

**DDT**  
**(*para,para'*-Dichlorodiphenyltrichloroethane)**  
**(addendum)**

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### Explanation

Several Joint Meetings between 1963 and 1984 evaluated DDT in order to establish an ADI (Annex 1, references 2, 3, 6, 8, 12, 16, 22, 28, 32, 40, and 42). An ADI of 0–0.02 mg/kg bw was allocated in 1984 for any combination of DDT, DDD, and DDE on the basis of data for both humans and experimental animals (Annex 1, reference 42). The 1994 JMPR converted the ADI to a provisional tolerable daily intake (PTDI) for several pesticides including DDT that are no longer used in agricultural practice but may be present in food commodities as contaminants (Annex 1, reference 71). Use of the term ‘provisional’ reflects the lack of reliable data on the consequences of human exposure to these pesticides, and submission of relevant data from any source is encouraged.

The compound was reviewed at the present Meeting within the CCPR periodic review programme. An extensive range of studies on the biochemistry and toxicology of DDT and related compounds, including hormone-modulating effects, *in vivo* and *in vitro* has been reported since the 1984 JMPR. The present Meeting considered numerous reviews of the toxicity of DDT that have been published recently, and summarized new data on the toxicologically relevant effects of DDT and its metabolites. Mixtures of the *para,para'* and *ortho,para'* isomers of DDT, DDE, and TDE are referred to as the ‘DDT complex’. Most of the studies that were reviewed by the present Meeting were published in the open literature and were not performed according to GLP. The new toxicological data were considered with special regard to the following aspects of the potential hazards of DDT to human health, which were identified by the 1984 Joint Meeting:

- storage of DDT and its metabolites in human body fat and accumulation of the pesticide in the human environment owing to its chemical stability;
- the presence of residues of DDT and its metabolites in human milk and other milk fed to infants and the possibility of greater hazard to neonates, who have a relatively undeveloped capacity to detoxicate chemicals;
- the potential carcinogenicity of DDT to humans, indicated by its reported tendency to induce hepatomas in mice given high doses.

## Evaluation for provisional tolerable intake

### 1. Biochemical aspects

#### *Rats*

The transfer of *para,para'*-DDE (purity, 99%) from pregnant or lactating Sprague-Dawley rats to their fetuses or suckling neonates was measured after administration of 10 or 100 mg/kg bw per day on days 14–18 of gestation. The fetal DDE concentrations were about threefold lower than the corresponding placental concentration. The contributions of transplacental and lactational transfer were compared in a cross-fostering design. DDE was detectable on postnatal day 10 in the livers of pups of dams given 100 mg/kg bw per day. The concentration of DDE in the group exposed during lactation (3.0 µg/g of liver) was about 50 times higher than that of pups exposed only *in utero* (0.06 µg/g of liver), indicating that lactational exposure is quantitatively far more important than exposure *in utero* (You et al., 1999).

The induction of microsomal monooxygenase systems (CYP2B; benzyloxyresorufin *O*-dealkylation activity) by DDT (purity, 98%), DDE (purity, 99%), and DDD (purity, 99%) was investigated in the livers of male Fischer 344/NCr rats and in cultured rat hepatocytes. The efficacies and potencies of DDT, DDE, and DDD for CYP2B induction appeared to be similar on the basis of DDT equivalents in total serum (EC<sub>50</sub> values: 1.5, 1.8, and ≥ 0.51 µmol/L, respectively) and in hepatic tissue (EC<sub>50</sub> values: 15, 16, and ≥ 5.9 µmol/kg of liver, respectively). The effects of DDT on CYP2B induction would not be expected to be substantially diminished as a result of metabolic dechlorination of the parent compound to DDE or DDD (Nims et al., 1998).

The induction of microsomal monooxygenase systems by technical-grade DDT (80% *para,para'*-DDT; 20% *ortho,para'*-DDT) was investigated in the livers of male and female Wistar rats. CYP3A, which is not normally expressed in females, was strongly induced, but no significant induction was seen in males. The effects on CYP2Bs and associated enzymes indicated that males had a lower threshold than females, which attained greater relative induction. It remains to be established if this modulation of the sexual dimorphism in rats has significance in exposed human populations (Sierra-Santoyo et al., 2000).

#### *Rabbits*

Technical-grade DDT (80% *para,para'*-DDT; 20% *ortho,para'*-DDT) was administered orally to sexually mature female rabbits at a dose of 3 mg/kg bw, three times per week for 12–15 weeks. Oviductal and uterine luminal fluid, cleavage-stage embryos (day 1 *post coitum*), blastocysts (day 6 *post coitum*), fetuses, exocoelic fluid, and placentae (day 11 *post coitum*) were analysed. DDT accumulated in uterine secretions but not in oviductal luminal fluid. It was found in preimplantation blastocysts, and the concentration of residues in fetuses (197 µg/kg) was 16-fold higher than that in blastocysts (10.8 µg/kg) (Seiler et al., 1994).

## 2. Toxicological studies

### (a) Short-term studies of toxicity

#### *Rats*

In male Wistar rats (Pzh:WIS) that received DDT (purity, 92.2%) in one, three, or five daily oral doses of 24 mg/kg bw, the hepatic changes included hepatomegaly accompanied by an increase in *para*-nitroanisole *O*-demethylase activity and hepatocyte proliferation. Administration of DDT for 3 or 5 days significantly increased the number of binuclear hepatocytes. After the single dose, the liver sections contained a high proportion of vacuolated cytoplasm, and inflammatory infiltrations suggested hepatocyte necrosis. These changes were more pronounced after three administrations, when distinct signs of necrosis in the central lobular zone and more pronounced cytoplasmic vacuolization were found. In addition, abnormal mitotic figures were observed. Vacuolated cytoplasm and focal necrosis suggest that the increased hepatocyte proliferation and the mitogenic effect reflected a regenerative response of the liver to DDT (Kostka et al., 1996).

Male Wistar (Pzh:WIS) rats received one, three, five, or 14 daily oral doses of DDT (purity, 100%) equivalent to 12 mg/kg bw. After five and 14 oral doses, the relative liver weight was increased. DDT stimulated a sustained increase in DNA synthesis, which was accompanied by increased mitotic activity of hepatocytes and increased hepatocyte binucleation. The findings provide evidence for the occurrence of abnormal mitoses in the hepatocytes of rats treated with DDT. The histological investigation indicated vacuolized cytoplasm and signs of cell necrosis. The authors suggested that the mitogenic effect of DDT is at least partly related to a regenerative liver response (Kostka et al., 2000).

### (b) Long-term studies of toxicity and carcinogenicity

#### *Rodents*

DDT fed to rats for 2 years caused hepatic lesions at all doses, with a LOAEL of 0.5 mg/kg bw per day (Fitzhugh, 1948). Both hepatocellular adenomas and carcinomas were observed in six studies in mice. Benign and malignant lung tumours were observed in two studies in which mice were exposed both *in utero* and throughout life. Three studies in rats given doses of 25–40 mg/kg bw per day showed increased incidences of benign liver tumours. On the basis of more recent long-term studies, the 1984 JMPR established an overall NOAEL for tumorigenicity in rats of 125 ppm (Cabral et al., 1982), equivalent to 6.25 mg/kg bw per day.

A working group convened by the IARC concluded that “there is sufficient evidence in experimental animals for the carcinogenicity of DDT”. DDT is considered to be a nongenotoxic rodent carcinogen and a potent liver tumour promoter (IARC, 1991).

#### *Monkeys*

DDT (purity not given) was administered orally to 13 cynomolgus and 11 rhesus monkeys at a dose of 20 mg/kg bw per day for 130 months. A control group of 17 monkeys received corn oil. The two cases of malignant tumour detected in the treated group were a metastasizing hepatocellular carcinoma in a 233-month-old male and a well-differentiated adenocarcinoma of the prostate in a 212-month-old male. Benign tumours detected in the treated group included three cases of leiomyoma, two of which were uterine and one, oesophageal. No tumours were found in the control group of 17 monkeys. Fatty changes in the liver were observed in 53% of the treated and 29% of the control group. More specific signs of hepatotoxicity were detected microscopically in seven treated monkeys. Severe tremors and histological evidence of central nervous system and

spinal cord abnormalities were observed in six DDT-treated monkeys. Possible estrogenic effects may be reflected by the observation of uterine leiomyomas and two cases of intraductal hyperplasia of the breast in the DDT-treated females, but not in the control group. It was concluded that DDT is neurotoxic and hepatotoxic and has estrogenic effects and that the occurrence of two malignant tumours of different types does not permit a conclusion with respect to the carcinogenic effect of DDT in nonhuman primates. The occurrence of two malignant and three benign tumours, together with the preneoplastic changes in the breast of two additional monkeys, may indicate carcinogenicity, confirming the carcinogenicity observed in rodents. The Meeting could not reach a conclusion about the carcinogenicity of DDT in monkeys on the basis of this 130-month study at one dose (Takayama et al., 1999; Tomatis & Huff, 2000).

(c) *Genotoxicity*

Comprehensive summaries of the genotoxic effects of DDT and its metabolites have been published. Conflicting data were obtained with regard to some genetic end-points. DDT induced chromosomal aberrations in human blood cultures, but, in most studies, DDT did not induce genotoxic effects in rodent or human cell systems, nor was it mutagenic to fungi or bacteria. *para,para'*-DDE weakly induced chromosomal aberrations in cultured rodent cells and mutations in mammalian cells and insects, but not in bacteria (IARC, 1991).

DDT induced structural chromosomal aberrations in the spleen cells of mice 6, 24, and 48 h after an intraperitoneal injection of DDT at a dose of 5.5 mg/kg bw. Maximal induction was found at 24 h (Amer et al., 1996).

(d) *Reproductive toxicity*

The effects of the DDT complex on reproduction and development in humans and experimental systems have been reviewed (Coulston, 1985; Agency of Toxic Substances and Disease Registry, 1994; Environmental Protection Agency, 1998). The effects on reproduction in animals include decreased fertility and abortions, and stillbirths. In multigeneration studies in rodents, DDT decreased fertility and gonadal weights, increased the length of the estrous cycle, decreased the number of implantations, increased the rate of embryo mortality, decreased litter size, and increased the length of gestation. In a three-generation study in rats, the mortality rate of offspring increased at all doses, the lowest of which corresponded to about 0.2 mg/kg bw per day (Laug et al., 1950). Three other studies in rats and mice showed no effects on reproduction at higher doses (1–6.5 mg/kg bw per day (Agency of Toxic Substances and Disease Registry, 1994).

The effects on development observed pre- or postnatally after DDT treatment that may be related to estrogenicity include embryoletality, decreased fetal growth, and prematurity in rabbits and dogs fed diets providing a dose of 5 mg/kg bw per day, and decreased ovarian weights, cystic ovaries, loss of corpora lutea, infertility, premature puberty, altered onset of vaginal opening, tail anomalies, and increased pup mortality rates in rodents. The lowest relevant NOAEL for developmental effects was reported to be 1 mg/kg bw per day in rats (Agency of Toxic Substances and Disease Registry, 1994; Environmental Protection Agency, 1998).

*Rats*

*para,para'*-DDE had effects on developing, pubertal, and adult male Long-Evans rats after oral administration to their dams at 100 mg/kg bw per day on days 14–18 of gestation. Male pups from the resulting litters had a reduced anogenital distance at birth and retained thoracic nipples. No antiandrogenic anomaly was observed in untreated rats. In order to establish the antiandrogenic effects of *para,para'*-DDE on male pubertal development, 21-day-old male rats were given the vehicle or *para,para'*-DDE at 100 mg/kg bw per day until day 57. Treatment significantly delayed

the onset of puberty, defined as the day the prepuce separated from the penis, by 5 days compared with control rats. In 120-day-old male rats that had been castrated and in which constant serum testosterone levels were maintained by implantation of testosterone-containing Silastic capsules, oral treatment with *para,para'*-DDE at 200 mg/kg bw per day by gavage for 4 days significantly reduced the androgen-dependent weights of the seminal vesicles and prostate, despite high serum testosterone concentrations. The results suggest that abnormalities in male sexual development induced by *para,para'*-DDE are mediated at the level of androgen receptors (Kelce et al., 1995).

The effects of *para,para'*-DDE on male sexual development were compared in Sprague-Dawley and Long-Evans rats by giving the compound to pregnant dams by gavage at 10 or 100 mg/kg bw per day on days 14–18 of gestation. The rats were examined for sexual developmental landmarks, and the effects of *para,para'*-DDE on androgen receptor expression were evaluated in the testis and other reproductive organs. A significant increase in the frequency of thoracic nipple retention was observed in male pups of both strains at the high dose. A much weaker response was observed only in Sprague-Dawley rats at the low dose. The higher dose also induced a significant reduction in the anogenital distance in Long-Evans rats. Alterations in expression of the androgen receptor in testicular tissue were described in both strains only at the high dose (You et al., 1998).

### *Rabbits*

The accumulation of orally administered technical-grade DDT (a mixture of 15–20% *ortho,para'* and 80–85% *para,para'* isomers) in tissues and fluids of the genital tract and the effect on reproductive functions were studied in female hybrid rabbits. DDT was given at a dose of 3 mg/kg bw three times per week by gavage for 12 weeks. The animals were then inseminated and were killed on day 1, 6, or 11 after insemination. The serum concentration of DDT increased to > 100 µg/L over 12 weeks. DDT-treated animals had a significantly reduced ovulation rate, but the decrease was within the range of the control animals in this study. Furthermore, the ovulation rate after combined treatment with DDT and  $\gamma$ -hexachlorocyclohexane was lower than that after treatment with DDT alone, but it was not significantly lower than that of the corresponding controls. The relative proportion of uteroglobin in uterine secretions decreased after treatment with DDT, but the total protein content and the electrophoretic pattern were unchanged. DDT did not affect the estradiol levels after insemination but tended to reduce the increase in progesterone. Nevertheless, the serum progesterone concentration on day 11 after insemination was comparable to that of the controls on day 11 and had no adverse biological consequences, as early embryonic development and implantation were not affected. No differences in ovarian or uterine morphology was seen in histological sections from control and exposed animals. The relevance of the slightly reduced ovulation rate and relative proportion of uteroglobin and the reduced increase in progesterone concentration for human reproduction is not clear (Lindenau et al., 1994).

### (e) *Special studies*

#### (i) *Hormonal effects*

#### *Mice*

Adult ovariectomized CD-1 mice received a single subcutaneous injection of *ortho,para'*-DDT (purity, 99%) and *para,para'*-DDD at a dose of 3.8, 7.5, 15, or 30 mg/kg bw. The regulation of the estrogen-responsive genes for lactoferrin and the progesterone receptor in the uterus by the estrogenic *ortho,para'*-DDT and the nonestrogenic *para,para'*-DDD were compared with the regulation by 17 $\beta$ -estradiol, which was administered at a dose of 10 µg/kg bw. The results showed modestly increased uterine concentrations of lactoferrin and progesterone receptor mRNA at

3.8 mg/kg bw and a maximal response at 7.5 mg/kg bw. The higher doses of DDT maintained the concentrations of lactoferrin and progesterone receptor mRNA. The responses after injection of DDT were much lower than those induced by estradiol. The authors suggested that alteration of lactoferrin and progesterone receptor genes by environmentally relevant doses of *ortho,para'*-DDT has a significant impact on uterine responses at the molecular level. As the responses reported in this study were acute effects, their relevance to the consequences of long-term exposure to xenobiotics in the environment remains to be determined (Das et al., 1998).

### *Rats*

The regulation of androgen receptor-dependent gene expression by *para,para'*-DDE (purity, 99%) was studied in adult male Harlan Sprague-Dawley rats that had been castrated and implanted with capsules containing testosterone. Treatment of these rats with DDE for 4 days at 200 mg/kg bw per day induced a decrease in the weights of the seminal vesicles and prostate and a reduction in immunohistochemical staining of the androgen receptor in epididymal nuclei when compared with vehicle-treated controls. The ability of DDE to induce a testosterone-repressed and/or repress a testosterone-induced prostatic message indicates specific androgen receptor antagonism. Thus, the results indicate that DDE acts as an antiandrogen *in vivo* by altering the expression of androgen-regulated genes (Kelce et al., 1997).

In a tier I screening battery designed to detect endocrine-active compounds in male CD (Crl:CD IGS BR) and Long Evans (Crl:(LE)BR) rats, *para,para'*-DDE (purity not given) was administered at a dose of 100, 200, or 300 mg/kg bw per day to CD rats and 200 or 300 mg/kg bw per day to Long Evans rats for 15 days. On the morning of day 15, the rats received the test compound and were killed 2 h later by exsanguination after anaesthesia with carbon dioxide. Organ weights were calculated relative to body weight. *para,para'*-DDE decreased the mean final body weights and increased the liver weight in all treated groups. The absolute unit weights of the epididymides and relative accessory sex glands was increased only in Long Evans rats at the highest dose. The hormonal responses—increased serum testosterone, estradiol, dihydrotestosterone, and thyroid-stimulating hormone concentrations and decreased thyroxine concentrations—were dose-dependent only in Long Evans rats. The results showed considerable differences in sensitivity by strain. *para,para'*-DDE was identified as a weakly endocrine-active androgen receptor-antagonist in Long Evans rats but not in CD rats (O'Connor et al., 1999).

### *In-vitro studies*

The capacities of various DDT isomers and metabolites to activate the human estrogen receptor transcriptionally were studied in MCF-7 cells and yeast expression-reporter systems. The results of competitive binding assays showed that *ortho,para'*-DDT, *ortho,para'*-DDD, *ortho,para'*-DDE, and *para,para'*-DDT bind specifically to the human estrogen receptor with an approximately 1000-fold weaker affinity than that of estradiol. Only *ortho,para'*-DDT bound to the rat estrogen receptor. In the yeast expression systems, an *ortho,para'*-DDT metabolite transactivated the human estrogen receptor with a 140- to 300-fold weaker potency than that of estradiol. The greater potency of DDT in the yeast cell system may indicate that the DDT-related compounds have greater biological activity than that indicated by their affinities for human estrogen receptor in a cell-free system. Thus, in MCF-7 cells and in yeast expression-reporter systems, certain DDT isomers and metabolites acted as direct agonists and transactivated human estrogen receptors at the concentrations found in human tissues (Chen et al., 1997).

The interaction of DDT and DDT-like compounds with the androgen receptor was characterized in the human hepatoma cell line HepG2. The chemicals were tested for androgen receptor agonist and antagonist activity in the presence and absence of 0.1  $\mu\text{mol/L}$  of

dihydrotestosterone, respectively. The concentration of *para,para'*-DDE, the most potent DDT metabolite, that inhibited androgen receptor-dependent activity by 50% was almost 50 times higher than the median concentration of DDE measured in serum in a study of women in the USA in 1986 (Maness et al., 1998).

(ii) *Neurotoxicity*

*Mice*

NMRI mice were treated with a single oral dose of 0.5 mg/kg bw of [<sup>14</sup>C]DDT (purity not given) between day 3 and day 20 after birth, a period of rapid development of the rodent brain (the 'brain growth spurt'). The amount of radiolabel found 24 h after treatment increased between 3 and 20 days of age, and the highest activity was found at day 24. The largest amount of radiolabel still retained 1 week after administration was found on day 10 after birth. Radiolabel was retained up to 7 days. One month after treatment, the amount of radiolabel was no different from the background level. The concentration of DDT found in brain between days 11 and 17 after birth was about 15 ng/g, representing approximately 2% of the total administered dose.

Administration of DDT on postnatal day 10 caused changes in the density of muscarinic cholinergic receptors in the cerebral cortex 7 days after treatment. No significant changes in the hippocampus were observed at any time after treatment. Concomitantly, a significant decrease in the percentage of high-affinity muscarinic binding sites and a corresponding increase in the percentage of low-affinity muscarinic binding sites was found. In adults, a significant decrease in the density of muscarinic cholinergic receptors in the cortex was found after DDT treatment neonatally, but this decrease was < 5% in two studies and was not observed in the hippocampus or the striatum.

Behavioural tests on adults at 4 months of age indicated disruption of habituation in mice treated on postnatal day 10. Habituation was defined as a decrease in locomotion, rearing, and total activity variables in response to diminishing novelty of the test chambers over a 60-min test period divided into three 20-min periods, with 12 animals in each group. The effects of DDT on behaviour and muscarinic cholinergic receptors in adult mice were not observed when DDT was administered to mice on postnatal day 3 or 19 and therefore appeared to be limited to a short induction period during neonatal development at about day 10. As the responses reported from this laboratory were found after administration of a single low dose of DDT to mice of one strain, exclusively when treated on postnatal day 10, their relevance to the consequences of exposure of humans to DDT remains to be determined (Eriksson, 1984; Eriksson & Nordberg, 1986; Eriksson et al., 1990, 1992; Johansson et al., 1995).

The toxicological significance of these findings for humans could not be fully evaluated by the Meeting. Differences in brain development between species were taken into consideration. In many mammalian species, the brain grows rapidly during perinatal development. In humans, this period is at its maximum at the end of the third trimester of pregnancy. Early postnatal exposure of rodents encompasses a time span equivalent to peri-neonatal exposure of humans. Therefore, postnatal days 10–16 in mice have no identical equivalent in humans but still represent a sensitive period of postnatal life. The neurodevelopmental effects of DDT should be investigated under conditions comparable to human perinatal exposure. Many of the studies of compounds tested during the 10–16-day postnatal period have not been reproduced under identical conditions with the same dose range (Federal Institute for Consumer Health Protection and Veterinary Medicine, 1997). Although rodents appear to be an appropriate model for testing postnatal neurotoxic effects, the use of a single mouse strain and only one dose was considered of limited relevance for risk assessment.

### (iii) Immune responses

A variety of DDT-induced effects on humoral and cell-mediated responses and modulation of non-specific host defences by DDT have been reported in rabbits, guinea-pigs, rats, and mice (Banerjee et al., 1996a). Because no validated study protocols were used with different species, doses, treatment periods, routes of exposure, or parameters evaluated, no NOAEL could be established for the immune system.

#### *Mice*

Male albino mice (Hissar strain) were given diets containing technical-grade DDT (purity, 95%) at a concentration of 20, 50, or 100 ppm, providing doses of 3, 7.5, and 15 mg/kg bw per day, for 3–12 weeks. No deaths or other obvious signs of general toxicity were seen, but significant changes in the weights of the spleen and liver were observed in a dose–time-dependent pattern at the two higher doses. Decreases in the primary antibody titre to sheep red blood cells and reductions in plaque-forming cell responses suggested depression of primary and secondary humoral immune responses in mice at 50 and 100 ppm. The inhibitory effects of DDT on the humoral immune response to a thymus-independent antigen (bacterial lipopolysaccharide) was also reported in mice exposed to 50 or 100 ppm DDT for 6–12 weeks (Banerjee, 1987a; Banerjee et al., 1986).

#### *Rats*

Male Wistar rats were given diets containing technical-grade DDT (purity, 95%) at a concentration of 20, 50, or 100 ppm, equivalent to 1, 2.5, and 5 mg/kg bw per day, for 4 or 8 to 22 weeks. No deaths or other obvious signs of general toxicity were seen, and the weights of the spleen and thymus were unchanged. The increase in immunoglobulin (Ig)G titres after immunization with tetanus toxoid was inhibited by treatment with 50 ppm for 22 weeks or 100 ppm DDT for 18–22 weeks. Cell-mediated parameters were inhibited in rats given DDT at the two higher doses and subsequently immunized with tetanus toxoid. Humoral and cellular immune responses were suppressed by DDT at the two higher doses after a relatively short exposure of 4 weeks in rats fed a diet containing only 3% protein, but not in rats on a diet containing 12 or 20% protein (Banerjee, 1987b; Banerjee et al., 1995).

The effects of DDT, DDE, and DDD (all, purity, 98%) on humoral and cell-mediated immune responses were compared in male Wistar rats. All three compounds suppressed humoral (IgM and IgG) and cellular immune responses (inhibition of migration factors, delayed-type hypersensitivity reaction) in rats fed 200 ppm over 6 weeks. The order of potency for decreasing antibody titres to ovalbumin and suppression of T-lymphocyte activity was DDE > DDD > DDT (Banerjee et al., 1996b).

### (f) Studies of metabolites

The metabolites *ortho,para*'-DDD, *para,para*'-DDD, and *para,para*'-DDE are included in the DDT complex. The persistent lipophilic DDT metabolite 3-methylsulfonyl-DDE appears to be formed in a pathway involving enterohepatic circulation and sequential metabolism of glutathione conjugates in the liver and intestinal microflora. Once formed, 3-methylsulfonyl-DDE is further metabolized by the mitochondrial CYP11 isoform in the adrenal cortex, to a reactive intermediate that binds covalently to cellular constituents in the adrenal zona fasciculata. 3-Methylsulfonyl-DDE is known to be excreted in human milk (Haraguchi et al., 1989).

### *Mice*

Treatment of C57B1 mice with a single dose of 3 mg/kg bw of 3-methylsulfonyl-DDE (purity, 99%) resulted in mitochondrial destruction in the adrenal zona fasciculata. The transplacental toxicity of this metabolite was studied in the developing adrenal cortex in pregnant C57B1 mice given a single injection of 25 mg/kg bw. The compound was readily transferred through the placenta to fetuses, where covalent metabolite binding and mitochondrial destruction were observed as early as the fetal adrenal cortex could be observed. Electron microscopy revealed mitochondrial degeneration and vacuolation in fetal adrenal cortical cells. The lesions were clearly visible on days 14–15 but were most pronounced on days 16–17. Transplacental transfer and irreversible binding to the fetal adrenal cortex were studied after a single injection of [<sup>14</sup>C]3-methylsulfonyl-DDE to pregnant C57B1 mice. Tape-section autoradiograms of fetuses on days 12–17 of gestation revealed high, tissue-specific accumulation of radiolabel in the fetal adrenal gland. On day 12 of gestation, the adrenal radiolabel could be extracted with organic solvents, whereas on days 13–17 the radiolabel was irreversibly bound in the adrenal gland. The uptake of 3-methylsulfonyl-DDE by the fetal adrenal glands increased continuously with gestational age.

3-Methylsulfonyl-DDE was also efficiently transferred from the dams' milk to suckling pups, which attained higher levels of bound adducts in their adrenal glands than the dams. Decreased corticosterone concentrations in the plasma of suckling pups were observed after administration of 3-methylsulfonyl-DDE to lactating dams. After injection of the compound at a dose of 12 mg/kg bw, the adrenocorticotrophic hormone-induced corticosterone concentrations in the offspring 8 days after injection were reduced to 50% of the control values. Thus, the transferred dose of 3-methylsulfonyl-DDE induced a functional disturbance of the adrenal cortex at doses at which no overt histological changes were seen in the adrenal glands postnatally, resulting in a reduced capacity to secrete corticosterone (Jönsson, 1994; Jönsson & Lund, 1994; Jönsson et al., 1995).

### **3. Observations in humans**

The health effects of DDT in humans have been reviewed (Hayes, 1982; Coulston, 1985; Agency of Toxic Substances and Disease Registry, 1989, 1994; Environmental Protection Agency, 1998). In several studies, volunteers ate diets containing measured amounts of DDT and DDE. A single dose of 6–10 mg/kg bw of DDT resulted in sweating, headache, and nausea, while a dose of 16 mg/kg bw led to convulsions. Persons who consumed DDT in these amounts usually recovered within 24 h. Volunteers ate 0.31–0.61 mg/kg bw per day for up to 21 months with no noticeable effects (Agency of Toxic Substances and Disease Registry, 1989). No changes in liver function were observed in workers exposed to 0.05–0.25 mg/kg bw per day (Agency of Toxic Substances and Disease Registry, 1994).

#### *(a) Exposure*

Pesticide applicators are exposed primarily to *para,para'*-DDT, whereas nearly all of the general population is exposed to the *para,para'*-DDE metabolite in the diet or drinking-water (Longnecker et al., 1997). Levels of exposure and the concentrations of DDT in human tissues, milk, and blood have been summarized by Ahlborg et al. (1995). The IARC (1991) and Smith (1999) reported that the mean concentrations of DDT in the population have declined in much of the world: from 5000–10 000 µg/kg to around 1000 µg/kg of milk fat or even lower over the last three decades. Although different means are found in different regions, the declines seen in various countries correspond to their restrictions on use of DDT.

(b) *Carcinogenicity*

Epidemiological studies on the association between exposure to DDT and cancer risk have been reviewed extensively (Ahlborg et al., 1995; Longnecker et al., 1997; Baris et al., 1998). The concentrations of DDE in population samples of adipose tissue from persons in 22 states of the USA in 1968 were compared with the age-adjusted rates of mortality from multiple myeloma, non-Hodgkin lymphoma, and cancers of the breast, corpus uteri, liver, and pancreas in 1975–94. The rate for mortality from liver cancer increased significantly with the concentration of DDE in adipose tissue in whites of each sex but not among African-Americans. No association was observed for pancreatic cancer or multiple myeloma. The rate of mortality from breast cancer was inversely correlated with the concentration of DDE among both white and African-American women. Significant inverse correlations were also observed for uterine cancer among white women, whereas no association was observed for African-Americans or for non-Hodgkin lymphoma among white and African-American women. The results for pancreatic cancer, multiple myeloma, non-Hodgkin lymphoma, breast cancer, and uterine cancer do not support the hypothesis of an association with past adipose tissue concentrations of the DDT derivative DDE. The association between liver cancer and DDE observed among whites warrants further investigation (Cocco et al., 2000).

(i) *Non-Hodgkin lymphoma*

In two large case-control studies in the USA, agricultural exposure to DDT, as assessed from questionnaires, was associated with an increased risk for non-Hodgkin lymphoma (Longnecker et al., 1997). No association was found in other studies, and a pooled analysis of three case-control studies in the USA provided no consistent evidence that exposure to DDT is associated with non-Hodgkin lymphoma among male farmers. Some excess risk was initially found for farmers with longer or more frequent exposure to DDT, but this largely disappeared after adjustment for use of other pesticides (Baris et al., 1998).

(ii) *Pancreatic cancer*

Among 5886 workers at a chemical manufacturing plant, exposure to technical-grade DDT was associated with an increased risk for pancreatic cancer in 28 verified cases. Exposure for more than 10 years and a latency of more than 20 years since the last exposure were also considered to be risk factors. Exposure to two DDT derivatives, Ethylan and DDD, was also associated with pancreatic cancer. Adjustment of the results for potential confounders for this disease did not lower the risks. The results may indicate that DDT can cause pancreatic cancer under conditions of heavy, prolonged exposure (Garabrant et al., 1992; Longnecker et al., 1997).

The concentrations of organochlorine compounds were measured in serum obtained at the time of enrolment into the study from 108 patients with pancreatic cancer and 82 control subjects aged 32–85 years in the San Francisco Bay Area (USA) between 1996 and 1998. Cases were identified by rapid case-ascertainment methods; controls were frequency matched to cases on age and sex by random-digit dialling and random sampling of health care financing administration lists. A weak association was found between the serum concentration of DDE and pancreatic cancer. However, the results are not directly comparable with the sevenfold increase in risk shown for DDT manufacturing workers, as the patients were more likely to have been exposed to DDE in food than to the parent compound DDT. Occupationally exposed persons would be more heavily exposed to DDT than those who are environmentally exposed, and they are more likely to be exposed by inhalation and dermal contact rather than ingestion (Hoppin et al., 2000).

(iii) *Prostate and testicular cancer*

National cancer registries in the Nordic countries show a continuous increase in the incidence of testicular cancer since the 1950s. It has been suggested that fetal and neonatal exposure to *para,para'*-DDE has caused this increase. In 1985–89, the annual incidence rates in the age group 20–24 were 14.5 in Denmark, 12.6 in Norway, 8.1 in Sweden, and 3.6 in Finland; however, no difference was found in the mean concentration of *para,para'*-DDE in breast milk in the four countries or in the rate of decline since the late 1960s (Ekbom et al., 1996).

The association between the mortality rate from prostate or testicular cancer and environmental exposure to DDT and *para,para'*-DDE in the USA in the period 1971–94 was explored by multiple linear regression analysis. The study provided no support for the hypothesis of a link between environmental exposure to DDT derivatives and cancer of the male reproductive tract (Cocco & Benichou, 1998).

(vi) *Endometrial cancer*

In a multicentre case–control study in five regions of the USA, the association between serum concentrations of organochlorine compounds, such as DDT, and risk for endometrial cancer was analysed on the basis of a sample of 90 endometrial cancer cases and 90 individually matched community controls. The adjusted relative risk for endometrial cancer for women in the highest quartile of exposure when compared with women in the lowest quartile was negligible for *para,para'*-DDE (Sturgeon et al., 1998).

In a population-based case–control study in Sweden, the serum concentrations of organochlorines, including DDE, were measured in 154 patients with endometrial cancer and 205 population controls. When logistic regression was used to calculate odd ratios as a measure of relative risk, no significant association was found between increasing concentrations of organochlorines and risk for endometrial cancer (Weiderpass et al., 2000).

(v) *Breast cancer*

A pilot study was undertaken to measure and compare the concentrations of chemical residues in mammary adipose tissue from 50 white women with malignant or non-malignant breast disease. Significantly higher concentrations of *para,para'*-DDE and polychlorinated biphenyls were found in tissue from 20 women with breast cancer than from those with benign breast disease, while the concentrations of *para,para'*-DDT did not differ between the two groups. However, because other important risk factors for breast cancer were not studied, the interpretation of these findings is uncertain. The authors suggested that the discrepancy with other findings may have been due to chance or to differences in the study groups, e.g. nationality (Falck et al., 1992).

The association between serum concentrations of DDE and breast cancer was analysed in archival serum samples collected between 1985 and 1991 from women who had been enrolled in the New York University Women's Health Study (14 290 participants) in the USA. The women were aged 35–65 years, and 80% were white. In a case–control study, the concentrations of DDE and of polychlorinated biphenyls in 58 women in whom breast cancer was diagnosed 1–6 months after they had joined the study were compared with those of 171 matched controls from the same population. After adjustment for such confounders as family history of breast cancer, history of lactation, and age at first full-term pregnancy, the authors found that women with the highest serum concentrations of DDE had a fourfold higher relative risk for breast cancer than women with the lowest DDE concentrations. They suggested that, in this population of New York City women, breast cancer was strongly associated with DDE concentration in serum (Wolff et al., 1993).

In a preliminary study, biopsy specimens from 41 women aged 40–69 years living in the Quebec City (Canada) region were investigated histologically and analysed for organochlorines; in addition, organochlorines were determined in plasma. Infiltrating mammary adenocarcinoma was diagnosed in 18 women (case patients), while benign breast disease was found in 17 other women (control subjects). Higher concentrations of *para,para'*-DDE were reported only in women with estrogen receptor-positive breast cancer and not in women with estrogen receptor-negative cancer. Since there were only nine cases of estrogen receptor-positive breast cancer, the suggestion that women with hormone-responsive breast cancer have a higher DDE body burden than women with benign breast disease needs further confirmation (Dewailly et al., 1994).

In a prospective study in California (USA), the concentrations of DDE and polychlorinated biphenyls were measured in blood samples drawn between 1964 and 1971 from 150 women who went on to develop breast cancer and from 150 matched controls. The development of breast cancer was not associated with the serum concentration of DDE. When women of different ethnic groups were considered separately, DDE appeared to be a risk factor for African-American women but not for Asian women (Krieger et al., 1994).

In a case-control study conducted between 1994 and 1996 in Mexico City, 141 histologically confirmed cases of breast cancer were compared with 141 age-matched controls ( $\pm 3$  years). No statistically significant difference was found in the mean DDE serum concentration in breast cancer patients (562  $\mu\text{g}/\text{kg}$ ) and controls (505  $\mu\text{g}/\text{kg}$ ) (López-Carrillo et al., 1997).

In a European multicentre case-control study, the concentrations of DDE in fat tissue from 265 postmenopausal women with breast cancer were lower than those of 341 controls matched for age and centre. The results of this large study are clearly incompatible with an increased risk for breast cancer at increased concentrations of DDE, although associations with other organochlorine compounds cannot be excluded (van't Veer et al., 1997).

In an analysis within the Nurses' Health Study in the USA, the concentrations of organochlorine compounds were measured in blood samples drawn in 1989 or 1990 from 240 women who developed breast cancer by 1992 and from matched control women in whom breast cancer did not develop. The concentrations of DDE were available for 236 pairs. The risk for breast cancer tended to be lower among women with higher serum concentrations of DDE, but the trends were not statistically significant. These results do not support the hypothesis that exposure to DDT increases the risk for breast cancer (Hunter et al., 1997).

In a prospective study in Denmark (Copenhagen City Heart Study), organochlorine compounds were measured in blood samples obtained in 1976 from 240 women who developed breast cancer by 1995 and from 477 matched control women. The development of breast cancer was not associated with the concentration of DDE or total DDT. In this study, 78% of the participants donated blood twice, in 1976–78 and in 1981–83. A nested case-control study was conducted of 155 women who had developed breast cancer by 1992 and 274 matched controls who had participated in both examinations. Information on risk factors for breast cancer was obtained from standardized questionnaires. Above-average body weight and use of hormone replacement therapy were associated with an increased risk for breast cancer. Significant decreases in the average serum concentrations of organochlorines were found between the two examinations, except for *para,para'*-DDT, and the high serum concentration of this compound was associated with an increased risk for breast cancer. However, the odd ratios and confidence intervals were not reported. The risk for breast cancer increased nonsignificantly with increasing serum concentration of all DDT isomers. No significant association was found between overall survival and the serum concentration of *para,para'*-DDT at the first examination. When the same analyses were performed with the average concentration from the two examinations, a weak dose-response

relationship was seen for *para,para'*-DDT, which was not significant when adjusted for tumour characteristics (Høyer et al., 1998, 2000a,b).

The relationship between serum concentrations of organochlorine pesticides, including five DDT compounds, and the risk for breast cancer was evaluated prospectively from samples in the breast cancer serum bank in Columbia, Missouri, USA. Samples from 105 women in whom breast cancer was diagnosed during up to 9.5 years of follow-up and who had donated blood in 1977–87 and 207 controls matched on age and date of blood collection were examined. Women with higher serum concentrations of DDT compounds (total DDT, *para,para'*-DDT, and *para,para'*-DDE) had no increased risk for breast cancer. The women with the highest concentrations of *para,para'*-DDT had a significantly reduced risk for breast cancer when compared with those with the lowest concentrations. The results of this study do not support a role of DDT in the etiology of breast cancer (Dorgan et al., 1999).

A case–control study was conducted in Connecticut, USA, between 1994 and 1997 to investigate the relationship between exposure to DDE and DDT and risk for breast cancer. A total of 304 women with newly diagnosed breast cancer and 186 women with benign breast disease, aged 40–79 years, provided surgical specimens of breast adipose tissue for gas chromatographic analyses. The age-adjusted geometric mean tissue concentration of DDE was similar in the case women (740 µg/kg) and the controls (780 µg/kg), as was that of DDT (52 µg/kg and 56 µg/kg). These results do not support an association between adipose tissue concentrations of DDE and DDT and risk for breast cancer (Zheng et al., 1999).

In a prospective study of the association between exposure to DDE and the development of breast cancer, the resources of two specimen banks established in Washington County, Maryland, USA, in 1974 and 1989 were used. It was considered that the serum concentrations of DDE were likely to be maximal in 1974, when organochlorine compounds were banned in the USA. The median concentrations of DDE were lower in women who developed breast cancer by 1994 than in controls in both periods. The risk for developing breast cancer of women with the highest concentrations of DDE was roughly half that of women with the lowest concentrations. Adjustment for family history of breast cancer, body mass index, age at menarche or first birth, and months of lactation did not alter these associations (Helzlsouer et al., 1999).

In most studies in which the relationship between exposure to organochlorine compounds and breast cancer was examined, residues were measured in serum, although they are higher in breast adipose tissue, which represents cumulative internal exposure at the target site for breast cancer. In a large study in which DDT and its metabolites DDE and DDD were measured in breast adipose tissue, the concentrations of DDE were higher than those of DDT in both breast cancer patients and controls. After adjustment for age, no relationship was found between the concentration of either DDT, DDE, or DDT + DDE + DDD and breast cancer (Bagga et al., 2000).

### (c) *Reproductive toxicity*

Studies on the reproductive effects of DDT in humans are rare. The few studies available showed no correlation between exposure to DDT and stillbirths, miscarriage, or premature rupture of fetal membranes (Coulston, 1985; Agency of Toxic Substances and Disease Registry, 1989).

In a study of 859 children in the USA who were tested at the age of 3, 4, or 5 years, exposure to DDT transplacentally or during breast-feeding did not affect psychomotor or mental behavioural patterns, tested on the McCarthy and Bayley scores, respectively, or measures of school performance in English and mathematics (Gladden & Rogan, 1991).

No confirmed adverse health effects have been reported in infants exposed to DDT while suckling, even in communities where the reference level was frequently exceeded (WHO, 1998).

### Comments

The hepatic effects of DDT in rats include increased liver weights, hypertrophy, hyperplasia, induction of microsomal enzymes, including cytochrome P450, cell necrosis, increased activity of serum liver enzymes, and mitogenic effects, which might be related to a regenerative liver response to DDT. The potencies of DDT, DDE, and DDD for induction of CYP2B are of the same order of magnitude. The effects on CYP2B and associated enzymes indicated that males have a lower threshold than females, which induced these enzymes to a greater extent.

Conflicting data were obtained with regard to some genotoxic end-points. In most studies, DDT did not induce genotoxic effects in rodent or human cell systems nor was it mutagenic to fungi or bacteria. *para,para'*-DDE weakly induced chromosomal aberrations in cultured rodent cells and mutation in mammalian cells and insects, but not in bacteria. The induction of structural chromosomal aberrations in mouse spleen cells was maximal 24 h after intraperitoneal administration of DDT.

The Meeting could not reach a conclusion about the carcinogenicity of DDT in monkeys, as a 130-month study at one dose in nonhuman primates showed a small number of tumours at various sites. A working group convened by IARC classified the DDT complex as a non-genotoxic carcinogen in rodents and a potent promoter of liver tumours. The 1984 JMPR estimated that the lowest relevant NOAEL for carcinogenicity in rats was 6.2 mg/kg bw per day and concluded that "there is no significant risk of DDT producing tumours in humans". The overall evaluation of the IARC group was that "DDT is possibly carcinogenic to humans" but that "there is inadequate evidence in humans for the carcinogenicity of DDT". Epidemiological studies on the association between exposure to DDT and cancer risk were reviewed for the 2000 JMPR. The association between exposure to DDT and/or DDE and breast cancer in women that was suggested in some case-control studies was not confirmed in later prospective studies. The results of studies of pancreatic cancer, multiple myeloma, non-Hodgkin lymphoma, and uterine cancer did not support the hypothesis of an association with environmental exposure to the DDT complex e.g. in food. Under circumstances of heavy, prolonged occupational exposure to technical-grade DDT, an increased risk for pancreatic cancer could not be excluded.

The 1984 JMPR concluded that "there is no firm evidence that DDT has any reproductive or teratogenic effects". The effects of DDT on reproduction and development in humans and experimental animal have been reviewed. After treatment of rabbits with 3 mg/kg bw for 12 weeks, increased serum concentrations of DDT were found, but no adverse effects on reproductive outcome were observed. The relevance for human reproduction of slight changes in the ovulation rate, the relative proportion of uteroglobin, and progesterone concentrations in rabbits is not clear. After perinatal exposure to *para,para'*-DDE, there was some evidence of impaired sexual development in male pups, including an increased frequency of thoracic nipple retention and a reduction in the male anogenital distance, with a NOAEL of 10 mg/kg bw per day. The Agency of Toxic Substances and Disease Registry concluded that the DDT complex could impair reproduction and/or development in mice, rats, rabbits, dogs, and avian species at doses  $\geq 5$  mg/kg bw per day. The lowest relevant NOAEL for developmental effects was reported to be 1 mg/kg bw per day in rats.

Data of limited usefulness for human risk assessment indicated changes in spontaneous behaviour and brain muscarinic receptors in mice receiving DDT by a single oral administration of a dose of 0.5 mg/kg bw on postnatal day 10. Similar effects were not observed when this dose was administered on other postnatal days. Three multigeneration studies in rats and mice showed no reproductive effects at doses of 1–6.5 mg/kg bw per day.

Quantitative measurements of the transfer of DDE from pregnant or lactating rats or rabbits to their fetuses or suckling neonates showed that the concentrations in rabbit fetuses were much higher than those in blastocysts and that, in rats, lactation is a quantitatively far more important route than transplacental. The persistent DDT metabolite in animals, 3-methylsulfonyl-DDE, is a potent transplacental and transmammary adrenal toxicant in mice. Treatment of mice with a single dose of 3 mg/kg resulted in mitochondrial destruction in the adrenal zona fasciculata.

Few data were available on reproductive effects in humans, and the few that were provided showed no correlation between exposure to DDT and stillbirth, miscarriage, or premature rupture of fetal membranes. In a study of 859 children in the USA who were tested at the age of 3, 4, or 5 years, neither transplacental nor lactational exposure to DDT affected psychomotor or mental behavioural patterns or measures of school performance, even when the PTDI was exceeded.

Activation of estrogen receptors and inhibition of androgen receptors may be mechanisms of the action of DDT-related compounds which lead to the observed perturbations of reproductive function. The *para,para'*-DDE metabolite acts as an antiandrogen. DDE binds to the androgen receptor *in vitro* and inhibits 5-dihydrotestosterone-induced transcriptional activation with a potency similar to that of the antiandrogenic drug hydroxyflutamide. The results of competitive binding assays showed that *ortho,para'*-DDT, *ortho,para'*-DDD, *ortho,para'*-DDE, and *para,para'*-DDT bind to the human estrogen receptor but with an approximately 1000-fold weaker affinity than that of estradiol.

Numerous studies have been conducted on the effect of DDT on the immune system of laboratory animals. Because no validated study protocols were used in different species, at different doses, application periods, and routes of exposure, and with evaluation of different parameters, a reliable NOAEL could not be stimulated for effects on the immune system.

Pesticide applicators are exposed primarily to *para,para'*-DDT, whereas it is the *para,para'*-DDE metabolite to which the general population is exposed in the diet or drinking-water. Summaries of data on exposure and DDT concentrations in human tissues, milk, and blood have shown that the mean concentrations in populations have declined in much of the world, and the declines seen in various countries correspond to restrictions on DDT use. The available data on humans do not show causal relationships for carcinogenicity in any organ system or significant adverse health effects after repeated exposure to concentrations up to 0.25 mg/kg bw per day.

The newer studies and reviews provided the basis for a change by the present Meeting of the PTDI established in 1984. The Meeting derived a PTDI of 0.01 mg/kg bw on the basis of the NOAEL of 1 mg/kg bw per day for developmental toxicity in rats and a safety factor of 100.

DDT is no longer used in agricultural practice but may be present in food commodities as a contaminant because of its persistence in the environment. As peaks of acute dietary intake above the PTDI are not likely to occur, an acute RfD was not allocated.

#### *Levels that cause no adverse toxic effects*

- Rat: 125 ppm, equivalent to 6.25 mg/kg bw per day (study of carcinogenicity; JMPR 1984)  
1 mg/kg bw per day (developmental toxicity; review by the Agency of Toxic Substances and Disease Registry in 1994)
- Monkey: 10 mg/kg bw per day (7-year study in the diet; JMPR 1984)
- Humans: 0.25 mg/kg bw per day (overall NOAEL for humans; JMPR 1984)

#### *Estimate of provisional tolerable daily intake for humans*

0.01 mg/kg bw

*Estimate of acute reference dose*

Unnecessary

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