enhanced conversion of other chemicals to active, carcinogenic forms mediated by microsomal enzymes induced by DDT. Several investigations indicate that DDT administered to animals along with a known carcinogen may result in either an increase or a decrease in tumor production relative to the carcinogen tested without DDT. A study by Walker et al. (1972) suggested that the liver enlargement was greater and the time to palpability of liver masses was earlier in mice fed dieldrin and DDT than those fed either pesticide separately. A potentiation of carcinogenic activity of dieldrin was suggested but not conclusively shown. It is possible that DDT could also promote the formation of hepatic tumors initiated by other carcinogens. DDT has been reported to promote the tumorigenic effects of several known carcinogens, such as 3-methyl-(4-dimethylamine)-azobenzene (Kitagawa et al. 1984), 2-acetylaminofluorene (2-AAF) (Peraino et al. 1975), diethyl-nitrosamine (DEN) (Diwan et al. 1994; Nishizumi 1979), and carbon tetrachloride (CCl_4) (Preat et al. 1986) when given after the putative carcinogen. The promoting effect of DDT in rats was reported to act in a dose-dependent fashion, with DDT decreasing the latency period of tumor development and increasing the incidence and yield of hepatic tumors, mainly hepatocellular carcinomas.

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

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

The effects of DDT on the nervous system can be altered when DDT is given in combination with certain neurologically-active pharmacological agents. Some pharmacological agents (hydantoin, phenobarbital), prevent some or all of the neurological effects seen in animals treated with DDT (see Section 2.15), while other agents (trihexyphenidyl, haloperidol, propranolol) enhance DDT-induced neurotoxicity (Herr et al. 1985; Hong et al. 1986; Matin et al. 1981). One of the effects of DDT is to hold sodium channels open, which probably contributes to DDT-induced neurological effects (tremors and hyperexcitability). Studies by Rubin et al. (1993) have shown that DDT analogues and metabolites, as well as several pyrethroids, modify radioligand binding of batrachotoxinin to sodium channels in mouse brain synaptosomes. DDT and pyrethroids do not, by themselves, stimulate Na^+ uptake, but they enhance activator-dependent uptake. DDT is more efficacious than the pyrethroids tested. Eriksson et al. (1993) have shown that the pyrethroid bioallethrin and DDT can interact *in vivo* in rats.

In an immature rat uterotrophic assay, mixtures of six synthetic chemicals with demonstrated estrogenic activities (*o,p'*-DDT and five other chemicals) were shown, at low concentrations, to not alter responses

induced by a mixture of phytoestrogens and to act in an additive manner when exposed in the absence of external phytoestrogens (Charles et al. 2007).

A series of studies examined the effects of oral exposure to binary mixtures of 1,4-dichlorobenzene and *p,p'*-DDE (Makita 2005, 2008a) or tributyl tin and *p,p'*-DDE (Makita 2008b; Makita et al. 2003b) on reproductive capabilities of immature male and female rats, but the designs of the studies were inadequate to conclude whether or not the components of these mixtures displayed joint actions that were additive, less-than-additive, or greater-than-additive.