

2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO DDT/DDE/DDD IN THE UNITED STATES

DDT is an organochlorine insecticide that has found a broad range of agricultural and nonagricultural applications in the United States and worldwide beginning in 1939. In 1972, DDT use was banned in the United States and in many parts of the world, except for use in controlling emergency public health problems. DDT is still used in certain parts of the world to control vector-borne diseases, such as malaria. The release of DDT into the environment occurs primarily through spraying applications onto agricultural crops, forest lands, other nonagricultural land, and homes (for the control of disease-bearing vectors). Exposures in the home occurred through the use of DDT as a mothproofing agent, to control lice and, in some parts of the world, to control mosquitoes and other disease-bearing vectors. Both DDD and DDE are degradation products of DDT. DDD was also manufactured and used as an insecticide, but to a much lesser extent than DDT. DDE has no commercial use, but is commonly detected along with DDT at concentrations in the environment that often exceed those measured for DDT.

Upon introduction into the environment, DDT will enter soil, water, or air. DDT and its metabolites are essentially immobile in soil, becoming strongly absorbed onto the surface layer of soils. Likewise, as a consequence of their extremely low water solubilities, DDT and its metabolites become absorbed onto particulates in water and settle into sediments. Because of its chemical characteristics, DDT can undergo long-range transport through the atmosphere in a process known as “global distillation” where DDT migrates from warmer regions to colder regions through repeated cycles of volatilization from soil and water surfaces followed by deposition of DDT onto surfaces through dry and wet deposition processes. This long-range transport of DDT results in the wide dispersion of DDT and its metabolites throughout the world, even into remote areas, such as the Arctic or Antarctic regions. The rate and extent of disappearance of DDT may result from transport processes as well as from degradation and transformation. Both the slow degradation of DDT and its metabolites and the ban on DDT use in the early 1970s in the United States and most of the world have contributed to a decrease in the levels of these compounds in the environment over the past 30 years. DDT can be degraded through atmospheric photooxidation in air or photolysis on the surface of water or soil. DDT can undergo slow biodegradation through reductive dechlorination to form DDE and DDD, and then be further degraded to form other metabolites. The persistence of DDT and its metabolites, in combination with their high lipophilicity, have contributed to the bioaccumulation (increasing concentration of a chemical in an organism which

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exceeds that in its environment) and biomagnification (increasing concentration of a chemical in an organism as a function of trophic level) of DDT and its degradation products in the environment. DDT, DDE, and DDD accumulate in fatty tissues, with tissue concentrations typically increasing with the trophic level of the organism. In addition to DDT, DDE, and DDD, methylsulfonyl derivatives of DDE can be formed in higher organisms, such as seals, polar bears, beluga whales, and humans, and are found predominantly in the liver and fatty tissues.

Exposure of the general public to DDT, DDE, and DDD has been declining since the ban on the use of DDT, at rates that depend on geographic location and environmental conditions. The predominant route of exposure of the general public to DDT and its metabolites is through the diet. Although DDT and its metabolites are ubiquitous in the atmosphere, they are present in such low concentrations that exposures through inhalation or dermal contact are considered to be negligible. From the standpoint of dietary exposures, the main exposure route is through the consumption of foods either obtained from areas of the world where DDT is still used or that have the potential to contain bioaccumulated residues of DDT and its metabolites (e.g., meat, fish, poultry, dairy products). Exposure to DDT in drinking water is considered negligible because of the extremely low water solubility of DDT and the efficiency of standard drinking water methods. With the ban on the use of DDT, occupational exposures that result from formulation, packaging, and application activities should be negligible, except in areas where DDT use remains. Activities that result in the mobilization of DDT (e.g., site remediation) may increase exposure of workers to DDT and its metabolites. With the discontinued use of DDT, exposure of populations living near areas of heavy DDT use or deposition may not be much greater than for the general public, except in areas of the world where DDT is still used to control disease-bearing vectors or under conditions where DDT and metabolites become mobilized (e.g., site remediation, sediment resuspension, erosion, etc.). Residents living near National Priorities List (NPL) sites that contain DDT may be exposed to higher ambient levels of DDT and its metabolites than the general population through dermal (deposition onto plants, objects), ingestion (contaminated food, water), and/or inhalation of DDT vapor, although inhalation exposures are thought to be insignificant compared to dietary sources of DDT. These exposures can lead to greater body burdens of DDT and its metabolites; for example, residents living near a pesticide dump site in Aberdeen, North Carolina, which is known to contain DDT, have higher age-adjusted mean levels of DDE in their blood than residents of neighboring communities (4.05 vs. 2.85 ppb).

Exposures of wildlife to DDT and its metabolites have been declining since the early 1970s, as evidenced by marked decreases in the levels of these compounds in fish, shellfish, aquatic mammals, birds,

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invertebrates and many other species. Yet, DDT and its metabolites were detected in 94% of whole fish samples in the 1990s even though total DDT concentration in fish continues to decline. This is due to the presence of DDT in stream beds and the continued input of DDT to streams through long-range aerial transport and deposition of DDT and erosion of soil. The DDT metabolite, *p,p'*-DDE, was detected with the highest frequency in fish, followed by *p,p'*-DDD and *p,p'*-DDT; accumulation of *p,p'*-DDE in fish is due to absorption of DDE from the diet rather than to recent exposures to DDT. Differences in exposures of benthic and pelagic feeders to DDT and its metabolites have been observed. In the marine environment, there is evidence to suggest that benthic organisms feeding at the bottom of the oceans accumulate higher levels of *p,p'*-DDT and its metabolites, *p,p'*-DDE and *o,p'*-DDE, than do surface feeders. This is attributed to the absorption of *p,p'*-DDT, *p,p'*-DDE, and *o,p'*-DDE onto organic particulates and detritus that then settle to the ocean floor. However, in a fresh water lake system, it was found that bioaccumulation of GDDT was greater in the pelagic food web than in the benthic food web due to the increased mobility of pelagic organisms and their ultimate reliance on benthos. The GDDT that has accumulated in these aquatic food webs can then be further accumulated in organisms in higher trophic levels of the food chain. Exposure of wildlife to DDT and its metabolites can also occur through DDT-contaminated soils and sediments.

Since the ban on DDT was instituted in the United States and most of the world in 1972, the environmental concentrations of DDT and its metabolites have been decreasing. Average adult intakes of DDT were estimated to be 62 µg/person/day in 1965 and 240 µg/person/day in 1970, before the DDT ban was instituted. The FDA Total Diet Studies show that the daily intakes have fallen since the ban, with daily intakes (for a 16-year-old, 70 kg male) averaging 6.51, 2.38, 1.49, and 0.97 µg/person/day for 1978–1979, 1979–1980, 1984–1986, and 1986–1991, respectively. As would be expected from the decline in the concentrations of DDT in the environment, the levels of DDT, DDE, and DDD measured in foodstuffs have also fallen over the last 30 years. Yet, there are still measurable quantities of DDT, DDE, and DDD in many commodities. The mean concentrations of *p,p'*-DDE within specific food groups ranged from 0.1 to 25.7 ppb in dairy products, 0.7–6.5 ppb in meat, 0.2–9.2 ppb in fish, and 0.3–0.7 ppb in poultry, as determined from the data collected in the FDA Total Diet Studies conducted between 1991 and 1999. DDT was detected in 312 out of 2,464 plain milk samples at a maximum concentration of 0.92 ppm and in 8 out of 180 vitamin D fortified milk samples at a maximum concentration of 0.10 ppm in 1985–1991. In a recent FDA Total Diet Study, the mean concentrations of *p,p'*-DDT, *o,p'*-DDT, and *p,p'*-DDE ranged from 0.0001 to 0.0257 ppm. People who eat fish caught in the Great Lakes were found to consume larger amounts of DDT in their diets. However, this route of exposure to DDT is expected to decline as the levels of DDT in the environment continue to diminish. Even so, it is anticipated that low

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levels of DDT and its metabolites will be present in the human diet for several more decades, especially in food items, such as fish, that are expected to contain bioaccumulated DDT residues.

Exposures of the general public to DDT and its metabolites result in the accumulation of these compounds in adipose tissue. Due to the persistence of DDT and its metabolites, the concentrations of these compounds in adipose tissue are determined by both past and current exposures. Estimated national means for DDT concentrations in adipose tissue on a lipid basis were 189, 123, and 177 ppb in 1982, 1984, and 1986, respectively. For DDE, the mean concentrations were found to be 1,840, 1,150, and 2,340 ppb, respectively. In a study of people from northern Texas, the concentrations of DDT and DDE in adipose tissues decreased from 7,950 ppb in 1970 to 5,150 ppb in 1974, and then to 1,670 ppb in 1983. More current measurements of the mean concentrations (lipid basis) of DDT, DDE, and DDD in breast adipose tissue collected during 1995–1996 in the United States were 267.3, 709.1, and 24.0 ppb, respectively, (DDT+DDE+DDD=1,000.4 ppb), which are consistent with the expectation of continuing declines in GDDT body burdens. The adrenocorticolytic agent, 3-methylsulfonyl-DDE, has been detected in the human liver (1.1 ppb), lung (0.3 ppb), and adipose tissue (6.8 ppb) (based on wet weight), and also in breast milk (0.1 ppb). In a study of Swedish women, concentrations of 3-methylsulfonyl-DDE in breast milk ranged from 0.4 to 5 ppb.

At risk populations include indigenous peoples of the arctic who have diets that are high in fatty tissues from the consumption of traditional foods like seal, caribou, narwhales, etc. Intake of GDDT averaged 24.2–27.8 µg/day for eastern Arctic communities and 0.51–1.0 µg/day for western Arctic communities. It is likely that fish-eating populations in the higher latitudes of the Southern Hemisphere may also be at risk; however, these exposures have not been well documented. Children are also at risk to exposure to DDT, mainly through dietary sources. The mean intakes of DDT and its metabolites during 1986–1991 have been estimated to be 0.0448 and 0.0438 µg/kg body weight/day for a 6–11-month-old infant and a 2-year-old child, respectively, which is roughly 4 times the intake per body weight for an adult. Increased infant exposure to DDT has been attributed to breast feeding. The average levels of DDT in human breast milk fat were about 2,000–5,000 ppb in the early 1970s, but have steadily declined at a rate of 11–21% per year since 1975. For example, Norén reported concentrations of *p,p'*-DDT in breast milk fat of 0.71, 0.36, 0.18, and 0.061 ppm for the years 1972, 1976, 1980, and 1984–1985, respectively. These investigators also reported concentrations of *p,p'*-DDE of 2.42, 1.53, 0.99, and 0.50 ppm for these same years, respectively. The mean concentrations of *p,p'*-DDE are roughly 10 times greater than those obtained for *p,p'*-DDT concentrations. Some more recent measurements taken in 1992 show a mean DDE concentration of 222.3 ppb in breast milk of Canadian women. Measurements of DDE and DDT in

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Swedish women in 1997 show levels of 129 and 14 ppb, respectively. Consumption of cow's milk is another route of potential exposure of DDT to children; however, the concentrations of DDT and its metabolites are typically lower in cow's milk than in human breast milk. The mean level of *p,p'*-DDE in cow's milk was measured at 96 ppb in 1970 and 16 ppb in 1985–1986, showing a sharp decline over the years.

2.2 SUMMARY OF HEALTH EFFECTS

Numerous studies have been conducted on the effects of DDT and related compounds in a variety of animal species, but the human data are somewhat limited. Most of the information on health effects in humans comes from studies of workers in DDT-manufacturing plants or spray applicators who had occupational exposure to DDT over an extended period and also from some controlled exposure studies with volunteers. Epidemiological studies of the general population are also available. Because of limitations inherent to all epidemiological studies, disease causality cannot be determined from them; however, epidemiological studies have been conducted that allow the evaluation of the potential role of DDT and related compounds in specific health outcomes.

DDT is an organochlorine pesticide whose best known effect is impairment of nerve impulse conduction. Effects of DDT on the nervous system have been observed in both humans and animals and can vary from mild altered sensations to tremors and convulsions. Humans have been reported to tolerate doses as high as 285 mg/kg without fatal result, although because vomiting occurred, the absorbed dose is not known. There are no documented unequivocal reports of a fatal human poisoning occurring exclusively from ingestion of pure DDT, but deaths have been reported following ingestion of commercial preparations containing also other substances. Death in animals following high exposure to DDT is usually caused by respiratory arrest. In addition to being a neurotoxicant, DDT is capable of inducing marked alterations on reproduction and development in animals. This is attributed to hormone-altering actions of DDT isomers and/or metabolites. Of all the DDT-related compounds, the *o,p'*-DDT isomer has the strongest estrogen-like properties, although it is still several orders of magnitude less potent than the natural hormone, 17 β -estradiol. *p,p'*-DDE, a metabolite of DDT and a persistent environmental pollutant, has antiandrogenic properties and has been shown to alter the development of reproductive organs when administered perinatally to rats. There have been studies in humans suggesting that high DDT/DDE burdens may be associated with alterations in end points that are controlled by hormonal function such as duration of lactation, maintenance of pregnancy, and fertility. High blood levels of DDE during

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pregnancy have also been associated with increased odds of having pre-term infants and small-for-gestational-age infants and height abnormalities in children.

Studies in animals have shown that DDT, DDE, and DDD can cause cancer, primarily in the liver. The possible association between exposure to DDT and various types of cancers in humans has been studied extensively, particularly breast cancer. Thus far, there is no conclusive evidence linking DDT and related compounds to cancer in humans. Possible genotoxic effects in humans have been reported in a few studies, but simultaneous exposure to other chemicals and lack of control for relevant confounders make the results inconclusive. For the most part, DDT and related compounds are not mutagenic in prokaryotic organisms. Studies in animals have shown that DDT can cause adverse liver effects, but studies of humans exposed occupationally or of volunteers given DDT have reported only mild liver alterations of no clinical significance. Very limited information exists on immunological effects of DDT in humans. A recent study of subjects residing near a waste site found that individuals with higher blood DDE levels had minor changes in some immune markers, which the authors considered of uncertain clinical importance. Studies in animals suggest that exposure to DDT may impair immunocompetence. There is sufficient information indicating that the adrenal gland is a target for *o,p'*-DDD toxicity in humans and animals and of the sulfonyl metabolite 3-MeSO₂-DDE in animals. *o,p'*-DDD has been used in humans to treat adrenocortical carcinoma and benign Cushing's disease.

Discussion of some end points affected by exposure to DDT/DDE/DDD has been expanded below. These include end points that have generated considerable interest due to public health implications such as various types of cancer and reproductive/developmental effects that may be caused by alterations of the endocrine system. Also included are neurological effects, since the main effect of DDT is on the nervous system, and hepatic effects, since the liver is significantly affected in several animal species.

Reproductive/Developmental Effects. In recent years, concern has been raised that many pesticides and industrial chemicals are endocrine-active compounds capable of having widespread effects on humans and wildlife. Hormones influence the growth, differentiation, and functioning of many target tissues, including male and female reproductive organs and ducts such as mammary gland, uterus, vagina, ovary, testes, epididymis, and prostate. Therefore, mimicking the effects of hormones or antagonizing hormonal effects can potentially affect a number of organs and systems, especially if this occurs at vulnerable times such as during development. Developing organisms respond to endocrine-disrupting chemicals very differently than adults. Fetuses lack feedback regulatory mechanisms and active metabolism of steroid hormones that regulate maintenance of secondary sex tissues, estrous cycling, and

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pregnancy. Consequently, low levels of exogenous hormones or xenobiotics may induce severe effects in the development of the reproductive organs. Thus far, there is no conclusive evidence that exposure to DDT/DDE/DDD at the levels found in the environment has affected reproduction and development in humans, but there is sufficient information from animal studies that these chemicals have the potential for doing so.

Several studies in humans have examined possible associations between body burdens of DDT and analogues and the incidence of alterations in reproduction and development and in other end points in which hormones may play a pivotal role such as lactation, infertility, miscarriages, and pre-term labor. For example, duration of lactation was inversely related to the concentration of *p,p'*-DDE in a group of women from Mexico who had lactated previously and similar findings were reported in a study in the United States. A plausible explanation for this decrease in the longevity of lactation is the antiandrogenic/weak estrogenic effect of DDE, as estrogens inhibit milk secretion. In a study of 240 women, there was no association between blood DDE levels (<6 ppb) and miscarriages, but there was suggestive evidence of an association in a study of 89 German women, 13 of whom had DDE blood levels >2.5 ppb; the highest DDE level was 8.6 ppb. A weak association between infertility and DDT blood levels was observed in an additional study of 489 German women. Studies in India and Israel have reported higher levels of DDT, DDE, and DDD in maternal blood and in placental tissue in mothers who gave birth to premature infants or who spontaneously aborted fetuses compared to mothers who gave birth to full-term infants, but the possible role of other potentially endocrine-disrupting chemicals, such as PCBs and other chlorinated pesticides, could not be ruled out. A smaller study in the United States of only 20 women did not find the same association between serum DDE levels and pre-term delivery. Yet another study found no differences in DDT levels in maternal blood at delivery between full-term babies and cases of pre-term delivery; however, they found that umbilical cord blood from pre-term newborns had higher levels of DDT than full-term newborns. A recent study of 361 pre-term infants and 221 small-for-gestational-age cases found that the odds for both outcomes increased steadily with increasing maternal blood concentrations of DDE in samples taken in the third trimester of pregnancy. The association was evident at DDE concentrations ≥ 10 ppb, but there was essentially no relation at lower DDE concentrations. It is important to point out that while levels of DDE in the general population in the United States continue to decline and current serum levels average <10 ppb, it is not unusual to find higher levels in populations from countries where DDT is still used for controlling vector-transmitted diseases. High blood levels of DDE during pregnancy have also been associated with height abnormalities in children.

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Technical DDT is a mixture of various isomers of which *p,p'*-DDT is the most prevalent (65–80%), followed by *o,p'*-DDT (15–21%). Although *p,p'*-DDT is more acutely toxic than *o,p'*-DDT, it has been known for several decades that the latter had marked effects on reproduction in animals. In most experiments, *o,p'*-DDT mimicked the action of the natural ligand, 17 β -estradiol. In general, results from *in vivo* studies indicate that DDT and analogues have much lower estrogenic potency (1,000–1,000,000-fold lower) than the endogenous hormone, 17 β -estradiol. Experiments in rats have shown that *o,p'*-DDT can induce delayed vaginal opening and increased uterine and ovarian weights. In another study in rats, using the uterine glycogen response assay, it was observed that *o,p'*-DDT was the most potent among several isomers. *p,p'*-DDT and *o,p'*-DDE were approximately 16 times less potent than *o,p'*-DDT, and *o,p'*-DDD was inactive in this assay. In yet another study, the lowest dose of *o,p'*-DDT needed to produce estrogenic effects in the immature rat uterus was about 1×10^5 times greater than that of DES. An *in vivo* assay with rats and mink showed that *o,p'*-DDT had uterotrophic activity, whereas *p,p'*-DDT had only slight activity, and the activity of technical DDT was dependent on the level of *o,p'*-DDT that it contained. Many other *in vivo* studies have shown the estrogenic potential of DDT and related analogues. Most recent studies have been conducted using a wide variety of *in vitro* assays and the results vary greatly. Still, the estrogenicity of DDT-related compounds in these tests has been determined to be 10^4 – 10^6 times lower than 17 β -estradiol, depending on the testing conditions.

Safe conducted a mass/potency balance exercise to assess the impact of environmental estrogens as causative agents of adverse health effects, primarily reproductive disturbances and breast cancer. Besides environmental estrogens (xenoestrogens), humans are exposed to several structural classes of naturally occurring estrogens including plant bioflavonoids and various mycotoxins. The estrogenic activities of these substances has been investigated in *in vivo* and *in vitro* cell systems and in ER binding assays; most elicit multiple estrogenic responses. Also, a number of foodstuffs contain 17 β -estradiol and estrone. At the same time, several different structural classes of chemicals present in the human diet show antiestrogenic activity, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related halogenated aromatics. Polynuclear aromatic hydrocarbons (PAH), found in cooked foods, and indole-3-carbinol (IC), a major component of cruciferous vegetables, have also shown antiestrogenic properties. From published data, Safe estimated a dietary estrogen equivalent level of 2.5×10^{-6} $\mu\text{g}/\text{day}$ from estrogenic pesticides and of 102 $\mu\text{g}/\text{day}$ from bioflavonoids. Studies from the literature also suggested that the estrogenic potencies of bioflavonoids relative to 17 β -estradiol (potency=1) are 0.001–0.0001, whereas for pesticides, the estrogenic potency is about 0.000001. Therefore, the estrogen equivalent from dietary intake of flavonoids was estimated to be 4×10^7 times higher than that from estrogenic pesticides. A similar exercise with the antiestrogenic compounds led Safe to the conclusion that the sum of antiestrogen

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toxic equivalents are orders of magnitude higher than the estimated dietary intakes of estrogenic pesticide equivalents. These conclusions are not necessarily contradictory to the recommendation that intake of pesticides should continue to be monitored and kept to a minimum for the protection of developing organisms, but rather point out the multiple sources of potentially endocrine-active substances. One additional fact to keep in mind is that DDT and analogues bioconcentrate in the food chain and accumulate in the body.

Recent studies *in vivo* have also demonstrated that *p,p'*-DDE, the most environmentally relevant DDT derivative, has antiandrogenic properties when administered to rodents at relatively high doses. Studies *in vitro* have confirmed the findings *in vivo*. In particular, *p,p'*-DDE functions as an antagonist after it has bound to the androgen receptor. This androgen antagonism or antiandrogenic activity can explain a number of reproductive and developmental effects seen in male rats of various ages exposed to *p,p'*-DDE. These effects include reduced anogenital distance and retention of thoracic nipples in pups exposed during gestation and lactation; delayed puberty in rats exposed either during juvenile development or at very high doses during gestation and lactation; and reduced accessory sex organ weights in exposed adult males. In addition to being antiandrogenic by an action on the androgen receptor, *p,p'*-DDE was shown to increase liver CYP enzyme systems that hydroxylate testosterone in perinatally exposed rats. This effect can be also considered an antiandrogenic effect since hydroxylated testosterone is more polar than testosterone and can be conjugated and excreted. It is not known, however, whether the increase in activity of enzymes that hydroxylate testosterone resulted in a reduction of circulating androgens since the authors did not measure serum testosterone. In general, altering steroid metabolism is another possible mechanisms by which DDT and related compounds can alter reproduction and development. *p,p'*-DDE also was shown to increase liver aromatase activity in adult male rats; aromatase plays a critical role in steroidogenesis by catalyzing the conversion of C19 androgens into estrogens. Thus, like DDT, DDE can have an overall feminizing effect on animals by antagonizing the androgen receptor at the same time that it increases estrogens concentration.

Administration of DDT *in utero* or to neonates during sensitive periods in nervous system development has caused behavioral and neurochemical changes in adult mice. In the series of experiments conducted by Eriksson and co-workers, exposure of 10-day-old mice to a single relatively low dose of 0.5 mg of technical DDT/kg resulted in altered motor behavior at the age of 4–5 months and in alterations in neurotransmitter receptors in the brain. The findings from the Eriksson's studies served as the basis for derivation of an acute oral minimum risk level (MRL) for DDT.

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Cancer. The possibility that exposure to DDT and related compounds might increase the risk of certain cancers in humans has been explored in many studies. Special attention has been devoted, but not limited, to breast cancer. Moreover, the interest is not limited to DDT/DDE only, but to persistent organochlorine compounds in general (e.g., PCBs, dieldrin, hexachlorobenzene). In addition to factors inherent to epidemiological studies that play a role in trying to establish associations between exposure and health outcome, for breast cancer, one must consider additional factors that are thought to play important roles such as menopause, estrogen receptor status, and concurrent exposure to other estrogenic and antiestrogenic compounds. Several methods for assessing combined current and past exposures to DDT have been used. Most DDT (and the most prominent environmental residue, DDE) is stored in the fat, so an accurate method of assessing body burden is measuring levels in adipose tissue samples. However, procedures for obtaining adipose tissue samples are usually invasive and not easy to obtain (unless done during cancer surgery). Alternatively, serum DDT/DDD/DDE levels can be measured; however, since DDT/DDD/DDE are fat soluble, the serum levels may vary with the fat content of the blood. Variability in serum DDT/DDE/DDD that is attributable to variability in serum lipids can be accounted for by using lipid-adjusted serum DDT/DDE/DDD levels. Adjusting for lipid content provides a standardized body burden estimation and makes comparisons between studies easier. Studies have shown that on a fat-weight basis, DDT residue levels in serum correlate well with adipose tissue samples, making serum an adequate biological medium for estimating DDT body burden.

The second issue related to appropriate exposure assessment in human breast cancer studies pertains to the timing of the exposure assessment relative to the etiology of cancer. Cancer is a chronic disease and can have a latency time of 15–20 years after initiation. Theoretically, DDT could contribute to breast cancer either by being a complete carcinogen, an initiator, or a promoter. Thus, for DDT to cause or contribute to breast cancer, exposure needs to occur at a time substantially before the time of diagnosis of the cancer. A common methodological approach in many of the cancer studies reported below has been to assess DDT body burdens at or shortly before the time of breast cancer diagnosis. This method is limited by the assumption that a “snapshot” of DDT body burden obtained near the time of diagnosis represents DDT exposures at the time of cancer initiation or early promotion, perhaps as much as 15–20 years earlier. This assumption could be in error for several reasons. First, a large amount of the DDT body burden measured could represent more recent exposures, but it could also represent residues originating from past high exposures to the pesticide. In developed countries where DDT use is banned and average exposures in food have declined considerably with time, current DDT burdens most likely represent past exposure. Second, the presence of the cancer itself or other conditions associated with the cancer might influence DDT metabolism, excretion, or the use of fat stores and the mobilization of fat-

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stored DDT/DDE/DDD to the blood, and hence, the concentration of DDT/DDE/DDD in blood, fat, and other tissues. Several studies have had access to blood samples collected several years before the diagnosis of cancer was made; however, there is no information available regarding possible exposures *in utero* or during adolescence that may have contributed to the development of cancer later in life. Third, recent reports have shown that obese and lean women metabolize organochlorines at a different rate. Therefore, there may be misclassification of exposure depending on time of measurement.

Thus far, the majority of the epidemiological studies conducted in the United States and abroad, particularly the most recent ones and those that included larger sample sizes, have found no association between DDT/DDE and breast cancer. However, some studies did find positive associations between DDT/DDE body burdens and breast cancer. The study of Canadian women by Demers et al. provided a new and important finding that deserves special attention. Demers et al. found that while mean plasma concentrations of DDT and DDE in women with breast cancer were not significantly different from those of controls, there seemed to be an association between DDE and cancer aggressiveness, as judged by increased tumor size and axillary lymph node involvement. A similar finding was reported by Woolcott et al., who found that the concentrations of DDE and DDT were higher in adipose tissue from cases with more poorly differentiated tumors than cases with moderately or well differentiated tumors. It seems that replication of these results is necessary before further speculating on the clinical significance of these findings. Details of these studies can be found in Section 3.2.2.7.

Possible associations with other cancers that have been investigated include pancreatic cancer, lymphoma, multiple myeloma, prostate and testicular cancer, endometrial cancer, and liver cancer. Overall, in spite of some positive correlations, there is no clear evidence that exposure to DDT/DDE causes cancer in humans. The case of pancreatic cancer deserves special mention because, as discussed by Hoppin et al., this cancer is characterized by cachexia, and the impact of this on serum organochlorine levels is difficult to predict. As the lipid pool decreases, the concentration of a fixed amount of lipid soluble chemical increases. While in the Hoppin et al. study, cases and controls had almost the same DDE serum levels before adjusting for lipid contents, adjusted values were much higher in cases than in controls. This points to the need to better understand changes in pharmacokinetics that occur as a result of a disease state and how these changes can influence the interpretation of the results. While there is no clear evidence of carcinogenicity of DDT/DDE/DDD in humans, there is sufficient evidence of carcinogenicity of these compounds in rodents, that developed tumors primarily in the liver following long-term oral treatment with these compounds. Two earlier studies in monkeys treated chronically with DDT reported no evidence of carcinogenicity, but a more recent study that involved Rhesus and Cynomolgus monkeys

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administered *p,p'*-DDT in the diet for up to 130 months reported that 2 out of 13 *Cynomolgus* monkeys developed malignant tumors, one hepatocellular carcinoma and one adenocarcinoma of the prostate. This corresponds to an incidence of 15%, which is significant compared to no neoplasms found in a group of nine contemporary untreated control monkeys.

The EPA has assigned DDT, DDE, and DDD to Group B2, probable human carcinogens. The International Agency for Research on Cancer (IARC) has determined that DDT, DDE, and DDD are possibly carcinogenic to humans (Group 2B). The Department of Health and Human Services (DHHS) has determined that DDT, DDE, and DDD may reasonably be anticipated to be human carcinogens.

Neurological Effects. The main neurophysiological effect of DDT is to prolong the sodium current in axons drastically, which accounts for the increase in depolarizing after-potential and the resultant repetitive afterdischarges in nerve fibers and synaptic junctions. As many basic functions such as respiratory and cardiovascular functions are controlled by the nervous system, exposure to high amounts of DDT is expected to produce a wide array of symptoms and central and peripheral signs of toxicity. High oral doses of DDT in humans result in paresthesia of the tongue, lips, and face, as well as apprehension, hypersusceptibility to external stimuli, irritability, dizziness, vertigo, tremor, and tonic and clonic convulsions. In general, results from studies in animals are consistent with observations in humans. Earlier studies of workers exposed to DDT (approximately 42 mg/man/day) and also studies with volunteers who ingested up to 35 mg DDT/man/day up to 18 months did not report adverse neurological effects, as assessed by tests of motor and sensory functions. However, a recent study of 27 workers in Costa Rica who used DDT for malaria control between 1955 and 1986 found that exposed subjects performed worst on tests of verbal attention and visuomotor speed and sequencing than a group of controls. However, because exposure levels were not available and current body burdens that could have been used to back estimate exposure were not determined, it is difficult to reconcile these findings with those from the earlier studies.

Hepatic Effects. Although there is no conclusive evidence in the available studies that the human liver is a primary target for DDT toxicity or that exposure to DDT causes liver toxicity in humans, many studies have reported hepatic effects in animals following exposure to DDT and related compounds. Effects observed include induction of microsomal enzymes, increased serum transaminase activities of hepatic origin, liver hypertrophy, hyperplasia, and necrosis, and liver cancer. Induction of microsomal enzymes is important because it can lead to altered metabolism of exogenous and endogenous substrates, including steroid hormones. In fact, as previously mentioned, alteration of hormone metabolism has been

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suggested to be at least a contributing factor in the DDT-induced reproductive and developmental effects. Based on induction profiles obtained in rats, DDT and related compounds are considered primarily, although not exclusively, phenobarbital-type inducers. Liver effects were the most sensitive effects observed in animals treated with DDT by relevant routes of exposure. Thus, an intermediate-duration study in rats that provided dose-response information for liver effects was used to derive an intermediate oral MRL for DDT.

2.3 MINIMAL RISK LEVELS

Inhalation MRLs

No MRLs were derived for inhalation exposure since adequate experimental data were not available by this route of exposure.

Oral MRLs

CAAn acute oral MRL of 0.0005 mg /kg/day was derived for DDT.

This MRL is based on a LOAEL of 0.5 mg/kg/day for neurodevelopmental effects in mice reported in a series of studies from the same group of investigators (Eriksson and Nordberg 1986; Eriksson et al. 1990a, 1990b, 1992, 1993; Johansson et al. 1995, 1996; Talts et al. 1998). An uncertainty factor of 1,000 was used (10 for use of a LOAEL, 10 for animal to human extrapolation, and 10 to account for intrahuman variation).

The most significant finding was the presence of altered motor behavior in adult mice treated with DDT perinatally. Groups of 10-day-old male NMRI mice were treated by gavage with a single dose of 0 (vehicle control) or 0.5 mg DDT/kg in a fat emulsion vehicle by gavage (Eriksson et al. 1990a) (this same single dose level was used in all studies from the series). At the age of 4 months, the mice were subjected to behavioral tests of spontaneous activity (locomotion, rearing, and total activity). Tests were conducted for 1 hour, and scores were summed for three 20-minute periods. During the last 40 minutes of testing, the treated mice showed significantly more activity than untreated controls. This was interpreted as disruption of a simple, nonassociative learning process (i.e., habituation), or a retardation in adjustment to a new environment. These same results were reported in a later paper (Eriksson et al. 1990b) in which the authors also reported results of neurochemical evaluations conducted 2–3 weeks after behavioral

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testing. They measured muscarinic acetylcholine (MACH) receptors density and choline acetyltransferase (ChAT) activity in the cerebral cortex and hippocampus (MACH also in striatum) and also measured K^+ -stimulated ACh release from cerebral cortex slices. In addition, five 10-day-old mice were administered 0.5 mg ^{14}C -DDT and the radioactivity in the brain was assayed 24 hours, 7 days, or 1 month after dosing. The results showed that K^+ -evoked ACh release in treated mice was significantly increased relative to controls, ChAT activity was not changed in the cerebral cortex or hippocampus, and the density of MACH was not significantly changed in the hippocampus or striatum, but a decreasing trend was seen in the cerebral cortex. DDT-derived radioactivity could be detected until day 7 after dosing, but none could be detected 1 month after dosing.

Previous studies have shown a significant increase in density of MACH in the cerebral cortex of 10-day-old mice 7 days after dosing, but not at 1 day postexposure compared to controls (Eriksson and Nordberg 1986). No increased binding was noted in the hippocampus either 1 or 7 days posttreatment. This was further investigated by evaluating the proportion of high- and low-affinity binding sites and affinity constants of the muscarinic receptors. A significant increase in the percentage of low-affinity binding sites accompanied by a significant decrease in high-affinity binding sites was measured in the cerebral cortex 7 days postexposure. No significant changes in affinity constants were noted. According to the authors, these low-affinity binding sites correspond to the M_1 receptor in the cerebral cortex, which are thought to be associated with neuronal excitation. No changes were observed in the sodium-dependent choline uptake system in the cerebral cortex 7 days postexposure.

In a follow-up study, Eriksson et al. (1992) treated 3-, 10-, and 19-day-old mice, and conducted behavioral testing and neurochemical evaluations at 4 months of age. As previously published, mice treated at 10 days old exhibited hyperactivity relative to controls and also had a significant decrease in the density of MACH in the cerebral cortex. No such changes were seen in mice treated at 3 or 19 days old. The authors suggested that the changes in MACH density and behavior might be the consequence of early interference with muscarinic cholinergic transmission specifically around the age of 10 days. In subsequent studies by the same group, mice were tested at 5 and 7 months old (Eriksson et al. 1993; Johansson et al. 1995). At both time points, mice treated with DDT perinatally showed increased spontaneous motor activity relative to controls, and decreased density of MACH in the cerebral cortex. No changes were seen regarding percentages of high- or low-affinity muscarinic binding sites in the cerebral cortex. Mice in these studies were also treated orally with the type I pyrethroid insecticide, bioallethrin, and tested for motor activity at 5 months old (Eriksson et al. 1993) and 7 months old (Johansson et al. 1995). In general, mice treated with DDT at the age of 10 days and later with

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bioallethrin showed increased motor behavior relative to those treated with bioallethrin alone, suggesting a DDT-induced increased susceptibility to bioallethrin. Mice treated first with DDT and later with bioallethrin showed increased difficulties in learning a skill, such as the swim maze test, compared with untreated mice, mice treated with DDT alone, or mice treated with bioallethrin alone (Johansson et al. 1995). Also, treatment with DDT followed by bioallethrin significantly increased the density of muscarinic receptors in the cerebral cortex relative to DDT alone. This increase was later attributed to increased expression of muscarinic receptor m4 mRNA (Talts et al. 1998). In yet another study from this group, paraoxon replaced bioallethrin, and the mice were tested at 5 and 7 months old (Johansson et al. 1996). In addition, acetylcholinesterase activity was measured in cerebral cortex of 5-month-old mice and MACH and nicotinic cholinergic receptors were measured in the cortex of 7-month-old mice. Relevant new findings include that: (1) DDT did not significantly alter acetylcholinesterase activity; (2) DDT did not alter the effects of paraoxon on acetylcholinesterase activity (decreased); (3) DDT altered (increased or decreased) some of motor responses due to paraoxon alone at 7 months, but not at 5 months; (4) none of the treatments altered performance in the swim maze test; and (5) none of the treatments altered the density of nicotinic cholinergic receptors in the cortex.

In this series of studies, two responses seem to be consistent from study to study in mice treated with DDT perinatally and tested as adults, a decrease in the density of muscarinic cholinergic receptors in the cerebral cortex and increased spontaneous motor activity. Whether or not there is a causal relationship is not clear. DDT also altered some motor responses induced by other pesticides, but a pattern was not always clear. The investigators interpreted the latter findings as DDT inducing changes early in the brain that translated into increased susceptibility to other pesticides later in life. The DDT-induced increase in spontaneous motor activity at the dose of 0.5 mg/kg is considered a less serious lowest-observed-adverse-effect level (LOAEL).

CAn intermediate oral MRL of 0.0005 mg/kg/day was derived for DDT.

This MRL is based on a no-observed-adverse-effect level (NOAEL) of 0.05–0.09 mg/kg/day for liver effects in Osborne-Mendel rats administered technical DDT in the diet at dosage levels of 0, 1, 5, 10, or 50 ppm for 15–27 weeks (Laug et al. 1950). In this dietary study, the amount of DDT added to the food was measured, but the actual food consumption and body weights of the rats were not measured. The calculated values for the NOAEL range from 0.05 to 0.09 mg/kg/day depending on the food consumption values used to calculate the actual dose of DDT consumed. Details about the dose calculation are discussed in Appendix A. The approximate doses provided in the diet in this study were 0.05–0.09,

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0.25–0.5, 0.5–0.9, and 2.5–4.6 mg/kg/day. An uncertainty factor of 100 was used (10 for interspecies extrapolation and 10 for human variability). The selected effects for the intermediate-duration oral MRL represent reliable effects that occurred at the lowest administered dose (including endocrine effects), as seen in Table 3-1 (see Chapter 3); therefore, the intermediate oral MRL is protective for endocrine effects and other non-cancer effects.

This study was essentially designed to examine whether DDT accumulates in adipose tissue and to what extent, how age and dose level affect accumulation, and how rapidly it is eliminated. Seventy-seven rats were used for microscopic evaluation of only the liver and kidney. This was based on findings from a previous study from the same group (Fitzhugh and Nelson 1947, see below) in which higher dietary levels of DDT had been used. Based on the previous findings, only the liver was expected to show microscopic changes. Although not explicitly stated, it is assumed that morphologic evaluations were conducted at the times when DDT levels in fat were determined (after 15, 19, 23, and 27 weeks of treatment).

There were no morphologic alterations in the kidneys. Liver alterations were noticed at the 5 ppm (0.25–0.5 mg/kg/day) dietary level of DDT and higher, but not at 1 ppm (0.05–0.09 mg/kg/day). Liver changes consisted of hepatic cell enlargement, especially in central lobules, increased cytoplasmic oxyphilia with sometimes a semihyaline appearance, and more peripheral location of the basophilic cytoplasmic granules. Necrosis was not observed. The severity of the effects was dose-related, and males tended to show more hepatic cell changes than females. Changes seen at the 5 ppm level (0.25–0.5 mg/kg/day) were considered by the authors as "minimal".

In the Fitzhugh and Nelson (1947) study, 16 female Osborne-Mendel rats were fed a diet containing 1,000 ppm technical DDT (approximately 96 mg/kg/day) for 12 weeks. Sacrifices were conducted at cessation of dosing and at various intervals after a DDT-free period. Liver changes were similar to those seen in the Laug et al. (1950) study, although of increased severity, and were still present in rats killed after 2 weeks in a DDT-free diet. Minimal liver changes were apparent after 4–6 weeks of recovery, and complete recovery was seen after 8 weeks. Hepatic effects ranging from increased liver weight to cellular necrosis have been reported in animals after chronic exposure to DDT in the diet.

An oral MRL for chronic-duration exposure was not derived because of the inadequacy of the available data on liver effects in animals to describe the dose-response relationship at low dose levels. The liver appears to be the most sensitive target of DDT for chronic duration exposures. In a brief communication, Fitzhugh (1948) stated that histopathological lesions occurred in the liver of rats fed 10 ppm DDT in the

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diet for 2 years, but no experimental details were given, so the quality of the study cannot be evaluated. This dietary level was the lowest level tested in the study, but was still higher than the lowest level resulting in hepatic effects in the Laug et al. (1950) study used for derivation of the intermediate-duration MRL.

